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Conception, mise en oeuvre et caractérisation de nouveaux bio-nano-matériaux fonctionnels.

Nathalie Lavoine

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THÈSE

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITÉ DE GRENOBLE

Spécialité : **Matériaux, Mécanique, Génie Civil, Electrochimie**

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préparée au sein du **Laboratoire Génie des Procédés Papetiers (LGP2),
UMR CNRS 5518**

dans l'École Doctorale **Ingénierie – Matériaux, Mécanique, Energétique,
Environnement, Procédés, Production (I-MEP2)**

Design, Processing and Characterization of innovative functional bio-nano-materials for packaging

Thèse soutenue publiquement le **15 novembre 2013**,
devant le jury composé de :

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HOW DO I LOVE YOU, THESIS? LET ME COUNT THE WAYS...



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Scientific Publications (2010 – 2013)

Scientific Journals

Nathalie Lavoine, Isabelle Desloges, Alain Dufresne and Julien Bras (2012) *Microfibrillated cellulose—its barrier properties and applications in cellulosic materials: A review*. Carbohydrate Polymers, 90(2), 735-764.

Nathalie Lavoine, Julien Bras and Isabelle Desloges. (2014) *Mechanical and barrier properties of cardboard and 3D packaging coated with microfibrillated cellulose*. Journal of Applied Polymer Science, DOI: 10.1002/APP.40106.

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Nathalie Lavoine, Isabelle Desloges, Bertine Khelifi and Julien Bras. *Impact of different coating processes of microfibrillated cellulose on the mechanical and barrier properties of paper*. Submitted to Journal of Materials Science, July 2013.

Nathalie Lavoine, Isabelle Desloges, Cécile Sillard and Julien Bras. *Controlled release and long-term antibacterial activity of chlorhexidine digluconate through the nanoporous network of microfibrillated cellulose*. Submitted to Applied Materials & Interfaces, July 2013.

Nathalie Lavoine, Isabelle Desloges, Brigitte Manship and Julien Bras. *Cardboard packaging with long-term antibacterial properties using microfibrillated cellulose*. Submitted to Food Control, September 2013.

Nathalie Lavoine, Nicolas Tabary, Isabelle Desloges, Bernard Martel and Julien Bras. *Controlled release fiber-based system using cyclodextrin and microfibrillated cellulose*. Submitted to Material Science Engineering-PartC, September 2013.

Nathalie Lavoine, Clara Givord, Nicolas Tabary, Isabelle Desloges, Bernard Martel and Julien Bras. *Elaboration of a new antibacterial bio-nano-material for food-packaging by synergistic action of cyclodextrin and microfibrillated cellulose*. Submitted to Journal of Controlled Release, September 2013.

Oral Presentations in Scientific Meetings

Nathalie Lavoine, Isabelle Desloges, Sandra Tapin-Lingua and Julien Bras (2012) *Nanocellulose films: Barrier properties and controlled release*. Intechfibres /Research in Fibres, Grenoble, France.

Nathalie Lavoine, Isabelle Desloges and Julien Bras (2012) *Functional Bio-nanomaterial for packaging applications*. MATBIM 2012, Dijon, France.

Nathalie Lavoine, Isabelle Desloges and Julien Bras (2013) *Microfibrillated cellulose: A nanoporous network for a controlled release of antimicrobial molecule*. 245th ACS, Division: Cellulose, New Orleans, USA.

Nathalie Lavoine, Clara Givord, Isabelle Desloges, and Julien Bras (2013) *Elaboration of a new antibacterial bio-nano-material for food-packaging application by grafting of cyclodextrine and coating of microfibrillated cellulose*. EPNOE Conference, Nice, France.

Communications in Scientific Meetings

Nathalie Lavoine, Isabelle Desloges and Julien Bras (2011) *Impact of different coating processes of MFC on barrier and mechanical properties*. Presented at the 2011 TAPPI International Conference on Nanotechnology for Renewable Materials, Arlington, USA (2nd Poster Award).

Nathalie Lavoine, Isabelle Desloges and Julien Bras (2012) *Caffeine release study in a microfibrillated cellulose nanoporous structure*. Presented at ACS - American Chemical Society Conference.

Raphaël Bardet, **Nathalie Lavoine**, Isabelle Desloges, Naceur Mohammed Belgacem and Julien Bras (2012) *Barrier properties of specialty papers coated with microfibrillated cellulose: influence of base paper and coating weight*. Presented at the 2012 TAPPI International Conference on Nanotechnology for Renewable Materials, Montreal, Canada.

Nathalie Lavoine, Virginie Bigand, Isabelle Desloges, Sandra Tapin-Lingua and Julien Bras (2013) *Use of different quality of MFC for producing controlled release films*. Presented at the 2013 TAPPI International Conference on Nanotechnology for Renewable Materials, Stockholm, Sweden (2nd Poster Award).

Julien Bras, Karim Missoum, **Nathalie Lavoine**, Artur Klamczynski, Isabelle Desloges, Naceur Belgacem, William J Orts and Alain Dufresne (2011) *Different Nanofibrillated celluloses and innovative strategies developed for their uses in coating or extrusion*. 16th International Symposium on Wood, Fiber and Pulping Chemistry, p. 407.

Abbreviations

AFM	Atomic Force Microscopy
βCD	Beta-Cyclodextrins
BC	Bar coating process
CA	Citric Acid
CNC	Cellulose nanocrystals
CHX	Chlorhexidine digluconate solution
FE-SEM	Field Emission Gun Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
LCA	Life Cycle Assessment
MFC	Microfibrillated cellulose
NCC	Nanocrystals of Cellulose
NFC	Nanofibrillated cellulose
OTR	Oxygen Transmission Rate
PE	PolyEthylene
PLA	PolyLactic Acid
SEM	Scanning Electron Microscopy
SP	Size press process
UV	Ultra-Violet
WVTR	Water Vapor Transmission Rate
WVP	Water Vapor Permeability

RESUME

RESUME FRANÇAIS – FRENCH EXTENDED ABSTRACT

De sa production à sa consommation, l'aliment représente aujourd'hui un bien particulièrement coûteux en énergie. Sa valeur moyenne a été estimée à 20 GJ, soit l'équivalent énergétique d'environ 40 000 barres de Mars ou 59 246 canettes de Coca-Cola (Figure 1).

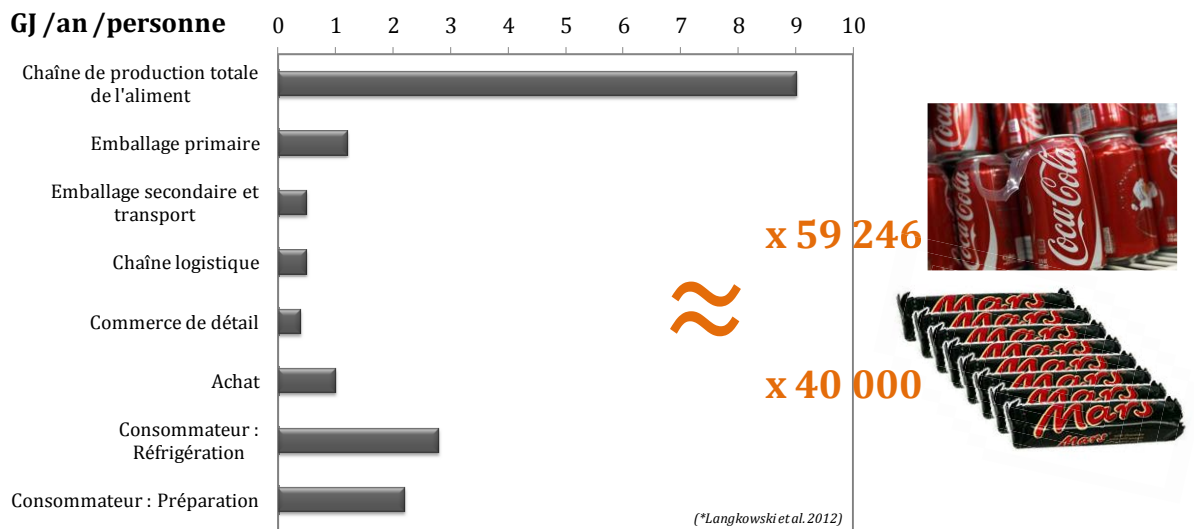


Figure 1. Estimation du coût énergétique d'un aliment (adapté d'une étude de Langkowski et al. 2012)

Malgré cela, chaque année, alors que 870 millions d'êtres humains ne mangent pas à leur faim tous les jours, un gaspillage de 1,3 milliards de tonnes de nourritures est enregistré. Ce gaspillage est non seulement une perte économique conséquente pour les producteurs (estimée à 750 milliards de dollars par an, d'après le dernier rapport de la FAO, Organisation des Nations Unies pour l'alimentation et l'agriculture¹), mais aussi un grave préjudice aux ressources naturelles dont l'humanité dépend.

D'après le rapport de la FAO, la nourriture produite et non consommée chaque année engloutit un volume d'eau équivalent au débit annuel du fleuve Volga en Russie, et est responsable du rejet dans l'atmosphère de 3,3 gigatonnes de gaz à effet de serre.

Ce gaspillage alimentaire à échelle mondiale aurait lieu pour 54% « en amont » de la chaîne, c'est-à-dire durant les phases de production, de manutention et de stockage après-récolte, et pour 46% « en aval », soit aux stades de la transformation, de la distribution et de la consommation. Selon l'étude de la FAO, plus la perte d'un aliment se produit tard dans la chaîne, plus l'impact environnemental est élevé. En effet, les « coûts » environnementaux occasionnés

¹ FAO. September 2013 Food waste harms climate, water, land and biodiversity – new FAO report. Online on: <http://www.fao.org/news/story/en/item/196220/icode/>.

lors de la transformation, du transport, du stockage et de la préparation se rajoutent aux coûts initiaux de production.

Un réel besoin ressort donc de cette première étude : la nécessité de protéger l'aliment. Malgré la diversité des matériaux d'emballages proposés aujourd'hui sur le marché, peu de solution existe. Ainsi, la recherche de nouveaux matériaux d'emballages est un sujet de première importance. Elle s'est tournée principalement vers le développement de matériaux bio-sourcés, biodégradables et recyclables, encouragée par des études d'analyses de cycle vie des matériaux, par la mesure de l'impact environnemental grandissant des déchets d'emballage et par la hausse du prix du pétrole ainsi que sa pénurie annoncée. La législation européenne, à cet égard, s'est affirmée par la parution de la Directive n°94/62/CE relative aux emballages et aux déchets d'emballages. Considérant qu'il convient d'éviter ou de réduire l'impact des emballages sur l'environnement, la législation impose une réduction du poids et du volume global d'emballages, encourage fortement le recours à des matériaux d'emballages réutilisables et recyclables et incite à l'utilisation de matériaux non nocifs ou de substances inertes pour l'environnement. A ce cahier des charges environnemental et législatif vient s'ajouter une exigence relative à la santé du consommateur. De récents évènements ont nourrit et accru l'inquiétude des consommateurs quant à leur sécurité alimentaire. Ces trois dernières années, des cas d'intoxications alimentaires ont été en effet révélés aux quatre coins du monde. En France, en 2011, huit enfants ont été hospitalisés suite à l'ingestion de steaks hachés surgelés contaminés par la bactérie *Escherichia coli*. Suite à cela, deux autres sont décédés après consommation de fast-food contaminé. Des cas similaires d'intoxication ont été relevés au Canada, et plus récemment (2013), en Inde, vingt-deux étudiants ont trouvé la mort après avoir ingéré des plats de cantines contenant des pesticides.

Le développement de nouveaux matériaux d'emballage bio-sourcés, biodégradables, recyclables, et dotés de nouvelles fonctionnalités permettant une meilleure protection de l'aliment et donc du consommateur, est par conséquent une problématique importante dans notre société actuelle. Bien que de nombreux projets de recherche soient aujourd'hui centrés sur cette thématique, très peu de ces matériaux ont encore vu le jour sur les marchés Européens. Dans la perspective d'apporter une nouvelle solution d'emballage répondant aux besoins sociétaux actuels, ce projet de thèse a été réalisé au sein du Laboratoire du Génie des Procédés Papetiers de Grenoble (LGP2, UMR CNRS 5518) dans le cadre d'un contrat doctoral avec mission d'enseignement.

Ce projet, intitulé, « **Conception, mise en œuvre et caractérisation de nouveaux bio-nano-matériaux fonctionnels pour le domaine de l'emballage** », a débuté le 1^{er} octobre 2010, et ce,

pour une durée de 3 ans. Il a été encadré par Dr. Julien Bras, Maître de Conférences à Grenoble INP-Pagora, et Dr. Isabelle Desloges, Enseignant-Chercheur à Grenoble INP-Pagora.

L'objectif de ce projet est de proposer une nouvelle solution d'emballage répondant aux principales exigences des consommateurs et de la société : bio-sourcé, biodégradabilité, recyclabilité, protection de l'aliment et du consommateur.

Dans l'optique de présenter un produit « vert », les matériaux papier et carton ont été choisis comme emballages de base.

L'apport de fonctionnalité et de propriétés a été conféré par l'utilisation stratégique d'un nouveau type de bio-nano-matériau, les microfibrilles de cellulose (ou MFC).

Produites initialement à partir de la fibre de cellulose suite à un traitement mécanique sous haute pression et fort cisaillement, les MFC ont l'apparence d'un gel visqueux blanc ou transparent (Figure 2), et présentent des dimensions nanométriques qui leur confèrent des propriétés intrinsèques extrêmement convoitées (diamètres compris entre 10 et 50 nm, et longueurs de l'ordre de 1 à 2 μm).

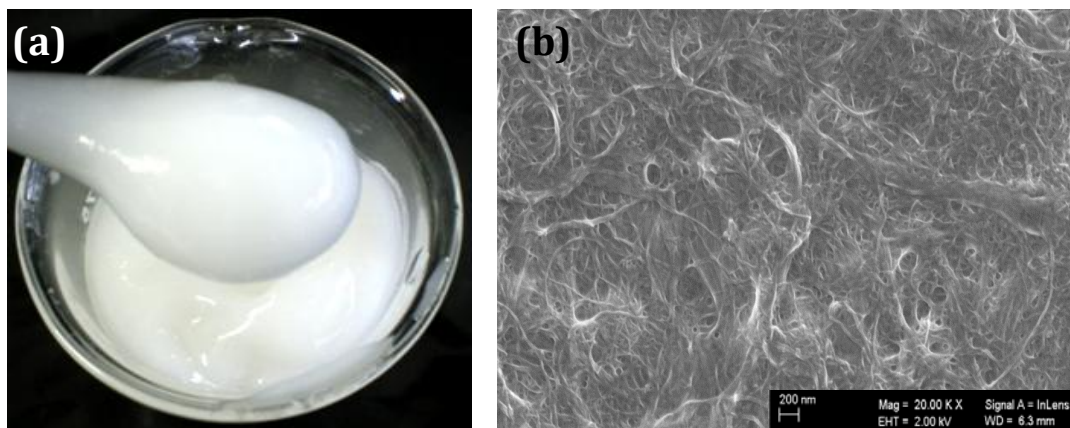


Figure 2. (a) Photo d'une suspension de MFC concentrée à 2% (en masse dans l'eau) produite à partir d'un mélange de pâte de pin et d'épicéa et (b) son image microscopique réalisée au microscope électronique à balayage (x 20.00K)

L'intérêt scientifique pour cette nouvelle matière augmente de manière exponentielle, comme le montre l'évolution du nombre cumulé de publications scientifiques sur le sujet (Figure 3), passant de 1403 publications/an à 4400 publications/an ces 5 dernières années (2007-2012).

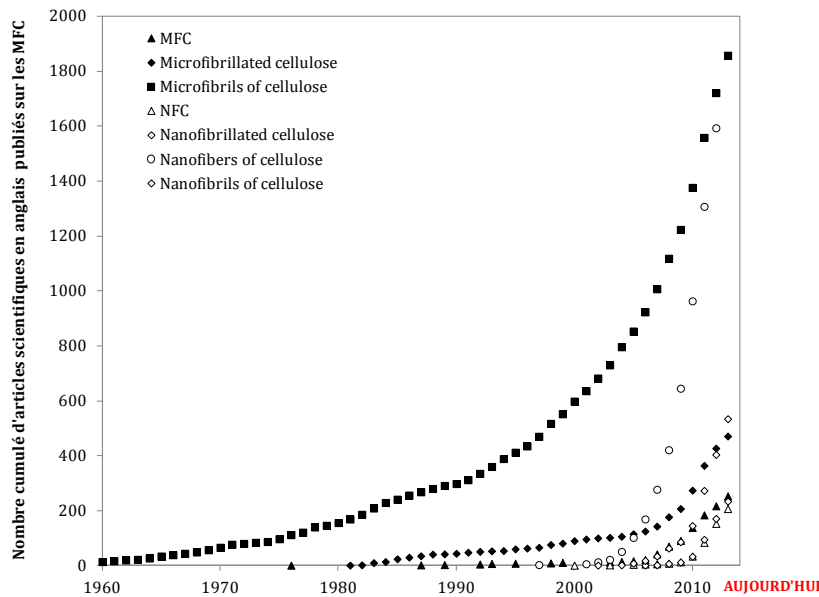


Figure 3. Nombre cumulé d'articles scientifiques publiés (en anglais) sur les MFC en fonction des mots clés employés pour les définir

Recherche effectuée en Septembre 2013 avec le moteur de recherche SciFinder

Aujourd'hui, de nombreuses sources cellulosiques sont étudiées et de plus en plus de procédés de fabrication sont mis au point pour produire une diversité de MFC considérable. Leur point commun reste leur structure géométrique, qui leur impose une configuration en réseau (Figure 2b). Ce réseau est extrêmement serré, maintenu par de nombreuses liaisons hydrogènes, et présente des nanopores qui rendent le matériau léger et flexible, quelle que soit sa forme (film, aérogel, suspension etc.).

Au début de ce projet de thèse, les MFC étaient essentiellement utilisées dans les composites pour renforcer leurs propriétés mécaniques et barrières, ou sous forme de films aux propriétés barrières et optiques compétitrices avec celles des polymères actuels. Leur utilisation avec les matériaux cellulosiques avait tout juste débuté avec quelques études portant sur l'enduction de formettes ou de papiers avec une suspension de MFC. Moins d'une dizaine d'études ont été recensées alors que les premiers résultats étaient prometteurs. En effet, les MFC déposées sur un matériau cellulosique améliorent considérablement les propriétés de résistance mécanique, de barrière à l'air ou aux graisses du matériau final.

En parallèle à ces applications, de premières études ont aussi commencé à entrevoir une utilisation possible des MFC dans l'élaboration de matériaux à haute valeur ajoutée, tels que les composés électroniques ou les aérogel pour usage médical. Tout l'intérêt des MFC repose sur leur réseau nanoporeux, conférant propriétés mécaniques, porosité et légèreté au matériau final souhaité.

Ainsi, face à ces constats, ce projet de thèse se veut innovant en proposant une toute autre application des MFC. Afin d'apporter une nouvelle fonctionnalité aux matériaux cellulosiques enduits de MFC, l'idée est d'incorporer dans ce réseau une molécule aux propriétés antibactériennes capable de se libérer au contact de l'aliment, afin de prolonger sa conservation. La stratégie principale de ce projet s'est divisée en trois étapes : la conception, la mise en œuvre et la caractérisation d'un nouveau bio-nano-matériau antibactérien (Figure 4).

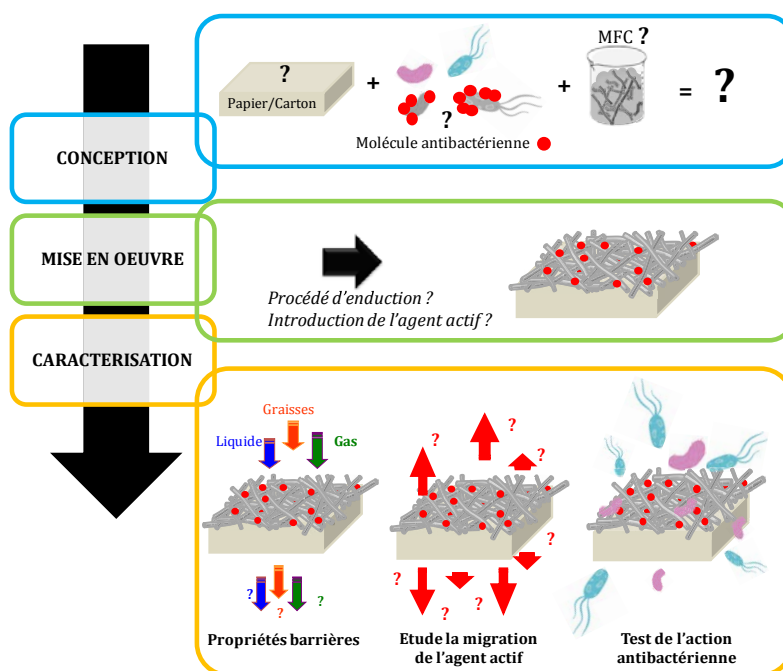


Figure 4. Stratégie globale du projet de thèse

La première étape consiste en la **conception du matériau** : comment réaliser un emballage antibactérien combinant les MFC, l'agent antibactérien et le substrat cellulosique tout en répondant aux exigences actuelles de la société ?

Cette étape a nécessité un travail bibliographique conséquent afin, dans un premier temps, de recenser les demandes et besoins actuels liés aux emballages alimentaires, et dans un deuxième temps, d'identifier les acteurs, les procédés et les propriétés attendus afin d'élaborer ce nouveau matériau.

La deuxième étape réside dans la **mise en œuvre** du matériau imaginé. Elaboration de nouveaux protocoles, recherche et application de nouveaux procédés sont les principales tâches constituant cette deuxième partie. L'idée est ici de sélectionner les procédés optimaux permettant la réalisation du nouveau matériau.

Enfin, le matériau préalablement conçu et mis au point est soumis à une dernière étape de **caractérisation**. L'objectif est ici de caractériser les propriétés actives finales du matériau pour s'assurer qu'il réponde bien au cahier des charges.

Cette stratégie de projet nous a finalement conduit à organiser ce manuscrit en quatre chapitres (Figure 5) : les trois premiers se réfèrent aux trois étapes évoquées, et le dernier offre des perspectives de développements au projet.

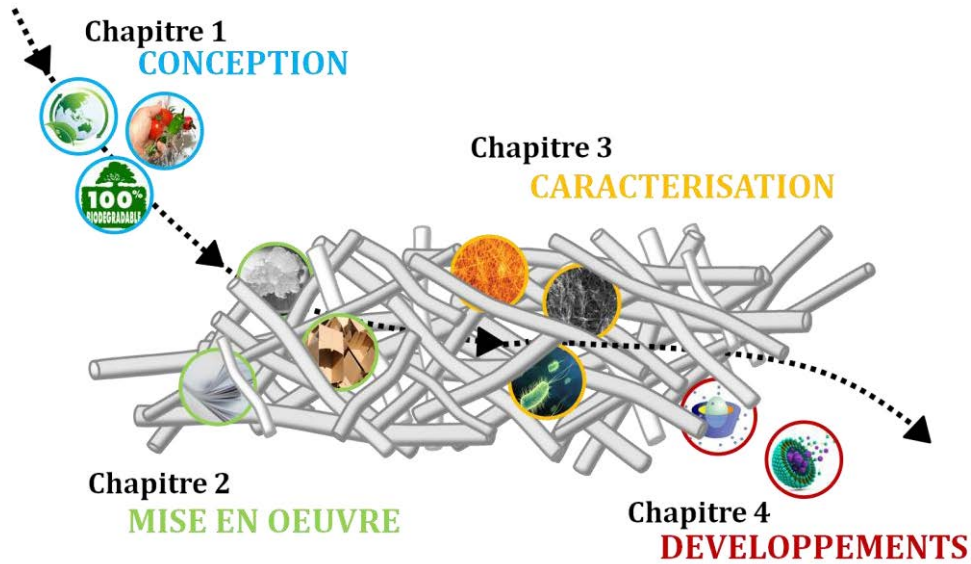


Figure 5. Organisation générale du manuscrit

Plus précisément, le **Chapitre 1** introduit le contexte de notre projet en rappelant les bases économiques, législatives, techniques et scientifiques liées aux emballages alimentaires et à leur développement. Ensuite les nouveaux biomatériaux ont été définis, pour finalement proposer une solution regroupant les principaux besoins sociétaux et palliant les difficultés rencontrées actuellement (Figure 6).

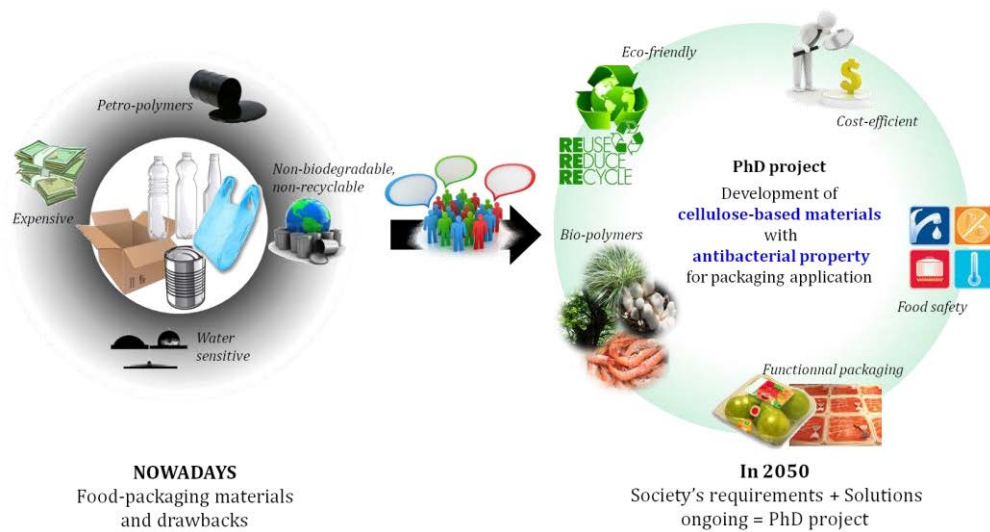


Figure 6. L'emballage alimentaire : des problématiques actuelles aux solutions envisagées

Ce chapitre s'est alors centré sur les microfibrilles de cellulose, bio-nano-matériau envisagé dans le cadre de ce projet comme composant de base d'un nouvel emballage alimentaire fonctionnel. Cette partie est extraite d'une revue détaillée (publiée dans *Carbohydrate Polymers*) sur les microfibrilles de cellulose et leurs propriétés barrières. Elle vise essentiellement à présenter les propriétés barrières des matériaux cellulosiques obtenues suite à leur association avec les MFC. Le chapitre 1 introduit enfin les premières études portant sur l'utilisation des MFC dans le développement de nouveaux matériaux fonctionnels, et fait référence aux tous premiers travaux (publiés ces trois dernières années) mettant en jeu le réseau nanoporeux des MFC dans la réalisation de nouveaux systèmes de libération de molécules actives ou de médicaments.

Ainsi, dans ce projet de thèse, les MFC seront utilisées pour 1) améliorer les propriétés mécaniques et barrières des matériaux papier/carton et 2) conférer à ces matériaux un système de libération contrôlée après introduction d'un agent actif.

Le **Chapitre 2** étudie en détail le premier point. L'objectif est double : (i) déterminer le procédé optimal d'enduction de MFC et (ii) analyser les propriétés mécaniques et barrières des matériaux enduits obtenus (Figure 7).

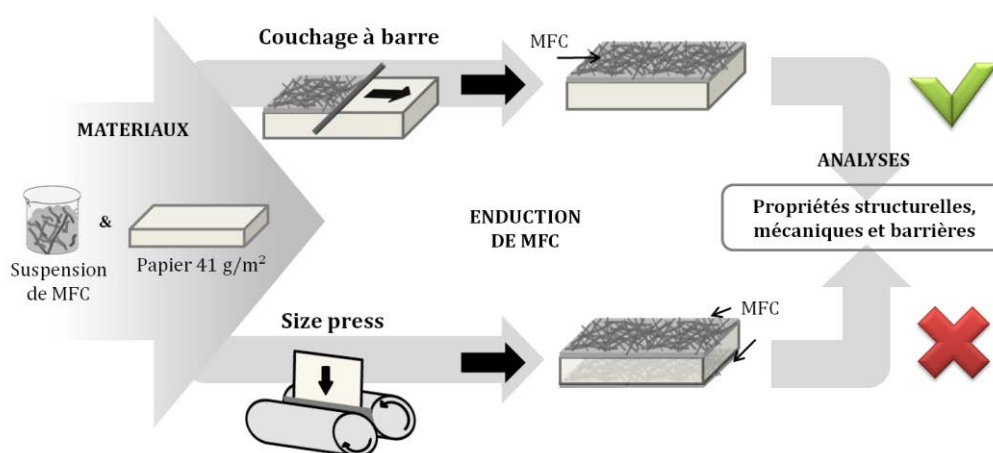


Figure 7. Stratégie du Chapitre 2, première partie

Deux matériaux cellulosiques ont été choisis : un substrat papier de 41 g/m² et un matériau carton de 300 g/m².

La première partie de ce chapitre (Article 1 soumis à *Journal of Materials Science*) consiste en la sélection du procédé optimal d'enduction des MFC. Deux procédés d'enduction ont été comparés : le couchage à barre et la size press (Figure 6). Une analyse détaillée des propriétés mécaniques et barrières des papiers couchés obtenus a été réalisée, sélectionnant le procédé de couchage à barre comme procédé optimal d'enduction en laboratoire des MFC. Ce procédé a en effet permis de déposer une quantité plus importante de MFC, bien que cinq

passages successifs soient nécessaires, afin d'améliorer les propriétés mécaniques et barrières du matériau final.

Cette première partie a aussi mis en évidence l'impact négatif des cycles de mouillage et séchage successifs imposés par le protocole de couchage. La suspension de MFC étant composée à 98% d'eau (pour 2% de microfibrilles), son couchage induit un apport en eau considérable au matériau papier qui peut être très préjudiciable selon le matériau fibreux support. Après le dépôt de cinq couches successives de MFC, le matériau cellulosique a donc subi cinq cycles de mouillage et séchage. Une perte importante de ses propriétés mécaniques a été observée, mais celle-ci fut en partie contrebalancée par le couchage des MFC.

Suite aux résultats obtenus avec le matériau papier, une étude similaire a été réalisée avec le matériau carton (Article 2 publié dans *Journal of Applied Polymer*). Le couchage à barre, procédé sélectionné dans la première partie, a donc été utilisé dans cette deuxième partie pour enduire le carton de MFC.

En raison du fort grammage du carton de référence (300 g/m²), l'apport de 8 g/m² de MFC (soit 5 couches successives comme précédemment étudié) n'améliore que légèrement les propriétés mécaniques et barrières de ce dernier. Cependant, malgré l'apport d'eau considérable, les propriétés finales obtenues ont été aussi bonnes que celles d'un même carton couché avec 17 g/m² de polyéthylène. Ainsi, le couchage des MFC a permis d'apporter une solution concurrentielle bio-sourcée à l'actuel produit complexe, sans pour autant augmenter le poids du matériau, répondant en cela aux besoins sociétaux de développement durable. Il permet donc d'envisager ainsi un emballage 3D identique mécaniquement au carton de référence mais qui pourrait alors être doté d'une nouvelle fonctionnalité via la couche de MFC.

Le chapitre 2 permet dans un premier temps de comprendre et de détailler l'influence des MFC sur les propriétés mécaniques et barrières des matériaux cellulosiques enduits. L'amélioration des propriétés grâce aux MFC sera d'autant plus importante que la quantité sèche déposée sera conséquente. Cependant, il est encore délicat aujourd'hui d'optimiser l'enduction de matériaux cellulosiques avec les MFC : l'objectif serait d'obtenir, en une seule fois, un poids de couche au moins égal à 8 g/m², ce qui est semble difficile partant d'une suspension de MFC à 2 % en masse.

Dans un deuxième temps, ce chapitre 2 souligne un autre intérêt du couchage de MFC. Dans le cas du carton, par exemple, cette couche a permis l'apport de matière sans augmenter la densité du matériau final. L'analyse microscopique de cette couche a par ailleurs révélé la formation d'un réseau nanoporeux de MFC couché à la surface des deux substrats cellulosiques (Figure 8).

Le réseau des MFC, présent en l'état de suspension, a donc été conservé lors du procédé de couchage. Ce résultat primordial n'avait pas été démontré jusqu'à présent.

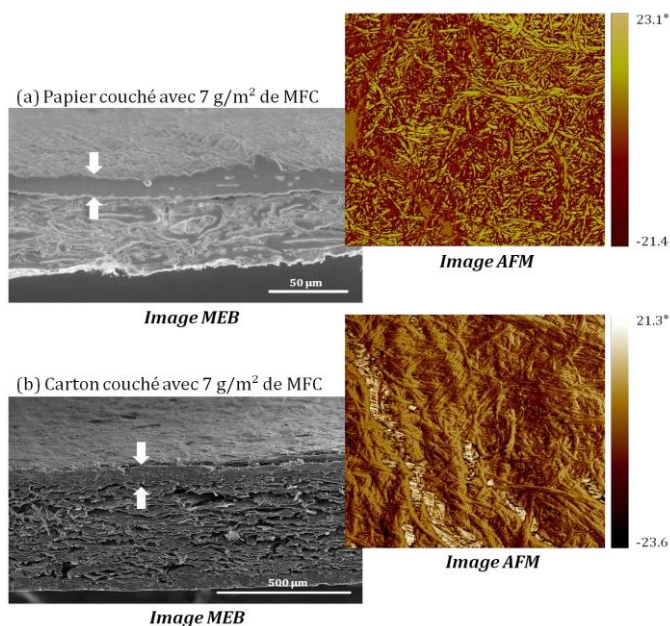


Figure 8. Analyses microscopiques du réseau de MFC couchées sur un papier (a) et sur un carton (b). Des images réalisées au microscope électronique à balayage (MEB) ont mis en évidence la couche de MFC en surface des deux matériaux cellulosiques. Les images réalisées au microscope à force atomique (AFM) ont souligné la configuration en réseau nanoporeux de ce couchage.

L'objectif du **Chapitre 3** est alors de démontrer que le réseau de MFC formé à la surface des matériaux cellulosiques couchés peut être utilisé comme système de libération contrôlée de molécules actives et conférer, dans le cas de cette étude, une fonctionnalité antibactérienne au support fibreux.

La première partie (Article 3, accepté pour publication à *Carbohydrate Polymers*) est une étude de faisabilité. A partir d'une molécule modèle, la caféine, il est démontré que le réseau de MFC est en mesure de libérer plus progressivement et plus lentement les molécules de caféine dans un milieu aqueux.

La caféine a été introduite dans le matériau papier suivant deux stratégies : par imprégnation du matériau papier suivi d'un couchage de MFC, et par couchage, après incorporation dans la suspension de MFC. Suite à la mise en place de deux protocoles expérimentaux, l'influence des MFC sur la libération de la caféine en milieu aqueux a été étudiée.

Ainsi, une cinétique de libération en fonction du temps a montré que la couche de MFC permettait de libérer des proportions moindres de caféine au cours du temps, prolongeant ainsi la durée totale nécessaire pour libérer toutes les molécules.

Le deuxième protocole (Figure 9) a permis de mettre en évidence la stratégie optimale d'introduction de caféine afin d'obtenir le système de libération le plus « efficace ».

Ce protocole consiste à étudier la libération de l'agent modèle (ici, la caféine) toutes les dix minutes, après renouvellement du milieu aqueux de libération. La Figure 9 présente ainsi la quantité libérée de caféine (rapportée à la quantité initiale introduite dans l'échantillon papier en %) en fonction du nombre accumulé de changements de milieu.

Cette représentation nous permet dans un premier temps de confirmer le résultat clé obtenu lors de la première cinétique de libération : la présence de MFC permet de libérer la caféine plus progressivement.

Par ailleurs, ce graphique permet d'opter pour un système optimal de libération contrôlée. En effet, la molécule, directement introduite dans la suspension de MFC (courbe noire), est plus longtemps et plus progressivement libérée que la molécule imprégnée dans un premier temps dans le matériau papier puis recouverte de MFC (courbe grise).

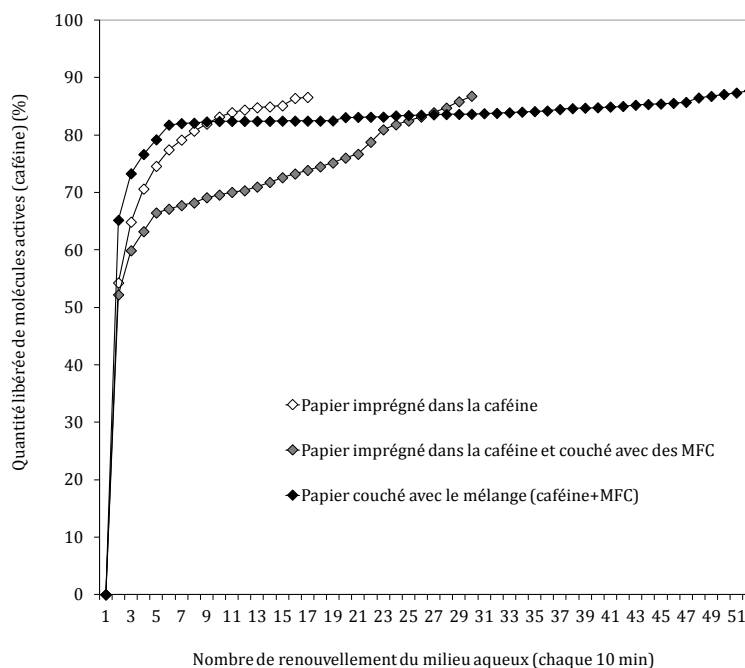


Figure 9. Libération par intervalle de temps successif de l'agent actif (caféine) dans l'eau en fonction du nombre de changement de milieu effectué chaque 1 h

La deuxième partie du chapitre 3 met en application cette preuve de concept avec une molécule modèle antibactérienne, le digluconate de chlorhexidine (CHX) (Article soumis à *Applied Materials & Interfaces*).

Les études de libération en milieu aqueux ont confirmé les premiers résultats obtenus avec la caféine : les MFC ont libéré plus progressivement et plus lentement le CHX (cinétique de libération divisée par trois). Ces résultats sont d'autant plus prometteurs que des tests similaires ont été réalisés avec une couche modèle de latex (liant le plus couramment utilisé dans les sauces de couchage développées en papeterie). Contrairement aux MFC, le latex a empêché la libération du CHX en milieu aqueux et a ainsi limité toute action antibactérienne.

En effet, suite aux tests antibactériens réalisés avec une bactérie non pathogène, *Bacillus subtilis*, les proportions libérées dans le cas de la couche de latex ont été insuffisantes pour conférer une action antibactérienne aux matériaux papiers. *A contrario*, les échantillons couchés avec les MFC ont présenté une forte activité contrôlée dans le temps. Elles ont ainsi montré un réel effet sur le contrôle de la libération du CHX : avec une couche de 7 g/m² de MFC, l'action antibactérienne a été prolongée de 9 jours. Lorsque le CHX a directement été introduit dans la couche de MFC, l'action antibactérienne a duré 15 jours supplémentaires (Figure 10).

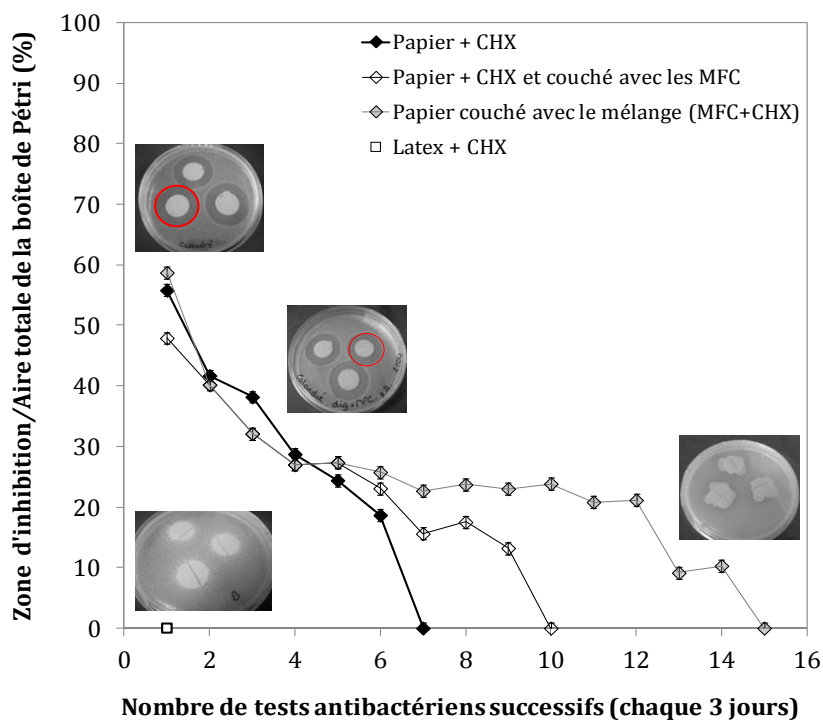


Figure 10. Tests antibactériens successifs réalisés avec des échantillons papiers contenant du CHX, avec et sans MFC. L'activité antibactérienne est observée par la présence d'une zone d'inhibition autour de l'échantillon papier (zone encerclée en rouge sur les boîtes de Pétri après 3 et 9 jours de tests respectivement).

L'échantillon témoin couché avec du latex n'a montré aucune activité antibactérienne malgré la présence du CHX.

Le protocole suivi est une adaptation de la norme AFNOR EN 1104 (méthode de diffusion sur agar).

Nous avons montré que la couche de MFC, grâce à son réseau nanoporeux, était en mesure de libérer progressivement une molécule active tout en conservant sur le long terme son action antibactérienne. Afin de relier ce système au domaine de l'emballage alimentaire, une troisième partie a donc été consacrée à l'élaboration d'un emballage en carton mis en volume à effet antibactérien prolongé. (Article soumis à *Food Control*)

La stratégie consistant en l'incorporation directe de la molécule active dans la suspension de MFC a montré des résultats plus prometteurs en termes de système de libération contrôlée. Par conséquent, le carton de 300 g/m², initialement étudié dans le chapitre 2, a été enduit, de façon similaire au matériau papier, avec un mélange (CHX+MFC).

Tout comme dans le chapitre 2, le matériau carton a été mis en 3D afin de réaliser des tests alimentaires. Ces tests ont été effectués sur le foie porc, viande très sensible dont la dégradation est rapidement et aisément caractérisable. Le carton couché avec les MFC et le CHX prolongea l'état de la viande plus efficacement et plus longtemps (durant 1 semaine au réfrigérateur) que le carton de référence ou le carton couché avec du polyéthylène (Figure 11).

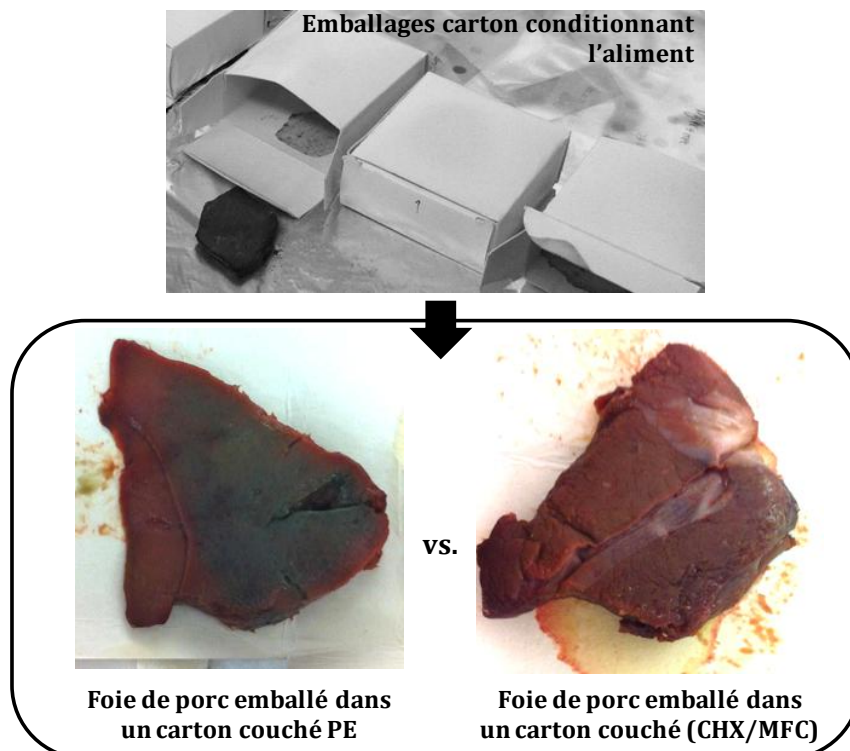


Figure 11. Tests alimentaires sur foie de porc. Le carton couché avec le CHX et les MFC a préservé la fraîcheur du foie (couleur rouge) contrairement au carton couché avec du polyéthylène (PE).

Suite à ces résultats prometteurs, nous avons souhaité, dans le **Chapitre 4**, améliorer la propriété de libération contrôlée de ce nouveau matériau afin d'élargir son champ d'application à d'autres secteurs tels que le biomédical.

Dans cette perspective, nous avons développé une collaboration avec le laboratoire UMET (Unité Matériaux et Transformation, UMR CNRS 8207), département d'Ingénierie des Systèmes Polymères, spécialisé dans l'étude et l'utilisation des cyclodextrines.

La cyclodextrine, oligosaccharide d'unités glucosidiques liées par liaison α -1,4, est généralement utilisée comme additif ou greffée sur des matrices polymériques ou des matériaux textiles. Par sa configuration particulière qui lui permet de se complexer avec des molécules hôtes par interactions chimiques (notion de « complexe d'inclusion »), elle a fait ses preuves dans le domaine de la libération contrôlée de médicaments (ex : application pour des prothèses), d'agents antibactériens (ex : textiles médicaux) ou d'insecticides (ex : moustiquaires plastiques). Son application avec les matériaux cellulosiques reste cependant encore rare. Un seul article portant sur l'élaboration d'un système de libération contrôlée basé sur un papier greffé avec des cyclodextrines a pour l'instant été recensé.

L'idée de ce chapitre 4 est donc d'associer les MFC avec la cyclodextrine afin de développer un système de libération contrôlée de molécules actives fonctionnant sur l'action synergique de ces deux composés.

Deux stratégies ont été envisagées :

- (i) Le couchage de la cyclodextrine sur le matériau papier avec et sans suspension de MFC (article soumis à *Material Science Engineering-PartC*)
- (ii) Le greffage du matériau papier avec la cyclodextrine, suivi du couchage de MFC. (article soumis à *Journal of Controlled Release*)

La stratégie (i) fait l'objet de la première partie du chapitre 4. Suite aux résultats obtenus dans le chapitre 3, il a semblé pertinent d'introduire dans le réseau nanoporeux de MFC le complexe d'inclusion comprenant la cyclodextrine et la molécule active. La solution de digluconate de chlorhexidine (CHX) a, ici encore, été utilisée. L'inclusion de ce composé actif dans la cyclodextrine a en effet été prouvée dans plusieurs études précédentes.

Des études de libération, basées sur les protocoles explorés dans le chapitre 3, ont été réalisées analysant l'influence des MFC, de la cyclodextrine et du mélange sur la libération du CHX dans l'eau.

Comme pressenti, les MFC et la cyclodextrine ont permis de libérer plus progressivement le CHX dans l'eau. Cependant, aucune synergie n'a été observée entre ces deux composés : la quantité libérée de CHX a été, avec les MFC tout comme la cyclodextrine, libérée en 24 h.

Cette étude a néanmoins révélé un résultat tout à fait surprenant qui a orienté l'objectif premier de cette partie vers une perspective d'application très prometteuse.

En effet, contre toute attente, l'échantillon couché avec le mélange (cyclodextrine + CHX) s'est révélé le plus efficace en terme de système de libération contrôlée, alors qu'aucun greffage

de la cyclodextrine n'a été réalisé. Ceci est dû au complexe (cyclodextrine :-CHX) qui bénéficie de beaucoup plus d'interactions avec la cellulose que les molécules seules de CHX ou de cyclodextrine (Figure 12). Cependant, il est aussi important de préciser que cette application n'aurait pu être possible sans la présence d'une molécule telle que le CHX, qui présente d'ores et déjà de fortes interactions avec la cellulose (hydrogène et ionique).

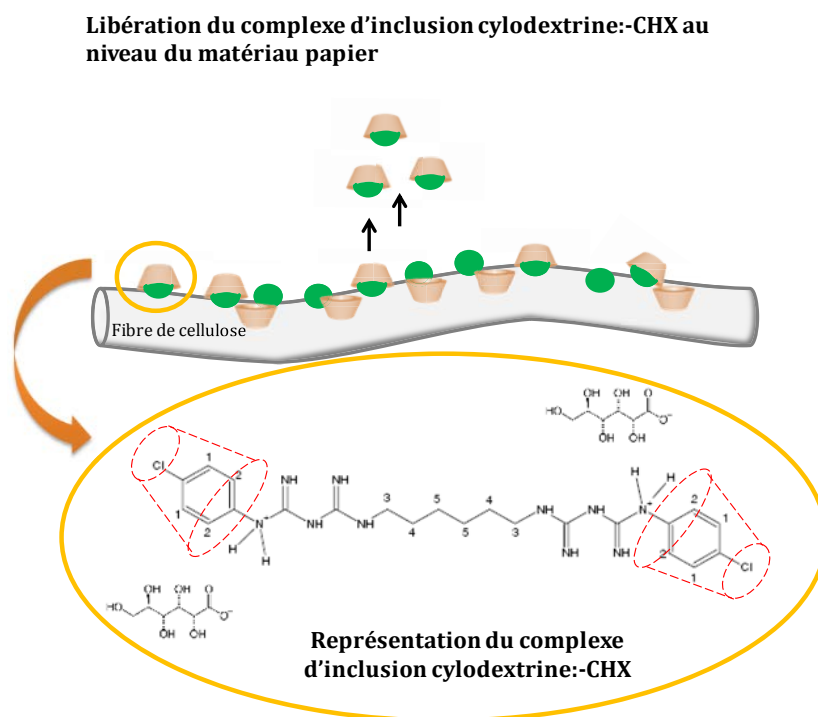


Figure 12. Mécanisme de libération du CHX complexé avec la cyclodextrine et couché sur le matériau papier. La structure chimique du complexe d'inclusion cyclodextrine :-CHX est représentée afin de souligner les interactions chimiques existantes.

Par ailleurs, dans ce cas particulier, la libération du CHX implique la libération de tout le complexe (qui reste toutefois antibactérien). Par conséquent, une fois la libération de l'agent actif terminée, le matériau papier perd sa fonctionnalité et ne peut, éventuellement, être réutilisé.

Le sous-chapitre 4.1 a donc montré qu'il est possible de couler simplement la cyclodextrine avec le CHX sur le matériau papier afin d'obtenir un système de libération contrôlée sur le long terme, mais visant une utilisation unique. Bien que les MFC aient une surface spécifique importante, aucune synergie n'a été observée avec la cyclodextrine, probablement due aux faibles quantités introduites en cyclodextrine et en CHX. L'activité antimicrobienne du complexe (cyclodextrine :-CHX) peut aussi être remise en question pour des bactéries plus résistantes que la *Bacillus subtilis* utilisée dans ce projet.

Afin de pallier la libération de la cyclodextrine et de tout le complexe en milieu aqueux, la **deuxième partie** de ce chapitre présente donc la stratégie (ii): le greffage du papier avec de la cyclodextrine, suivi de son couchage avec les MFC.

Le greffage du matériau papier avec la cyclodextrine a été réalisé selon un protocole développé par le laboratoire UMET, qui consiste en l'imprégnation du matériau papier dans une solution de cyclodextrine, d'acide citrique et d'un catalyseur, suivi d'un séchage à haute température (160°C). Ce protocole de greffage est effectif, puisque 10% de cyclodextrines peuvent être greffées sur un papier de 40 g/m². Cependant, notre étude a montré que l'acide citrique utilisé dans ce procédé dégrade considérablement les propriétés mécaniques du matériau final et le couchage des MFC n'a pu que très légèrement contrebalancer cet impact.

Pourtant, contrairement à la première stratégie, le greffage de la cyclodextrine suivi du couchage des MFC aboutit à une synergie entre les deux composants.

Dans cette deuxième partie, une autre molécule antibactérienne a été utilisée, le carvacrol. Cette molécule naturelle, extraite des huiles essentielles de thym ou d'origan, a des propriétés antibactériennes reconnues. Cependant, son caractère hydrophobe et volatile ralentit fortement son utilisation avec des matériaux hydrophiles tels que le papier. Son inclusion avec la cyclodextrine constitue donc une solution.

Le carvacrol a été introduit dans le matériau papier greffé avec de la cyclodextrine selon deux stratégies. La première consiste en la diffusion dans l'air de la molécule dans le matériau greffé. Cependant, la quantité de carvacrol diffusée dans le matériau papier ne lui confère aucune propriété antibactérienne.

La deuxième stratégie donne des résultats plus concluants. Le matériau papier greffé a été imprégné dans un bain de carvacrol et d'éthanol. L'éthanol, un solvant du carvacrol, a permis de contrôler la concentration de la solution d'imprégnation en agent actif et de limiter l'aspect « huileux » du papier final (sans pour autant altérer les propriétés antibactériennes conférées au matériau final).

Afin de simuler le contact alimentaire dans la perspective d'une application dans le secteur de l'emballage alimentaire, des tests de libération sur agar (considéré comme aliment modèle) ont été réalisés avec les échantillons papiers greffés contenant du carvacrol, avec et sans MFC. Si le papier simplement greffé avec de la cyclodextrine est actif pendant près de 14 h, l'ajout de MFC prolonge son activité de 2 h de manière reproductible (Figure 12).

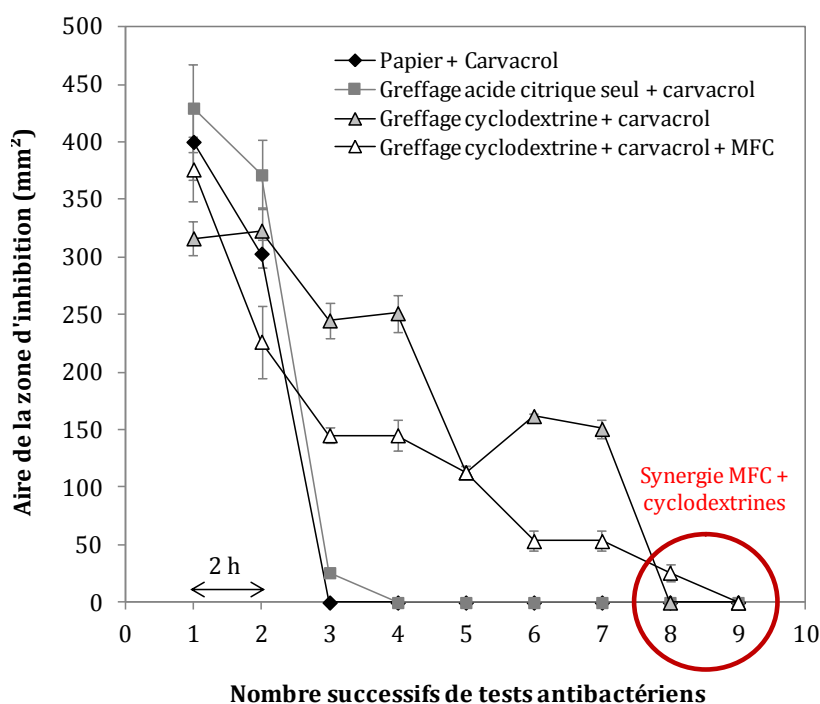


Figure 13. Tests antibactériens soulignant la synergie obtenue entre le greffage de la cyclodextrine et le couchage des MFC sur la libération du carvacrol.

L'aire de la zone d'inhibition proportionnelle à l'action antibactérienne des échantillons est exprimée en fonction du nombre de tests réalisés successivement jusqu'à épuisement du carvacrol.

Le papier simplement imprégné dans le carvacrol et la référence greffée avec l'acide citrique (sans cyclodextrines) ont été actifs pendant une période équivalente à 4h. Le papier greffé avec la cyclodextrine (incluant aussi le greffage de l'acide citrique) a été actif pendant une période d'environ 14h, période prolongée de 2h par le couchage des MFC.

Une légère synergie de libération est donc observée entre les MFC et la cyclodextrine (zone encerclée sur la Figure 12). La cyclodextrine permet le contrôle de la libération du carvacrol dans toute l'épaisseur du papier, et les MFC agissent par la suite exclusivement en surface, prolongeant davantage l'action antibactérienne de l'échantillon.

Cette complémentarité aurait été d'autant plus efficace si la couche de MFC avait été réalisée dans des conditions de séchage à température plus élevée. En raison de la présence de carvacrol, les couches de MFC n'ont pas pu être séchées à 105°C (température prévue dans le

protocole développé dans le chapitre 2), afin d'éviter la perte massive d'agent antibactérien. Par conséquent, le réseau nanoporeux des MFC s'est révélé être beaucoup moins lié, ce qui s'est confirmé lors des études de libération en milieu aqueux : la couche de MFC a commencé à se déliter.

Ce chapitre 4 propose un nouveau modèle de système de libération contrôlée fondé sur l'utilisation conjointe des MFC et de la cyclodextrine.

Un compromis reste cependant à trouver pour 1) développer un système de libération prolongée reposant sur l'action complémentaire des MFC et de la cyclodextrine et pour 2) obtenir un matériau final aux propriétés mécaniques et barrières nécessaires et suffisantes pour une application dans l'emballage alimentaire ou dans le biomédical.

Ce projet de thèse a mis en avant une toute nouvelle utilisation des MFC couchées sur matériaux cellulosiques pour des applications dans le secteur de l'emballage alimentaire ou le biomédical. Grâce au réseau de MFC, structure clé de ce bio-nano-matériau, un nouveau système de libération contrôlée de molécules actives a en effet été développé à partir de matériaux papier/carton.

Ce système a été testé et validé avec différents types de molécules actives et différents substrats cellulosiques, montrant, à chaque fois, une réelle efficacité en matière de libération prolongée et progressive d'un agent actif.

Ce projet de thèse a donc mis en évidence plusieurs points, ouvrant sur des perspectives nouvelles d'élaboration de bio-nano-matériaux fonctionnels :

- 1) Le **Chapitre 2** met en exergue l'importance de certains paramètres à considérer dans le couchage de MFC sur substrats cellulosiques. Non seulement la concentration initiale de la suspension est un facteur clé (essentiellement due à sa haute teneur en eau), mais les propriétés initiales du matériau de référence à enduire sont aussi déterminantes sur la qualité du matériau final souhaité.
- 2) Le **Chapitre 3** montre que le réseau nanoporeux des MFC, observé dans la suspension, est conservé et préservé une fois la suspension couchée et séchée sur le matériau cellulosique. Ce réseau se révèle être l'élément clé dans l'élaboration d'un système de libération prolongée de molécules actives.
- 3) Enfin, le **Chapitre 4** suggère une perspective d'amélioration pour ce nouveau bio-nano-matériau fonctionnel. L'association de cyclodextrines et de MFC a en effet montré une synergie possible, ouvrant la voie vers des développements de systèmes de libération contrôlée encore plus performants.

Enfin, ce projet de thèse n'aurait pu être réalisé et aboutir sans les nombreuses collaborations que nous avons initiées au cours de ces trois années.

Une première collaboration avec le FCBA (Grenoble), nous a permis d'avoir des suspensions de MFC variées et de très bonne qualité.

Une deuxième collaboration a été réalisée avec le laboratoire UMET de Lille, permettant le développement et l'aboutissement de l'étude avec les cyclodextrines (Chapitre 4).

Enfin, dans la continuité de ce projet, une dernière collaboration a été mise en place avec l'Université de Montpellier II afin de développer une étude plus fondamentale et théorique sur les mécanismes de libération et de diffusion des molécules actives étudiées.

Si ce projet de thèse établit les bases du développement expérimental d'un nouveau système de libération, nous avons souhaité poursuivre par une analyse plus fondamentale des phénomènes de transfert réalisés lors de la diffusion des molécules actives en milieu aqueux.

Ce dernier projet est actuellement en cours, mais le contexte de l'étude ainsi que des premiers résultats sont présentés en annexe de ce manuscrit.

Nous espérons que cette dernière partie contribuera au développement plus poussé de tels systèmes de libération, et aidera à la compréhension des phénomènes de diffusion s'effectuant au sein d'un réseau si complexe, que représente les MFC.

Outre cette modélisation des phénomènes de diffusion, plusieurs perspectives s'offrent à nous :

- (i) Une optimisation du couchage des MFC en travaillant sur le taux de matière sèche, le type de procédé (couchage rideau, couchage mousse) mais aussi, sur les phénomènes de séchage des couches de MFC (notion de sur-séchage et de densité de réseau) ;
- (ii) Une évaluation des différentes qualités de MFC et notamment, les MFC fortement chargées, de type TEMPO ;
- (iii) La réalisation de tests antibactériens avec des bactéries plus résistantes, mais aussi avec d'autres couples antibactérien/MFC et d'autres situations alimentaires (autre que le foie) ;
- (iv) Une optimisation du greffage de la cyclodextrine (avec des MFC TEMPO par exemple) pour limiter l'impact de l'acide citrique ou l'utilisation de polymère de cyclodextrine en mélange avec les MFC.

Tout ceci prouve à la fois le caractère innovant et l'intérêt des résultats présentés dans ces travaux de thèse qui devraient permettre un développement de nouveaux biomatériaux fonctionnels, répondant ainsi aux attentes de notre société.

GENERAL INTRODUCTION

General Introduction

Food is scarce and very valuable. Ever since time began, people have fought to grow it, to produce it and to share it; firstly for a matter of survival; more and more for a matter of commercialization.

According to many studies, the total energy used per consumer for the food production to its consumption is high. It indeed reached about 20 GJ per year and per person (Langowski, 2012), i.e. an equivalent to about 40,000 Mars bars or 12,070 km in a car with a 1.4 L engine (Figure 1). We must, thus, protect the food that we grow and convert into high added value product.

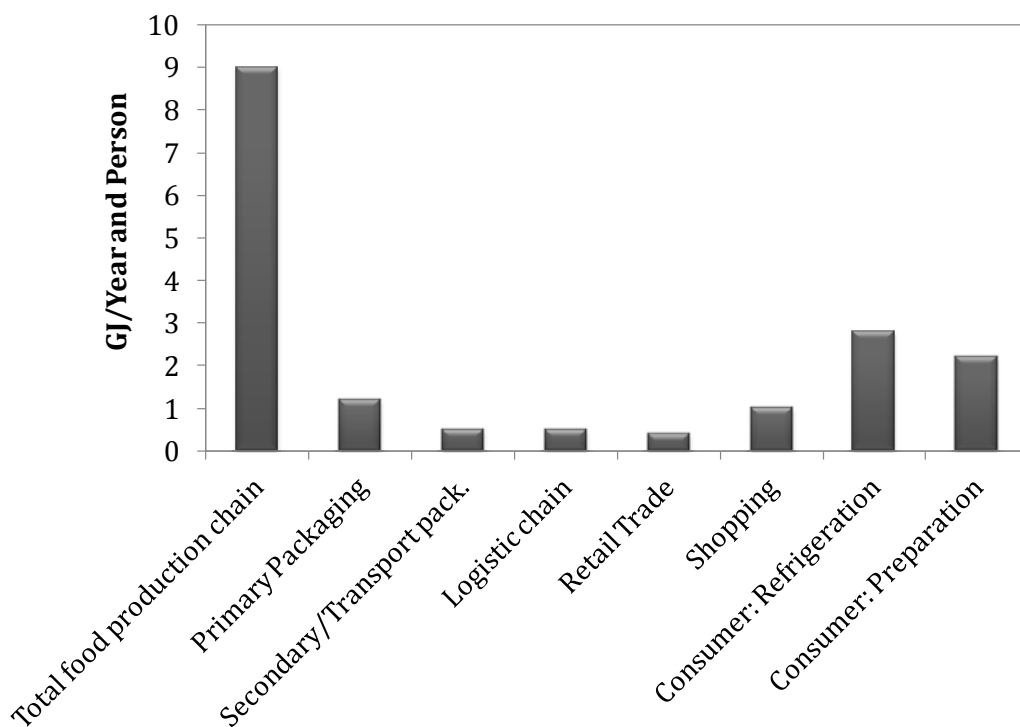


Figure 1. Energetic consumption of food (adapted from Langowski, 2012)

Whereas the current society blames the packaging materials for being responsible of the arising amount of wastes and pollution, different studies proved that the Life Cycle Assessment (LCA) of the food product is much higher than the LCA of the packaging itself (Büsser and Jungbluth, 2009). Furthermore, a very recent study (September 2013) of the Food and Agriculture Organization of the United Nation highlighted the environmental impact of the global food wastage (FAO, 2013). Its key findings showed for example that the volume of food produced and not eaten is equivalent to the annual flow of Russia's Volga and is responsible for

adding 3.3 billion tones of greenhouse gases to the planet's atmosphere. Beyond this environmental impact, the producers are also subjected to a direct economic loss, which stands at \$750 billion annually.

Solutions are thus required to enhance the protection of these valued nutritional substances. It is necessary to take care of the foodstuff, even more when 870 million people go hungry every day (FAO, 2013). One solution could be the development of new packaging materials.

The elaboration of new kinds of food-packaging materials is a current concern with several targets. On one hand the **arising amount of packaging material wastes** and their **environmental impact** on the planet could limit their development. As a result, the European Union has instituted legislation on the packaging and packaging waste (1994) focusing on the limitation of weight and volume of packaging, the reduction of the content of hazardous substances and materials in the packaging material and its components, and the design of reusable and recoverable packaging. In parallel, each country has also established complementary laws on this thematic focusing on the improvement of the environment protection such as the Grenelle laws in France (Ministère de l'Ecologie, 2007-2012) or the municipal waste management of the Norwegian Pollution Control Act (EIONET, 2013, Kjaer, 2013).

On the other hand, the **protection of the consumer health** is another main purpose promoting innovative solutions like active packaging. Indeed, despite the growing worldwide industrialization and standard way of life, the risk of food poisoning is still present with new cases registered over these last years. For example, in 2011, in France, eight hospitalized children were contaminated by the bacteria *Escherichia coli* after eating frozen beef steak (Louet and Savary, 2011) and two children died after eating junk food. More recently, in Canada, another case of food poisoning was also registered on at least twelve people, who became seriously sick after eating the famous "cronut burger" produced in Toronto (Hiscott, 2013). In a small village in the poverty-stricken state of Bihar (Eastern Indian), the consequence of the food-poisoning was more drastic: twenty-two students died and dozens were hospitalized after eating school meals that may have been contaminated with pesticides (Burke, 2013).

The food-poisoning is thus a serious and current concern, affecting both developed and developing countries.

Food wastage, environmental impact, food and consumer safety are the three main issues, which lead us to develop a new kind of functional bio-material for food-packaging within the framework of this PhD.

This project, titled “**Design, processing and characterization of innovative functional bio-nano-material for packaging**”, was developed at the Laboratory of Pulp & Paper and Graphic Arts (LGP2), a joint research center (UMR 5518) of CNRS (National Center for Scientific Research), in Grenoble (France). It was conducted by a 3 year doctoral contract financed by the Grenoble Institute of Technology (with a monitorship subcontract as assistant professor), and supervised by the Associate Professor Julien Bras and the research engineer Dr. Isabelle Desloges.

The objective of this PhD project was, thus, to design and develop a sustainable, biodegradable paper-based bio-material with antibacterial properties, which aims to improve, extend or maintain the shelf-life of the food.

In this regard, we selected three cellulosic materials: two papers of about 40 g/m², one was not treated and the second one was produced for a food-packaging application; and one cardboard of 300 g/m² intended for food contact.

Different kinds of antibacterial agents were also targeted and tested such as a solution of chlorhexidine digluconate used in pharmaceutical application, or carvacrol, an antibacterial essential oil.

A main concern regarding the development of antibacterial material is the release of the active compounds. From this perspective, we developed new kinds of controlled release system based on the use of nanocelluloses, and especially microfibrillated cellulose (MFC).

The MFC is a new innovative bio-nano-polymer extracted from the cellulosic fibers (10-50 nm of diameter and exceed 1 μm in length), which has been increasingly studied for around one decade. More and more scientific works, reviews (Khalil, *et al.*, 2012, Klemm, *et al.*, 2011, Lavoine, *et al.*, 2012, Missoum, *et al.*, 2013, Siqueira, *et al.*, 2010, Siró and Plackett, 2010), conferences (TAPPI Nano Conferences, ACS Cellulose division), European projects (FlexpakRenew, SustainComp, SUNPAP, NewGenPak) and even a full book (Dufresne, 2012) deal with this topic and were mainly published during the first two years of this PhD.

The Figure 2 shows the cumulative number of scientific works published on MFC. The interest is exponentially increasing, and especially since 2005. Indeed, during the last decade, the number of scientific papers has multiplied by a factor of six, with an increase from 859 papers in 2003 to 5149 in 2013. Today, almost one paper-a-day is published on the topic reaching a total amount of 4400 scientific papers in 2012. As noted on the legend of the graph, various kinds of name exist to describe the same bio-nano-material. Until today, no standards exist regarding the exact

definition of what we called in this whole manuscript, microfibrillated cellulose (MFC). As it will be explained in the first chapter, the name does not really matter: the most important is to exactly know the manufacturing process and the final dimension of the material obtained.

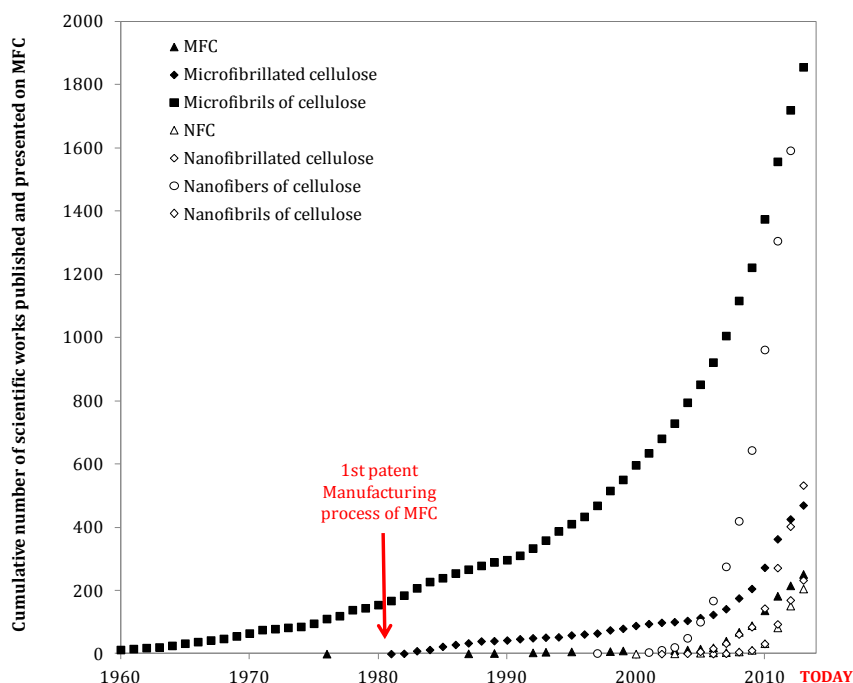


Figure 2. Cumulative number of scientific works on the MFC (data obtained with SciFinder, September 2013)

In spite of the huge amount of scientific works on MFC, the use of MFC with cellulosic substrates, and especially as controlled delivery systems is new. The combination of MFC and cellulosic materials only began in 2008-2009, and the use of MFC as delivery system only started at the end of this PhD, in 2013.

This PhD is consequently a very innovative research project studying the impact of the MFC onto the cellulosic substrates properties and their use as a new controlled delivery system of antibacterial molecules.

Within the context of this research project, three collaborations have been developed. Firstly, one collaboration was established within the Intechfibres consortium, and more precisely with the FCBA (Grenoble). The objective of this partnership was scientific exchanges on nanocellulose manufacturing strategies and on the elaboration of nanocellulose films as new controlled release system. The results are not presented in this PhD report, but were recently valued with a poster presentation at the TAPPI International Nanotechnology Conference on Renewable Materials (2013) (cf. Appendix).

We conducted a second collaboration with another research laboratory, the UMET laboratory (UMR CRNS 8207), in Lille (France). Working with Professor Bernard Martel and Associate Professor Nicolas Tabary, we developed a research project (with a short-term mission of three weeks) during the two last years based on the use of cyclodextrin for synergetic effect on drug delivery system. The results of this project will be detailed in the last Chapter of this manuscript. Finally, a last collaboration started in 2013 with the laboratory of Agropolymers Engineering and Emergent Technologies (UMR INRA 1208) at the University of Montpellier II. Professor Nathalie Gontard and Associate Professor Valérie Guillard took part in this project aiming at better understanding of the mass transfer phenomena within our new functional bio-nano-materials. The context of this collaboration as well as the first results will be detailed in the Appendix of the manuscript.

The manuscript is thus organized in four main parts (Figure 3):

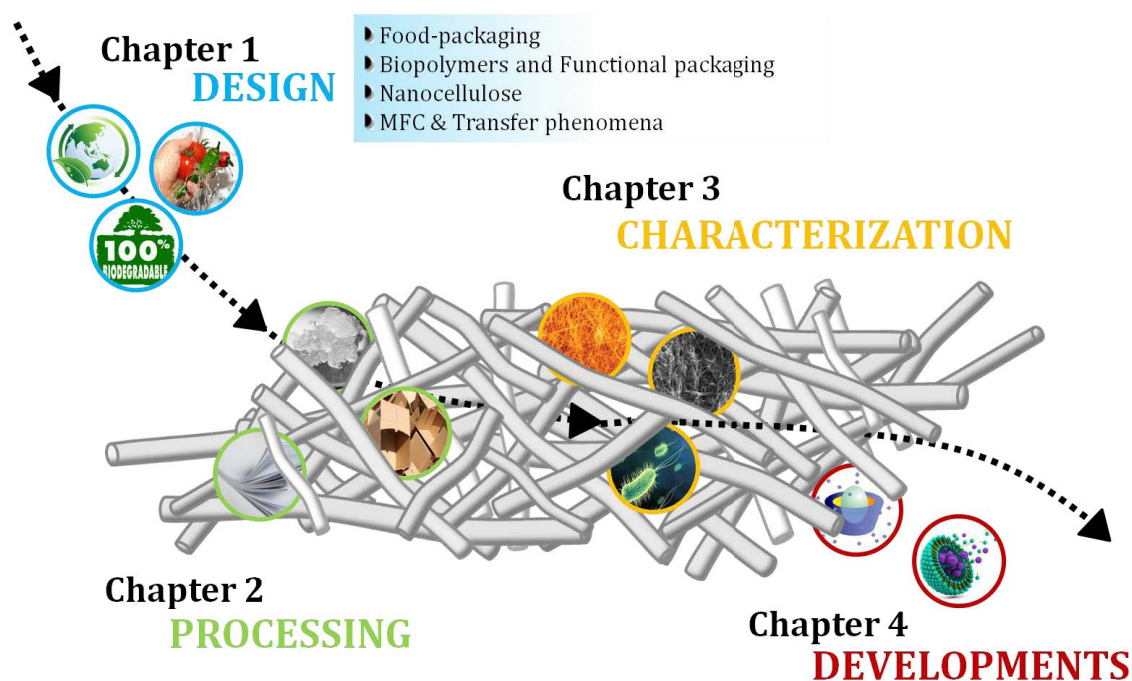


Figure 3. Manuscript organization

Chapter 1 develops the context of the study, which lead us to **design** the objectives of this 3 year project. A specific focus on MFC will be presented, adapted from our review (*Paper 1*) published in 2012 (Lavoine, *et al.*, 2012).

Chapter 2 aims to **process** the cellulosic substrates in a new bio-nano-material. A specific coating protocol of MFC was, thus, implemented, and the properties of the MFC-coated papers (*Paper 2*) and cardboard (*Paper 3*) were studied in details.

Chapter 3 consists in the **characterization** of the functional bio-nano-materials. After proving that the MFC coating is an efficient controlled delivery system (*Paper 3*), the development of paper-based (*Paper 4*) and cardboard-based bio-nano-material (*Paper 5*) with long-term antibacterial properties was conducted.

Finally, **Chapter 4** broadens out this innovative material to other kinds of application such as biomedicine. This chapter is the **development** of a more efficient controlled release system using the synergistic action of MFC and cyclodextrin, either applied as paper coating (*Paper 6*) or as grafted into the cellulosic substrate (*Paper 7*).

The chapters are mainly based on scientific papers or on parts following structured as such, in order to present a homogeneous manuscript. To specify, or complete the original versions of each work, some comments or illustrations might have been added and are indicated by a *grey bold italic font*.

Due to the specific presentation of this manuscript, we tried to link each chapter to each other, and to avoid some data repetitions, so that, you can appreciate the work which has been done during these three last years.

To begin with, let yourself be immersed in the context of this project with an extensive literature review focusing on the current situation of the packaging industry, which lead us to use microfibrillated cellulose, and then, to define the objectives of the PhD.

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CHAPTER 1

Literature Review

Chapter 1: Summary

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Chapter 1-I Literature Review

The present chapter aims to set the context in which this PhD project has evolved. It begins with a first introduction about food-packaging market from industrial and consumer perspectives. This analysis of the current situation regarding food-packaging materials leads us to list their main requirements for the future. A second part is thus focused on the screening of current solutions investigated in responds to these requirements. Use of bio-polymers and development of functional packaging are the two main solutions in progress, but still present some drawbacks. A new solution is thus proposed within the framework of this PhD: the use of nanocelluloses. Consequently, the third part consists in an exhaustive description of this raw material, focusing onto microfibrillated cellulose. Their barrier properties and the transport phenomena occurring through their matrix are then detailed in a last chapter in order to explain and precise the main challenges of the PhD project.

Parts of the two last chapters are adapted from our review published in Carbohydrate Polymers (Lavoine, et al., 2012). Some sentences are written in italics to enhance the relationship between this first and the subsequent chapters, dealing with our results.

I-1. Food packaging

I-1.1. Different kinds of packaging materials on the current market

Excluding packaging machinery, the global packaging industry turnover is around \$564 billion in 2009 (Council, 2013). Within consumer packaging, food packaging represent the largest element, valued at \$228 billion in 2009, i.e. about 40% of the global packaging industry (Pearson, 2008). Since then, these numbers are increasing (Council, 2013), keeping at the head of the market a high consumption and strong demand for food packaging materials. With the increasing diversity of food products, the current food packaging market is indeed expanding. There is nowadays a real need for more and more packaging solutions adapted to more and more various products, and to a consumer demand for a better and longer storage and shelf-life of the product.

The current international market already offers various kinds of packaging materials. Whatever the packaged product, they assume a same role: (i) protect food product from outside, (ii) help for transportation, and (iii) provide consumers with ingredients and nutritional information. In addition to these three actions, the development of food packaging must consider other specifications. Indeed, the food-packaging should now contain food in a cost-effective way that satisfies industry requirements and consumer desires, maintain the food safety and minimize the environmental impact (Kenneth and Bugusu, 2007), as shown in Figure 1-1.1.



Figure 1-1.1. Food-packaging material requirements

Within the global packaging market, 40% of packaging materials are devoted to food and 14% to beverages (Rexam, 2011). The 46% left includes cosmetics, healthcare, industrials packaging etc. Among all materials used, four categories can be defined: (i) glass, (ii) metal,

(iii) plastics and (iv) papers/paperboards. It is quite difficult to obtain the updated data regarding the market share of food-packaging materials of the current year. Nevertheless, according to a recent market study of a company for beverage packaging (Rexam, 2011), plastics and papers/paperboards packaging materials represent the main part of the packaging-materials market with respectively 37% and 34% (Figure 1-I.2).

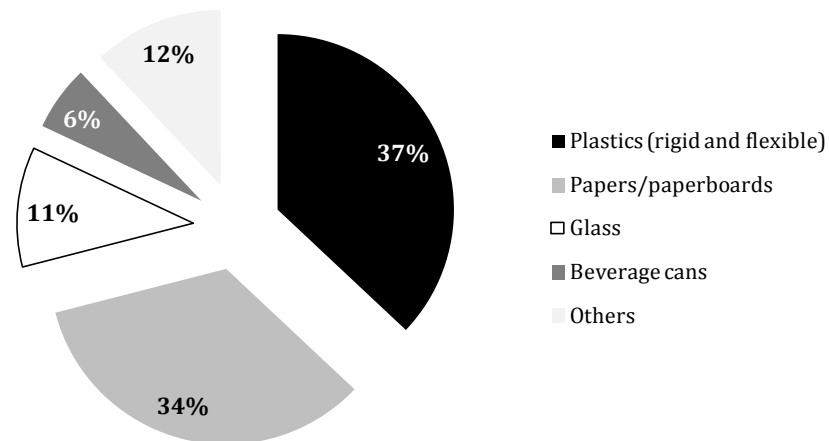


Figure 1-I.2. Worldwide market shares of food-packaging materials (2011)

Each food-packaging material owns distinct properties which made them attractive for specific uses. Table 1-I.1 resumes the strengths and weaknesses of each category and presents their specific markets in food-packaging.

Glass has a long history as food packaging. It has been used and reused during long time for various applications. Nowadays, its use is limited to beverages only. Because of its price and weight, this material is less used with food and its application with beverage tends also to decrease in favor of plastics.

Metal packaging has also been devoted to a specific use: cans and beverages. The price of this raw material is the main weakness and with the development of our society, its incompatibility with microwaves decreases its notoriety.

Plastic packaging has been a revolution within the food packaging in the 1980's. Its diversity made it very attractive and usable for various kinds of products. With good mechanical and barrier properties, it could be the best food packaging responding to the whole society's requirements. However, its environmental impact is a strong weakness in a sustainable society. Furthermore, with the increasing prices of petroleum and its coming soon shortage, its use tends to be avoided more and more and alternative solutions are expected.

On the contrary, **papers and paperboards** are environmentally-friendly and less expensive. They also present good properties in terms of mechanical properties, printability but they are too much sensitive to humidity and water that reduces clearly their range of applications.

	Strenght	Weakness	Specific uses in food-packaging
Glass	Stiffness	Heavy	Beverage
	Impermeable	Breakable	
	Transparency	High energy consumption for its production	
	Recyclable/reusable	Costly transport	
	Inert		
Metal	Stiffness	Expensive	Cans
	Barrier	Non thermoforming possibility	Beverage
	Recyclable	No use with microwaves	
	Aluminium: light		
Plastics	Light	Petroleum polymers	Food (meat, vegetables, fruits, cheese...)
	Transparency	Prices	Beverage
	Stiffness	Non-biodegradable	
	Impermeable (gas, liquid)	Migration issues	
	Thermoforming/Heat sealing (various shapes)		
	Shelf-life		
	Recyclability		
	Microwaves		
Printability			
Papers/paperboards	Biodegradable	Permeability to liquid and gaz	Food (dried food, meat, cheese, vegetables, fruits)
	Recyclability	Stiffness	Liquid as multilayers (milk)
	Stiffness	Heat sealing/Thermoforming	Secondary packaging (yoghourt)
	Light		
	Cheap		
	Printability		
	Easy to handle, cut and fold		

Table 1-I.1. Strength and weakness of the main food packaging materials

Regarding the properties of each material, it seems obvious that none of them answers to the entire goal of food packaging (Figure 1-I.1). A carefully study of the industry's and consumers' requirement is thus needed to propose new packaging solutions which could consider the protection of food in a cost-effective and sustainable ways. *For the following, we will focus our study on plastics and cellulosic packaging materials, and especially on solid food packaging materials (excluding thus beverages), since they represent the main target of this PhD.*

I-1.2. Society's requirements

Packaging is a substantial part of our everyday life and the use of packaging materials has shown a continuous increase over time. That is why the society's requirements have to be considered in the development and choice of new packaging materials. Today, the packaging industry relies strongly on the use of petroleum-derived plastics materials, whereas it is raising both environmental and economic concerns (Johansson, *et al.*, 2012a). Within an industrial point of view, the continuing use of oil resources implies an increasing cost of raw material and, within a consumers' point of view, an increasing price of food packaging. Moreover, the limited oil resource will lead to a lack of raw material and thus an extinction of the current plastics packaging if no new solutions are found.

One society's requirement is related to the environmental impact. Moreover, food-packaging materials must adhere to new regulations settled in each country. For example, the European Union has instituted regulations regarding the wastes management of packaging materials, and this, depending on the material (1994). The USA follow also the regulations instituted by the EPA Guidelines (Kenneth and Bugusu, 2007). Other countries have specified detailed laws to manage better the environmental impact of packaging (Grenelle laws in France, 2009). Recyclability and biodegradability are becoming two key specifications for food-packaging materials and their regulations. From these requirements come out other needs: (i) the decrease of the packaging materials number (e.g. secondary packaging), (ii) the request for lightweight, smaller and "monomaterial" packaging and (iii) the need of new packaging materials (Johansson, *et al.*, 2012a).

These requirements agree with a recent French survey (Glineur, 2012). This survey interviewed companies interested in purchasing food-packaging and asked them to classify the main lines of innovation of their future packaging (Figure 1-I.3):

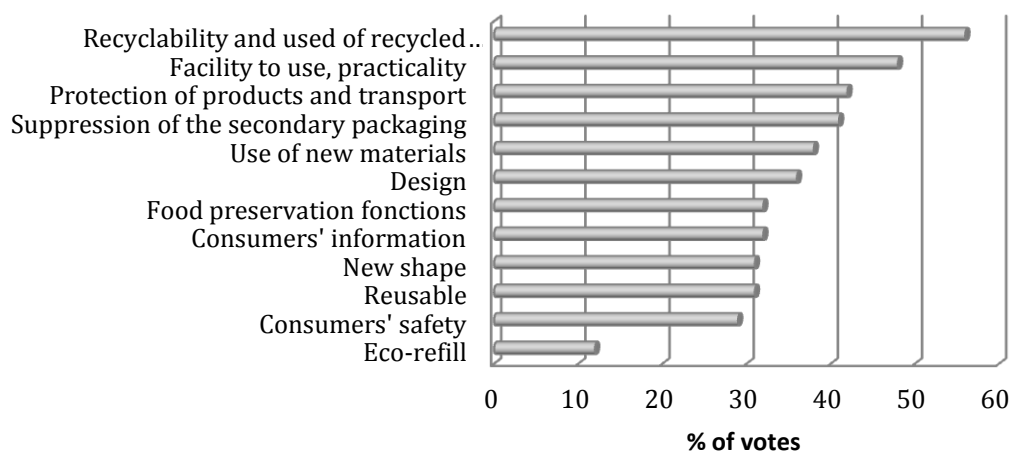


Figure 1-I.3. Main innovation lines of packaging companies (Glineur, 2012)

Recyclability represents 56% of the vote. It is clearly the main requirement from an industrial perspective. In a second place, the facility to use and practicality of the packaging seem also to be essential with 48% of the votes. The protection of the product and the suppression of the excess of packaging materials come at the third place (42-41%). Surprisingly the use of new materials and the improvement of the food preservation come later with 38 and 36% of the votes, respectively. Finally, the packaging companies do not seem to consider the consumer safety as a priority since only 29% chose it.

This survey is quite interesting and permits to have a clear vision on the future packaging from companies' perspective. The results can be compared with another survey organized by DuPont Packaging and Polymer Industries (PackagingDuPont and WorldPackaging, 2012). They

interviewed about 500 industries in the European Union and North America in 2012. Their first question was to attribute the two key trends that will most impact the packaging world within the next ten years. The first unanimous trend is the sustainability. The second trend differs according to the country: from the North America perspective, the cost will be the most influent whereas for the European Union, the food safety and security comes right after the sustainability. Their second question dealt with the two most important packaging attributes to the consumers today and in ten years. The results are shown Figure 1-I.4.

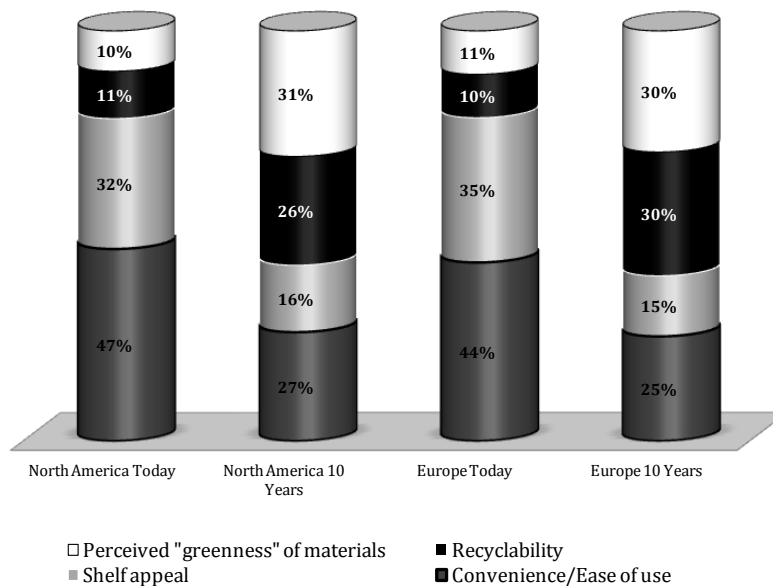


Figure 1-I.4. Survey of future packaging trend
(adapted from DuPont Packaging and Polymer Industries, 2012)

Unanimously, convenience/ease of use and shelf appeal are considered the most crucial today. In ten years, however, this dynamic will completely change, with consumers valuing sustainability features, specifically “greenness” of the materials (increasing 20 percent) and recyclability (increasing 15-20 percent).

These two surveys present similar results and give clear tendencies. For next years, the new packaging solutions will have to take into account the following specifications: (i) sustainability, recyclability and biodegradability, (ii) convenience, ease of use and reusability, (iii) food safety/security, and (iv) low prices.

The research of new packaging materials solutions responding to these previous requirements has already begun and some are nowadays marketed.

The **first solution** concerns **environmental aspects** and can be considered with the replacement of petroleum polymers by bio-based polymers (named biopolymers). The family of biopolymers is quite expanded and gathers widely used polymers such as cellulose, but also new polymers (like polylactic acid) as detailed later. Their increasing development is mainly due to

the intensifying industries and consumers requirements. Nevertheless, despite the use of new and original biopolymers as packaging materials, a global opinion tends to come back to classic packaging bio-based materials such as paper. In 2007, the French company IPSOS made a survey interviewing 7,000 Europeans from 7 countries (Belgium, France, Germany, Poland, UK, Spain and Sweden) about using paper as packaging materials (Bras, *et al.*, 2011a). They had to give their opinion about two comments: (1) “if for the same product I could choose between paper and plastic packaging or labels, I’d choose paper based” and (2) “manufacturers should use more paper based packaging because these are more environmentally friendly”. The results, presented hereinafter Figure 1-I.5, clearly confirm the current behavior of consumers.

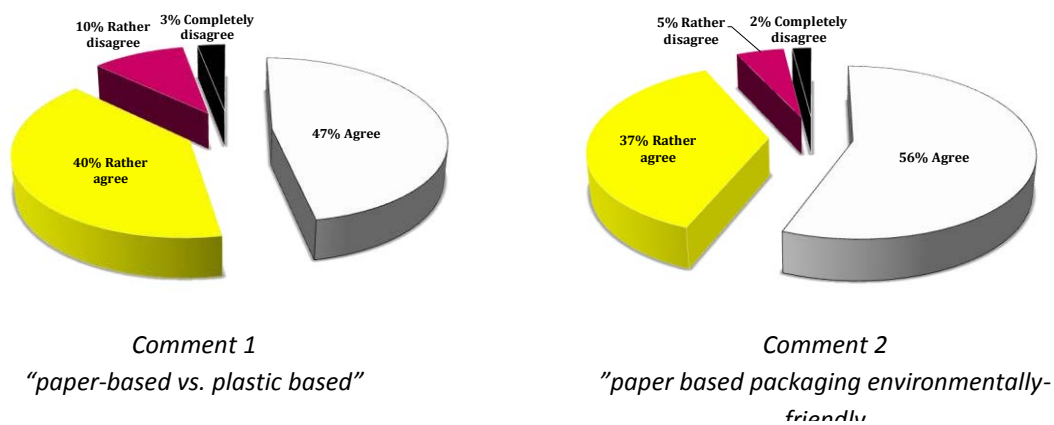


Figure 1-I.5. European consumers' opinion over the use of paper-based packaging materials (IPSOS, 2007)

Consumers are more and more environmentally-friendly and have more and more an “eco-behavior”. According to this survey, the majority supported the use of paper as packaging materials. A more recent survey the consumer expectations (Legentil, 2011). As regards of innovation, two main expectations were also highlighted in this survey: (1) decreased quantity of packaging materials and (2) development of reusable packaging. In conclusion, consumers wished a more convenient and recyclable packaging keeping all basic functions. The development of biopolymers and especially cellulosic-based packaging materials with improved properties are then expected.

The **second solution** investigated in response to industry and consumer requirements is the **development of functional packaging**, i.e. packaging materials which are able to monitor, handle or improve the shelf-life of packaged product. Indeed, a more and more involved society about its welfare and health is emerging. From consumer point of view, the protection of food is one of the three main needs as described above. From industry point of view, a recent survey analyzed future expectations about new technology (Canadean, 2012). According to this survey, the leading drivers of an increased demand for functional packaging in 2012 – 2013 are “consumer convenience”, “safety and traceability” and “enhanced product performance”.

Industries understand perfectly the demand growth for functional packaging materials, and among them, 39% of executives expect “moisture control packaging” to be in the top three of technology oriented packaging types and 56% of respondents expect growth in the demand for “RFID smart labels” in 2012-2013. The industrial expectations for 2012-2013 are then focused on functional packaging and with the growing consumers’ demands and the expanding market internationalization, the focus will be increasingly opted for these new kinds of packaging materials.

Figure 1-I.6 summarizes the evolutions expected as regards packaging materials.

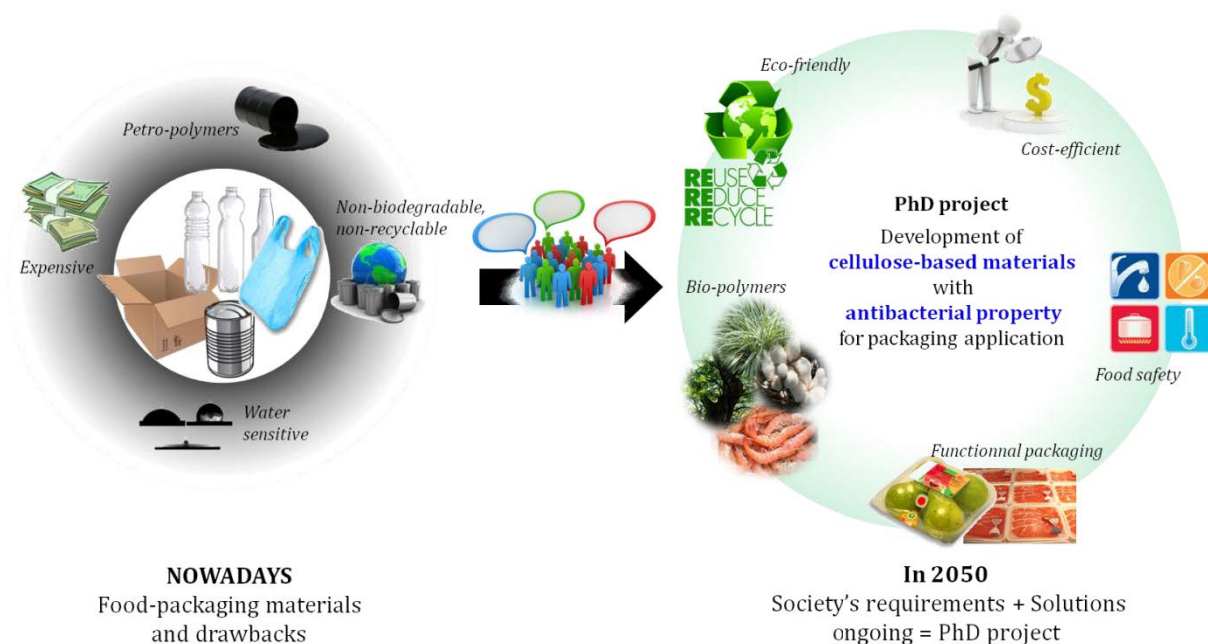


Figure 1-I.6. Food-packaging materials: from current issues and society requirements to ongoing solutions.

In response then to the current society’s requirements, two main answers are apparent: use of recyclable and bio-based materials and development of functional packaging. *Within the framework of this PhD, we will try to answer to these requirements by proposing a cellulose-based packaging material with antimicrobial properties. Some solutions already exist as regards bio-based and functional packaging. They will be described in the next subchapter.*

I-2. Bio-based and functional packaging

I-2.1. Biopolymers for packaging

The term “bio-polymers” is used to classify polymers derived from renewable materials. The prefix “bio” refers to “bio-based” since the end of 1990’s (Petersen, *et al.*, 1999). Biopolymers are generally sorted according to their origin and are gathered in three main categories (Van Tuil, *et al.*, 2000a) (Figure 1-I.7).

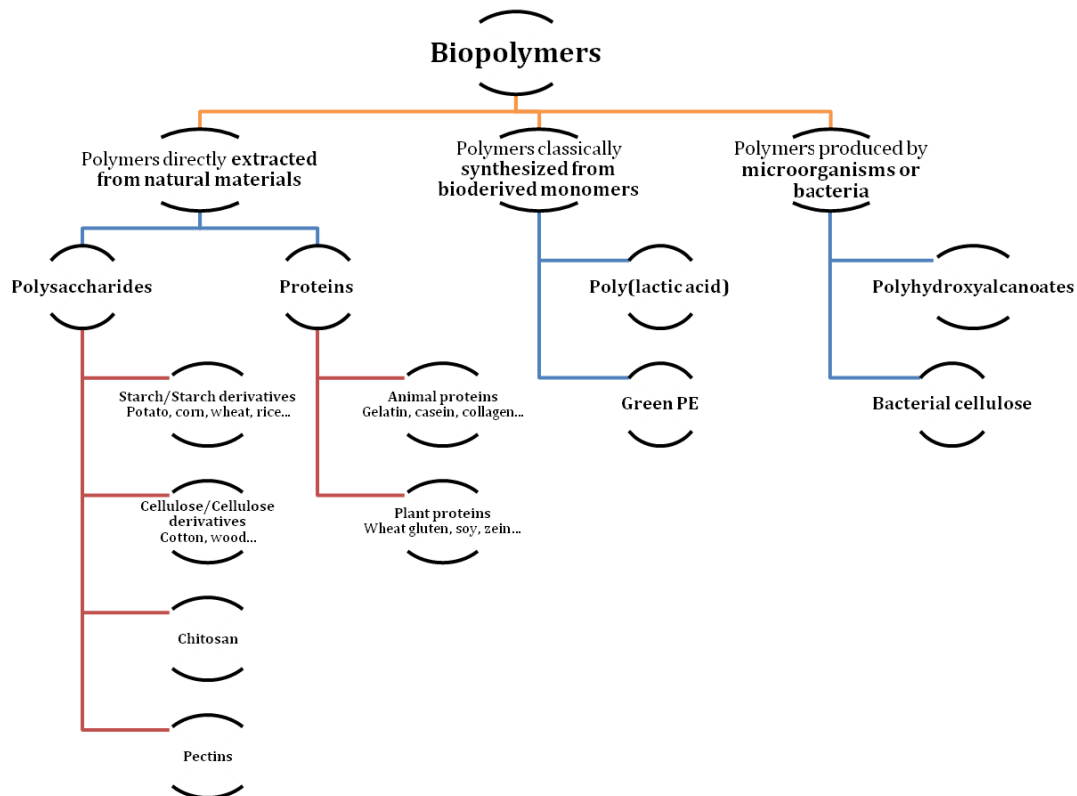


Figure 1-I.7. Schematic overview of the classification of biopolymers (Van Tuil, *et al.*, 2000b)

Their production is constantly increasing since last decade and more and more sorts exist. Two other categories are now usually admitted and added to the classical classification: (i) blends of bio-based polymers and biodegradable petro-based polymers, and (ii) petro-bio-based polymers using one petrol-based monomer with one bio-based monomer. These two categories are called petro-bio-based polymers.

Compared to synthetic plastics, biopolymers can offer two main advantages: (i) biodegradability and/or compostability, and (ii) availability from renewable resources. However, some fully bio-based polymers are not biodegradable, and some fully petro-based polymers are biodegradable as shown in Figure 1-I.8. Among these various biodegradable and/or bio-based polymers, those belonging to the “fully biodegradable” and “fully bio-based” respond to society’s expectations and are developing in that purpose. *We will then focus our study on this category.*

Among them, polylactides have the greatest impact on the marketplace for packaging materials and is now produced on a large scale (main supplier: Natureworks LLC®). Compared to petroleum polymers, its way of production presents numerous advantages such as (i) the use of renewable agricultural resources, (ii) the ability to make hybrid paper-plastic which is compostable and (iii) the possibility to recycle back to lactic acid by hydrolysis (Jamshidian, *et al.*, 2010). If its price was quite expensive in the previous years, it tends today to decrease considerably and begins to be competitive with petroleum polymers. PLA offers high mechanical strength, transparency and low toxicity (Rodriguez, *et al.*, 2008) that make it competitive with polymers such as PE, PP or PS. Nevertheless, the barrier properties of PLA remain low: its water vapor permeability is 3-5 times higher than PET or LDPE (Robertson, 2008) (at 100% of relative humidity) and its oxygen transmission rate (at 0% RH) is 20 times higher than PET (Rodríguez, *et al.*, 2008). Several researches are applied to improve its properties like for example, the use of nanocelluloses (Fukuzumi, *et al.*, 2009), which will be detailed later.

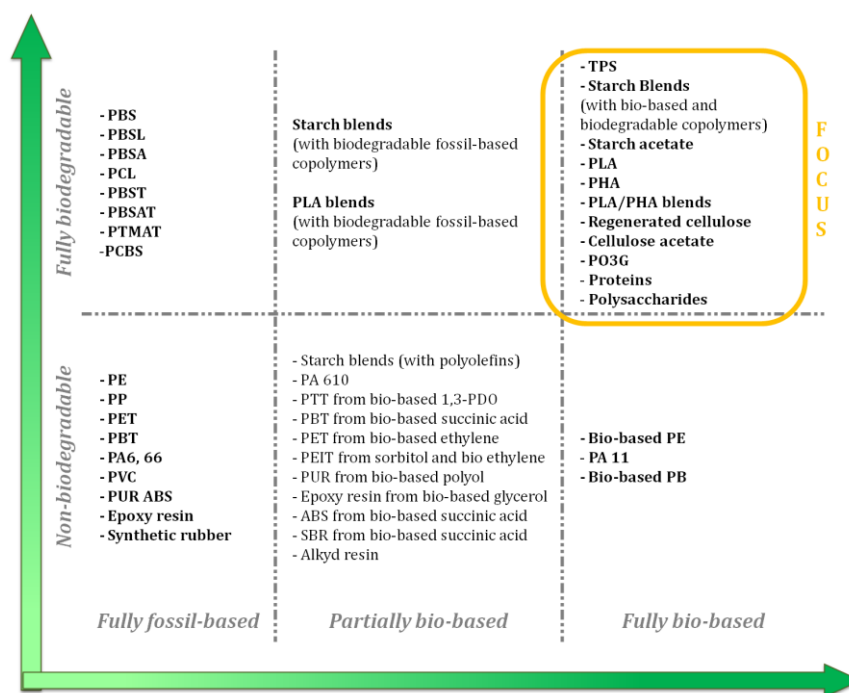


Figure 1-I.8. Current and emerging bio-based plastics and their biodegradability (Shen, *et al.*, 2009)

The PHAs are another expanded bio-based polymers family, which is produced by controlled growth within certain bacteria under nutrient-limited conditions. The first end-use product launched in Germany by Wella AG (1990) was a biodegradable, injection blow-molded bottle for hair and skin products. Nowadays about 20 companies worldwide are pursuing PHA commercialization (Johansson, *et al.*, 2012a). Even if PHAs family presents a wide variety of mechanical properties (from hard crystalline plastics to elastics rubbers with melting

temperature of 50-180°C (Sudesh and Doi, 2005)) and good barrier properties (water vapor transmission rates similar to those of petroleum-derived polymers (Rodríguez, *et al.*, 2008)), their synthesis by bacteria batch remains costly. Nevertheless, two key future applications are targeted for PHAs: packaging end-uses and high-value added applications in medicine (e.g.; drug delivery, implants) (Johansson, *et al.*, 2012a, Sudesh, 2013).

Bacterial cellulose also belongs to the family of biopolymers produced by microorganisms or bacteria. This new biopolymers is more and more developed and studied, essentially in terms of bacterial nanocelluloses. *This new family of bio-based polymers will be further discussed in the chapter Nanocelluloses of this manuscript.*

Polymers directly extracted from natural materials also belong to the category of “fully biodegradable and bio-based polymers” and gather the most numerous family. The materials the most widely used as food-packaging in this category are cellulose and cellulose derivatives. Paper and board substrates as well as regenerated cellulose films (referred to as Cellophane® in many countries) are commonly used as food-packaging materials and, as described before, represent 34% of the worldwide food-packaging market. They are not directly competitive with petro-based polymers, since they have their own market. Nevertheless, their use as well as their market share are wished to be increased, and innovations are currently looked to bring them new functionalities or improve their properties (e.g. hydrophilic nature) by the means of bio-based and biodegradable polymers (e.g. coating of biopolymers, blend of fibers and biopolymers). Besides, their manufacturing process is continuously improved either by decreasing the use of chemicals or by replacing current raw materials by “greener” one (e.g. used of recycled fibers, development of “green” bleaching (Pouyet, *et al.*, 2013), etc.). Paper and board materials perfectly match with the perspective to elaborate more recyclable, biodegradable, lightweight and convenient packaging materials. Furthermore, their manufacturing process is already well-known, widely expanded and cost-effective. *Within the framework of this PhD, these both materials have been thus chosen as packaging materials. More details will be given in the following about their current innovations and challenges.*

The application of cellulose as films is also widely developed and some cellulose-derivative plastics are already available and represented in the current market by, for example, Innovia Films (Cellophane™ and Natureflex™ products), which offers food packaging materials aimed to microwave uses or baked products (Johansson, *et al.*, 2012a). Related to this family, it is also necessary to mention that there is an on-going research into the use of hemicelluloses for bio-based packaging materials. Xylan (polysaccharides made from units of xylose, C₅H₈O₅) isolated from bleached kraft pulp in combination with nanoclays has shown very promising water vapor and aroma barrier properties when coated on low-grammage papers (Talja and Poppius-Levlin, 2011). Xylans and mannans have also a film-forming capacity, which make them

good competitors for petroleum-derived polymers films since they showed a very low oxygen and grease permeability (Cha and Chinnan, 2004, Saxena, *et al.*, 2011).

After cellulose and cellulose derivatives, another commonly used polysaccharide is starch. Starch is usually used with plasticizers such as water or glycerol that confers it thermoplastic properties. However its sensitivity to water vapor and its poor mechanical properties make it unsuitable for many applications. The idea is then to blend starch with synthetic polymers: for example, blending starch with aliphatic polyesters improve their processability and keep their biodegradability; the combination of starch with a water-soluble polymer such as PVOH is also used as a substitute for expanded PS (Nassar and Youssef, 2012). The leader in this field is the Italian company Novamont well-known for its line of Mater-Bi® products.

Complementing PLAs, PHAs, cellulose, cellulose derivatives and starch, other biopolymers such as zein, gluten as well as chitosan have been investigated for their potential as food packaging materials. Although their water sensitivity can be an issue, they attracted attention with their good oxygen barrier and, in the case of chitosan, its antimicrobial activity. They are often used as films, coating or in combination with other biopolymers as they do not own the sufficient properties to make a food packaging material on their own (Dutta, *et al.*, 2009, Han, *et al.*, 2010, Joerger, 2007).

Table 1-I.2 summarizes main properties for each biopolymers family. Only the main properties targeted for food packaging applications are represented and efficiency of biopolymers is qualitatively compared. According to Table 1-I.2 and despite considerable research and development, the use of bio-based packaging materials for food packaging remains limited. Biopolymers such as PLAs or PHAs are already on the current market, but do not still enter in competition with petroleum-polymers because of mainly two features: (i) their poor barrier properties and (ii) the prices. The poor barrier properties (especially at high relative humidity) are well-known for the traditional and most widely used bio-based materials. It is thus often necessary for them to be coated with synthetic polymers in order to achieve the desired barrier properties for food packaging, but in this case, the environmentally-friendly aspect is lost.

MAIN PROPERTIES FOR PACKAGING APPLICATIONS		Mechanical properties			Thermal resistance		Barrier properties			Others
BIOPOLYMERS		Young's Modulus	Tensile Strength	Elongation at break	Thermoforming	O ₂ barrier	Water vapor barrier	H ₂ O barrier	Grease barrier	
Directly extracted from natural materials	Starch	---	--	+++/-	+++ (Zhang, <i>et al.</i> , 2008), (Mali, <i>et al.</i> , 2002)	+++ at dry conditions (McHugh and Krochta, 1994)	-- (Zhang, <i>et al.</i> , 2008)	- (Bastoli, 2005)	-	(+) Biodegradable (-) Food resources competition
	Cellulose ethers	++	++	++	---	+++	--	--	+++	(+) Cheap raw materials (+) Edible
	Chitosan	+++ (Arvanitoyannis, 2008, Rhim, <i>et al.</i> , 2006, Srinivasa, <i>et al.</i> , 2007)	+++ (Arvanitoyannis, 2008, Rhim, <i>et al.</i> , 2006, Srinivasa, <i>et al.</i> , 2007)	+++	---	+++ (Arvanitoyannis, 2008)	+	+	++	(+) Antimicrobial biopolymer (+) Low cost
Synthesized from bio-derived monomers	Proteins	-- (Chiellini, <i>et al.</i> , 2008, Wolf, <i>et al.</i> , 2006)	-- (Chiellini, <i>et al.</i> , 2008, Wolf, <i>et al.</i> , 2006)	--	---	+++ (Aulin, <i>et al.</i> , 2010a)	--	---	++	(+) Film-forming ability (-) Price, availability
	PLA	++ (Clarival and Halleux, 2005)	++ (Clarival and Halleux, 2005)	++	+++ (Clarival and Halleux, 2005)	+	+	+	++	(+) Already marketed (-) Cost
Produced by microorganisms or bacteria	Green PE	++	++	++	+++	+	++	+++	+++	(+) Decrease CO ₂ release (-) 30% only of bio-based polymer
	PHA	++/- (Clarival and Halleux, 2005, Sudesh and Doi, 2005a)	+	++/- (Clarival and Halleux, 2005, Sudesh and Doi, 2005a)	+++/- (Sudesh, 2013a, Sudesh and Doi, 2005a)	+++	+++ (Miguel and Iruin, 1999)	++	+	(+) Large range of properties (-) Expensive, availability

Table 1-1.2. Main properties of biopolymers for packaging applications. (+++) means excellent, (++) good, (+) enough in comparison with (---) very bad, (--) bad and (-) not enough.

Figure 1-I.9, adapted from Aulin, *et al.*, 2010a, shows the barrier properties for petroleum and bio-based materials proving the higher water vapor permeability of bio-based polymers compared to petroleum polymers.

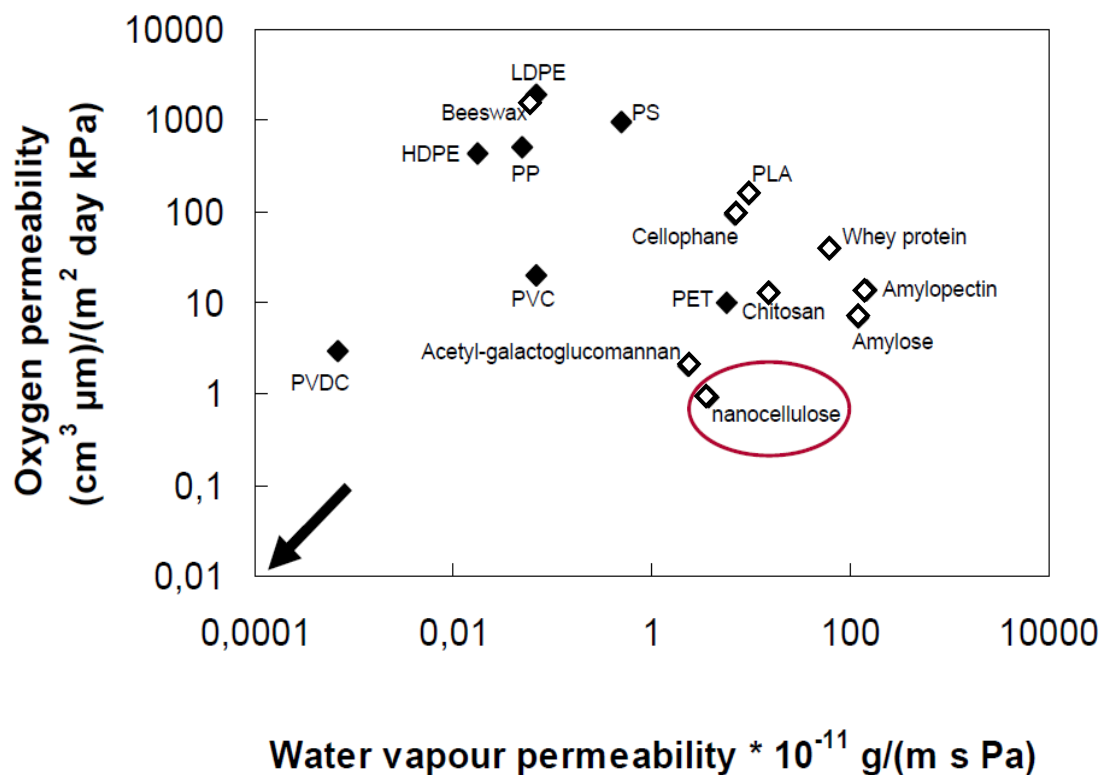


Figure 1-I.9. Oxygen permeability as a function of water vapor permeability for petroleum (*black diamond*) and bio-based (*white diamond*) polymer (adapted from Aulin, *et al.*, 2010a)

Finally, when focusing on the overall mechanical and barrier properties of the biopolymers, they are not always competitive with the petroleum ones. Nevertheless, the development of bio-composites can be a solution, which is by now under on-going research. From an economic perspective, the prices of bio-based polymers are still high compared to petroleum-derived polymers, but with the coming years, will tend to decrease with the increase of production capacity (EuropeanBioplastics, 2012).

Another challenge to consider for the successful use of bio-polymers is achieving the desired food shelf-life meanwhile keeping efficient biodegradation after disposal. The use of biopolymers is thus a first answer to society's requirements. They offer (i) sustainability and biodegradability, (ii) an ease of use but, still have to confirm (iii) food safety and (iv) low prices. A new kind of packaging materials may help to answer to the food safety requirement. This new category has just started to be commercialized in the beginning of the XXIst century in the

European Union and is still mainly ongoing from a research perspective. They are called “functional packaging” and regroup two main families of packaging named: “intelligent packaging” and “active packaging”. Both families will be described in the following part with a specific highlight on active packaging as they aim to improve the shelf-life of packaged product. *Besides, active packaging, especially antimicrobial packaging, are the main topic of this PhD and will be thus more detailed.*

1-2.2. Intelligent packaging

Two kinds of functional packaging can be distinguished: (i) intelligent packaging and (ii) active packaging. According to the last regulation of the European Parliament (Regulation (EC) No 1935/2004), intelligent food-packaging materials “means materials which monitor the condition of packaged food or the environment surrounding the food”. In comparison, active food-packaging materials “means materials that are intended to extend the shelf-life or to maintain or improve the condition of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food”.

The regulation regarding these new kinds of packaging is quite recent (2004). The development of functional packaging in the European Union did not begin before the XXIst century. In other countries, such as Japan, Australia or the United States, the development and commercialization of functional packaging, especially active packaging, appeared in the 1970s. The regulation is usually one of the main obstacles to the marketing of such packaging, and especially the concept of “inert packaging material” specific to the European Union. They seem nevertheless to be the response to some consumers’ demand. They tend towards “safer” products that can be stored longer despite containing lower quantities of preservatives.

Intelligent packaging (or smart packaging) aims to inform consumers of specific changes of the packaged food or its surrounded environment. They can report the conditions on the outside of the packaging or measure the quality of the food product inside the packaging.

Nowadays, different kinds of intelligent packaging are developed. The most used is the Time-Temperature Indicator (TTI). It enables to register the accumulated time-temperature history of the product, signals the end of the shelf-life as defined by the supplier and acts as a freshness indicator. The current market offers a variety of TTI with different mode of action:

(i) Some are based on a polymerization reaction: by a thermal polymerization of a colorless monomer, the colored conjugated polymer is created (Fresh-Check® from LifeLines Technology, USA)

(ii) Others, like the 3M™ MonitorMark™ in the USA (Figure 1-I.10), are based on dye diffusion. These labels, for example, use a colored ester and phthalate mix with the desired melting point that is colored with a blue dye. Above its melting point it diffuses along a wick, and the progress along this wick gives an indication of how long the indicator has been liquid. A polyester strip keeps the liquid away from the wick, until it is pulled out by the operator to start the device.



Figure 1-I.10. 3M™ MonitorMark™ labels - The blue dye continues to diffuse while the melting point is exceeded

(iii) Enzyme chemistry is also a solution (Vitsab® TTI labels from Vitsab AB, Sweden). By mixing an enzyme (which is active above a specific temperature) and a pH indicator dye with an appropriate lipid, the enzyme liberates acid from lipid and decreases then the pH of the solution. The indicator changes thus of color, which informs consumers that the product has been exposed to an excess of temperature.

After TTI labels, others sort of smart packaging have been recently marketed. Each focuses on one parameter which is responsible of the degradation of the product. For example, biological change sensors can lead to off-tastes and odors which are repellent to the consumer and, in worst case can lead to serious illness. They provide therefore a rapid indication of goods that have excessive biological activity. Then, gas indicators especially oxygen indicators also exist and can be used when preparing packaging as well as at the consumer end of the chain. Oxygen is mainly responsible of the degradation of foods, it is thus necessary to detect it during the conditioning and once marketing. Finally, other smart packaging such as moisture indicators or chemical change indicators are also emerging, providing each time information to the consumer regarding the quality of the packaged product (LeGood and Clarke, 2006). *No more details will be given regarding intelligent packaging since this PhD work is more focused on active packaging.*

I-2.3. Active packaging

The concept of active packaging is not really new: the use of lemon to prevent apples or mushrooms from oxidation, or the use of leaves to cover products and transfer them the aroma compounds or enzymes that are responsible of their preservation, are examples of ancestral

active packaging. By instinct, each of us employs the basics to preserve better and longer the food products.

Nowadays, the concept of active packaging is redefined as detailed previously, and recently accepted by the international food safety regulations (2009a). It is then possible to rethink and design new packaging technologies.

As said by Catalá and Gavara, 2001, an active packaging is “a food/package/environment system that works in a coordinated way to improve the quality and safety of the packaged product and increase its shelf-life.” Its purpose is to protect the food from agents that cause physical, chemical, microbial or enzymatic changes. There are essentially two ways of making a packaging active (Figure 1-I.11): either (i) the active material is placed inside the sealed packaging, a *1 in 2 solution*, or, (ii) the active element is a part of the packaging material itself, a *2 in 1 solution*.

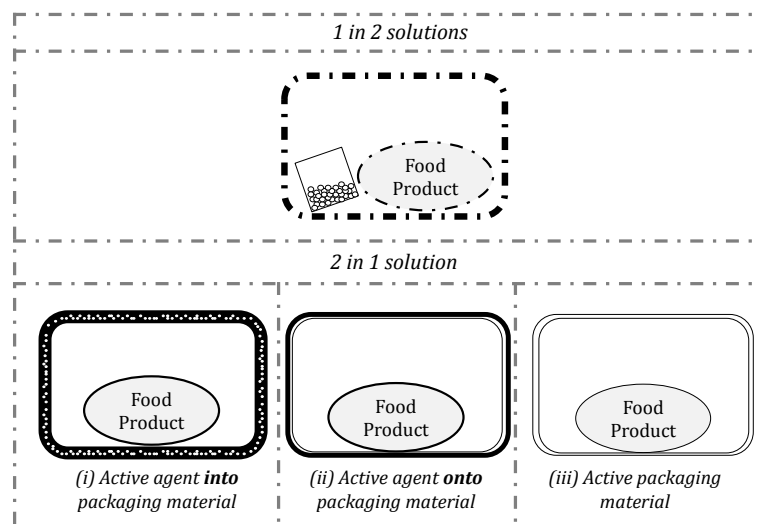


Figure 1-I.11. Two main ways of making a packaging active: *1 in 2 solution*, an active material (*white circles*) is placed inside the sealed packaging or *2 in 1 solution*, the active element is a part of packaging (into *(i) -white spots-* or onto *(ii) -white layer*) or is the packaging material itself *(iii)*.

Then, two kinds of activity can also be distinguished: (j) the scavenger/absorber packaging, which aims to absorb or release a specific element (oxygen, ethylene, moisture etc.) and (jj) the antimicrobial packaging, which aims to inhibit the microbial growth inside the packaging. Both activities intend to improve the shelf-life of the food product.

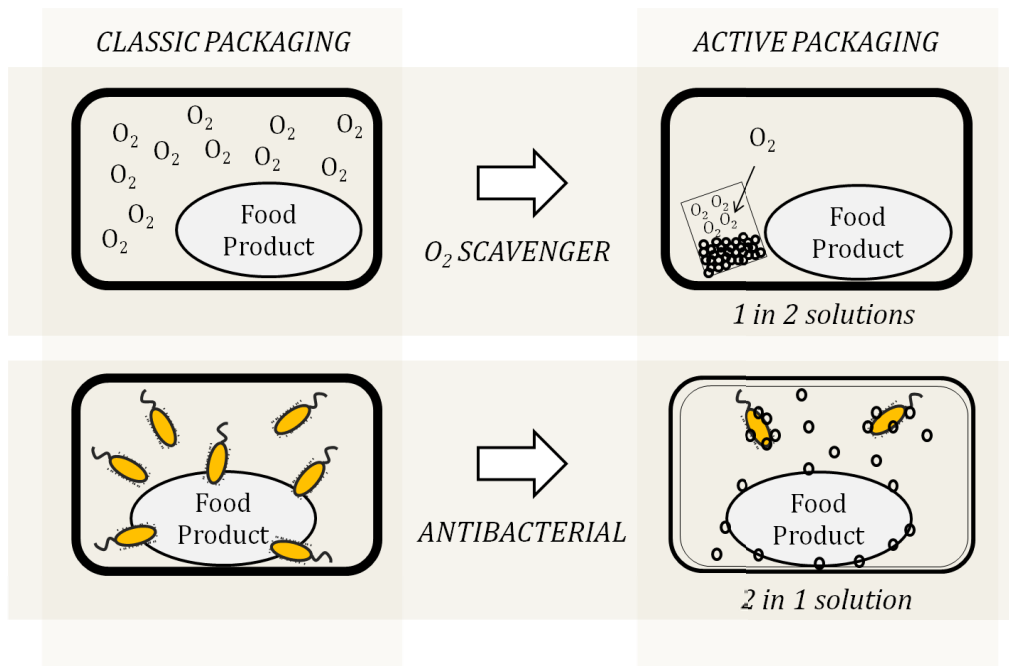


Figure 1-I.12. Classic vs. active packaging: (i) Oxygen scavenger with 1 in 2 solutions (up) and (ii) Antibacterial packaging with 2 in 1 solutions (down).

Figure 1-I.12 schemes example of scavenger and antibacterial packaging. Both strategies (i.e. 1 in 2 or 2 in 1) can be used indifferently for each activity (scavenger or antibacterial). However, most of the time, 1 in 2 solution is applied for scavenger packaging, and 2 in 1 solution for antibacterial packaging. *These two strategies are detailed in coming subchapters.*

- “1 in 2” solution, 1 active packaging in 2 pieces

The first activity ((j) scavengers) has been favored at the beginning of active packaging and is still a widely used technique. The usual method is the introduction of the active element in a sachet or a label and their insertion inside the food packaging before sealing. The most widely used system is oxygen scavengers. It has been engineered to remove oxygen from the interior of package. The first major commercial oxygen scavengers were from Japan’s Mitsubishi Gas Chemical Company during the 1970s. Their most famous system, based on iron salt, was sold under the tradename Ageless® (Brody, *et al.*, 2001a). It has also been commercialized in the

United State at the same period. This system was soon followed into the market by Japan's competitor Toppan Printing Company with ascorbic acid-based oxygen scavenger systems. At the beginning of the XXIst century, Japan became the first country to use and produce oxygen-scavengers. At that time, more than two billion oxygen-scavengers sachets were used annually on the Japanese market. Nowadays, Japan still remains the country with the highest global market for active packaging (not only gathering oxygen scavengers) as shown by Figure 1-I.13 (Restuccia, *et al.*, 2010).

Following this success, other companies emerged in different countries. In the United State, companies such as Multisorb Technologies or Air Liquid developed also iron-based oxygen-scavengers (e.g. FreshMax®). These systems used highly resistant material for the sachets such as tear-proof polypropylene network (Tyvek® materials) or protective films impermeable to liquids (Schvester, 1989). It is only from 2000 that the French company STANDA ATCO marketed for the first time in the European Union ferrous iron powder in sachets placed inside cheese packages.

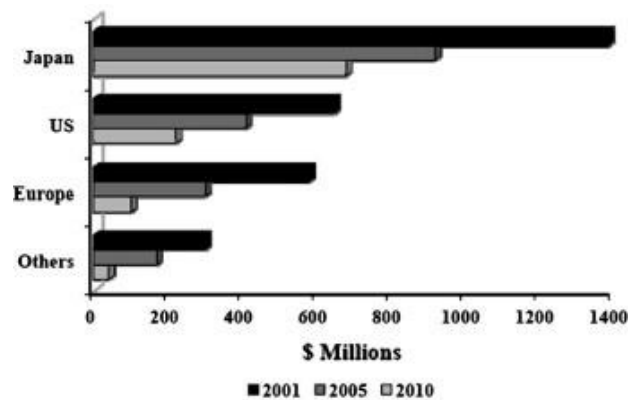


Figure 1-I.13. Global market for active packaging 2001-2010 (Restuccia, *et al.*, 2010)

Oxygen is not the only agent responsible for the food degradation. Ethylene, moisture, carbon dioxide or even odors are also elements linked to the modification of the organoleptic properties of foods. That is why new active packaging appeared on the international markets from these first oxygen-scavengers sachets or labels. Table 1-I.3 lists some of these currently marketed active packaging with their corresponding action.

Manufacturer	Country	Tradename	Function
Mitsubishi Gas Chemical Company	Japan	Ageless®	
Toppan Printing Co.	Japan	Freshlizer™	
Keplon Co.	Japan	Keplon™	
Oji Kako Co.	Japan	Tamotsu™	
Powdertec	Japan	Wonderkeep™	
Ueno Seiyaku Co.	Japan	Oxyeater™	
Dai Nippon	Japan	Sequel®	
Bioka Ltd	Finland	Bioka®	O ₂ scavenger
Standa Atco	France	Atco®	
Multisorb Technologies Inc.	USA	FreshPax™, FreshMax®	
Johnson Matthey Plc	UK	n/a	
EMCO Packaging System	UK	ATCO	
Sand-Tech Enterprise Co. Ltd	Taiwan	Agelong	
Hsiao Sung Preservative Technologies (Chang Shu) Co, Ltd	China	O-Buster	
Lipmen	Korea	OXYGEN ABSORBER	
O2CONTROL BV	Holland	Freshcare®	
Mitsubishi Gas Chemical Company	Japan	Ageless®, Fresh Lock	O ₂ and CO ₂ scavengers
Ethylene Control	USA	n/a	
Air Repair Products	USA	n/a	Ethylene Absorbers
Mitsubishi Gas Chemical Company	Japan	Sendo-Mate	
Toagosei Chemical Co.	Japan	Vitalon®	CO ₂ generator
SARL Codimer	France	Verifrais®	
Freund Industrial Co.	Japan	Ethicap®	
Freund Industrial Co.	Japan	Negamold®	Ethanol generator
Techno Intl. Inc.	USA	Fretek®	

Table 1-I.3. Currently commercialized active packaging as sachet or label

Most of the systems that made up the first generation of active packaging used this technique of *1 in 2 solutions*, it means the development of one active packaging with two pieces. This technique is still used, but a more attractive alternative is developed. The principle is to add the active agent in the packaging material itself, either as part of the polymer or deposited onto it as a second layer (Kruijff, *et al.*, 2002), i.e. the *2 in 1* solution.

- *“2 in 1” solution, 2 actions in 1 packaging*

This strategy makes positive use of migration and sorption mechanisms: instead of releasing undesirable substances into the food or absorbing desirable ones, substances with a beneficial effect are incorporated into the package and transferred to the food, or undesirable food components are removed by sorption (Catalá, *et al.*, 2008). It is also the most attractive form within a consumer point of view: they will not be disturbed by the presence of an unknown (sometimes toxic) material inside the packaging. Besides, from an industrial perspective the *2 in 1 solution* will simplify the packing technology.

Three methods can be carried out to introduce the active compound in the packaging material itself (Figure 1-I.14):

- (i) The active compound can be directly introduced in mass and mixed with the packaging material (e.g. during extrusion of polymer)
- (ii) It can be coated onto the packaging material (e.g. on polymers, films or papers)

(iii) It can have the ability to form a film and then, be used directly under the food shape (e.g. edible films of biopolymers such as chitosan or cellulose ethers)

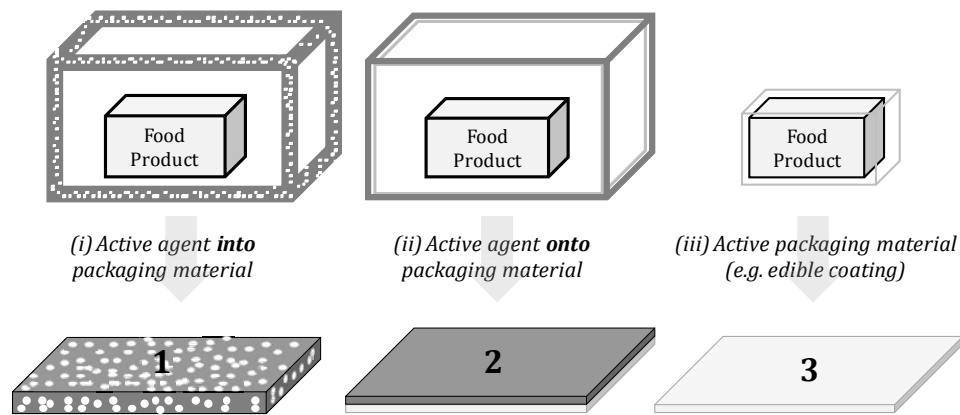


Figure 1-I.14. Active agent (*white spots*) as part of the packaging material. Three methods: (1) Direct introduction and/or mixing with packaging material, (2) coating onto the packaging material and (3) as packaging material itself.

The materials used as substrates are usually conventional synthetic polymers. Polyolefin are widely used when there are no particular gas or vapor barrier requirements. Otherwise, alternatives are polyesters or polyamide and multi-layer structures that include high barrier materials such as EVOH, ethylene vinyl alcohol polymer. Some such as PET or PE are already marketed and gather mainly oxygen scavengers and antimicrobial packaging. Table 1-I.4 presents a non-exhaustive list of the current active packaging marketed based on the use of synthetic polymers as packaging material. Many reviews and books have already detailed and described the commercialized active packaging based on the use of a synthetic polymers matrix (Brody, *et al.*, 2001a, Catalá, *et al.*, 2008, Gontard, 2000, Pereira de Abreu, *et al.*, 2012, Restuccia, *et al.*, 2010, Suppakul, *et al.*, 2003).

However, from consumers' perspective, the development of active packaging with petroleum polymers does not match with the current requirements (detailed previously). *That is why we will focus our study on biopolymer-based active materials for food-packaging.*

	Format	Tradename	Manufacturer	Country	Packaging material	Active compound	
Oxygen scavenger	Film	Multi layer	Bioka®	Bioka Ltd	Finland	EVA modifier PE + LDPE + Polyamide	Enzyme
		Laminate	OS2000®	Sealed Air Corporation	USA	PVDC	Activated by UV light
		Multilayer	ZERO2™	CSIRO and VisyPak	Australia	PE, PET, EVA or PP	Photosensitive dye
	Concentrate		Shelfplus	Ciba Specialty chemicals	Switzerland	PET bottle	Iron based
			Amosorb®	Amoco Chemical	USA	PET bottle	n/a
		Multilayer	Oxyguard™	Toyo Seikan Kaisha Ltd.	Japan	EVOH and PP layer	Iron based
		Closure	Celox™	Grace Darex Packaging Technologies	USA	Beverage bottles	n/a
Liner	Daraforms 649C	Grace Darex Packaging Technologies	USA	Polyolefin	Sodium sulfite and sodium ascorbate		
Antimicrobial	Concentrate	Multilayer	Zeomic®	Sinanen Zeomic Co. Ltd.	Japan	Dispersed in PP or PE layers	Synthetic zeolite with silver ion
			Agion	Agion Technologies	USA	n/a	Silver ions
			MicroFree™	E. I. du Pont de Nemours and Company	USA	Powder for coating, fibers or polymers	Zinc, silver and copper
		Microban®	Microban Products	USA/Europe/Asia	Every applications	n/a	
	Extract		Citrex™	Quimica Natural Brasileira	Brazil	n/a	vitamin C, citric acid, lactic acid, ammonium propionate
			Nisaplin®	Integrated Ingredients	USA	Food preservatives	Nisin
	Film		MicroGard™	Rhone-Poulenc	USA	Touch surface application	n/a
		Piatech	Daikoku Kasei Co.	Japan	n/a	Silver	

Table 1-1.4. Current active packaging commercialized. The active compound is introduced directly in the packaging material.

As already said, two main actions are targeted with the development of active packaging materials (scavengers and antibacterial), and some bio-based materials already exist and are used for their elaboration:

- **Scavengers and emitters active bio-materials**

Active packaging controlling oxidation problems in packaged food is the most developed, as detailed previously in the *1 in 2 solutions*. Other actions such as ethylene control, moisture regulation, the addition of aromas received attention, but remain less developed. That is why the following will be focused on oxygen scavenger bio-based materials.

Up to our knowledge, none of them is marketed in spite of excellent prospects (Catalá, *et al.*, 2008). The main application, at lab scale, consists in the development of edible coating for foods with biopolymers such as whey protein (Ramos, *et al.*, 2011), zein (Güçbilmez, *et al.*, 2007), carrageenan (Lee, *et al.*, 2003) or beeswax (Perez-Gago, *et al.*, 2006) including an antioxidant (e.g. ascorbic acid). Then, other studies have been investigated on the development of films with the addition of antioxidant compound. Oussalah, *et al.*, 2004 worked on milk protein films including oregano essential oil as antioxidant and antimicrobial agent for preserving beef. Others (Byun, *et al.*, 2010) tried to develop antioxidant PLA films with α -tocopherol and buthylated hydroxytoluene (BHT). Finally, other works have been published on the elaboration of oxygen scavenger papers or paperboards. For example, within the framework of a research project at the LGP2 (Grenoble), starch or PVA with ascorbic acid has been coated onto paper to increase with success shelf-life of meat. Johansson, *et al.*, 2012b proposed also latex- or starch-based coatings including laccase and liginosulfonates as oxygen-scavenging system. However, the development of scavenger materials using the strategy 2 in 1 is quite rare due to toxicity of classical oxygen scavengers and difficulties for controlling oxygen migration in bio-materials.

- ***Antimicrobial active bio-materials***

Controlling microbiological contamination is one of the applications of active packaging that is increasingly developed as proved by the number of recent reviews and books published on this thematic (Appendini and Hotchkiss, 2002, Cha and Chinnan, 2004, Cooksey, 2001, Cooksey, 2005, Gontard, 2000, Han, 2003, Joerger, 2007, Pereira de Abreu, *et al.*, 2012, Quintavalla and Vicini, 2002, Suppakul, *et al.*, 2003, Vermeiren, *et al.*, 2002). Various antimicrobial substances are usually applied directly on the surface of the product to control the micro-organisms. However, direct application of antimicrobial substances is not always effective, as their activity may be reduced by their rapid diffusion inside the product, and is sometimes not recommended within human health.

The idea is thus to incorporate the antimicrobial compound into the package itself. Then, the antimicrobial action will be based on

(i) the **emission** of volatile substances into the headspace of the package (e.g. essential oil); or

(ii) the **migration** of the active component into the packaged food (e.g. organic acids, bacteriocins, enzymes); or

(iii) **direct contact** of the bacteria on the active packaging surface (e.g. chitosan).

Within this PhD, the actions (i) and (ii) are investigated with the use of different kinds of antibacterial agents.

Various kinds of antimicrobial agents exist and are used in the elaboration of antimicrobial packaging. Table 1-I.5 gives a short list of these commonly used compounds. They are classified in two main categories: those produced by nature and those synthesized and produced by humans. Both regroup a large amount of antimicrobial agents that is why it is impossible to list them all. Furthermore, because of the different regulations implemented in each country, some antibacterial agents are only allowed in specific countries. For example, allyl isothiocyanate is forbidden in the USA and Europe, but is still allowed to be used in Japan providing that it is extract from natural resources.

Natural antimicrobial agents		Non-natural antimicrobial agents			
Produced by microorganisms	Plant origin	Animal origin	Metals	Chemicals	Polymers
<p>Bacteriocins</p> <ul style="list-style-type: none"> - Nisin (Coma, <i>et al.</i>, 2001, Imran, <i>et al.</i>, 2010) - Pediocin (Rodríguez, <i>et al.</i>, 2005) <p>Antibiotics</p> <ul style="list-style-type: none"> - Natamycin (Miller, <i>et al.</i>, 2002) - Penicillin (Pérez-Pérez, <i>et al.</i>, 2006) <p>Organic Acids & Salts</p> <ul style="list-style-type: none"> - Sorbic acid (Guillard, <i>et al.</i>, 2009, Silveira, <i>et al.</i>, 2007) - Potassium sorbate (Flores, <i>et al.</i>, 2007, Shen, <i>et al.</i>, 2010, Zactiti and Kieckbusch, 2006) - Benzoic acid, Benzoate (Pérez-Pérez, <i>et al.</i>, 2006) <p>Enzymes</p> <ul style="list-style-type: none"> - Lysozyme (Arcan and Yemencioğlu, 2013, Gemili, <i>et al.</i>, 2009) - Lactoferrin (Jenssen and Hancock, 2009) - Lactoperoxidase (Pérez-Pérez, <i>et al.</i>, 2006, Tenovu, <i>et al.</i>, 1977) 	<p>Essential oils</p> <ul style="list-style-type: none"> - Thymol (Mastromatteo, <i>et al.</i>, 2009) - Cinnamaldehyde (Ben Arfa, <i>et al.</i>, 2007, Rodríguez, <i>et al.</i>, 2007, Ramos, <i>et al.</i>, 2012) - Carvacrol (Ben Arfa, <i>et al.</i>, 2007, Ramos, <i>et al.</i>, 2012) - Eugenol (Sanla-Ead, <i>et al.</i>, 2012) - Rosemary oil (Pérez-Pérez, <i>et al.</i>, 2006) - Garlic oil (Pranoto, <i>et al.</i>, 2005) - Grapefruit seed extract (Pérez-Pérez, <i>et al.</i>, 2006) - Allyl isothiocyanate (Pérez-Pérez, <i>et al.</i>, 2006) 	<p>Chitosan (Arvanitoyannis, 2008, Dutta, <i>et al.</i>, 2009)</p>	<p>Silver (Ag) ions (Liu, <i>et al.</i>, 2012)</p> <p>Ag nanoparticles (Drogat, <i>et al.</i>, 2011, Jung, <i>et al.</i>, 2009)</p> <p>Silver zeolites (Dogan, <i>et al.</i>, 2009)</p> <p>Copper ions (Pérez-Pérez, <i>et al.</i>, 2006)</p>	<p>Imazalil (Vartiainen, <i>et al.</i>, 2003)</p> <p>Chlorhexidine digluconate (Jeansson and White, 1994, Şenel, <i>et al.</i>, 2000)</p> <p>Benomyl (Quintavalla and Vicini, 2002)</p> <p>Sulfur dioxide (Pérez-Pérez, <i>et al.</i>, 2006)</p> <p>Chlorine dioxide (Pérez-Pérez, <i>et al.</i>, 2006)</p> <p>Hexamethylene-tetramine (Devlieghere, <i>et al.</i>, 2000)</p> <p>Ethylene diamine tetra-acetic acid (EDTA)(Güçbilmez, <i>et al.</i>, 2007, Vartiainen, <i>et al.</i>, 2003)</p>	<p>Chitosan derivatives (Kenawy, <i>et al.</i>, 2007)</p> <p>Quaternary ammonium salts (Muñoz-Bonilla and Fernández-García, 2012)</p>

Table 1-I.5. Classification of antimicrobial agents commonly used

According to the literature, various systems exist for delivering antimicrobials compounds: sphere, films and coatings (Balasubramanian, *et al.*, 2009).

Microencapsulation of enzyme, flavors, antioxidants or preservatives has been developed and used commercially for two decades (Gibbs, 1999, Madene, *et al.*, 2006). This technique contributed to the boom of the antimicrobial packaging. One of the first antimicrobial materials based on encapsulation is the product Bioswitch® (2008) of TNO, the Netherlands Organization for Applied Scientific Research. The Bioswitch particles are biopolymers cages containing an antimicrobial component. These particles are usually coated onto the surface of the food package. In the case of microbial contamination, the bacteria will start to secrete enzymes that break down the polysaccharides matrix and then release the antimicrobial compound. Different techniques of microencapsulation exist nowadays and permit the use of biopolymers such as starch, gelatin, carboxymethylcellulose, chitosan, gums or proteins. These micro-capsules or cages aim thus to contain the antimicrobial compound and release it progressively. It is especially favored with the use of volatile antimicrobial compounds, e.g. essential oils (Abreu, *et al.*, 2012, Hosseini, *et al.*, 2013, Wu, *et al.*, 2012). Nevertheless encapsulation of bacteriocins or proteins such as nisin (Malheiros, *et al.*, 2012) or lysozyme (Li, *et al.*, 2009) is also under investigation. Although microencapsulation is now a well-known technique, some drawbacks slow down its development: cost, use of toxic chemicals for its manufacturing and non-control of the active agent release.

That is why, films and coatings are promising systems for the release of antimicrobial compounds. They are especially well adapted to the food packaging, where changes often take place on the surface or in the headspace of the packaged food. The antimicrobial compound can then be combined with the polymeric materials to form films, or introduced in coating slurries and coated on films, foils or cellulosic substrates. According to the review of Cha and Chinnan, 2004, biopolymers based on polysaccharides such as cellulose, cellulose derivatives, starch, alginates and chitosan, as well as proteins (e.g. zein, wheat gluten or casein) have been widely used. Cellulose derivatives have particularly received the most attention as matter of renewable packaging material. Coma, *et al.*, 2001 investigated edible hydroxypropyl methylcellulose (HPMC) films as matrix for the antimicrobial compound, nisin. Similar works were published on the release of nisin added to methyl cellulose (Boluk, *et al.*, 2011), HPMC films and other biopolymers such as carrageenan or chitosan (Cha, *et al.*, 2003, Grower, *et al.*, 2004). Gemili, *et al.*, 2009 used another kind of cellulose derivatives, cellulose acetate films, to introduce lysozyme. Other biopolymers were also tested, but more and more as films or coating slurry. For example, Zhai, *et al.*, 2004 developed a starch/chitosan blend films with antimicrobial properties due to the presence of chitosan. Pelissari, *et al.*, 2009 also used starch and chitosan to produce films, but they incorporated oregano essential oil to reinforce the antimicrobial property. Starch

is generally used in coating slurry of papers. Mixing starch with nanometric zinc oxide, Prasad, *et al.*, 2010 developed a coating slurry for paper substrate with anti-fungal property. Various authors investigated also research on antibacterial zein films. (Mastromatteo, *et al.*, 2009) incorporated thymol in zein based-films in order to release more progressively the essential oil. Arcan and Yemenicioğlu, 2013 chose to develop flexible zein-composite as release matrix for lysozyme. The listing of all the current research about antibacterial biopolymers films and coating is quite consequent. For more details, very exhaustive reviews have been published and give all the details relating to the different studies (Appendini and Hotchkiss, 2002, Balasubramanian, *et al.*, 2009, Cha and Chinnan, 2004, Cooksey, 2001, Cooksey, 2005, Han, 2003, Joerger, 2007, Khwaldia, *et al.*, 2010).

Nowadays, it seems clear that several possibilities are investigated to propose key solutions to the society's requirements.

Not only active packaging is needed but also a "controlled release packaging". It means "an active system that uses packaging materials as delivery vehicle to efficiently bring the active compound in specific controlled rates, over prolonged periods, into the food, to enhance the food quality and safety" (Balasubramanian, *et al.*, 2009). The purpose is not only to inhibit the growth of bacteria or to kill them, but also to release progressively the active component in order to reach a prolonged efficiency of the activity. Proof is that each study ends with release studies of their new antibacterial materials to conclude on their effectiveness as a function of time. At lab scale, effective results are indeed obtained (Brody, *et al.*, 2001b, Quintavalla and Vicini, 2002, Suppakul, *et al.*, 2003) but a concern still remains: when will arise the extrapolation of this efficiency into the complexity of the industrial world?

A positive result at lab scale with food simulant will not necessarily give positive results with a real food. Real foods mean more nutrients, a lower water activity, a higher salt content, fats or proteins which can interact with the active compounds etc. Besides, the storage and transport conditions can influence the quality of the food by changing the temperature or the pH.

Thus the development of an universal effective antibacterial packaging seems for now a little bit compromised. However, the objective to elaborate a controlled release bio-material for food packaging is still effective. The previous studies raised different issues regarding their active materials: poor mechanical or barrier properties, poor release of the antibacterial agent, or manufacturing process too complicated etc. Then, to complete the previous works and help finding a key solution to society's requirements, we propose within the framework of this PhD, a work on the use of new biomaterials which could limit the previous drawbacks and allow a controlled release of active agents: the nanocellulose.

I-3. Nanocellulose: preparation and properties

The part 1.3. of this manuscript is an adaptation of a review published in 2012 by the same authors (Lavoine, *et al.*, 2012).

I-3.1. From cellulose to nanocellulose

Cellulose

Cellulose is considered to be the most abundant organic compound derived from biomass. The worldwide production of this biopolymer is estimated to be between 10^{10} and 10^{11} tons each year (Azizi Samir, *et al.*, 2005). Of this, only about $6 \cdot 10^9$ tons are processed by industries such as paper, textile, material and chemical industries (Simon, *et al.*, 1998).

Although cellulose is the main building material out of which woods are made, there are other major sources such as plant fibers (cotton, hemp, flax, etc.), marine animals (tunicate), or algae, fungi, invertebrates, and bacteria. Regardless of the plant sources, cellulose can be present in the leaf (e.g., sisal), in the fruit (e.g., cotton) or in the stalk or the rigid structure of plants (e.g., wood, flax). Figure 1-I.15 describes how cellulose is present in nature. Irrespective of its source, cellulose is a white fiber-like structure with no odor and has a density of around 1.5.

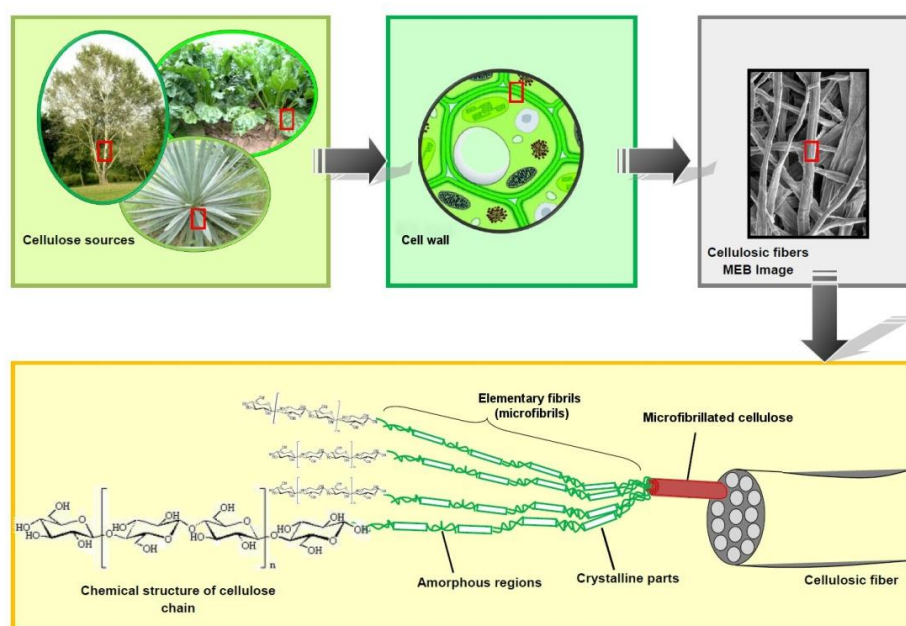


Figure 1-I.15. From the cellulose sources to the cellulose molecules: details of the cellulosic fiber structure with emphasis on the cellulose microfibrils

Since its discovery by Payen, 1838, the physical and chemical aspects of cellulose have been intensively studied. Today, its unique hierarchical structure no longer holds any secrets: cellulose is a linear homopolysaccharide of β -1.4-linked anhydro-D-glucose units (Habibi, *et al.*,

2010, Siqueira, *et al.*, 2010b) with a degree of polymerization (Robertson, 2008) of approximately 10,000 for cellulose chains in nature and 15,000 for native cellulose cotton (Azizi Samir, *et al.*, 2005). The basic chemical structure of cellulose, which is presented in Figure 1-I.15, shows a dimer called cellobiose that appears as a repeated segment. The monomer, named anhydroglucose unit (AGU), bears three hydroxyl groups. These groups and their ability to form strong hydrogen bonds confer upon cellulose its most important properties, in particular its (i) multi-scale microfibrillated structure, (ii) hierarchical organization (crystalline vs. amorphous regions), and (iii) highly cohesive nature (with a glass transition temperature higher than its degradation temperature).

There are four different polymorphs of cellulose: cellulose I, II, III, and IV (Figure 1-I.16).

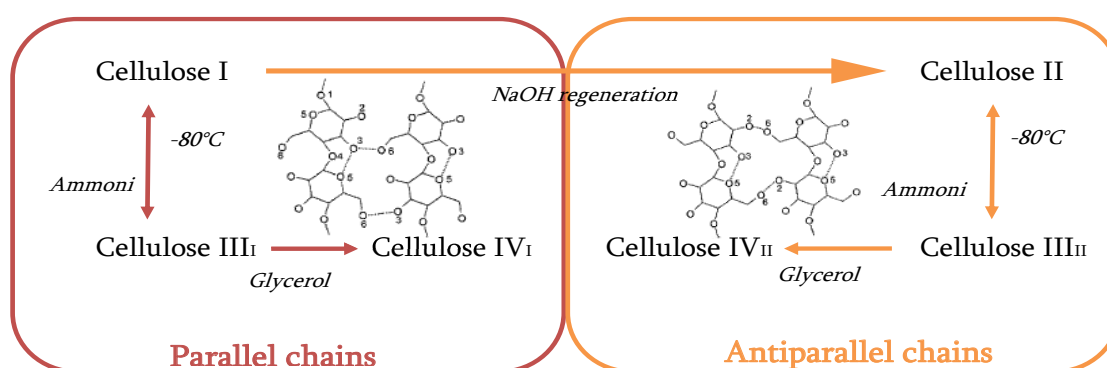


Figure 1-I.16. Polymorphs of cellulose and the main steps to obtain them

Cellulose I, native cellulose, is the form found in nature, and it occurs in two allomorphs, I_α and I_β. Cellulose II, or regenerated cellulose, emerges after re-crystallization or mercerization with aqueous sodium hydroxide. It is the most stable crystalline form (Aulin, 2009, Siqueira, *et al.*, 2010b). The major distinction between these two forms of cellulose lies in the layout of their atoms: cellulose II has antiparallel packing, whereas the chains in cellulose I run in a parallel direction (Aulin, 2009).

Cellulose III_I and III_{II} are obtained by ammonia treatment of cellulose I and II, respectively, and with the modification of cellulose III, cellulose IV is finally produced (Figure 1-I.16).

Within the framework of this PhD, only native cellulose I is considered. This semicrystalline fibrillar structure is indeed the main source of nanocellulose.

Nanocellulose

According to Habibi, *et al.*, 2010 about 36 individual cellulose molecules are brought together by biomass into larger units known as elementary fibrils or microfibrils, which are packed into larger units called microfibrillated cellulose. The latter are in turn assembled into familiar cellulose fibers, which are presented schematically in Figure 1-I.15. The diameter of

elementary fibrils is about 5 nm whereas the microfibrillated cellulose (also called nanofibrillated cellulose) has diameters ranging from 20 to 50 nm. The microfibrils are formed during the biosynthesis of cellulose and are several micrometers in length. Each microfibril can be considered as a flexible hair strand with cellulose crystals linked along the microfibril axis by disordered amorphous domains (Azizi Samir, *et al.*, 2005). The ordered regions are cellulose chain packages that are stabilized by a strong and complex network of hydrogen bonds (Habibi, *et al.*, 2010) that resembles nanocrystalline rods. This is why the two main types of nanocellulose are: (i) cellulose nanocrystals and (ii) cellulose microfibrils. Very recently, a book has been published entirely dedicated to nanocellulose (Dufresne, 2012).

There is yet another source of nanocellulose: bacterial cellulose. These cellulose nanofibers (Klemm, *et al.*, 2011, Klemm, *et al.*, 2009, Siró and Plackett, 2010) are secreted extracellularly by specific bacteria, mainly by *Gluconacetobacter* strains. For example, *Gluconacetobacter xylinus* secretes microfibrillated cellulose having a width of about 3.5 nm (Kose, *et al.*, 2011). Its unique fibrillar nanostructure endows it with excellent physical and mechanical properties such as high porosity, high elastic modulus (Guhados, *et al.*, 2005, Saheb and Jog, 1999, Siró and Plackett, 2010), and high crystallinity (up to 84–89% (Czaja, *et al.*, 2004)). Nowadays, bacterial cellulose is the subject of investigation on applications in numerous areas: biomedical applications (Czaja, *et al.*, 2004, Klemm, *et al.*, 2006, Oscar M, *et al.*, 2004), reinforcement in nanocomposites (Juntaro, *et al.*, 2008, Nakagaito and Yano, 2003, Nogi and Yano, 2008), electronic papers (Shah and Malcolm Brown, 2004), and fuel cell membranes (Evans, *et al.*, 2003). However, only a few studies have focused on barrier and packaging applications and their industrial use does not seem imminent. *For these reasons, we have decided not to focus this PhD on bacterial cellulose.*

The structure of native cellulosic fibers consequently results in two families of cellulosic nanoparticles which, depending on the researcher, can be referred to by many different names. Indeed, because different terminologies are used to describe these two nanocelluloses, this leads to some misunderstandings. The clearest way to distinguish between these cellulose nanoparticles is to consider the steps involved in their preparation. The main steps involved in the preparation of cellulose nanocrystals and microfibrillated celluloses, along with the terminologies, are presented in Figure 1-I.17.

Concerning cellulose nanocrystals, the first reference to the existence of definite crystalline zones in the amorphous structure of cellulose materials originates with Nageli and Schwendener, 1870. They confirmed the optical anisotropy of plants both in cell walls and in fibers. Many decades later, Rånby and Ribl, 1950 were the first to produce a stable suspension of colloidal cellulose crystals via the sulfuric acid hydrolysis of wood and cotton cellulose. The nanocrystals obtained were 50–60 nm in length and had a diameter of about 5–10 nm. Since then, an exponential number of researchers have focused their work on such materials. Pioneering work in cellulose nanocrystals mainly comes from Canada (Marchessault, Gray) and France (Dufresne, Cavail ), as shown by the date and the number of scientific papers of each author.

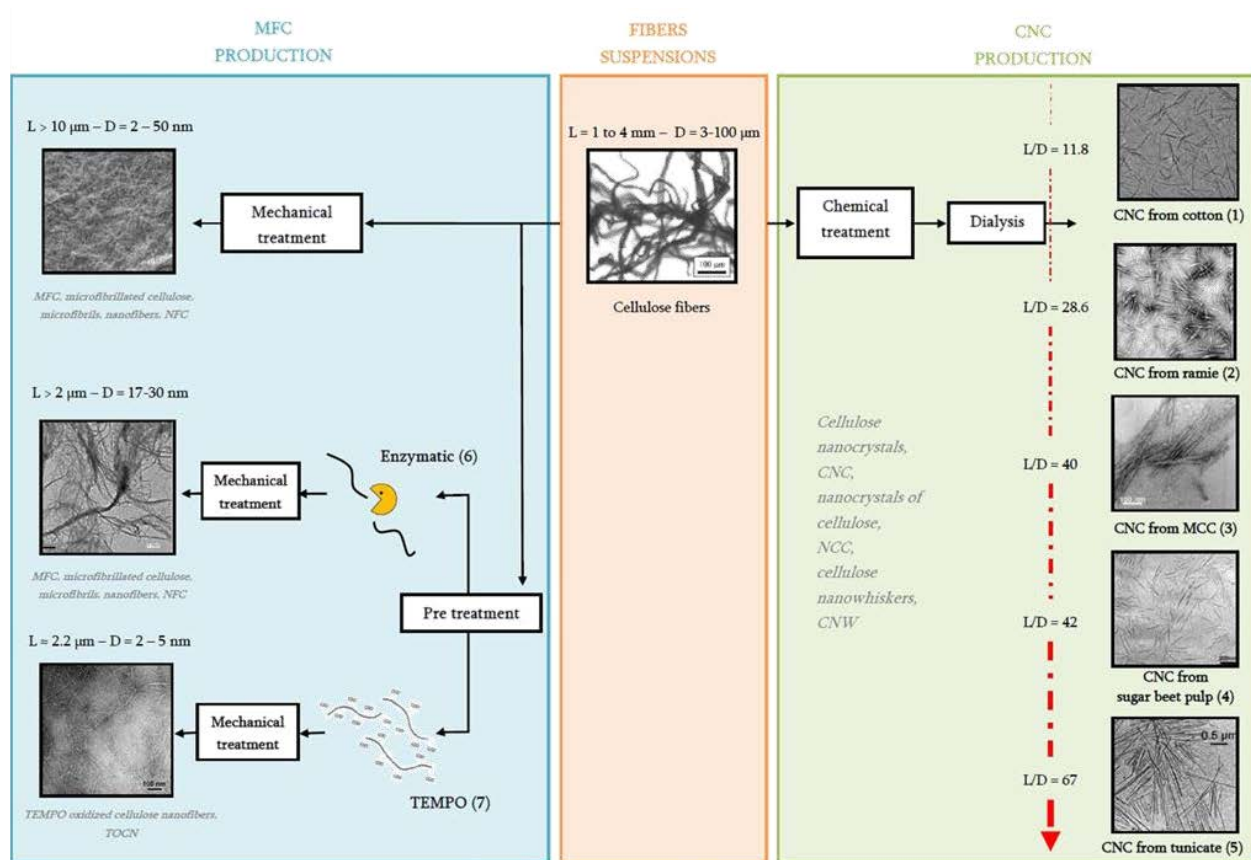


Figure 1-I.17. From fibers suspensions to nanocelluloses with their various terminologies

- (1) Fleming K., Gray D., Prasannan S., Matthews S (2000) *J. Am. Chem. Soc.*, 122:5224
- (2) Habibi Y., Goffin A. L., Schiltz N., Duquesne E., Dubois P., Dufresne A. (2008) *J. Mater. Chem.*, 18:5002
- (3) Siqueira G., Bras J., Dufresne A. (2009) *Biomacromolecules*, 10:425
- (4) Azizi Samir M.A.S, Alloin F., Paillet M., Dufresne A. (2004) *Macromolecules*, 37:4313
- (5) Angles M. N., Dufresne A. (2000) *Macromolecules*, 33:8344
- (6) Hassan M. L., Hassan E. A., Oksman K. N. (2011) *J. Mater. Sci.*, 46:1732-1740
- (7) Saito T., Nishiyama Y., Putaux J.-L., Vignon M., Isogai A. (2006) *Biomacromolecules*, 7(6)1687-16

Many terms are used to refer to cellulose nanocrystals (Figure 1-I.17): rod-like colloidal particles, nanocrystalline cellulose, cellulose whiskers, cellulose microcrystallites, microcrystals, microfibrils, etc. The recent review of Habibi, *et al.*, 2010 gives an overview of these nanocelluloses. In this chapter, the term “cellulose nanocrystals” (CNC) will be used. As described in another review by Azizi Samir, *et al.*, 2005 cellulose nanocrystals are cellulose structures grown under controlled conditions, leading to the formation of high-purity single crystals. The main process in the preparation of CNC is based on strong acid hydrolysis under strictly controlled conditions of temperature, agitation, and time (Bondeson, *et al.*, 2006). The amorphous regions, which are considered as structural defects, are attacked under acid hydrolysis, leaving the crystalline regions, the more resistant domain, intact. The resulting suspension is washed by successive centrifugations and dialysis is performed using distilled water to remove any free acid molecules. Different sources of cellulose were studied and used for producing CNC: tunicin (Šturcová, *et al.*, 2005), valonia (Sugiyama, *et al.*, 1994), cotton (Revol, *et al.*, 1994), wood pulp (Boluk, *et al.*, 2011), microcrystalline cellulose (Bondeson, *et al.*, 2006), sugar-beet pulp (Azizi Samir, *et al.*, 2004), etc. A recent study (Bras, *et al.*, 2011b) shows the influence of sources on the dimensions and structure of cellulose nanocrystals.

The precise morphological characteristics of CNC are typically studied using microscopic techniques (TEM, AFM, SEM) or light scattering techniques (SANS, polarized and depolarized dynamic light scattering, DLS, DDLS)(Habibi, *et al.*, 2010). Recently, a study showed that intrinsic viscosity measurements could also be used to calculate the aspect ratio of CNC rods (Boluk, *et al.*, 2011).

Concerning mechanical properties, the Young's modulus of CNC was estimated to lie between 130 GPa (Sakurada, *et al.*, 1962) and 250 GPa (Zimmermann, *et al.*, 2004), a value that is close to the modulus of a perfect crystal of native cellulose (which has a modulus of 167.5 GPa, according to (Habibi, *et al.*, 2010) and (Tashiro and Kobayashi, 1991)). Modelization strategies have also been developed, as detailed in another recent review (Moon, *et al.*, 2011).

Such studies on nanocrystals are on the rise with an increasing number of scientific publications and conferences such as the International Conference on Nanotechnology for Renewable Materials (TAPPI, 2011-2013), not to mention new reviews each year, such as that by (Ramires and Dufresne, 2011) or the publishing of book (Dufresne, 2012). In 2012, we even anticipate the industrial-scale production of such materials, by a joint venture formed between Domtar and FPInnovation in Canada, called CelluForce. In 2013, the University of Maine has launched its production of CNCs from wood and is now supplying them to academic, public and private research groups interested in evaluating this material. The research on CNC is thus promoted and expanding thanks to recent implementation of pilot plants able to scale-up the production of CNC in order to sell them after.

However, this PhD specifically focuses on the other family of nanocellulose, i.e., microfibrillated cellulose. Indeed, microfibrillated cellulose has been strongly studied last decade with almost one scientific paper every two days since 2011. Their intrinsic properties (e.g. gas barrier) and their nanostructure entanglement lead us to consider them as very good candidate for active bio-based packaging materials.

Microfibrillated cellulose

Microfibrillated cellulose (MFC), also called cellulose microfibril, microfibrillar cellulose, or more currently, nanofibrillated cellulose (NFC), has been reviewed quite recently, particularly in terms of nanocomposite applications (Klemm, *et al.*, 2011, Siqueira, *et al.*, 2010b, Siró and Plackett, 2010).

MFC can be viewed as a cellulosic material, composed of expanded high-volume cellulose, moderately degraded and greatly expanded in surface area, and obtained by a homogenization process (Herrick, *et al.*, 1983, Turbak, *et al.*, 1985). MFC actually consists of aggregates of cellulose microfibrils. Its diameter is in the range 20–60 nm and it has a length of several micrometers. If we consider that the microfibrils have a 2–10-nm-thick fibrous cellulose structure and a length of several tens of microns (Siró and Plackett, 2010), then MFC is composed of 10 to 50 microfibrils.

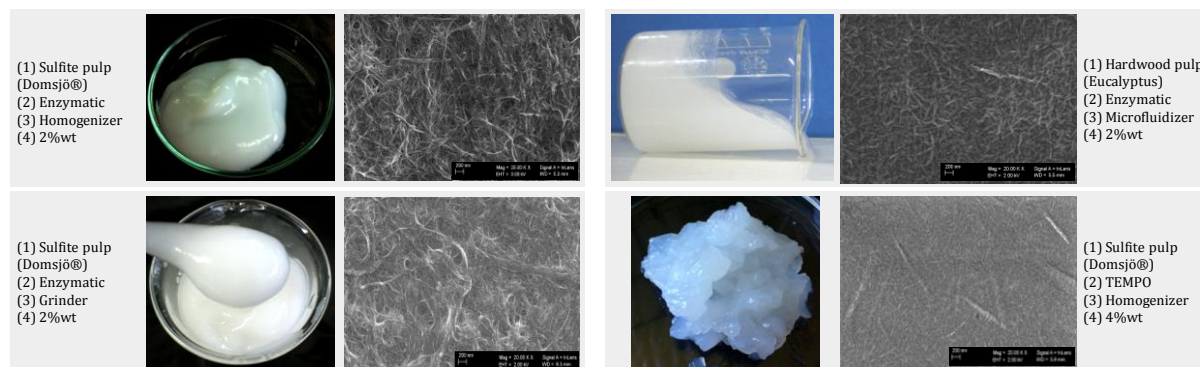


Figure 1-I.17. Pictures and FE-SEM images of different kinds of MFC suspensions. Steps of their manufacturing process are described nearby with (1) cellulosic source, (2) applied pre-treatment, (3) mechanical treatment and (4) concentration of final suspension.

Unlike CNC, MFC exhibits both amorphous and crystalline parts and presents a web-like structure (Lu, *et al.*, 2008). Furthermore, the ratio L/d of MFC is very high, which endows it with a very low percolation threshold (Figure 1-I.17). Microfibrillated cellulose thus has a very good ability to form a rigid network. It is obtained by the mechanical disintegration of cellulosic materials without the use of hydrolysis.

Turbak, *et al.*, 1985 were the first to patent a new process to produce a new kind of cellulose, which they named microfibrillated cellulose (MFC). In that process, a wood fiber suspension was passed several times through a narrow gap under high pressure, thus forming a viscous gel (Figure 1-I.18). This low-cost and totally new kind of cellulose already showed attractive applications such as a binder for paper or as a viscosity modifier. This patent marked the emergence of many significant research studies focusing on either optimizing such a mechanical process, or analyzing newly found properties and applications. Very recently, the industrial production of MFC has been launched by fiber companies such as Booregaard, UPM, and Innventia (Chauve and Bras, 2013). Moreover, very important European projects such as SUNPAP, 2010 and FlexPakRenew, 2007 focused recently on the scaling up of MFC productions and new possible applications.

The first cellulosic material that was used to produce MFC was wood, as reported by Herrick, *et al.*, 1983 and Turbak, *et al.*, 1985. Wood pulp was disintegrated many times in a high-pressure homogenizer in order to obtain a viscous and shear thinning aqueous gel at a very low concentration (between 2 and 7% w/w). This is one of the two main characteristics of such a nanomaterial, and the other is its ability to form a transparent film once it is dried. Both these key properties are linked to its high specific area (at least ten times larger than that of cellulose fibers) and its extensive hydrogen-bonding ability.

Increasingly, various mechanical treatments have been used to manufacture MFC, depending on the type of MFC that is desired (Figure 1-I.18, 19), as detailed in the next subchapter.

1-3.2. Microfibrillated cellulose preparation

Microfibrillated cellulose is currently manufactured from a number of different cellulosic sources. Wood is obviously the most important industrial source of cellulosic fibers, and is thus the main raw material used to produce MFC. Bleached kraft pulp is most often used as a starting material for MFC production (Iwamoto, *et al.*, 2005, Saito, *et al.*, 2007, Saito, *et al.*, 2006a, Spence, *et al.*, 2010a, Spence, *et al.*, 2010b, Taipale, *et al.*, 2010), followed by bleached sulfite pulp (Ahola, *et al.*, 2007, Stenstad, *et al.*, 2007). The demand for such raw materials has increased, as has the competition among the many sectors that use wood (the building, furniture, pulp and paper industries, etc.).

Therefore, interest in other sources such as agricultural crops and their by-products, is increasing: they offer environmental benefits owing to their renewable nature and their low energy consumption in production (Alemdar and Sain, 2008). Unlike wood, whose cellulose fiber is present in a plant's secondary wall, cellulose microfibrils from agricultural fibers are easier to

separate from the primary wall (Dufresne, *et al.*, 1997), and the fibrillation of this pulp demands less energy.

In the literature, we find that diverse non-wood sources are already being used to produce MFC. For example, it can be extracted from sugar beet pulp (Dinand, *et al.*, 2002, Habibi and Vignon, 2007), wheat straw and soy hulls (Alemdar and Sain, 2008), sisal (Siqueira, *et al.*, 2009) or even bagasse (Bhattacharya, *et al.*, 2008), palm trees (Bendahou, *et al.*, 2010), jute, flax and hemp (Alila, *et al.*, 2012), carrots (Siqueira, *et al.*, 2010c), etc.

Irrespective of the source, MFC is manufactured from a pulp suspension mainly using a mechanical treatment.

In the 1980s, research groups used a Gaulin homogenizer with a pressure of 8000 psi (Herrick and Wash, 1984a, Herrick and Wash, 1984b, Turbak, *et al.*, 1985). In this approach, the wood pulp is passed through a small-diameter orifice under considerable pressure. It is exposed to a high shearing action followed by a high-velocity decelerating impact. Several passes of the suspension are necessary in order to obtain a substantially stable gel: 8 to 10 (Herrick and Wash, 1984a). In order to maintain a product temperature in the range 70–80°C during the homogenization treatment, cooling water is used. Other equipment was subsequently produced that offered alternative approaches. One example is the Microfluidizer®, from the Microfluidics Corporation. Unlike in the homogenizer, in the microfluidizer, the wood pulp is forced through thin z-shaped chambers under pressures as high as 30,000 psi (Figure 1-I.19) (Siqueira, *et al.*, 2010b). As a consequence, it is possible to produce more uniform nanocellulose that has thinner dimensions. In this approach, though, the repetition of the mechanical treatment with different sizes of chambers remains a necessary step in order to increase the degree of fibrillation (Herrick, *et al.*, 1983).

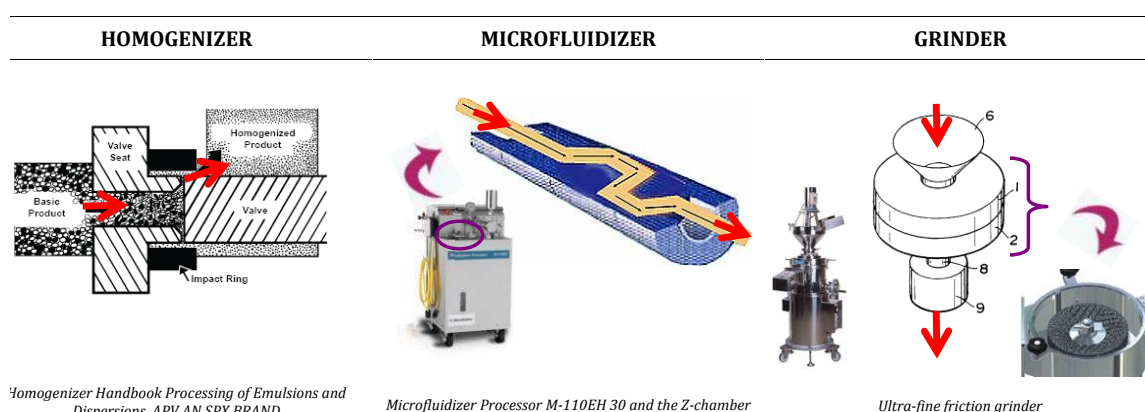


Figure 1-I.19. The most applied mechanical treatment processes used in the fabrication of microfibrillated cellulose: the homogenizer, the microfluidizer and the grinder

Some other mechanical treatments exist such as cryocrushing (Chakraborty, et al., 2005, Lavoine, et al., 2012) and electrospinning (Lavoine, et al., 2012, Li and Xia, 2004), but they will not be detailed in this manuscript as they have not been used to produce the MFC suspensions exploited during this PhD. Because of the numerous mechanical passes necessary to obtain well-defibrillated fiber suspensions, each mechanical process creates a high demand for energy. To address this energy problem, researchers have developed some pre-treatment processes for pulp. These treatments and pre-treatments are discussed next.

This manuscript will focus especially on enzymatic pre-treatment and mechanical treatments made with homogenizer and microfluidizer, as they have been applied to produce the MFC suspensions used in this PhD.

As stated above, several types of equipment currently exist to defibrillate the pulp and obtain nanocellulose. An exhaustive list of different possibilities is presented in Tables 1-I.6, 7, and 8; unfortunately, numerous authors have not provided the precise specifications of the equipment used for the production of MFC.

Mechanical treatments

- Homogenizer and microfluidizer

The first mechanical treatment, which was applied by Turbak, *et al.*, 1985 was the Gaulin homogenizer, which is still often used to manufacture all kinds of microfibrillated cellulose (Dinand, *et al.*, 1999, Dinand, *et al.*, 2002, Iwamoto, *et al.*, 2005, Leitner, *et al.*, 2007, Nakagaito and Yano, 2004, Uetani and Yano, 2011). In this treatment, cellulose slurry is pumped at high pressure and fed through a spring-loaded valve assembly. The valve opens and closes in rapid succession, so the fibers are subjected to a large pressure drop under high shearing forces. This combination of forces promotes a high degree of fibrillation of the cellulose fibers (Nakagaito and Yano, 2004). *This mechanical treatment was applied, in our case, to produce the MFC suspension (2% w/w) from sulfite pulp (Domsjö®). This suspension was produced and kindly supplied by the Centre Technique du Papier (CTP, Grenoble, France), who used their homogenizer GEA Niro Soavi ARIETA NS3075H.*

As mentioned earlier, a recent alternative to the Gaulin homogenizer is the microfluidizer, in which the wood pulp passes through thin z-shaped chambers (with channel dimensions that are usually 200–400 μm) under high pressure, i.e., 2070 bar (Siqueira, *et al.*, 2010b). The shear rate applied is thus very high (up to 10^7s^{-1}), which results in the formation of very thin cellulose nanofibers. This equipment is increasingly being used, since it makes it possible to obtain more uniformly sized fibers (Aulin, 2009). However, application of a mechanical treatment with chambers of different sizes remains a necessary step to increase the degree of fibrillation. The number of passes required easily runs to 10 (Leitner, *et al.*, 2007,

Taipale, *et al.*, 2010) and can even reach as high as 30 (Iwamoto, *et al.*, 2005). This requirement limits the possible scaling-up of production and results in a negative environmental impact with high energy consumption. *The MFC suspension (2% w/w) from eucalyptus pulp used in this PhD was produced by the CTP using a Microfluidizer M-110EH-30 (4 passes through the chamber 200 nm, 5 passes through the chamber 100 nm). To limit the high energy consumption, an enzymatic pre-treatment was applied and will be detailed further.*

- *Grinding process*

Another category of equipment is based on a grinding process. Masuko© was the first to build and sell apparatus using this approach, which is usually called a grinder. The principle consists of the breakdown of the cell wall structure owing to the shearing forces generated by the grinding stones. The pulp is passed between a static grind stone and a rotating grind stone revolving at about 1500 rpm. The nanofibers that compose the cell wall in a multi-layer structure are thus individualized from the pulp. Iwamoto, *et al.*, 2007 fibrillated pulp fibers from *Pinus radiata* with a grinder and observed the influence of multiple passes through the grinder on the MFC morphology. From one to three passes, most of the fibers were turned into sub-micron-size and nano-sized fibers. At five passes, most of the fibers became nano-sized fibers. With a higher number of passes, no significant changes were observed in the fiber morphology. It was deduced, then, that with five passes through the grinder, the fibrillation of pulp fibers was almost complete.

Unlike the homogenizer process, the grinding process apparently requires fewer passes to obtain MFC. However, this process can degrade the pulp fibers and decrease their length, which might affect the reinforcement and physical properties of MFC (Iwamoto, *et al.*, 2007). Unfortunately, as detailed later, length is difficult to measure and such properties cannot be studied and monitored in detail. Other authors tried to measure the chain length based on the measurements of the intrinsic viscosity in cupriethylene diamine. They proved with this technique that the microfluidizer shortens the chain length more than the grinder (Pöhler, *et al.*, 2010).

Sources	Pre-treatment	Mechanical treatment	Post-treatment	References
Sugar beet pulp Bich hardwood cellulose Potato pulp Kraft pulp (Norway spruce) Southern pine sulphite pulp Kraft pulp from Lodgepole Pine (<i>Pinus contorta</i>) White Spruce (<i>Pinus glauca</i>) and Douglas-fir (<i>Pinus menziesii</i>) Sugar beet pulp Fully bleached spruce sulphite pulp Kraft pulp of Norway spruce Sugar beet pulp Sugar beet pulp Bleached kraft pulp Fully bleached spruce sulphite pulp Fully bleached spruce sulphite pulp Bleached softwood sulphite pulp Kraft and sulphite pulp Bleached spruce sulphite cellulose	- Acetylation - Silylation (IPDM-SiCl) Silylation (IPDM-SiCl) Octadecyldimethyl(3-trimethoxysilylpropyl)-ammonium chloride (ODDMAC) Silylation with chlorodimethyl isopropylsilane - Grafting of glycidyl methacrylate (Polymerisation reaction) Coating with hexamethylene diisocyanate + functionalization with 2 differents amines Grafting of anhydrides (Diisopropylamine) -			Dinand et al. EP 0726356A1, 07.02.1996 Diez et al. (2011) Macromolecular Bioscience, 11:1185-1191 Dufresne et al. (1998) Macromolecules, 31:2693-2696 Rodionova et al. (2011) Cellulose, 18:127-134 Franklin et al. US 4,481,077, 06.11.1984 Nakagaito et al. (2004) Applied Physics A, 78:547-552 Nakagaito et al. (2005) Applied Physics A, 80:155-159 Dufresne et al. (1997) Journal of Applied Polymer, 64(6):1185-1194 Syvend et al. (2009) Cellulose, 16:75-85 Rodionova et al. (2010) Cellulose, 18:127-134 Goussés et al. (2004) Polymer, 45:1569-1575 Agoda-Tandjawa et al. (2010) Carbohydrate Polymers, 80:677-686 Hult et al. (2010) Cellulose, 17:575-586 Andresen et al. (2007) Biomacromolecules, 8:2149-2155 Chinga-Carrasco et al. (2010) J Nanopart Res, 12:841-851 Andresen et al. (2006) Cellulose, 13:665-677 Turbaek et al. (ITT Industries, Inc.) CH 648 071 A5, 28.02.1985 Stenstad et al. (2008) Cellulose, 15:35-45 Quiévy et al. (2010) Polymer degradation and stability, 95:306-314 Syvend et al. (2011) Carbohydrate polymers, 84:1033-1038 Spence et al. (2010) Bioresource Technology, 101:5961-5968 Spence et al. (2010) Cellulose, 17:835-848 Spence et al. (2011) Cellulose, 18:1087-1111 Wenshuai et al. (2010) Advanced materials research, 87:88-393-397 Matsuda et al. US 6,183,586 B1, 06.02.2001 Iotti et al. (2011) J Polym Environ, 19:137-145 Dinand et al. (1999) Food Hydrocolloids, 13:275-283 Syvend et al. (2011) Carbohydrate polymers, 84:1033-1038 Bilbao-Sainz et al. (2011) Carbohydrate Polymers, 86:1549-1557 Ivaneto et al. (2009) Biomacromolecules, 10:2571-2576 Xhanari et al. (2011), Cellulose, 18:257-270 Mikkonen et al. (2011) Cellulose, 18:713-726 Sidi et al. (2011) Journal of Applied Polymer Science, 119:2652-2660 Minelli et al. (2010) Journal of Membrane Science, 358:67-75 Plackett et al. (2010) Journal of Applied Polymer Science, 117:3601-3609 Wichmann (Borregaard Industries Limites) EP 2 196 579 A1, 09.12.2008 Svagan et al. (2009) Composites Science and Technology, 69:500-506 Beiglund et al. (KTH Holding AB) WO 2011/059398 A1 Plackett et al. (2010) Journal of Applied Polymer Science, 117:3601-3609 Minelli et al. (2010) Journal of Membrane Science, 358:67-75 Hollan et al. (Boregaard Industries Limited) WO 2010/105847, 23.09.2010 Wenshuai et al. (2010) Advanced materials research, 87:88-393-397 Henriksson et al. (2007) European Polymer Journal, 43:3434-3441 Liu et al. (2011) Biomacromolecules, 12:633-641 Hamada and Boufield (2010) TAPPI 11th Advanced Coating Fundamentals Symposium, Germany, 11-13.10.2010 Dufresne et al. (1997) Journal of Applied Polymer, 64(6):1185-1194
Bleached wood pulp Bleached kraft pulp (<i>Eucalyptus</i> and <i>Pinus radiata</i>) Unbleached and bleached hardwood and softwood kraft pulp Kraft wood pulps after chemical treatments and TMP Bleached and unbleached kraft hardwood pulps Bleached kraft pulp Bleached kraft pulp Elemental chlorine free (ECF) fully bleached sulphate pulp (<i>Picea abies</i>) ECF fully bleached sulphate cellulose (<i>Picea abies</i>) with up to 5% w/w pine (<i>Pinus sylvestris</i>) Sugar beet pulp Bleached kraft pulp (<i>Eucalyptus</i> and <i>Pinus radiata</i>) Softwood cellulose fibers Tunicate (<i>Halicynthia papillosa</i>) Fully bleached softwood kraft pulp Sulfite dissolving pulp (Domsjö Dissolving Plus) Sulfite dissolving pulp (Domsjö Dissolving Plus) Sulfite softwood dissolving pulp (Domsjö Dissolving Plus) Sulfite softwood dissolving pulp (Domsjö Dissolving Plus) Wood pulp Bleached softwood sulphite pulp Wood pulps Bleached sulfite softwood (Domsjö ECO Bright) Bleached sulfite softwood (Domsjö ECO Bright) Wood pulp Bleached kraft pulp Bleached wood sulphite pulp based on Norway Spruce (<i>Picea abies</i>) Bleached wood sulphite pulp based on Norway Spruce (<i>Picea abies</i>) Wood pulp Sugar beet pulp	Grinders comprising abrasive grains Cutting with an office shredder Waring Blender TEMPO-Mediated Oxidation Carboxymethylation Enzymatic Acid hydrolysis (mild and strong conditions) Magnesium Chloride Cyclocrossing	Homogenizer		

Table 1-I.7. Pre- and post-treatments applied in literature to different sources with Homogenizer as mechanical treatment

Sources	Pre-treatment	Mechanical treatment	Post-treatment	References
Bleached hardwood kraft pulp		Successive stirring		Ishii et al. (2011) Biomacromolecules, 12:548-550
Moso Bamboo Wheat straw Flax fibers				Chen et al. (2011) Cellulose, 18:433-442
Bleached southern pine kraft pulp Kraft pulp		Sonication		Li et al. (2010) Cellulose, 17(1):57-68
Softwood bleached kraft pulp			Alkali treatment Methylation	Li et al. (2009) Cellulose, 16(6):1025-1032 Fukuzumi et al. (2010) Polymer degradation and stability, 95:1502-1508
Tunicin and bacterial cellulose	TEMPO-Mediated Oxidation	Ultra turrax		Saito et al. (2006) Biomacromolecule, 7(6):1687 - 1691
Fully bleached softwood kraft pulp Fully bleached softwood kraft pulp Bleached hardwood kraft pulp				Xhanari et al. (2011) Cellulose, 18:257-270 Sveoud et al. (2011) J. Nanopart Res, 13:773-782 Saito et al. (2007) Biomacromolecules, 8:2465-2491
Japanese Cedar (<i>Cryptomeria japonica</i>) pulp		Grinder		Uelani et al. (2011) Biomacromolecules, 12:346-353
ECF-bleached birch kraft pulp				Vartiainen et al. (2011) Cellulose, 18:775-786
Bleached hardwood pulp			Spence et al. (2011) BioResources, 6(4):4370-4388	
Never - dried holocellulose pulp (<i>Sitka spruce</i> , <i>Picea sitchensis</i> Carr.)				
Once - dried holocellulose pulp (<i>Sitka spruce</i> , <i>Picea sitchensis</i> Carr.)				
Never- dried alkali treated pulp (<i>Sitka spruce</i> , <i>Picea sitchensis</i> Carr.)				
Once - dried alkali-treated pulp (<i>Sitka spruce</i> , <i>Picea sitchensis</i> Carr.)				
Wood pulp (<i>Pinus radiata</i>)				
Wood powder from Hinoki cypress (<i>Chamaecyparis obtusa</i>)				
Wood powder (<i>Pinus radiata</i>)	Refining			
Bleached and unbleached kraft hardwood pulps	Acetylation			
Wood powder from Douglas fir (<i>Pseudotsuga menziesii</i>)	Enzymatic			
Southern softwood bleached pulp				
Bleached northern black spruce pulp	Refining	Cryocrushing		
Wheat straw	Acid and alkali treatments			
Wheat straw and soy hulls		High-speed blender		
Japanese Cedar (<i>Cryptomeria japonica</i>) pulp		Blender + Homogenizer Gaulin		
Potato pulp				
Softwood and hardwood bleached kraft pulps		Blender-type homogenizer		
		Double cylinder homogenizer		
		Cylinder-type homogenizer		
		Blade agitation		
		Waring Blender		
Softwood bleached kraft pulp	TEMPO-Mediated Oxidation	Refiner		
Bleached sulfite pulp from <i>Pinus pinaster</i>		Extruder		
Wood pulp	Enzymatic			
Kraft pulp	Chemical (hydrogen peroxide) or enzymatic			
Cellulosic fibers				
Hadwood bleached kraft pulp	TEMPO Mediated Oxidation	No mechanical treatments		
Cotton linters				
Bleached ramie fibers				
Spruce holocellulose (<i>Picea abies</i>)				
Softwood bleached kraft pulp				

Table 1-I.8. Pre- and post-treatments applied in literature to different sources with others and less used mechanical treatments

- *Energy consumption and new processes*

Each of the previously mentioned mechanical treatments requires high energy consumption. Eriksen, *et al.*, 2008 determined, for example, that the consumption required by a homogenizer reaches as high as 70,000 kWh/t.

Zimmermann, *et al.*, 2010 also estimated the energy consumption of their mechanical treatment. The microfluidizer needs 8.5 kWh, assuming a processing pressure of 1500 bar. If we consider that 10L of cellulose pulp at 1–2wt% takes about 15 minutes to pass through a microfluidizer once, then the crucial parameter that has a strong influence on energy consumption is the number of passes. With four passes, the energy consumption was estimated to be 8.5 kW, and its value increased to 14,875 kW with only three passes more. Spence, *et al.*, 2011a conducted a very precise comparative study of the energy consumption and physical properties of MFC produced by different processing methods, namely a homogenizer, a microfluidizer, and a grinder. For bleached and unbleached kraft hardwood pulps, they compared the consumption as a function of the mechanical treatment, the number of passes, the pressure, and the speed. They concluded that the homogenizer resulted in MFC with the highest specific surface area and films with the lowest water vapor transmission rate, in spite of its high energy consumption. In addition, films produced by a microfluidizer and a grinder presented superior physical, optical, and water interaction properties, which suggests that these materials could be produced in a more economical way for packaging applications.

The development of disintegration methods that are less energy-consuming also becomes a priority in securing the industrialization of MFC production. Thus, some combinations of pre-treatments and mechanical treatments have been suggested, as shown in Tables 1-1.6, 7, and 8. Moreover, every year new equipment is being studied or developed in order to obtain MFC with low energy consumption or via a faster process. Testing with extrusion, for example, has recently been performed by Heiskanen, *et al.*, 2011. Other processes available for commercial use such as Cavitron®, which was further developed by the Papiertechnische Stiftung (PTS) in Germany as part of the project SUNPAP, 2010, and the GEA Niro Soavi ARIETA NS3075H, which was used for MFC sample preparation by Centre Technique du Papier (CTP) in France, as part of the same project SUNPAP, 2010, have also been recently proposed. Another strategy that has increasingly been developed in recent years is the fractionation of slightly ground MFC. For example, using a specific process for the extraction of wood MFC, Abe, *et al.*, 2007 produced MFC with a width of 15 nm with only one pass through a grinder. Similar research is being performed in Scandinavian countries, and Tanaka, *et al.*, 2011, recently presented a novel fractionation device, which was used to classify different MFC qualities.

Pre-treatments

Several strategies have been proposed in order to obtain fibers that are less stiff and cohesive, thus decreasing the energy needed for fibrillation, as detailed in Tables 1-I.6, 7, and 8. There are three alternatives: (i) limit the hydrogen bonds, and or (ii) add a repulsive charge, and or (iii) decrease the DP or the amorphous link between individual MFCs.

- Enzymatic pre-treatment

The last strategies (iii) consist in an enzymatic pre-treatment of cellulose pulp. Specific enzymes that hydrolyze crystalline cellulose into soluble sugar molecules are mixed with cellulose pulp. These enzymes, named cellulases, have three main types of activity. They can (1) cut randomly the amorphous part of cellulose to produce new extremity of chains; they can (2) act progressively onto the extremity of cellulose chains to free glucose or cellobiose; or they can (3) hydrolyze cellodextrin and cellobiose into glucose. The types (1) and (2) are the most efficient to produce MFC and are carried out by enzymes named respectively, endoglucanase and exoglucanase (Figure 1-I.20).

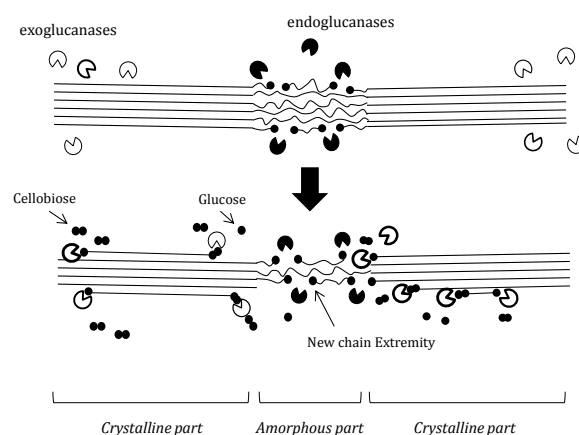


Figure 1-I.20. Enzymatic pre-treatment of MFC. Action modes of endoglucanases and exoglucanases

Pääkkö, *et al.*, 2007 obtained MFC that had a well-controlled diameter in the nanometer range and a high aspect ratio by combining enzymatic hydrolysis with mechanical shearing and high pressure homogenization (105 and 170 MPa). Between two refining steps, they performed an enzymatic treatment with endoglucanase before passing the pulp slurry through the microfluidizer. Such enzymatic hydrolysis is less aggressive than acid hydrolysis, and it allows for selective hydrolysis of the non-crystalline cellulose, which facilitates the mechanical disintegration (Engström, *et al.*, 2006, Pääkkö, *et al.*, 2007). The addition of the enzyme also promotes cell wall delamination, and thus prevents the z-shaped chamber in the microfluidizer from becoming blocked. In a more specific investigation, Henriksson, *et al.*, 2007 studied enzymatic pre-treatment using C-type endoglucanase, a type that requires some disorder in the

structure in order to attack the cellulose. They compared enzyme-pre-treated MFC with non-pretreated MFC, as well as with a gentle and strong hydrolysis of pretreated MFC. In each case, the mechanical treatment was done using a Gaulin homogenizer. Endoglucanase pre-treatment facilitates the disintegration of cellulosic wood fiber pulp by increasing its swelling in water. Moreover, this environmentally friendly pre-treatment confers a more favorable structure on the MFC, as it reduces the fiber length and increases the extent of fine material, as compared to the result of acid hydrolysis pre-treatment. *This kind of enzyme has been used to produce both MFC suspensions used in this PhD.*

In another study, Engström, *et al.*, 2006 further studied the mechanism of endoglucanase attack and its consequences for the reactivity of cellulose fibers. Their results showed that enzymatic pre-treatment seems to be a very promising method for industrial applications. It is also one of the key steps in the first pilot production of MFC that was announced by Lindström's group from Innventia ("Press Release: Nanocellulose - for the first time on a large scale - Innventia," 2011). A quite recent study that was subdivided into two scientific papers (Siqueira, *et al.*, 2010e, Siqueira, *et al.*, 2010d) gave a more detailed analysis of the impact of enzymatic treatment on the final properties of the MFC obtained. In their experiments, two kinds of enzymes at different concentrations were tested as post- and pre-treatments. The results showed the importance of precisely detailing such post- or pre-treatment. Indeed, the Young's modulus of ensured natural rubber nanocomposites with 6 wt% of MFC content could be completely different, yielding values of 31.7 ± 9.9 MPa versus 2.3 ± 0.4 MPa for an exoglucanase post- and pre-treatment, respectively; and 3.9 ± 0.1 MPa versus 0.84 ± 0.06 MPa for endoglucanase post- and pre-treatment, respectively (Siqueira, *et al.*, 2010e).

- *TEMPO mediated oxidation pre-treatment*

Currently, the more commonly used pre-treatment is TEMPO-mediated oxidation. Indeed, the TOCNs, or TEMPO-oxidized cellulose nanofibers, represent an entire category of nanocellulose worthy of consideration, in addition to CNC and MFC.

TEMPO-mediated oxidation is the most promising method for effecting the surface modification of native cellulose, in which carboxylate and aldehyde functional groups can be introduced into solid native cellulose under aqueous and mild conditions (Gert, *et al.*, 2005, Kitaoka, *et al.*, 1999, Montanari, *et al.*, 2005, Saito and Isogai, 2004, Saito, *et al.*, 2006a, Saito, *et al.*, 2006b). Moreover, compared to the energy consumption of repeated cycles of a high pressure homogenizer (700–1400 MJ/kg), TEMPO-mediated oxidation pre-treatment dramatically decreases the consumption to values less than 7 MJ/kg (Isogai, *et al.*, 2011a).

The basic principle of this form of pre-treatment consists in the oxidation of cellulose fibers via the addition of NaClO to aqueous cellulose suspensions in the presence of catalytic amounts of

2,2,6,6 tetramethyl-1-piperidinyloxy (TEMPO) and NaBr at pH 10–11 at room temperature. The C6 primary hydroxyl groups of cellulose are thus selectively converted to carboxylate groups via the C6 aldehyde groups, and only the NaClO and NaOH are consumed (Saito and Isogai, 2006). As a result, the nanofibrils within the fibers separate from each other better due to the repulsive forces among the ionized carboxylates, which overwhelm the hydrogen bonds holding them together (Eichhorn, *et al.*, 2010). The more NaClO is added, the more carboxylate groups are formed on the surface of the MFC. Correspondingly, the oxidation time also increases (Saito, *et al.*, 2007). With an increase in the addition of NaClO from 3.8 to 5.0 mmol/g, the carboxylate content increased from only 0.2 to 0.3 mmol/g, whereas the oxidation time increased from 40–45 min to 115–130 min.

Saito, Nishiyama, *et al.* 2006a and Saito, Okita, *et al.*, 2006b applied this treatment to many diverse sources: wood pulp, cotton linters, tunicate, bacterial cellulose, ramie, and even spruce holocellulose.

They defined the oxidation efficiency of their pre-treatment by the following equation (Saito, *et al.*, 2006b):

$$\text{Oxidation efficiency (\%)} = 100 * \frac{2 * (C_T - C_0) + (A_T - A_0)}{M_{\text{NaClO}}}$$

where M_{NaClO} is the quantity of NaClO added (mmol/g), C_0 and C_T are the carboxylate contents (mmol/g) before and after oxidation, respectively, and A_T and A_0 are the corresponding aldehyde contents (mmol/g), respectively.

For sulfite pulp, cotton linters, ramie, and spruce holocellulose, the oxidation efficiencies were 70–95%, 62%, 85%, and 96%, respectively.

Another TEMPO-mediated oxidation system has been reported by Hirota, *et al.*, 2009; Isogai, *et al.*, 2011a and Saito, *et al.*, 2009. This system is based on the same principle as the first one, except that it takes place at pH 7, NaClO replaces NaBr, and the primary oxidant is NaClO₂ instead of NaClO. The details of the differences between these two processes are summarized in a very recent review paper by Isogai, *et al.*, 2011a.

Recently, Isogai, *et al.*, 2011b developed an alternative method to oxidize the C6-primary hydroxyls of cellulose: a TEMPO electro-mediated reaction. They applied two new systems to softwood bleached kraft pulp: electro-mediated oxidation with TEMPO at pH 10, and 4-acetamido-TEMPO at pH 6.8 in a buffer solution. This is a new sustainable method to produce MFC that has carboxylate and aldehyde groups on its surface, and it could well replace the first two systems, although longer oxidation times are required. The yield is quite high (more than 80%). Moreover, it preserves the main characteristics of TEMPO-oxidized MFC produced from bleached softwood kraft pulp (Isogai, *et al.*, 2011a, Isogai, *et al.*, 2011b).

- *Carboxymethylation*

Another chemical pre-treatment is carboxymethylation. This pre-treatment increases the anionic charges in the formation of carboxyl groups on the surface of the MFC.

Aulin, 2009 produced carboxymethylated MFC and compared its dimensions with that of non-pretreated MFC. The carboxymethylation treatment makes the fibrils highly charged and easier to liberate. Moreover, Taipale, *et al.*, 2010 measured the net specific energy consumption of such treatment. They found that after carboxymethylation, the required energy of fluidization was 2.2 MWh/t per pass through a microfluidizer, whereas 5.5 MWh/t per pass was needed to obtain MFC without pre-treatment.

As regards such pre-treatments, Figure 1-I.21 gives a good overview of the situation to date. It highlights the novelty of the pre-treatment strategies and puts a particular emphasis on the research being conducted on enzymatic treatments.

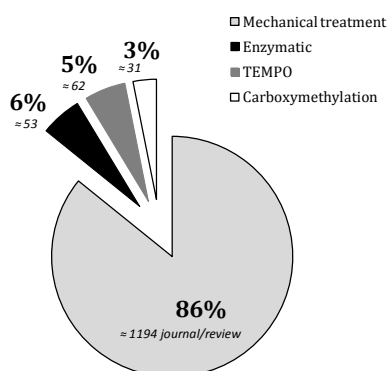


Figure 1-I.21. Quantification of the most frequently applied pre-treatments used for MFC production according to a bibliography research with the appropriate key words.

(SciFinder, Scientific documents found with the following key words, June 2013)

Combined with pre-treatments and mechanical treatments, a number of post-treatments are increasingly being carried out in order to enhance the properties of microfibrillated cellulose (Tables 1-I.6, 7 and 8). The use of post-treatment still remains small as compared to the numerous pre-treatment approaches. The objectives of the two treatments are actually completely different, since the primary aim of pre-treatment is to reduce the energy consumption of MFC production, whereas post-treatment primarily seeks to improve the MFC or endow it with new properties, from the perspective of new possible applications.

Within the framework of this PhD no post-treatments were applied on both MFC suspensions. For that reason, we will not detail the literature dealing with post-treatments. For further information, the authors send the reader to the original review (Lavoine, et al., 2012). To appreciate and well understand the relevant interest in using MFC as new biomaterials for food-packaging applications, its properties and characterization will be further detailed, keeping in mind its possible use as packaging materials.

I-3.3. Properties and characterization of MFC

Morphology

Depending upon the source of the cellulose and the method of production, MFC displays similar morphologies but various dimensions.

Techniques such as Scanning Electron Microscopy (SEM) (with and without the use of a Field Emission Gun (FEG)), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM) are the main approaches used to observe microfibrillated cellulose and to measure its diameter. Indeed, the length of long nanoscale material is rather complicated to determine, as detailed later.

Mechanical treatment	Pre-treatment	Diameter (nm)	References
Homogenizer	-	20-40	Siró I. and Plackett D. (2010) <i>Cellulose</i> , 17 (3): 459-494.
Grinder	-	20-50	Siró I. and Plackett D. (2010) <i>Cellulose</i> , 17 (3): 459-494.
	-	15	Abe K., et al. (2007) <i>Biomacromolecules</i> , 8 (10): 3276-3278.
Blender	-	15-20	Uetani K. and Yano H. (2011) <i>Biomacromolecules</i> , 12 (2): 348-353.
	TEMPO	3-5	Saito T., et al. (2006) <i>Biomacromolecules</i> , 7 (6): 1687-1691. Eichhorn S. J., et al. (2010) <i>Journal of Materials Science</i> , 45 (1): 1-33.
Microfluidizer	Carboxymethylation	10-15	Aulin C. (2009) Doctor Thesis. Sweden: KTH Chemical Science and Engineering, 55 p.
	-	10-30	
	Enzymatic	20-30	Pääkkö M., et al. (2007) <i>Biomacromolecules</i> , 8 (6): 1934-1941.

Table 1-I.9. Comparison of diameter of different MFC suspensions

The diameter as well as the length of MFC varies according to the mechanical treatment and pre-treatment that are applied. Table 1-I.9 lists some diameter measurements carried out for different kinds of MFC suspensions.

As shown by the above results (Table 1-I.9), mechanical treatment and pre-treatment seem to have a certain impact on the morphology of MFC. It remains difficult, though, to accurately compare all the different dimensions obtained since the raw materials are different. Indeed, depending on the source of the cellulose, the initial fibers have different qualities, lengths, microfibril angles, and amounts of residual lignin and hemicelluloses, or other characteristics when they come from wastes.

Alemdar and Sain, 2008 measured the dimensions of MFC extracted from different agricultural residues. The diameters of MFC from wheat straw and soy hulls were 10–80 nm and 20–120 nm, respectively, with lengths of about 1,000 nm. These dimensions are thus higher than those of MFC from wood. Nevertheless, other researchers have obtained MFC from sisal (Siqueira, *et al.*, 2010e, Siqueira, *et al.*, 2010d), carrots (Siqueira, *et al.*, 2010c), beet pulp (Dufresne, *et al.*, 1997, Leitner, *et al.*, 2007) and *Luffa cylindrical* (Siqueira, *et al.*, 2011) that has smaller diameters, of 20 to 65 nm, 3 to 36 nm, 30 to 100 nm, and 55 ± 15 nm, respectively. It is therefore difficult to draw a clear overall picture, since each source is specific and individual, and the strength of mechanical treatment differs from one study to another. Moreover, until now, the raw material used for producing MFC does not contain lignin, or if it does, it is only in low quantities. This is

why Spence, *et al.*, 2010a studied the influence of the presence of lignin on the morphological property of MFC. With lignin-containing pulp, the diameter of MFC produced was larger, regardless of the origin of the pulp used. For a low-lignin-content unbleached softwood (about 9%), the width was 30.8 nm, whereas for a higher lignin content (about 14%), the diameter was 34.4 nm. Hemicelluloses also influence the MFC diameter distribution by limiting the association between cellulose nanofibers.

Using ^{13}C NMR (CP/MAS), Pääkkö, *et al.*, 2007 obtained a lateral fibril aggregate measurement of 17.3 ± 0.7 nm, which confirmed the results obtained using AFM (20–30 nm) and TEM imaging (10–20 nm). Their results are shown in Figure 1-I.22.

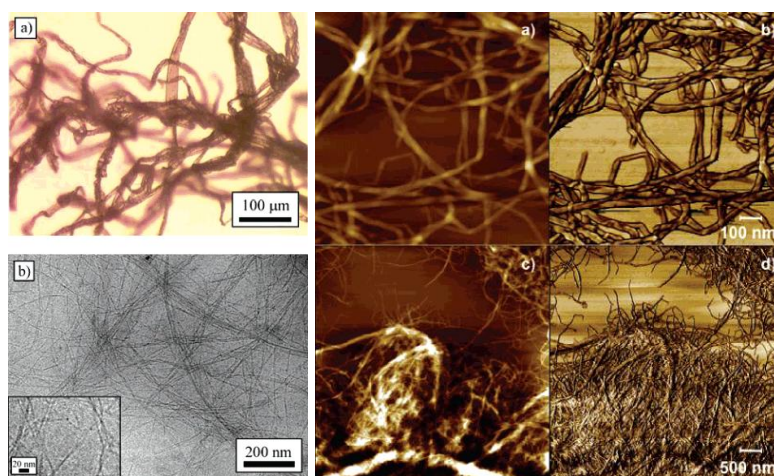


Figure 1-I.22. Left (a) Optical micrograph of original sulfite cellulose macroscopic fibers of sizes of several tens of μm . (b) Cryo-TEM of the frozen 2% w/w MFC gel after enzymatic pre-treatment and homogenization process (diameter of ca. 5-6 nm)

Right AFM Images of MFC on mica after frying: topographical images (a and c) and phase contrast images (b and d). Scan size $1 \times 1 \mu\text{m}$ (a and b) and $5 \times 5 \mu\text{m}$ (c and d). Dried fibrils have width of ca. 20-30 nm.

These images are extracted from the article of Pääkkö et al. (2007) Biomacromolecules, Vol. 8, n°6.

In this case, the ^{13}C NMR method gives a more accurate value of the fibril dimensions. Only a few studies have compared the values that are obtained using different techniques. Of these, most agreed that TEM obtains the highest degree of precision in MFC characterization, followed by SEM-FEG and then AFM. Depending on the measurement technique, the preparation steps are different, which can also have an impact on the measurement accuracy. Indeed, the steps involved in TEM preparation are delicate, though the technique gives precise images. In contrast, with AFM the preparation step is easier, but the resolution and precision of the images are not as good as those of TEM.

As regards CNC characterization, Bondeson, *et al.*, 2006 and Fukuzumi, *et al.*, 2009 pointed out that fibril diameters may be overestimated by FE-SEM because it requires that the surface be covered with a conductive metallic layer. Image analysis and sample preparation are

also important, which makes it advisable to find ways to obtain improve accuracy. (Chinga-Carrasco and Syverud, 2010), in particular, developed a new method for acquiring FE-SEM images of MFC surfaces without the use of a conductive metallic layer, thus gaining valuable information for describing the important structural properties of MFC films. The application of a low acceleration voltage (< 1kV) and short working distance (< 1mm) allows the nanofibril structure to be observed at a high degree of magnification (> 50 000x) (Chinga-Carrasco and Syverud, 2011).

In spite of this fine tuning, though the width of microfibrillated cellulose is now well characterized, its length remains an approximation. Indeed, the length of a given MFC is typically too long to be observed in its entirety within the microscope reading section. If the area of the reading section is increased, though, the magnification becomes too low for individual MFCs with a nanoscale diameter to be seen. The current practice of all researchers is to estimate the MFC length within several micrometers.

Recently, Ishii, *et al.*, 2011 sought to obtain a viscoelastic evaluation of the average length of MFC that was prepared by TEMPO-mediated oxidation. In measuring the storage and loss moduli of the TEMPO oxidized MFC, they concluded that each nanofiber behaves like a semiflexible rod-like polymer. Thus, they applied the theory of linear viscoelasticity:

$$\tau = \frac{\pi * \eta_s * L^3}{18 * k_B * T * \ln\left(\frac{L}{d}\right)}$$

where τ is the longest relaxation time of a semiflexible polymer chain, and L , d , T , η_s and k_B are the chain length (μm), chain diameter (nm), absolute temperature (K), solvent viscosity (mPa.s) and Boltzmann constant, respectively.

Employing this equation, they determined a length of 2.2 μm (no standard deviation has been proposed by the authors) for the TEMPO-oxidized microfibrillated cellulose produced in their study.

MFC-Physical properties and chemical structure

To better understand the influence of MFC in nanocomposites, in films, and mostly in paper coating, it is essential to first study and analyze its physical properties and chemical structure. *Only properties related to the PhD project are hereinafter presented. Others are detailed in the corresponding review (Lavoine, et al., 2012).*

- *Degree of polymerization and strength*

It has been reported that the degree of polymerization is strongly correlated with the aspect ratio of the nanofibers, and also with their length. To determine the DP of MFC, research

groups generally use a viscosity method with a cupri-ethylene diamine solution (Iwamoto, *et al.*, 2007, Zimmermann, *et al.*, 2010), based on ISO standard 5351. The average degree of polymerization of MFC samples is calculated from the viscosity using the Mark-Houwink equation.

In this way, researchers have proved that the disintegration process clearly reduces the DP of cellulose. MFC produced from softwood sulfite pulp had a DP of 825, whereas the initial pulp had a DP of 2,249 (Zimmermann, *et al.*, 2010). In addition, depending upon the source of the wood pulp, the DP varies. It is interesting to note that the strength properties of the ensuing film decrease with a decrease in the DP of the cellulose. Thus, the DP might be a tool that can be used to evaluate the performance of MFC as a reinforcing component in various matrices (Zimmermann, *et al.*, 2010), since the determination of the strength of microfibrils remains a work in progress.

Yano and Nakahara, 2004 first estimated a strength of at least 2 GPa, based on their results concerning the tensile strength of kraft pulp, in which 70–80% of the cellulose microfibrils are distributed parallel to the fiber direction. This value was quite low, considering that the elastic modulus of a perfect crystal of native cellulose is estimated at between 130 GPa and 250 GPa (Sakurada, *et al.*, 1962, Zimmermann, *et al.*, 2004). This large discrepancy in results can be explained by either the impact of various cellulose sources on the crystal structure, or the use of different methods to measure the E-modulus. Since the time of those experiments, new measurement systems have been created that give a better estimation of the elastic modulus. From a three-point bending test using an AFM cantilever, Iwamoto *et al.*, 2009 determined an elastic modulus of 145 GPa for a single TEMPO-oxidized tunicate cellulose nanofiber. Very recently, Saito, *et al.*, 2013 applied another method to determine the mechanical strength of native cellulose nanofibrils. This latter is based on a model for the sonication-induced fragmentation of filamentous nanostructures. They succeed to determine mean strength of TEMPO-oxidized wood and tunicate cellulose nanofibrils. Depending on the measured values of diameters, the mean strength ranged from 1.6 to 3 GPa for wood nanofibrils and from 3 to 6 GPa for tunicate. Compared to the estimation of Iwamoto *et al.*, 2009, these previous values are very low. The right method to use is still uncertain and more studies are needed to corroborate one of these values.

- *Specific area*

The other key property of MFC is its high specific area. This property is usually measured by means of modeling tools that use the length and/or diameter. The resulting measurement is often an approximation, and according to Siqueira, *et al.*, 2010a, the specific area of sisal MFC is around 50 m²/g, which is about 10 times greater than that of the fiber. It is quite difficult to

measure this property due to the strong aggregation of MFC upon drying. Berlioz, 2007 attempted a BET strategy, but the values obtained were highly underestimated due to aggregations in the dried state. More recently, others have tried to link the specific area to viscosity or film transparency, but in these cases, the surface chemistry has to be considered as well.

The Congo red adsorption method can also be used. The objective of this method is to determine the maximal absorbed amount of Congo red by means of UV measurements, and to then calculate the specific surface area using the following equation (Spence, *et al.*, 2010b):

$$SSA = \frac{A_{max} * N * SA}{MW * 10^{21}}$$

where A_{max} is the maximum absorbed amount, N is Avogadro's constant, SA is the surface area of a single dye molecule (1.73 nm^2), and MW is the molecular weight of Congo red (696 g/mole).

Regardless of the origin of the pulp, the specific surface area (SSA) increases from the original pulp to the homogenized MFC. The increase is quite significant. According to the results of Spence, *et al.*, 2010b the multiplication factors vary from x2 to x55, depending on the source and process. Among the different sources of wood pulp, softwood samples show the most significant increase in SSA, with increases ranging from 100 to 200 g/m^2 .

With the lignin content, the SSA values decrease for every class of pulp fiber. This can be explained by the modification of the internal and external pore structures that accompanies the lignin removal during the bleaching process, and by the less hydrophilic character of the fibers, which may improve the adsorption of the Congo red molecules at the surface (Spence, *et al.*, 2010b).

- Toxicity

As any new nanomaterials, the questions of toxicity and the impacts on the environment have to be addressed, especially for a future food-packaging application.

Concerning the nanoparticles, the interest is growing and recent. More and more studies give their full attention to their possible risks on the environment and the human health (Foster, *et al.*, 2011, O'Connor, 2011, Stone, 2010, Treye, 2011).

The first advanced studies concerning nanopolysaccharides were related to cellulose nanocrystals. They reported low toxicity and low environmental risk potential (Kovacs, *et al.*, 2010) according to ecotoxicological tests with several aquatic species (e.g. Daphnia, rainbow trout and fathead minnow). Other communication (Foster, *et al.*, 2011) demonstrated that cytotoxicity (intracellular toxic effect) and pro-inflammatory response for CNC were significantly lower than for MWCNT (multiwalled carbone nanotubes) and CAF (crocyllodite

asbestos fibers). Other works on cell proliferation, DNA damages and cell death are still ongoing, but these first results do not reveal a potential danger for health and environment. Very recently, CNC has been added as the first cellulose nanomaterial in the Domestic Substances List (DSL) in Canada. The DSL is an inventory of substances that may be imported and/or manufactured in Canada. An assessment of risk to the environment and to human health from this surface modified CNC concluded no suspicion of toxicity (Sultan, 2012).

Concerning the potential risks of MFC, similar studies have been carried out. These studies are, however, still less numerous and detailed than those about CNC. The raw material, the pre-treatment, and the mechanical treatment have indeed to be considered in the toxicity studies since, as explained before, MFC will have different properties and characteristics. The question of toxicity attracts European projects such as Scale-up Nanoparticles in Modern Papermaking (SUNPAP (2009b)). A first complete literature study report was published in 2010 Rouhiainen, *et al.*, 2010 and emphasized the fact that no studies concerning ecotoxicology, biodegradation, toxicity and toxicokinetic of MFC were still reported. Few months later, a study about the cytotoxic and genotoxic properties of MFC from birch pulp and Arbocel MF40 (produced by Rettenmaier & Sönhe GmbH+CO.KG) was published (Pitkänen, *et al.*, 2010). The tests showed the absence of toxicity and genotoxicity for both MFC suspensions according to the in-vitro methods carried out with human and mouse cells. In 2011 another study was published about the health and environmental safety aspects of two processes using MFC: the friction grinding and spray drying (Vartiainen, *et al.*, 2011). No toxicity was also noticed.

Nowadays, no clear responses exist to the question of the toxicity of MFC. Many hazard assessment methods are already scheduled (Rouhiainen, 2011) and more details will be certainly given in the coming years. *Regarding to the first conclusions, therefore, the results are very promising and encourage the investigation of MFC in new applications such as food-packaging materials and its use under different aspects.*

Properties of MFC and MFC assembly (suspension and films)

MFC has interesting intrinsic properties that make it very attractive for many applications. As was mentioned earlier, it exhibits a particularly high specific area, flexibility, and crystallinity, and contains a high amount of hydroxyl groups. All these properties influence their interactions, whether as a suspension in liquid, or as a film.

- Rheological properties

Regardless of the source of the suspension, in an aqueous environment MFC has specific rheological properties that can be described in terms of pseudoplasticity and shear thinning behavior (Herrick, *et al.*, 1983, Pääkkö, *et al.*, 2007).

Herrick, *et al.*, 1983, who first studied the rheological behavior of MFC, discovered a pseudoplastic behavior for a 2% MFC suspension characterized by a viscosity of 17,400; 264; and 136 mPa at 10, 1000, and 5000 s⁻¹, respectively.

Following the first results obtained in 2001 by Lowys, *et al.*, 2001, Pääkkö, *et al.*, 2007 studied the rheological behavior of enzymatic pretreated MFC. Regardless of the degree of concentration of the suspension, which was from 0.125 to 5.9 wt%, the MFC suspensions displayed a gel-like behavior (Figure 1-I.23).

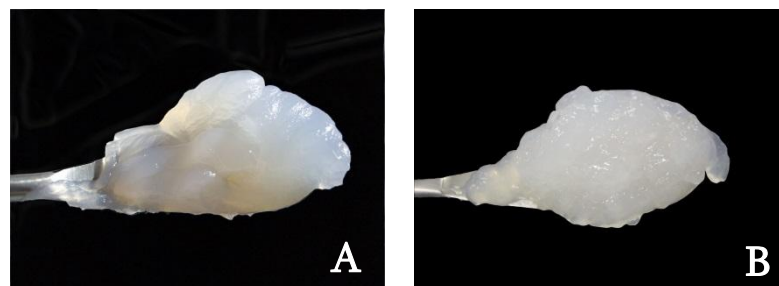


Figure 1-I.23. Pictures of two different MFC suspensions

- (A) A- 2wt% MFC from eucalyptus, enzymatically pre-treated, furnished by FCBA, France
 (B) B- 3wt% MFC TEMPO oxidated from dömsjö, furnished by CTP/FCBA, France

In addition, the values of the storage modulus were rather high. For a 3 wt% MFC suspension, the storage modulus was about 10⁴ Pa, as compared to the storage modulus of a 3 wt% suspension of nanoscale cellulose crystallites, which was $G' \approx 10^2$ Pa (Pääkkö, *et al.*, 2007). According to Pääkkö, *et al.*, 2007, this high elastic modulus is due to the long fibrils, which form an inherently entangled network structure. Moreover, they observed that, regardless of the suspension concentration, the storage moduli were almost 10 times higher than the loss moduli. These results show that even at the lowest concentration, the MFC suspensions form a rather strong network.

Other experiments have confirmed the pseudoplastic behavior of MFC gels. Indeed, in several published reports, all suspensions showed a large decrease in viscosity with increasing shear rate, in a dynamic that is referred to as shear thinning (Herrick, *et al.*, 1983, Pääkkö, *et al.*, 2007, Siqueira, *et al.*, 2009). It is still difficult to precisely explain this phenomenon, but it might be due to a packing aggregation of MFC, which limits the formation of a continuous network. More recent studies have confirmed this hypothesis (Missoum, *et al.*, 2010a, Missoum, *et al.*, 2010b).

Iotti, *et al.*, 2010 conducted a detailed study of MFC water dispersion at various concentrations (1–4%) and temperatures (between 25 and 60°C). Unlike the other studies, they observed and emphasized a shear rate-viscosity hysteresis loop, and measured the MFC network-forming capacity in slow dynamics studies. In addition, they investigated measurements obtained at high shear rates (above 100,000 s⁻¹), which revealed a dilatant behavior of MFC suspensions.

According to their results, a 1% MFC water dispersion cannot be used for high shear coating applications.

The study of the MFC suspension viscosity remains however sensitive because of the fibrils aggregates occurring. This has been proved with a recent rheological study of Puisto, *et al.*, 2012. They proposed a new model for describing the shear thinning behavior of highly anisotropic nanoparticles. A relation between the viscosity and the aggregation in MFC suspension has been established drawing attention to interpretations of rheology data.

As it will be presented in more detail later, an increasing number of researchers are trying to coat MFC onto films or cellulosic materials like in this PhD project. The viscosity of MFC suspensions is thus a significant parameter that influences MFC coating and may prevent the scaling-up of this application. However, it remains difficult to measure it and the suspension stability is still under discussion. Moreover, too many parameters can influence it.

As regards enzymatic pre-treated MFC, its shear thinning behavior depends on the pH. In a number of studies, the total charge of the original pulp was preserved following enzymatic treatment (Pääkkö, *et al.*, 2007). Practically all charges are due to the presence of hemicelluloses. During the MFC preparation process, the hemicellulose content remained constant and thus the charge number did as well. At lower pH values, Pääkkö, *et al.*, 2007 observed a higher degree of interfibrillar interaction and a higher viscosity, as the OH-ions neutralize the charges of the hemicelluloses associated with the MFC, which reduces the electrostatic repulsions. In contrast, at higher pH values, the MFC gel presents a lower degree of interaction and a lower viscosity due to a higher electrostatic repulsion.

Recently, Zimmermann, *et al.*, 2010 sought to calculate the intrinsic viscosity of MFC suspensions. Applying ISO standard 5351, they determined the limiting viscosity number using the following Schulz-Blashke equation (Sanches, *et al.*, 2005):

$$\frac{\eta_{rel}-1}{c} = [\eta] + K_{SB} * [\eta] * (\eta_{rel} - 1)$$

where η_{rel} is the relative viscosity, $[\eta]$ the limiting viscosity number, and K_{SB} the Schulz-Blashke constant.

The limiting viscosity number, $[\eta]$, which is also called the intrinsic viscosity, is a viscosity parameter related to infinitely diluted polymer solutions (Matusinovic, *et al.*, 2005). It is a dimensionless number that is calculated for ideal polymer solutions. (Zimmermann, *et al.*, 2010) considered that each cellulose molecule of MFC acts independently as MFC is infinitely diluted (even if 0.5 to 2% w/w are not infinite dilutions) in an aqueous environment. Depending on the source of the MFC, the intrinsic viscosities vary between 100 and 390 ml/g.

After considering various “wet” properties, we will next consider the properties of MFC in a dry state.

- *Thermal properties and drying process*

Only a few research groups have studied the thermal degradation behavior of MFC films. Although this behavior depends mainly on the process of MFC preparation, it also depends on the drying process (which affects the quantity of hydrogen bonds). Concerning the first point, it has been reported that TEMPO-oxidized cellulose undergoes multiple degradation events (Johnson, *et al.*, 2008). In the range 225–231°C, the largest degree of degradation takes place. Depending upon the sonication time, the weight loss varies from 60 to 80%. With increasing sonication time and temperature, the weight loss increases significantly (Johnson, *et al.*, 2008). This thermal behavior could be explained by the presence of a high surface area that is developed during the nanofibrillation process with sonication, which increases the effective mass of the cellulose consumed per unit rise in temperature.

Quiévy, *et al.*, 2010 studied the influence of the drying process on the thermal stability of MFC obtained by homogenization. They tested three drying processes: oven-drying at 80°C for 72 h; freeze-drying of a frozen cellulose suspension at 40°C for 48 h; and atomization, which consists of spraying the suspension by passing it through a nozzle and then drying the wet particles in a hot air flow of 200–205°C.

After freeze-drying, MFC gel globally formed a mat corresponding to the microfibril aggregates, in which some of the microfibrils remained distinct. The freezing step at -20°C seemed to play an important role in the formation of agglomerates. Other researchers concluded that this step even changes the rheological properties of the MFC suspension (Agoda-Tandjawa, *et al.*, 2010). While oven-drying also creates a mat of microfibrils, in this case none of them remained individualized. Finally, in the case of atomization, no microfibrils or bundles were observed, but only fibers with smaller dimensions than those of the original fibers. During this drying process, MFC had already been subjected to thermal degradation. During pyrolysis in the two first drying processes, water was removed, causing microfibril bundle formation and drawing aggregates close to each other. Because of the increasing number of contacts, the degradation of one microfibril led to the degradation of the surrounding fibers. The dried MFC thus presented a lower degree of thermal stability than that of the original fibers, since the degradation began at lower temperatures. Some very recent works Missoum, *et al.*, 2012 have showed that post-treatments such as hydrophobization can strongly improve this thermal stability, a development that opens the door to new possible applications.

Another challenge, which is completely different, is the interaction of MFC in a dried state. MFC suspensions have always been used directly after their preparation because they are

difficult to preserve or redisperse after drying. Obtaining redispersable MFC in a dried state to facilitate its storage and transport is perhaps the most important challenge for developing applications of such materials. Solutions dealing with this issue have been yet patented in 1984, by Herrick and Wash, 1984b and more recently in 2013, by Bras, *et al.*, 2013, but without any application up today.

The addition of water-soluble polymeric additives before drying, such as hemicelluloses, sodium carboxymethylcellulose, sodium polyacrylate, or a cationic polyacrylamide derivative has been presented as a solution to prevent the loss of MFC properties (Agoda-Tandjawa, *et al.*, 2010, Centola and Borruso, 1967, Kohnke and Gatenholm, 2007, Lowys, *et al.*, 2001, Rebuzzi and Evtuguin, 2005). This procedure, though, is either too costly to be practical or creates other problems, e.g., it is impractical for use with the commodity of recycled paper (Agoda-Tandjawa, *et al.*, 2010). To this day, it still remains a challenge to find a process that is applicable in industry and does not affect the MFC properties.

Indeed, once dried, MFC assemblies are either aerogels (MFC suspension in air) or films (an MFC aggregate structure) and cannot be redispersed. This is why their preparation and properties are of interest. *Within this PhD, we will mostly focus on the ability of MFC to form films. MFC films can indeed be investigated as packaging materials, and are considered as model to describe the behavior of MFC coated on cellulosic substrates.*

- *Film properties*

When MFC is dried, films or aerogels can be obtained (Figure 1-I.24). *In spite of the greatly increased interest in aerogels, we will focus our study on films for the reasons described above. That said, we also consider it important to offer a few key information on aerogels, since MFC aerogels have also been considered for antimicrobial activity.*

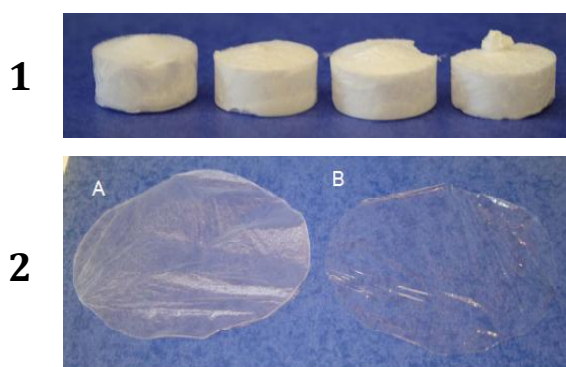


Figure 1-I.24. MFC aerogels made by freeze-drying (1) (Aulin, *et al.*, 2010b) and MFC films made by casting method of (A) enzymatically pre-treated MFC and (B) TEMPO oxidated MFC (Aulin, 2010).

MFC aerogels are a new class of materials that have stimulated interest in a number of applications (catalysis, filtrations, grafts, cushioning, liquid storage, etc.) (Aulin, *et al.*, 2010b,

Fischer, *et al.*, 2006, Liebner, *et al.*, 2010, Sehaqui, *et al.*, 2011). Pääkkö, *et al.*, 2008 mechanically prepared robust aerogels using two different freeze-drying methods with cellulose nanofiber suspensions. The first method, called cryogenic freeze-drying, consists in placing the aqueous gel in a mold, dipping it in liquid propane at a temperature of about -180°C , and then transferring it into a vacuum oven. In the second, more simple method, called the vacuum freeze-drying method, the aqueous gel is placed at room temperature in a vacuum oven at room temperature. These two methods are alternatives to the usual technique used to produce aerogels, called supercritical drying, and both offer the advantage of reduced cost.

Whatever the fabrication method, the resulting aerogels present a very low density (0.02 g/cm^3) and a high porosity of 98%. When the specific surface area was determined by N_2 adsorption, the first method yielded a value of $70\text{ m}^2/\text{g}$, while the second method gave a lower value of $20\text{ m}^2/\text{g}$, due to its greater number of aggregations.

Aerogels produced using the usual technique or produced from other sources such as cellulose derivatives (Fischer, *et al.*, 2006, Tan, *et al.*, 2001) and silica gels (Soleimani Dorcheh and Abbasi, 2008) showed different properties. Generally, the specific surface area of aerogels derived from cellulose varies in the range from 250 to $389\text{ m}^2/\text{g}$ (Pääkkö, *et al.*, 2008). Lower porosity (41 to 82%) and higher densities (0.15 to 0.85 g/cm^3) have also been observed. Moreover, MFC aerogels show increased ductility and flexibility compared to the usual brittle aerogels. According to their stress-strain curves, a linear behavior lasts up to a strain of about 40% (Pääkkö, *et al.*, 2008) and the maximum compression strain is about 70%. Pääkkö, *et al.*, 2008 suggested a number of applications for MFC aerogels, such as functional packaging, high performance biodegradable nanocomposites, filtration, and even, as was shown by demonstration, their functionalization to achieve electrical conductivity.

However, as was mentioned earlier, MFC suspensions have mainly been converted to MFC films, which are sometimes called “nanopaper” structures (Henriksson, *et al.*, 2008). MFC gels can be converted into films by several methods (casting method, vacuum filtration, etc.). When the water is removed from the MFC gel, a cellulose nanofiber network is formed through interfibrillar hydrogen bonding. The quality of the film will vary, depending upon the film formation process and the kind of MFC used.

Chinga-Carrasco and Syverud, 2010 and Syverud, *et al.*, 2010 developed computer-assisted methods to quantify the multi-scale structure of MFC and MFC films. They demonstrated the potential of several image acquisition techniques for complementing the characterization of MFC films. Using a simple desktop scanner analysis, they studied the texture of films at a macro-level, which revealed two major components: fibers and wiremarks. Thanks to laser profilometry (LP) and scanning electron microscopy (SEM), they were able to estimate the micro-characteristics of

MFC films, i.e., quantification of the specific surface area, thickness measurements, and observation of cross-sectional structures.

In another study, an overview of the fibril dimensions and the surface pore structure of MFC was obtained by a combination of FE SEM images and computer-assisted electron microscopy. Syverud, *et al.*, 2010 estimated the average surface porosity of TEMPO-oxidized MFC film produced from bleached kraft pulp at 10%, and an average nanofibril diameter of 12 nm, corresponding to microfibril bundles. More recently, Fukuzumi, *et al.*, 2011 also analyzed the pore structures of TEMPO-oxidized MFC films from bleached kraft pulp by another method, the positron-annihilation lifetime spectroscopy (PALS). At 0%RH, the TEMPO-MFC film from wood presented a constant pore size of about 0.47 nm from the film surface to depths up to about 2 μm from the surface. Compared to the TEMPO MFC suspension (with COOH groups) used by Syverud, *et al.*, 2010, Fukuzumi, *et al.*, 2011 studied in this case a TOCN-COO⁻Na⁺ suspension. Moreover it has been prepared with another process (surnatant) and the film-making technique was not similar (casting process but different drying temperature and time). This could explain the different pore size values measured.

A number of different processes have been developed to obtain these MFC films. Some that were prepared by vacuum filtration had thicknesses in the range 60–80 μm . With a dynamic sheet former, homogeneous films can be obtained (Rodionova, *et al.*, 2010, Sehaqui, *et al.*, 2010, Syverud and Stenius, 2009). This is a fast method, and with the use of an appropriate wire, very highly transparent films are obtained. Other films based on a solvent exchange process have also been produced, which displayed various porosities with thicknesses in the range 70–90 μm (Henriksson, *et al.*, 2008). Still others were prepared by spraying (Wågberg, *et al.*, 2008) or by the classical solvent casting method (Aulin, *et al.*, 2010a, Spence, *et al.*, 2010a). In this latter method, the objective is to evaporate the solvent with a combination of controlled temperature, relative humidity, and time. In most cases, the concentration of MFC is not higher than 1% wt. Depending upon the drying method used (oven, room temperature), the drying of MFC films lasts from two hours (Xhanari, *et al.*, 2010) to five days (Chinga-Carrasco and Syverud, 2010, Spence, *et al.*, 2010a, Spence, *et al.*, 2010b).

The spin-coating (Ahola, *et al.*, 2008, Aulin, 2009) is another method which, unlike the others, allows MFC films to form directly on a suitable substrate. This method is used in QCM-D balance experiments, for example.

Finally, more recently, the VTT Research Centre and the Aalto University have developed a method which for the first time enables manufacturing of a wood-based and plastic-like material in large scale. According to their method, MFC films are manufactured by evenly coating fibril cellulose on plastic films so that the spreading and adhesion on the surface of the plastic can be controlled. All the phases in the method can obviously be transferred to industrial

production processes. More details about this method are coming. The partners are indeed applying for a patent for the production technology of MFC films.

These film-like structures with nanoscale pores have led researchers to anticipate their eventual use in barrier applications, and therefore, to investigate the influence of their preparation on this property. This point is discussed in greater detail later.

Owing to all the intrinsic properties described above, MFC continues to attract many researchers who are planning for their use in various applications. Many challenges, however, are still being investigated, such as the hydrophilic nature, preservation, redispersion, and thermal stability of MFC, among other issues. Once dried, MFC films are moreover not easy to handle, that can limit their applications. Their good mechanical properties, film-forming capacity, astonishing rheological properties, and even their high specific area still do not qualify MFC for application in all fields. The difficulty of their use as non-thermoplastic films with the packaging thermoforming, or with other applications, has, for example, still to be considered.

Among its promising properties and associated challenges, MFC shows good barrier characteristics whether as film, in nanocomposites, or associated with the other cellulosic materials. These functions open the door to other possible uses in sectors such as the food industry and packaging, and they will be developed in response to new and evolving societal requirements.

Since barrier properties are the main characteristics required for food-packaging materials, we will focus the further discussion on the barrier properties of MFC as films and associated with other cellulosic materials. The use of MFC in nanocomposites will not be detailed in this manuscript. This study indeed focuses on the elaboration of packaging materials based on cellulose only in order to propose a new solution for controlled release packaging. Nevertheless, further information can be obtained in various reviews (Lavoine, et al., 2012, Siqueira, et al., 2010b, Siró and Plackett, 2010).

I-4. Microfibrillated cellulose and transfer phenomena

I-4.1. Theoretical background

Before dealing with MFC and barrier properties, a slight theoretical background will follow to further understand the transfer phenomena occurring in the case of our study.

MFC will be first used as barrier layer for our cellulose-based packaging materials, and then, within the perspective of an antibacterial application, MFC will be used as release matrix of an active agent. Thus, transfer phenomena of both gases and liquid have to be understood since MFC will be in contact either with gases such as oxygen or water vapor, or with liquid like nutrient media.

The active agent migration may be achieved by direct contact between food and packaging material, or through gas phase diffusion from packaging layer to food surface (Mastromatteo, *et al.*, 2010). Different phenomena can occur as described by Figure 1-I.25 :

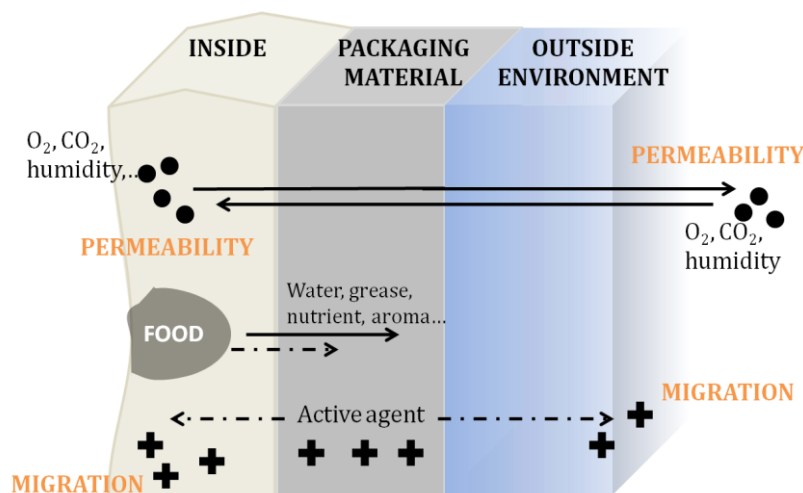


Figure 1-I.25. Environment/packaging material/food interactions

*adapted from (Gavara, 2012)

The permeability of a packaging material is the ability of this material to transmit any solute through itself. The permeation of a solute (e.g. gases or liquid) is the exchange of solute molecules between the external and internal through the packaging material.

The permeation of a solute through a layer is governed by its solubility and its diffusion coefficient, i.e. the rate at which transport occurs and is given by:

$$P = D \times S$$

where P is the permeability coefficient, D the diffusion coefficient and S is the solubility of the solute.

These parameters have already been measured and calculated in the literature for MFC films in contact with different gases: carbon dioxide, nitrogen and oxygen (Belbekhouche, *et al.*, 2011).

Regarding the migration phenomena of molecules, the literature suggests different kinds of mathematical description depending on the delivery system and types of mass transport (e.g. diffusion of the active agent, diffusion of water into the system, active agent dissolution, packaging material swelling etc.) (Siepmann and Siepmann, 2011). Usually, diffusional mass transport is always involved in the molecule release out of a matrix, and in various cases, is the predominant step (Siepmann and Peppas, 2012).

In order to quantify diffusional mass transport, Fick's laws of diffusion can be used. The first Fick's law of diffusion states that the migration of molecules through a cross-section of a matrix is proportional to the concentration gradient (Crank, 1975). Thus, the flux will go from regions of high concentration to regions of low concentration with a magnitude that is proportional to the concentration gradient. In one dimension, this law is given by:

$$F = -D * \frac{\partial C}{\partial x}$$

where F is the rate of transfer per unit area of section; C is the concentration of the diffusing species, and D is the diffusion coefficient.

Derived from Fick's first law and mass balance considerations, the Fick's second law of diffusion predicts how diffusion induces the concentration to change with time. Thus:

$$\frac{\partial C}{\partial t} = D * \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2} \right)$$

where C is the concentration of the diffusing species; t denotes time, D the diffusion coefficient and x, y, z are the three spatial coordinates.

Both equations can thus be used to describe the diffusional mass transport of a molecule from its matrix. However, no "analytical solutions" can be derived as too many parameters can influence the migration of molecules. For example, the diffusion coefficient can be constant or not, but the mathematical treatment will be much simpler if D is constant. Besides, depending on the matrix, phenomena such as erosion, swelling and/or degradation can also occur and thus influence strongly the molecules' migration and make the resolution of Fick's law difficult. Thus, in literature, other models adapted from previous equations have been established for specific conditions of migration.

Based on specific hypothesis, Siepmann and Siepmann, 2011 reviewed mathematical modeling of controlled drug delivery diffusion. Figure 1-1.26 present a classification of

predominantly controlled drug delivery systems, which could be applied in our project. The model will differ according to (i) the inner structure of the device, i.e. if the molecule is completely physically separated of the matrix (*reservoir system*) or, if they are more or less homogeneously distributed throughout the matrix (*monolithic system*), (ii) the initial molecule content c_i (in relation to drug solubility c_s) and (iii) the geometry.

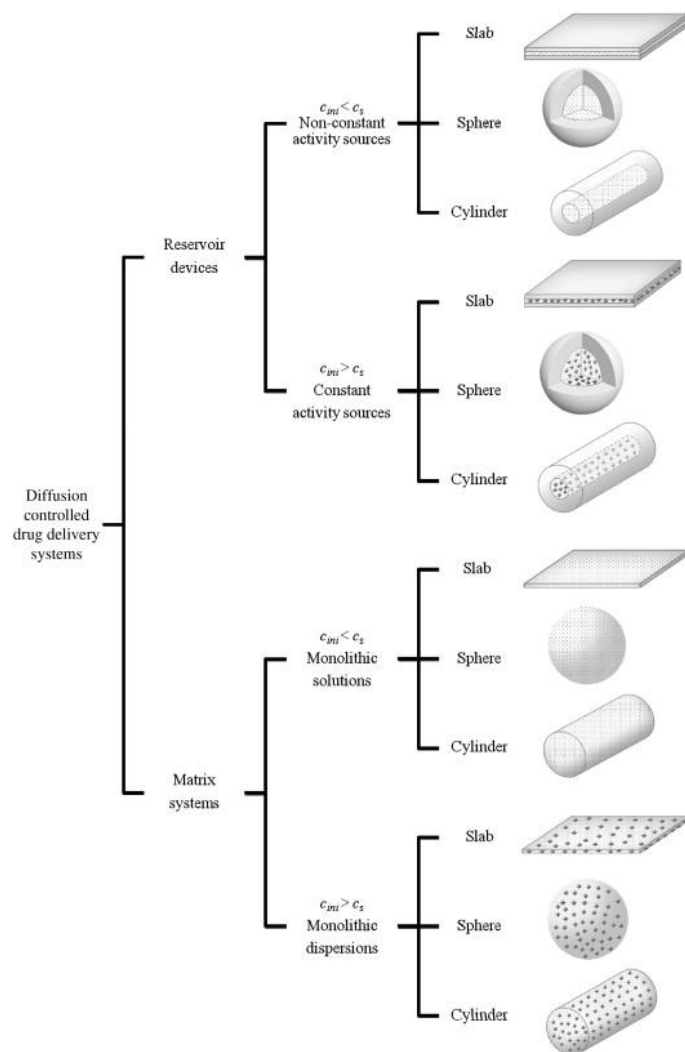


Figure 1-I.26. Classification scheme of predominantly diffusion-controlled drug delivery system (Siepmann and Siepmann, 2011). The dots indicate dissolved drug molecules, the circles non-dissolved drug particles.

These models are applied for “simplest” cases of molecule migration, but could in a first time be considered within this PhD. *No more details will be given in this chapter regarding modeling of transfer phenomena since none unique model have been proposed when MFC is used as delivery system. Nevertheless, two very recent studies (Cozzolino, et al., 2013, Kolakovic, et al., 2012b) have tried to establish a model explaining the migration phenomena of molecules introduced in MFC films. This scientific paper will be described further in this chapter.*

I-4.2. Microfibrillated cellulose and barrier properties

As was mentioned earlier, we anticipate the increasing use of the barrier properties of bio-based materials in our society in order to develop environmentally friendly and efficient materials for different applications. In this regard, MFC offers one of the most promising prospects, in the form of (i) MFC films and (ii) paper coating. Table 1-I.10 presents a review of the barrier properties that have been developed for each of the following avenues of development

Raw materials	Manufacturing process	Process	Conditions	OTR (mL.m ⁻² .day ⁻¹)	Oxygen permeability (mL.mm.m ⁻² .day ⁻¹ .atm ⁻¹)	Conditions	WVTR (g/m ² .day)	WVP (g.mm/kPa.h.m ²)	Air permeance (nL.Pa ⁻¹ .s ⁻¹)	References
Bleached spruce sulphite pulp	MT		23°C, RH 0% top side, 50% bottom	17-18	0.352 to 0.505	-	-	-	11 to 13	Syrenus and Stenius (2009) Cellulose, 16:75-85
BSP			-	-	-	23°C, RH 50%	240 +/- 0.7	-	-	Spence et al. (2010) Cellulose, 17:835-848
BHP	MT		-	-	-	-	200 +/- 0.0	-	-	
BHP			-	-	-	-	2.1	-	-	
BHP & Paraffin coat			-	-	-	-	0.4 to 0.5	-	-	
BHP & Beewax coat	MT		-	-	-	23°C, RH 50%	0.05 to 0.5	-	-	Spence et al. (2011) Bioresources, 6(4):4370-4388
BHP & Cooked starch coat			-	-	-	-	0.5 to 1.6	-	-	
			RH 0%	-	below detection limit	-	-	-	-	
Bleached wood sulphite pulp	MT		RH 50%	-	0.048	-	-	-	-	Liu et al. (2011) Biomacromolecules, 12:633-641
			RH 95%	-	17.8	-	-	-	-	
	Acetylation			5.86 to 11.1	-	-	170 to 265	-	-	
Kraft pulp	MT	Films	RH 0%	4.20	-	23°C, RH 50%	234	-	-	Rodionova et al. (2011) Cellulose, 18(1):127-134
	MT after solvent exchange			4.10	-	-	91.0	-	-	
Bleached sulfite softwood pulp	ET		35°C, RH 0%	≈ 10 ⁻¹¹ (mol.m/(m ² .s.Pa))	2.64E10 ¹⁵ +/- 0.87 (mol.m/(m ² .s.Pa))	-	-	-	-	Minelli et al. (2010) J. Membr. Sci., 358(1-2):67-75
Bleached sulfite softwood pulp	ET		23°C, RH 50%	-	0.016 +/- 0.001	-	-	-	-	Plackett et al. (2010) J. Appl. Polym. Sci., 117:3601-3609
Sulfite softwood dissolving pulp &	CT		23°C, RH 50%	-	0.013 +/- 0.005	-	-	-	-	
Sulfite softwood dissolving pulp	CT		23°C, RH 50%	-	0.050 to 0.037	-	-	-	-	Siro et al. (2011) J. Appl. Polym. Sci., 119:2652-2660
Sulfite softwood dissolving pulp			23°C, RH 0 to 80%	-	6E10 ⁵ to 0.085	-	-	-	-	Aulin et al. (2010) Cellulose, 17:559-574
Sulfite softwood dissolving pulp	CT			≈ 10 ⁻¹¹ (mol.m/(m ² .s.Pa))	6.26E10 ¹⁵ +/- 1.27 (mol.m/(m ² .s.Pa))	-	-	-	-	Minelli et al. (2010) J. Membr. Sci., 358(1-2):67-75
BS and BH kraft pulp & PLA coat	TOCN		dry conditions	-	1 (mL.m ⁻² .day ⁻¹ .Pa ⁻¹)	-	-	-	-	Fukuzumi et al. (2009) Biomacromolecules, 10:162-165
BSP	TOCN - NaOOH		23°C, RH 0%	-	1.7E10 ⁻⁵	-	-	-	-	Fujisawa et al. (2011) Carbohydr. Polym., 94:579-583
	TOCN - COOH		23°C, RH 0%	-	0.0049	-	-	-	-	
			RH 0%	-	below detection limit	-	-	-	-	
Bleached wood sulphite pulp & Clay	MT		RH 50%	-	0.045	-	-	-	-	Liu et al. (2011) Biomacromolecules, 12:633-641
			RH 95%	-	3.5	-	-	-	-	
BHP & Kaolin Clay			-	-	-	-	1.0 to 1.75	-	-	
BHP & Calcium carbonate			-	-	-	-	1.4 to 2.5	-	-	
BHP & Cooked starch	MT	Nanocomposites	-	-	-	23°C, RH 50%	2.5 to 2.7	-	-	Spence et al. (2011) Bioresources, 6(4):4370-4388
BHP & Uncooked starch			-	-	-	-	2.1 to 3.1	-	-	
Bleached kraft pulp & shellac	MT		25°C, RH 50%	about 4998	-	25°C, RH 50%	about 7.45	-	0.0923	Hult et al. (2010) Cellulose, 17:575-586
Eucalyptus sulphite wood pulp & HPNC	TOCN		-	-	-	about RH 81%	about 1064	0.49 to 0.70	-	Bilbao-Sainz et al. (2011) Carbohydr. Polym., 86:1549-1557
			-	-	-	about RH 82%	about 1026	0.75 to 0.79	-	
Bleached sulfite softwood pulp & Amylopectin	ET		23°C, RH 50%	-	0.020 to 0.037	-	-	-	-	Plackett et al. (2010) J. Appl. Polym. Sci., 117:3601-3609
Sulfite softwood dissolving pulp & Amylopectin	CT		23°C, RH 50%	-	0.013 to 0.034	-	-	-	-	
Bleached kraft pulp	MT	Coated paper	25°C, RH 50%	about 35000	-	25°C, RH 50%	about 63	-	0.635	Hult et al. (2010) Cellulose, 17:575-586
			23°C, RH 50% Kraft paper	-	-	-	-	-	100000 to < 1	Aulin et al. (2010) Cellulose, 17:559-574
Softwood pulp	CT		23°C, RH 50% Greaseproof paper	-	-	-	-	-	1000 to < 0.5	

Table 1-I. 10. Comparison of the barrier properties of MFC films, nanocomposites with MFC and MFC coated papers depending on the MFC sources and manufacturing process

BSP: bleached softwood pulp

ET: enzymatic pre-treatment

BHP: bleached hardwood pulp

CT: carboxymethylation pre-treatment

MT: mechanical treatment

TOCN: TEMPO oxydized cellulose nanofibers

100% MFC Films

Most papers written about 100% MFC films address their mechanical and optical properties (Siró and Plackett, 2010). Many applications such as transparent films for food packaging, electronic devices, or reinforcement in nanocomposites have therefore been proposed. 100% MFC films also exhibit excellent barrier properties, but thus far, only a few papers have presented research that addresses these properties.

- *Water vapor transfer rate and water vapor permeability*

The water vapor transfer rate (WVTR), i.e., the volume of water vapor passing through a film per unit area and time under specific conditions, and water vapor permeability (WVP) were the first barrier properties that were studied with regard to MFC films. One objective was to use these films in barrier packaging to replace the modified atmosphere packaging (MAP) materials that are currently in use. From this perspective, interesting results were observed that were dependent upon different parameters, such as the sources of MFC, the production treatment, and chemical modifications of MFC.

Spence, *et al.*, 2010b studied the influence of the types and chemical compositions of wood sources on the WVTR. They prepared MFC films from diverse wood pulp sources, using softwood and hardwood, both with and without lignin. They first compared the WVTR of the initial pulps with those of the corresponding MFC. Processing to convert microfibrils to MFC resulted in a clear decrease in the WVTR (from -20 to -30%). Next, among the different sources of wood, MFC from bleached hardwood showed the highest water vapor barrier property (200 g.m⁻².day⁻¹). However, regardless of the source, all the WVTR values obtained were higher than the value obtained for low density polyethylene (20 g.m⁻².day⁻¹). Finally, they observed an increase in the WVTR for lignin-containing samples, which may be due to the larger pores (due to the lower quality of the hydrogen bonds) in the films (from 300 to 500 g.m⁻².day⁻¹). Even if it is less hydrophilic, the film structure is obviously the most important criterion in the consideration of barrier properties. The chemical composition of the wood pulps used to produce MFC thus plays a major role in the study of barrier properties, whereas the kinds of wood do not act consequentially upon it.

Moreover, the physical structure of MFC also has an impact on the water permeability of films. The degree of crystallinity strongly influences the barrier properties of a cellulosic material. MFC usually has a slightly higher crystallinity than fibers, but compared to CNC, it still remains largely amorphous. Very recently, Belbekhouche, *et al.*, 2011 compared the barrier properties of CNC films with those of MFC films. Surprisingly, CNC films absorbed as much water as MFC films. In addition, the diffusion coefficient of CNC films was higher than that of MFC films. Because of their more organized and highly crystalline structure, at first glance, CNC films would

seem to provide more of a barrier to water. Other parameters, though, such as entanglement and nanoporosity, can be seen to exert a more considerable influence on water barrier than crystallinity.

As highlighted here, the study of water barrier properties entails the study of water sorption, particularly in kinetic and isotherm sorption studies. Many researchers have first analyzed these properties before carrying out the usual WVP or WVTR tests (Aulin, *et al.*, 2010a, Belbekhouche, *et al.*, 2011, Dufresne and Vignon, 1998, Minelli, *et al.*, 2010). In this way, they can establish a film's behavior as a function of water activity, and determine the water uptake, the diffusion coefficient, etc. With this information, the WVP can then be easily obtained by multiplying the diffusion coefficient and the solubility of water vapor ($P=D*S$). This approach is suitable for the development of a theoretical model that can then be correlated with the usual WVP results.

According to the previous results, the chemical composition of wood pulps as well as the physical structure of MFC films have an impact on the water barrier of MFC films. Now, let us see the influence of the pre-treatments that are used in the MFC production.

Minelli, *et al.*, 2010 compared two other kinds of MFC films, which had a similar source but to which two different pre-treatments were applied: enzymatic pre-treatment and carboxymethylation pre-treatment. The research group prepared MFC films by drying the cast gels in an incubator under controlled humidity. According to FE-SEM images, carboxymethylated MFC films consist of a closely packed nanofibril network. They possess a lower water diffusivity than enzymatic pretreated MFC films, and thus presented lower water vapor permeability (WVP) values (about 10^{-14} mol.m/(m².s.Pa), 35°C, 0% RH against 10^{-13} mol.m/(m².s.Pa)). Although carboxymethylated MFC films presented different barrier properties than enzymatic pretreated MFC films, the differences between the two remained slight. Thus, it was clear that the pre-treatment applied did not play a major role in their water vapor properties.

Post-treatments applied to MFC can also influence the water vapor permeability. Rodionova, *et al.*, 2010 studied the impact of acetylation on the WVTR. Depending on the reaction time, the WVTR values first decreased and then increased during a 3-h reaction. Compared to pure MFC films (234 g.m⁻².day⁻¹), the values obtained for 0.5 and 1 h of acetylation were lower (167 g.m⁻².day⁻¹). This decrease can be explained by the increasing fraction of acetylated hydrogen bonds that gradually prevented the amorphous parts of the MFC from water. Then, during a 3-h acetylation, the degree of substitution continued to increase, which Rodionova, *et al.*, 2010 suggested might indicate that the network structure was slightly more open, thus inducing an increase in the WVTR.

Acetylation thus improves the WVTR of MFC films and consequently lowered these values. Parallel to these results, another method that also decreased the WVTR was highlighted. After a complete solvent exchange that successively used water, acetone, and toluene, pure MFC films showed a WVTR of $91 \text{ g}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$, which was drastically lower than the $234 \text{ g}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ obtained for neat MFC. This result emphasized the role of surface hydroxyl group passivation and surface chemistry control.

In spite of all these treatments, the WVTRs of MFC films still remain high as compared to those of other polymer films. The improvement of this property was then studied by (Spence, *et al.*, 2011b). After coating MFC films with cooked starch, beeswax, and paraffin using a dipping method, they obtained WVTRs that were approximately half that of low density polyethylene. MFC films had already displayed a low WVTR at an equivalent thickness (about $220 \text{ (g/m}^2\cdot\text{day)/m}$), but after coating, the WVTR decreased to values between 100 to $50 \text{ (g/m}^2\cdot\text{day)/m}$, which was likely due to surface pore closure and filling of the pore network.

Improvement of the water vapor permeability of MFC films depends upon many parameters. The initial pulp characteristics as well as the treatments used to produce MFC play a major role in barrier property strategies. The MFC remains cellulose, however, and therefore still retains its affinity with water. When coupled with other fillers or coating agents, this disadvantage becomes reversible, but this can then change the film structure and porosity. The study of other barrier properties, such as the oxygen barrier property, gives rise to new parameters that also influence the measured properties.

- *Oxygen barrier*

Like water vapor permeability, the oxygen barrier plays a key role in food packaging. MAP was the first form of packaging to bring new security to food storage and distribution, as it prevented foodstuff oxidation by providing the recommended Oxygen Transmission Rate (OTR) value of less than $10\text{--}20 \text{ mL/m}^2/\text{day}$. Recently, Syverud and Stenius, 2009 studied the barrier properties of MFC films made from bleached spruce sulphite pulp, and their oxygen permeability in particular. They were among the first to publish results that linked MFC and barrier properties. They prepared non-oriented MFC films with a 0.1% MFC suspension using a cylindrical mold, and let them dry by evaporation at room temperature. This series had a thickness between 20 and $33 \mu\text{m}$, which corresponds to basis weights between $15\text{--}30 \text{ g/m}^2$. The OTR values (at 0% RH) measured were in the range $17\text{--}18 \text{ ml}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$, whereas EVOH or oriented polyester or PVdC-coated have an oxygen permeability of about $3\text{--}5 \text{ ml}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ and $9\text{--}15 \text{ ml}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$, respectively. MFC films therefore seem to be competitive with many well-known

synthetic polymer films of the same thickness, due to their dense and nanoporous network (Table 1-I.11).

Sample	Grammage (g/m ²)	Thickness (μ m)	Air permeability (nm/Pa s)	Oxygen permeability in the material (ml m ⁻² day ⁻¹)
MFC film A	17 \pm 1	21 \pm 1	13 \pm 2	17.0, 18.5
MFC film C	29 \pm 1	30 \pm 1	11 \pm 3	17.0, 17.0
Polyester, oriented	–	25	–	50–130 ^a
Polyester, oriented, PVdC coated	–	25	–	9–15 ^a
EVOH	–	25	–	3–5 ^a
Polyethylene LD	–	25	–	7800 ^a
Polyethylene HD	–	25	–	2600 ^a
Cellophane	–	21	–	3 ^b

Table 1-I.11. Permeabilities of MFC films and literature values for films of synthetic polymers and cellophane.

Results are extracted from Syverud et al. (2009) Cellulose, 16:75-85.

^a(Parry, 1993)

^b(Kjellgren and Engström, 2006)

Following these results, Aulin, *et al.*, 2010a showed the positive influence of carboxymethylation pre-treatment on oxygen permeability: at 50% RH, carboxymethylated MFC films presented a value of 0.085 mL.mm.m⁻².day⁻¹.atm⁻¹, as compared to values between 0.352 and 0.505 mL.mm.m⁻².day⁻¹.atm⁻¹ for non-pretreated MFC films (Syverud and Stenius, 2009). Due to this pre-treatment, the fibrils were highly charged and easier to liberate. The films thus had a dense network formed by fibrils with smaller and more uniform dimensions than those of untreated low-charged MFC.

In addition, Aulin, *et al.*, 2010a highlighted the impact of different parameters during MFC production. First, they noticed that increasing the relative humidity from 0% to 50% induced a considerable increase in the oxygen permeability, from 6.10⁻⁵ to 0.085 mL.mm.m⁻².day⁻¹.atm⁻¹, respectively. Such low OTR values at a low relative humidity can be attributed to the crystalline structure of MFC and its ability to form a dense network with inter- and intra-fibrillar hydrogen bonds. At a higher RH (70%), water limits the hydrogen bonds, creating films that are less packed. Even at such an elevated RH, however, they maintain competitive OTR values that are competitive with those of other biopolymer and polymer films (Lenes and Rodionova, 2010).

The next parameters studied were the basis weight and the thickness of films. As the weight and thickness increased, so did the difficulty of the path that oxygen must traverse, which resulted in a decrease in the OTR value. Moreover, the degree of oxygen permeability also decreased with increasing film thickness. Obviously, most pores are located at the surface of MFC films, and as

concluded by Minelli, *et al.*, 2010, the pores are not interconnected, and thus contribute to the impermeability of films when they are prepared at a sufficient thickness.

The last parameter arousing interest has been the number of homogenization steps. As was previously done by Aulin, *et al.*, 2010a and Siró, *et al.*, 2011 increased the number of homogenization steps. Both research groups concluded that an increasing number of steps produced no significant decrease in the OTR values (from 0.050 to 0.037 mL.mm.m⁻².day⁻¹.atm⁻¹ for 0 to 2 passes more).

After the grafting of MFC with acetylated groups, Rodionova, *et al.*, 2010 also analyzed the influence of this post-treatment on the OTR. They prepared films by simple filtration through a paper filter and then drying them for at least 2 h at 105°C until they were completely dry. They then studied the microstructure and pore size dimensions of the films using scanning electron microscopy. They found that while the pore dimensions remained constant regardless of the reaction time, the pore area fraction varied between 6 and 10% for each sample, and increased with an increase in the acetylation time (from 0.5 to 3 h). This result is attributed to the lower amount of hydrogen bonds. Pure MFC films made from kraft pulp had an OTR value of about 4 mL.m⁻².day⁻¹ for a basis weight of 30–40 g/m² (Syverud and Stenius, 2009). Acetylated MFC films presented higher OTR values depending on the reaction time (from 6 to 11 mL.m⁻².day⁻¹). Although both kinds of films fulfil the requirement of modified atmosphere packaging, the acetylated MFC films should perform better at a high RH (though this has not been studied).

Regardless of the treatment or the experimental condition used to produce MFC, MFC films are seen as a new biomaterial to realize the concept of good-barrier food packaging. Compared to other renewable and synthetic polymers, MFC films possess low barrier properties (Figure 1-I.8).

Due to their higher degree of crystallinity, they even exhibit an OTR that is much superior to that of cellophane. When compared with petropolymers, their WVP is still higher but their OTR remains lower.

To limit MFC's affinity with water but still preserve its good gas permeability, recent studies have suggested that polymer films be coated with MFC.

Fukuzumi, *et al.*, 2009, for example, prepared films from TEMPO oxidized softwood and hardwood pulps. Casting a TOCN/water dispersion onto a plasma-treated PLA film, they obtained a TOCN-coated layer that was 0.4 µm thick on a PLA film that was 25 µm thick. Despite this ultra-thin MFC layer, they observed a significant decrease in the oxygen permeability between an unmodified PLA film and a PLA film (from 7.4 mL/m².day.kPa to <0.001 mL/m².day.kPa). Similar conclusions have recently been reported, based upon the coating of

PET films with 1 μm of a TOCN dispersion, which decreased the OTR value from 29.3 to 0.08 $\text{mL}\cdot\text{m}^{-2}\cdot\text{day}$ (Rodionova, *et al.*, 2013).

The same year, a similar coating process for PET films and two kinds of TEMPO-mediated oxidation were proposed by Fujisawa, *et al.*, 2011. They prepared two different forms of MFC: (i) the usual TEMPO/NaBr/NaClO oxidated cellulose nanofibrils (called TOCNs) with sodium carboxylate groups (COONa), and (ii) TEMPO-oxidized cellulose nanofibrils HCl washed with free carboxyl groups (TOCN-COOH). The PET films were then coated with a thin layer of TOCN film in order to access their oxygen barrier properties. The oxygen permeability of the PET films decreased from 0.31 to 0.049 $\text{mL}/\text{m}^2\cdot\text{day}\cdot\text{kPa}$ when coated with TOCN-COOH and to 0.0017 $\text{mL}/\text{m}^2\cdot\text{day}\cdot\text{kPa}$ with TOCN-COONa. Thus, the presence of sodium carboxylate groups improved the oxygen barrier of the films compared to TOCN-COOH, proving the strong impact of the hydrogen bond quality on the OTR of MFC films. The reason for these results remains to be clarified, though as it was not completely explained by the authors.

The coating of MFC films on polymer films is a new way to produce a good barrier material and may also provide be a means of preserving the advantages of both MFC and polymers. However, the use of petro-polymers remains an issue for biodegradability. Another solution in this regard could be to use MFC with cellulosic substrates such as papers or cardboard. *As part of this PhD, we opted for that combination to elaborate a new food-packaging material. The first results were indeed promising as shown hereinafter.*

Paper coated with MFC

The first applications of MFC gel were as films or in nanocomposites. The combination of MFC and paper is quite recent, and only a few scientific publications are now addressing this theme. MFC may well succeed in improving the mechanical and barrier properties of paper, and the first studies in this regard have shown interest in applying this combination in fields such as food packaging and printing.

- Food-packaging applications

As early as 1983, Turbak, *et al.*, 1985 suggested the use of MFC in paper, but it took 25 years for the first applications of this use to appear.

Syverud and Stenius, 2009 were among the first to report upon the preparation of a coated paper coated with MFC. MFC was deposited on the top side of a wet-base paper by a dynamic sheet former. The deposited top layer and the base paper were thus combined, wet in wet. The basis weights of the deposited top layers were from 2 to 8 g/m^2 for a sheet whose total basis weight was 90 g/m^2 .

The results showed that the air permeability decreased drastically with an increase in the weight of the MFC coating.

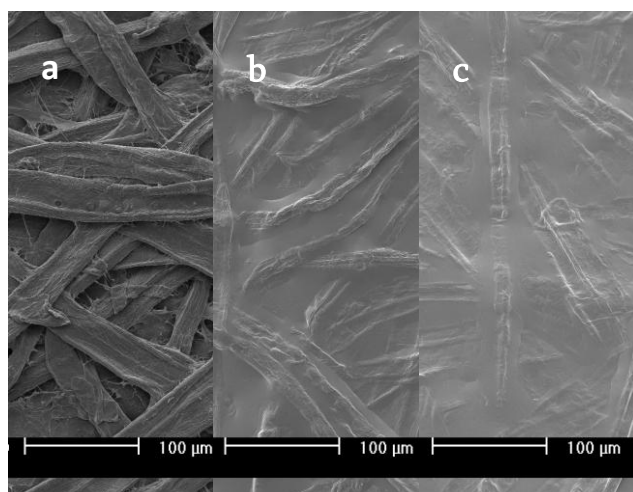


Figure 1-I.27. MFC coatings on paper/board

(a) reference paper, (b) 1 g/m² MFC coat, (c) 1.8 g/m² MFC coat

*Aulin, Gällstedt, et al., 2010

For a 2 g/m² MFC coating, the air permeability was about 3.10⁴ nm.Pa⁻¹.s⁻¹, whereas for an 8 g/m² MFC coating, the permeability dropped to about 360 nm.Pa⁻¹.s⁻¹—a value that is 100 times lower. The improved barrier properties were correlated with a reduction in the surface porosity. This explanation was later confirmed by Aulin, *et al.*, 2010a. Using another coating process (Print Coat instruments with a rod coater for sheets), they coated two different kinds of paper, kraft paper and greaseproof paper, with a 0.85%wt MFC suspension (Figure 1-I.27).

The air permeability decreased considerably for the two papers: from 69,000 nm.Pa⁻¹.s⁻¹ to less than 1 nm.Pa⁻¹.s⁻¹ for the kraft paper; and from 660 nm.Pa⁻¹.s⁻¹ to 1 nm.Pa⁻¹.s⁻¹ for the greaseproof paper (with a coating weight between 1 and 2 g/m²). The same group also showed that the use of carboxylated MFC formed denser coating layer and has superior air barrier property compared to enzymatically pre-treated MFC (Nygårds, *et al.*, 2011). The reduction of the surface porosity not only decreased the air permeability, but also improved their oil resistance.

Although the oil barrier property is rarely measured, it is often an essential consideration in the food and packaging industries. To the best of our knowledge, Aulin, *et al.*, 2010a published the first and only paper that studied this property in MFC-coated papers. Various standards are currently employed to measure oil resistance. Aulin, *et al.*, 2010a employed the Tappi T-454 in comparing the penetration time of turpentine oil and castor oils for each material. From their results, they concluded that when the air permeability decreased, the oil resistance increased. Thus, the MFC coated greaseproof paper exhibited better oil resistance.

In an approach different from that of previous works, Hult, *et al.*, 2010 proposed another method to improve the barrier properties of paperboard and paper, using MFC combined with shellac. Shellac is a natural resin that possesses properties such as oil resistance and biodegradability, acts as a good moisture barrier, and has a hydrophobic character.

In their study, Hult, *et al.*, 2010 tested two different combinations of MFC and shellac. In the first combination, MFC and shellac were coated first as a coating color, and in the second, as a multilayer structure with two coating layers (MFC and then shellac). The two coating methods were then carried out and the results were compared for a bar coater and a dynamic sheet former.

The combination of MFC and shellac in the bar-coated process, as either a blend or a multilayer system, produced a lower air permeability than paperboard and paper that were coated with shellac alone. MFC thus brought about a reduction in the air permeability. MFC coated using a dynamic sheet former obviously did not cover the entire surface of the paperboards and papers. The additional shellac layer that was a second coat thus brought about an additional reduction in the air permeability, which was already low after the first MFC coating (an additional decrease of 80 to 98%).

Concerning the oxygen transmission rate, the MFC layer first decreased the OTR values, and the second shellac layer, which closed the surface nanopores on the MFC layer, reduced these values even further. The OTR values obtained, however, still remained too high (around 5,000 cm³/m²day) to consider that these coated cellulosic materials provide a high oxygen barrier (3 cm³/m²day). These results were probably due to the non-homogeneous coating of MFC, a hypothesis that their study confirmed in a number of SEM images.

The ability of the coated materials to act as a moisture barrier was also investigated by measuring the water vapor transmission rate (WVTR). The substrates coated with MFC and shellac showed a very low WVTR (7–8 g/m².day), which is close to the WVTR value of a high moisture barrier material, i.e., 5 g/m².day (for a 25- μ m-thick film). Here, the coat of shellac played a major role in the decrease in the WVTR. As was concluded in the previous paragraph, MFC does not have a significant impact on WVTR, due to its hydrophilic nature. In this case, however, the MFC coating created a dense substrate that then permitted the laying of a more homogeneous shellac coating.

The three materials used in these studies presented good affinities, in that the adhesion between shellac and MFC, and between MFC and paper, is as strong as the internal bond strength of the paper substrate used. Thus, this new combination could be used in applications such as food packaging.

Another area widely investigated is printing. Recently become the subject of increasing study, the use of MFC brought very promising results. *These latter will not be detailed in this chapter*

as it is not the targeted application of this PhD. Nevertheless, further information can be found in our review (Lavoine, et al., 2012).

Studies dealing with MFC and cellulosic materials are limited in numbers in comparison to studies about nanocomposites, MFC films or MFC aerogels. Besides, the main application targeted is printing and less than ten scientific papers are focused on packaging application.

MFC is though a good candidate, since it gathers the main properties requested for food-packaging materials: (i) biodegradability and recyclability, (ii) renewable and cheap resources, (iii) non-toxicity, (iv) lightweight materials, (v) barrier properties against gases and grease, and (vi) mechanical reinforcement of any substrate. In addition, MFC is now commercialized and available in large amount. Its price is competitive with other biopolymers and cheaper.

The development of MFC as new food-packaging materials seems thus promising. MFC already fulfill the main society's requirements, and associated with cellulosic substrates, the request of more "convenient" and "ease of use" packaging is achieved. Nevertheless, still remains the need for better protection of the packaged product.

Consequently, we propose in this PhD to develop an active bio-material for food-packaging applications by the means of MFC.

I-4.3. New functional bio-nano-materials

To sum-up the previous paragraph, the development of active packaging materials is necessary to achieve a better protection of packaged food. Meanwhile, bio-based materials are expected and among them, MFC is considered as very promising.

However, the association of MFC with active materials is quite scarce and very new. According to the literature, two ways can be followed to elaborate active materials with MFC: either (i) MFC has to be functionalized by grafting or adsorption of an active polymer/molecule, or (ii) an active molecule has to be introduced in the MFC nanoporous network.

Both strategies will thus be described in the following part, leading then to the objectives of this PhD.

Functionalization of MFC

Functionalization of MFC is usually achieved by its surface modification. This strategy has been initially investigated to overcome main issue related to the surface chemistry of MFC such as (i) the high number of hydroxyl groups and the gel-like structure once produced, or (ii) the high hydrophilic-nature of this material, which limits its uses in several applications like in composites (tendency to form agglomerates in petro-chemical polymers) (Missoum, *et al.*, 2013a). A very recent review focused on the surface modification of MFC and details each strategy (Missoum, *et al.*, 2013a). According to it, the surface of the nanofibers can be modified either (j) by physical interactions or adsorption of molecules or macromolecules onto their surface or (jj) by using a chemical approach to achieve covalent bonds between cellulosic substrates and the grafting agent (Figure 1-I.28).

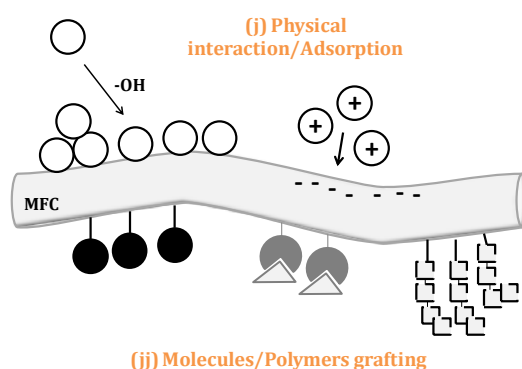


Figure 1-I.28. Strategies of MFC surface modification

Both strategies have been investigated to modify MFC in “active” or either “antimicrobial” MFC. Andresen, *et al.*, 2007 used the chemical approach to achieve covalent bonds between MFC and a grafting agent. They were the first to modify the MFC surface for achieving antibacterial

properties. Through an adsorption-curing process, they grafted the quaternary ammonium compound alkoxysilane octadecyldimethyl(3-trimethoxysilylpropyl)- ammonium chloride (ODDMAC) onto the surface of MFC. The MFC used in their study was produced at 2% w/w from bleached spruce sulfite cellulose by mechanical treatment with a Gaulin homogenizer. The antibacterial activity was tested according to the Japanese Industrial Standard Z 2801 against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Despite the few quantity of ODDMAC grafted onto MFC, the films produced from the modified MFC showed an efficient antibacterial activity against each kind of bacteria. Indeed, the killing efficiency was higher than 98% for an atomic concentration of ODDMAS nitrogen in the films surface of at least 0.14%.

Very recently, Martins, *et al.*, 2012 developed the second strategy to produce an antibacterial nanopaper using polyelectrolytes as binder between MFC and silver nanoparticles. MFC was modified using Layer-by-Layer technique. Cationic and anionic polyelectrolytes of different sorts were successively adsorbed onto MFC. Then, the modified MFC was mixed with the silver (Ag) colloid. This approach was successfully carried out and conferred antibacterial properties to MFC. Indeed, the slurry containing the highest Ag content was added to starch slurry and coated on paper sheet using size press. With coat weights of 2-3 g/m² containing 10 to 30% w/w of modified MFC, the coated papers presented an antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Others studies have also been investigated dealing with thematic such as Missoum, *et al.*, 2013b dealing with the antibacterial activity of chemically grafted MFC. Furthermore, more and more European projects have been developing around this issue, with for example the beginning of the NewGenPack project, in 2012, aiming to develop new functional cellulose fiber based packaging materials for sustainability.

However, each of these studies do not use antibacterial agents adapted to food contact, and in the case of silver nanoparticles, the question of toxicity has to be considered. Furthermore, the two processes of functionalization induce the use of toxic chemicals and regarding a food-packaging application, further tests have to be carried out to guarantee the absence or the non-migration of potential toxic substances into the packaged product.

The migration topic is a consequent problematic, since it regulates the use of any components in food-packaging materials. Indeed, according to European regulations, the packaging material intended to come into contact with food must be inert toward the packaged product and the consumers. Thus, some “unhealthy” components are allowed to be used and introduced into packaging materials, while their release into the packaged product remains below a certain amount, which must not modify the organoleptic properties of the packaged product and harm the consumers’ health.

Consequently, this first strategy (MFC functionalization) is still rare and seems not adapted today.

MFC nanoporous network as active agent carrier

The second strategy to elaborate active materials with MFC consists in introducing an active agent in the MFC nanoporous network. This strategy implies necessarily the release study of the active agent incorporated in MFC.

The use of MFC as molecule carrier or drug delivery system (DDS) is very recent and up to our knowledge, we only found an amount of six scientific papers dealing with this thematic from 2011 (Figure 1-I.29). When this PhD has started in 2010, no paper dealt with this topic, proving the novelty of our research. Compared to the increasing research about MFC since the XXIst century, this field is just started and already presents promising future applications.

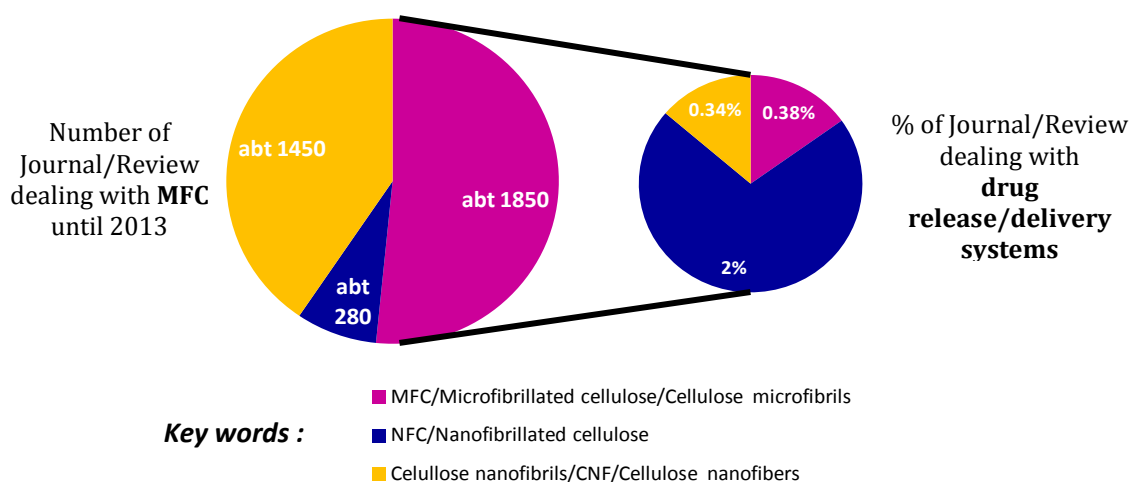


Figure 1-I.29. Number of journal/review published about MFC/NFC/CNF and percentage of these scientific works dealing with drug release/delivery systems. Statistic obtained with SciFinder (June 2013)

Among the five studies, two were performed by Valo et al. (Valo, *et al.*, 2011, Valo, *et al.*, 2013). This group used MFC either as stabilizer of nanoparticles (Valo, *et al.*, 2011) or as drug carrier aerogel (Valo, *et al.*, 2013). In their first paper (Valo, *et al.*, 2011), they studied a hydrophobic drug compound, itraconazole, which was precipitated to form nanoparticles with either hydrophobins (group of surface active proteins secreted by filamentous fungi) or genetically modified fusion proteins (fusion of hydrophobins with cellulose binding domains). To enhance stability of the precipitation, the drug nanoparticles were immobilized to MFC by mixing in water. The suspensions were either freeze-dried or stored. Dissolution tests have also been carried out on the powders loaded in gelatin capsules. They concluded on the ability of MFC to well stabilize the nanoparticles and prevent them from aggregations. However, MFC did not prevent fast drug release from bound nanoparticles in spite of stronger binding to cellulose

microfibrils. One explanation, provided by the authors, arises from the large surface area provided by the largely porous cellulose network. Indeed, the proteins and the particles can be bound to the cellulose, but the drug molecules themselves will be free to dissolve from the cellulose fiber dispersed particles, much like from free particles. In their second paper, Valo, *et al.*, 2013 prepared MFC aerogels from various sources (quince cellulose (QC), bacterial cellulose (BC), red pepper (RC), microcrystalline cellulose (MCC) and birch cellulose with a TEMPO oxidation (TC)). They introduced and studied the release of a model drug, beclomethasone dipropionate, which was also precipitated with hydrophobins. The aerogels made from RC and MCC released the drug immediately (about 60% released in five minutes), whereas the three others showed sustained drug release (during the first 10 minutes, 10-40% released for BC and only 10% released for QC and TC). Thus, depending of the MFC sources, the nanoporous network formed during the aerogel manufacturing process has a different effect on the drug release.

The three other studies are extracted from the PhD of Ružica Kolaković (Kolakovic, 2013), titled “Nanofibrillar cellulose in drug delivery”. This group, from pharmaceutical university, evaluated the potential of MFC to be used as an excipient for immediate release tablets and compressions (Kolakovic, *et al.*, 2011), for providing sustained drug release profiles from microparticles (Kolakovic, *et al.*, 2012a) and for film-type controlled release systems (Kolakovic, *et al.*, 2012b).

Spray-drying process was developed to produce drug loaded MFC microparticles for longer-lasting controlled drug release. During the drying process, the drugs (indomethacin, nadolol, atenolol, metoprolol tartrate, verapamil hydrochloride and ibuprofen) were entrapped in the nanoporous network formed by MFC. This tight MFC network was effective and sustained the release of the drugs for up to two months. Nevertheless, the spray-drying process has some drawbacks within the elaboration of drug delivery systems: (i) limitation to water soluble compounds and (ii) low loadings due to the difference drying kinetics of drugs and MFC. A better manufacturing process to overcome these mentioned problems was the production of drug loaded MFC films.

Kolakovic, *et al.*, 2012b produced MFC films loaded with indomethacin (IND), itraconazole (ITR) or beclomethasone dipropionate (BE) by filtration method through a PVDF membrane (Figure 1-I.30).

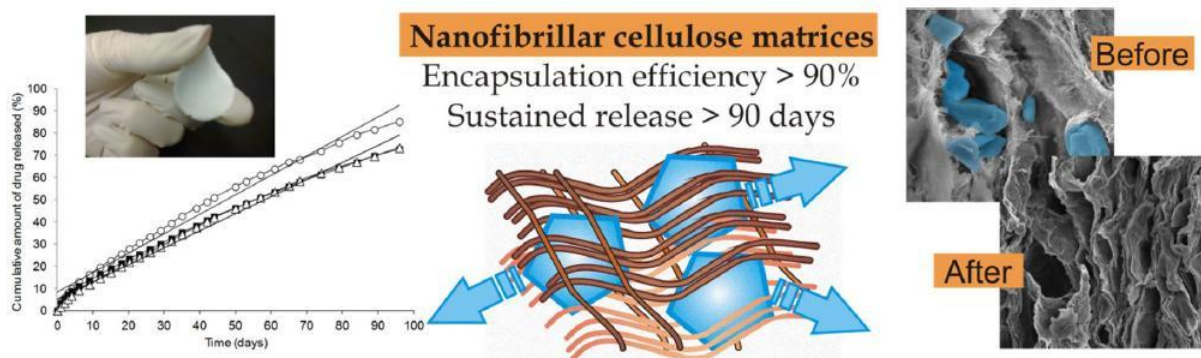


Figure 1-I.30. Schematic drawing of the matrix structure of the films (middle), appearance of the film and dissolution profiles of beclomethasone dipropionate loaded films (left), SEM image of indomethacin loaded film before (up) and after (bottom) the dissolution studies.

Figure extracted from the PhD of (Kolakovic, 2013)

Interestingly, the drug release did not affect the morphology of the films and their structure remained unchanged after the drug had been released. Each drug showed a different release, either a diffusion limited release (IND) or a zero-order release kinetics (ITR and BE). This was a consequence of the different drug solubilities in the dissolution medium, as well as of the different drug binding to MFC. Nevertheless, MFC films gave sustained release even up to 90 days for each drug. In this study, Kolakovic, *et al.*, 2012b tried, for the first time, to establish a model to better understand the drug release through the MFC nanoporous network. They chose to apply the following Higuchi equation:

$$Q = A \sqrt{\frac{D\varepsilon}{\tau} (2\rho - \varepsilon C_s) C_s t}$$

where Q is the amount of drug released, A is the surface area of the film, D is the diffusion coefficient of the drug inside the film, ε is the porosity of the films, τ is the tortuosity of the film, ρ is the density of the drug material in the film, C_s is the saturated solubility of the drug inside the film, and t is the time.

They supposed thus that the release mechanism was only due to drug diffusion. Besides, they also assimilated MFC films to a disc, i.e. with height \ll thickness, and that the drugs were homogeneously distributed within the films at an initial concentration that exceeds drug solubility (i.e. a monolithic dispersion (Siepmann and Siepmann, 2011)). The model fitted well with their experimental data regarding the indomethacin loaded MFC films only. Indeed, some others parameters such as drug/fibers interactions, drug dimensions or films swelling had to be considered for both other drugs. The authors suggested that the release of these latter was not

only controlled by a simple drug diffusion process from the films, but a zero order kinetic could be rather applied.

This model and conclusions will be thus considered as the base of our work on modeling and will be thus detailed further in our experimental results.

In a last study –not published yet- Kolakovic, 2013 studied the factors, which could influence the drug release from the MFC matrix. As they tested different kinds of drugs, they have measured the influence of many parameters: molecular size, pH of the release medium, and interactions (mechanisms of binding) between drugs and matrix. *These different parameters are necessary to keep in mind and this study will help us to better understand what happened in our case.*

These works proved the relevant interest in using MFC either as drug carrier or as drug delivery system. Whatever the method investigated, i.e. spray-dried MFC, MFC films or MFC aerogels, all allowed releasing longer the drug tested. Whatever the “shape” of MFC, the entanglement of nanofibers is thus preserved and thanks to this nanoporous network, a controlled and longer release of molecules can be achieved.

Both groups targeted the pharmaceutical and biomedical fields by testing specific drugs. Indeed, beclomethasone dipropionate is generally used for the prophylaxis of asthma or as treatment of rhinitis and sinusitis. Indometacin is an anti-inflammatory drug reducing fever or pain. Itraconazole is prescribed to patients with fungal infection. None study was thus focused on food-packaging applications.

Before these results, the astonishing properties of MFC and their network, as well as the current society's requirements regarding food-packaging materials, lead us to focus this PhD on the following subject: “Design, processing and characterization of innovative functional bio-nano-materials for packaging”.

PhD objectives

The aim of this PhD is to develop new functional bio-nano-materials for packaging application.

Bio-materials are one of the main society's requirements and for this reason we will work with papers and cardboard substrates, since they are biodegradable, recyclable and produced from renewable resources.

Then, to improve the shelf-life of packaged product, we will elaborate functional bio-materials by conferring antibacterial property to these cellulosic substrates. This function will be conferred by the addition of antibacterial molecules. Different kinds of active molecule will be tested throughout this PhD, so that we could consider the influence of parameters such as their molecule dimension or chemical properties on their release.

The release of these antibacterial agents will be controlled and handled by microfibrillated cellulose. Contrary to the simultaneous studies using MFC as powders, aerogels or films to release drugs (Kolakovic, *et al.*, 2012a, Kolakovic, *et al.*, 2011, Kolakovic, *et al.*, 2012b, Sudesh and Doi, 2005), the innovation of this work consists in using the MFC nanoporous network formed during the coating process of MFC onto cellulosic substrates to introduce and release progressively an antibacterial agent. As described above, the MFC suspension looks like a gel and their coating onto substrates is not so obvious. Thus, a screening of different coating methods will be performed to select the right process achieving the best mechanical and barrier properties of the final material.

Different strategies will be also implemented to introduce the antibacterial agent into MFC-coated materials. These strategies will be compared experimentally with release studies and antibacterial tests. Furthermore, a theoretical approach will help to better understand the molecule migration through the coated substrates.

Figure 1-I.31 resumes the global PhD strategy described previously:

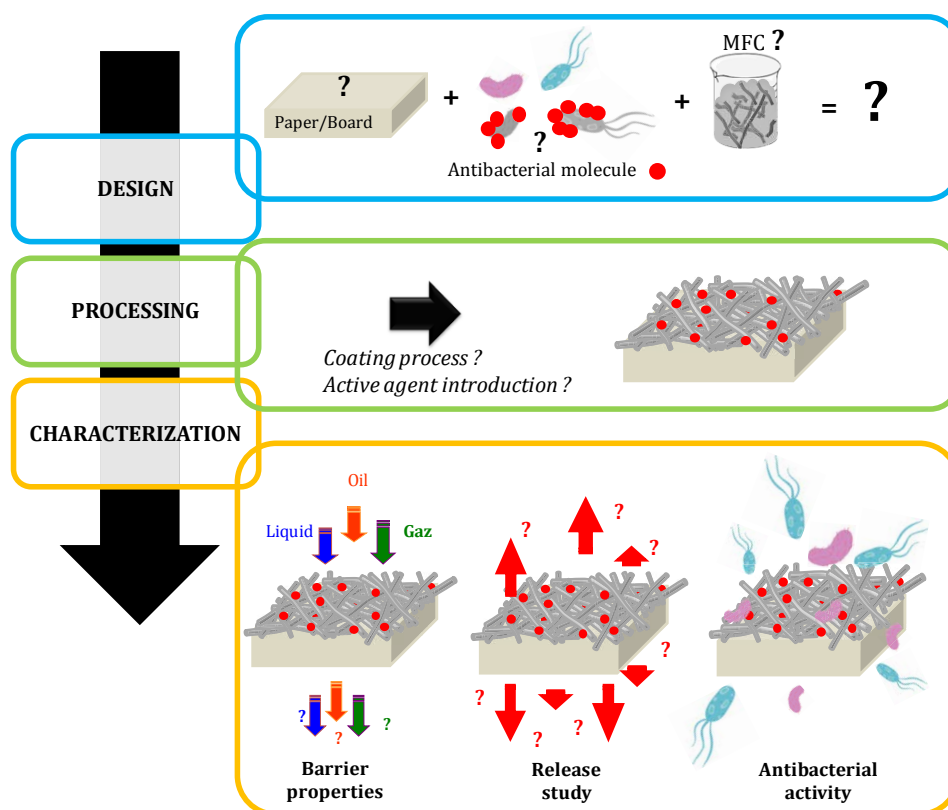


Figure 1-I.31. "Design, processing and characterization of functional bio-nano-materials for packaging":
Global strategy.

Further to this strategy, the manuscript will be divided in three main parts. The first part, **Chapter 2**, will discuss the mechanical and barrier properties brought by MFC coated on

cellulosic substrates. After screening of coating processes, one has been selected and used throughout the project to coat MFC either on paper or cardboard.

The second part, **Chapter 3**, will deal with the release study of an antibacterial molecule introduced into cellulosic substrates prepared Chapter 2. The effect of MFC on the molecule release will be studied in details from experiments to theory. The antibacterial activity of the bio-nano-materials developed will be then tested on model, but also on real food.

The final part, **Chapter 4**, will present a new strategy of introduction of the antibacterial agent into cellulosic substrates combining the effect of MFC and cyclodextrine on molecule release. This part is the result of collaborations between LGP2 and UMET laboratory (Lille, France).

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CHAPTER 2

Influence of MFC coating onto cellulosic materials

CHAPTER 2. Summary

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RESUME FRANÇAIS – FRENCH ABSTRACT

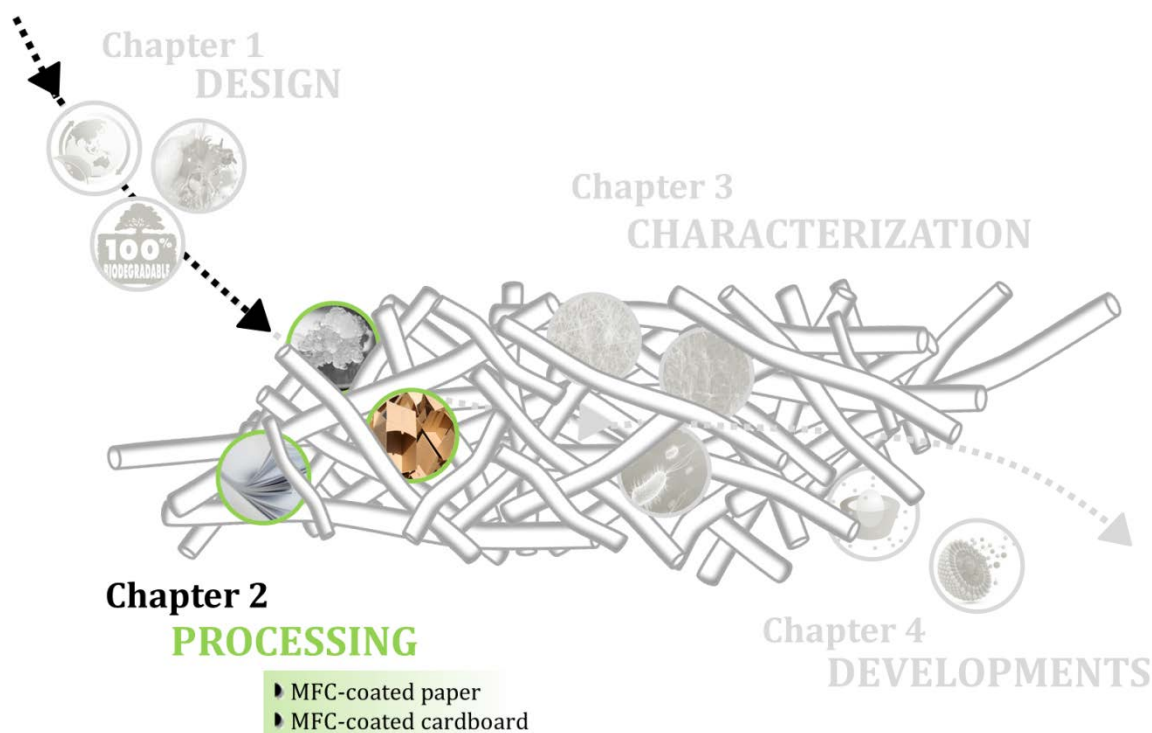


Figure Chapitre 2. Représentation graphique de l'organisation du manuscrit

Dans une société de plus en plus attentive aux contraintes économiques et aux besoins écologiques de la planète, les demandes en matériaux biodégradables, recyclables et d'un rapport performances/coûts optimisés se multiplient.

Le chapitre précédent a souligné ces nouveaux besoins dans le domaine de l'emballage alimentaire. En réponse à cela, les industries et centres de recherche proposent de nouvelles solutions basées sur l'utilisation de biopolymères et le développement d'emballages fonctionnels. Le chapitre 1 a ainsi explicité les atouts de ces deux solutions, mais a aussi clairement mis l'accent sur les quelques points, qui aujourd'hui, empêchent le développement commercial de ces emballages.

L'objectif de ce projet de thèse est donc de proposer un nouveau biomatériau fonctionnel pour l'emballage alimentaire répondant aux besoins de la société, et palliant aux problématiques actuelles des biopolymères et emballages fonctionnels.

L'idée est de transformer un matériau papier/carton en matériau antibactérien ayant la capacité de libérer de manière contrôlée et prolongée l'agent actif introduit. L'élaboration de ce système de libération sera réalisée à l'aide de microfibrilles de cellulose (MFC). Le chapitre précédent a identifié les principaux atouts des MFC et leurs applications majeures. L'utilisation des MFC avec

des matériaux cellulosiques n'a été que peu étudiée (une trentaine d'articles seulement), et leur usage en tant que système de libération n'a pas fait l'objet de plus de 7 publications. Pourtant, l'addition des MFC (en surface ou en masse) apporte une amélioration nette des propriétés mécaniques et barrières des papiers. De plus, les premières études relatant leur utilisation pour des systèmes de libération ont démontré un réel intérêt dans le milieu pharmaceutique.

Ainsi, dans ce projet de thèse, les MFC seront utilisées pour : 1) améliorer les propriétés mécaniques et barrières des matériaux papier/carton et 2) conférer à ces matériaux un système de libération contrôlée après introduction d'un agent actif.

Le premier point constitue le sujet du Chapitre 2. L'objectif est double : (i) déterminer le procédé optimal d'enduction de MFC et (ii) analyser les propriétés mécaniques et barrières des matériaux enduits obtenus. L'enduction de MFC sur matériaux cellulosiques a déjà été reportée dans la littérature. Contrairement à ces études, nous avons analysé des suspensions de MFC et des supports cellulosiques différents, et utilisé d'autres procédés d'enduction. Il était donc utile et nécessaire d'effectuer une analyse complète des propriétés de nos matériaux une fois les MFC ajoutées.

Dans la **première partie** (Article 2, soumis à *Journal of Materials Science*), deux procédés d'enduction de MFC ont été utilisés et comparés sur un même papier (40 g/m² et calandré) : le couchage à barre et la size press.

Dû à la viscosité particulière de la suspension de MFC (2% en matière sèche), l'enduction de plusieurs couches successives (1 à 10) fut nécessaire afin d'obtenir un couchage homogène sur toute la surface du papier. A partir de 5 couches, environ 8 g/m² de MFC ont été déposés avec le couchage à barre contre seulement 1 g/m² avec la size press.

L'influence du poids de couche des MFC a ainsi été étudiée pour les échantillons couchés 1, 5 et 10 fois avec les deux procédés. Analyses microscopiques (MEB, AFM), structurelles (grammage, épaisseur) et tests de propriétés mécaniques (module d'Young, rigidité à la flexion, allongement, longueur à la rupture, éclatement) et barrières (absorption à l'eau, perméabilité à l'air, résistance aux graisses) ont été réalisés. Par ailleurs, dû à la haute teneur en eau de la suspension de MFC, des papiers références traités de la même manière, mais avec de l'eau uniquement, ont également été étudiés afin de mesurer l'impact des cycles de mouillage/séchage successifs.

Ces cycles ont en effet fortement dégradé les propriétés mécaniques du papier de référence. Cependant, à partir d'un poids de couche de MFC de 8 g/m² (atteint avec le couchage à barre uniquement), ces effets ont clairement été contrebalancés. Des propriétés telles que la rigidité à la flexion et la résistance à l'air ont quant à elles significativement été améliorées (+70%).

L'action des MFC fut d'autant plus conséquente que le poids de couche déposé fut important. Suite à cette étude, le couchage à barre a été opté comme procédé d'enduction de cinq couches de MFC à 2%, i.e. d'environ 8 g/m², dans tous les chapitres suivants.

Cette première étude se focalise sur le matériau papier, ce qui limite une application finale à des emballages souples. Dans l'optique de créer des emballages rigides pour la mise en volume, le procédé d'enduction sélectionné fut donc appliqué sur un autre matériau cellulosique : un carton alimentaire de 300g/m². La **deuxième partie** de ce chapitre 2 (Article 3, soumis à *Journal of Applied Polymer*) détaille donc l'influence des MFC et des cycles de mouillage/séchage successifs sur les propriétés mécaniques et barrières de ce carton. C'est la première fois qu'une analyse complète des propriétés d'un carton couché avec des MFC a été menée.

Toutefois, avec 8g/m² seulement de MFC, l'amélioration des propriétés mécaniques ne fut pas drastique dans le cas spécifique d'un carton de 300g/m². Les propriétés barrières ne furent par ailleurs pas améliorées. Cependant une augmentation conséquente de l'absorption à l'eau fut constatée, due majoritairement à l'importante surface spécifique des MFC et leur nature hydrophile. Enfin, le matériau carton couché avec des MFC et mis en 3D présenta des propriétés mécaniques égalant celles d'un même carton 3D couché avec du polyéthylène. Ainsi, la réalisation d'un matériau plus léger, 100% biodégradable et recyclable est possible par l'utilisation de MFC. Ces dernières auront aussi l'avantage, par la suite (chapitre suivant), d'être utilisées comme matrice de libération de molécules actives pour des emballages antibactériens.

Ce chapitre 2 propose donc une analyse détaillée des propriétés structurales, mécaniques et barrières de matériaux cellulosiques couchés avec différents poids de couche de MFC. Contrairement à la littérature, l'amélioration des propriétés mécaniques ne fut pas aussi conséquente. La différence provient d'une part du type de suspension de MFC utilisée, mais principalement du support cellulosique de référence. Cependant, la forte chute de perméabilité à l'air et l'augmentation radicale de l'absorption à l'eau sont deux résultats qui se trouvent ici confirmés. Grâce à leur échelle nanométrique et leur réseau nano-poreux, les MFC sont à même d'augmenter la tortuosité et de diminuer la porosité du matériau offrant une barrière à l'air efficace.

Si certains résultats obtenus dans ce chapitre étaient plutôt attendus au vue de la littérature existante à ce sujet, l'analyse microscopique de ces matériaux cellulosiques couchés a permis de mettre en évidence le réseau nano-poreux des MFC, conservé lors du processus de couchage. Ceci était mis en doute par certaines études. Ce résultat apporte donc une information supplémentaire à notre communauté scientifique. Les images AFM présentés font en effet partie

des toutes premières parmi les articles déjà publiés. Elles soulignent clairement l'enchevêtrement des microfibrilles de cellulose et laisse entrevoir une ouverture sur d'autres applications possibles de ces matériaux cellulosiques, comme par exemple, la réalisation de systèmes de libération contrôlée.

Impact of different coating processes of microfibrillated cellulose on the mechanical and barrier properties of paper

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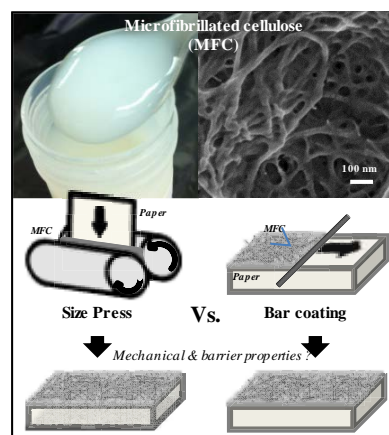
Abstract

This study presents a comparison of mechanical and barrier properties of papers coated with microfibrillated cellulose (MFC) by two different coating processes: (i) bar coating and (ii) size press. Due to the high water content of MFC, water-treated papers are taken as references to highlight effects of MFC on papers properties.

Structural, mechanical and barrier properties of ensued materials are performed respectively with AFM, FE-SEM, tensile and stiffness testers, and air permeability equipments.

Properties of water-treated papers are damaged compared to base paper that underlines the negative effect of both coating processes on papers structure.

With MFC, air barrier and bending stiffness are considerably improved (+90% and +50% respectively), especially when bar coating is used, i.e. with 7 g.m⁻² of MFC. Size press is indeed not able to considerably improve papers properties as the MFC coat weight barely reached 4 g.m⁻² resulting from ten successive MFC layers.



Inspired from Paper 2:

Nathalie Lavoine, Isabelle Desloges, Bertine Khelifi and Julien Bras (2013) Impact of different coating processes of microfibrillated cellulose on the mechanical and barrier properties of paper. Submitted to Journal of Materials Science.

I-1. Introduction

Paper is widely used as packaging material for its biodegradability and its eco-efficiency. Its hydrophilic nature, nevertheless, prevents it from some applications since water weakens its structure and thus, its mechanical and barrier properties.

Paper is thus often used with other materials such as plastics or aluminium essentially to improve its barrier properties (López-Rubio, *et al.*, 2005, Zhang, *et al.*, 2001). EVOH, ethyl vinyl alcohol, is for example often coated onto paper for its excellent oxygen-barrier properties. PE, polyethylene, is also a classical polymer used to coat papers and bring barrier properties (Choi, *et al.*, 2002). Many petroleum waxes (Touey, 1962) or latex (Duan, *et al.*, 1999, Wu, *et al.*, 2001) have also been investigated as protective layers for the development of high barrier packaging materials. These coating slurries improve considerably the barrier properties of papers. Nevertheless, its main and most attractive properties, i.e. sustainability and biodegradability, are then partially lost.

To overcome these issues, one idea consists in replacing synthetic polymers with more sustainable materials such as biopolymers (Khwaldia, *et al.*, 2010). Several biopolymers are nowadays investigated as paper-coating materials such as whey proteins (Gällstedt, *et al.*, 2005, Han, *et al.*, 2010), chitosan (Fringant, *et al.*, 1998, Ham-Pichavant, *et al.*, 2005, Kjellgren, *et al.*, 2006), starch (Rhim, *et al.*, 2006, Yan, *et al.*, 2005) or alginates.

More recently, the development of nanocelluloses has offered a new alternative to the polymer coating. In particular, microfibrillated cellulose (MFC) has been used as coating slurry for paper and paperboard. In 1983, Herrick, *et al.*, 1983 and Turbak, *et al.*, 1985 discovered and patented the manufacturing process of MFC. As subdivision of cellulosic fiber, MFC has diameters in the range of 10 - 50 nm and length of several micrometers. Due to its nano-scale dimensions, its high aspect ratio, its entangled network and its intrinsic high mechanical properties, MFC showed a high reinforcing potential and was used in nanocomposites (Siró and Plackett, 2010). Then, its ability to form films interested many researchers as mechanical and barrier properties of MFC films are competitive with those of current good quality polymers films (Syverud and Stenius, 2009). Even the coating of MFC and its combination with petro- and bio-polymers films showed its interest by increasing drastically the barrier properties of the initial films (Fukuzumi, *et al.*, 2009). Unlike to these applications, the use of MFC with cellulosic materials has just started and still remains rare (Lavoine, *et al.*, 2012). The first study relating to the MFC coating onto paper was investigated in 2009 (Syverud and Stenius, 2009). A 1%wt MFC suspension was deposited onto paper with dynamic sheet former. Coat weights between 2 to 8 g/m² were reached by this process, but the authors highlighted the disorder and discontinuities of the MFC layers. The mechanical properties of the sheet were improved (increase by 28% regarding the tensile strength) and the air permeability decreased drastically compared to initial

sheet: from 6.5×10^4 to $360 \text{ nm.Pa}^{-1}.\text{s}^{-1}$ with a coat weight of 8 g/m^2 . Using also a dynamic sheet former, Hult et al. (Hult, *et al.*, 2010) coated 0.1%wt MFC suspension on base paper and reached a coat weight of 5 g/m^2 . They also decreased the air permeability by 98% (e.g. from 49 to $0.752 \text{ nm.Pa}^{-1}.\text{s}^{-1}$) compared to base paper. However, they obtained very high oxygen permeability values ($> 10,000 \text{ cm}^3.\text{m}^{-2}.\text{d}^{-1}$) explained by a non-homogeneous MFC coating. To solve this problem of homogeneity, Aulin, *et al.*, 2010 coated a 0.85%wt MFC suspension with a rod coater and applied 6 different coats weights (up to a maximum of 2 g/m^2) as shown Figure 2-I.1.

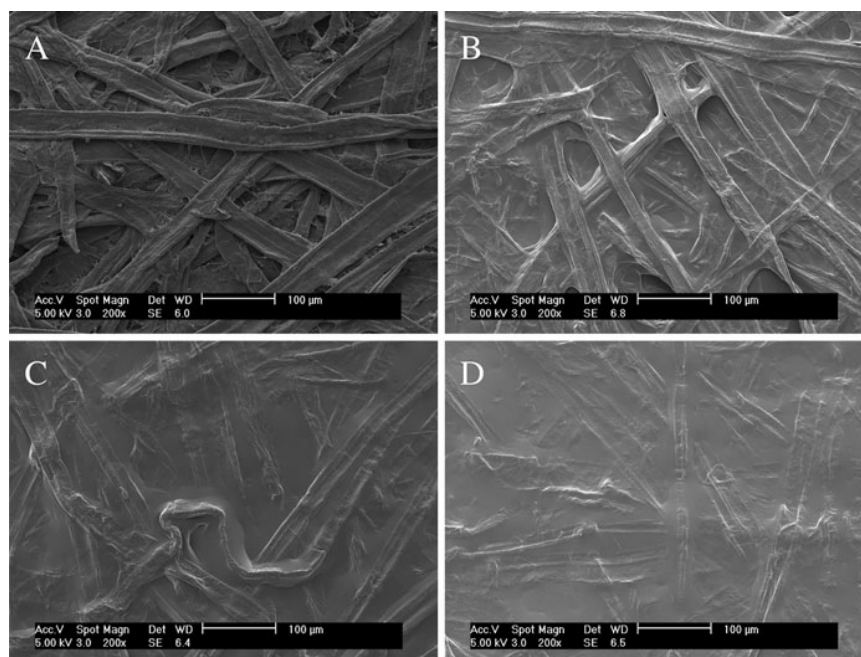


Figure 2-I.1. E-SEM micrographs of uncoated (a) and MFC-coated unbleached papers with coat weights of ca. 0.9 (b), 1.3 (c) and 1.8 g/m^2 (d), respectively. The scale bar is $100 \mu\text{m}$ (from Aulin, *et al.*, 2010)

They obtained very low air permeability but also very good oil resistance, that they explained by the formation of continuous and homogenous films on paper surface. Using a similar coating process (rod coater and drying at room temperature), Sofie Nygård's (Nygård's, *et al.*, 2011) developed MFC-coated papers for printing application. With also 6 g/m^2 of MFC coat weight, an air permeability closed to $0 \text{ nm.Pa}^{-1}.\text{s}^{-1}$ was reached with two kinds of MFC suspensions (one enzymatically pre-treated, the second carboxymethylated). Nevertheless, the improvement of printability was not concluded by using MFC in coating colors for offset printing. MFC showed yet its efficiency for flexography (Hamada, *et al.*, 2010) and inkjet printing (Luu T, *et al.*, 2011). Interestingly another coating process has been used to coat MFC, the size press process. Compared to the dynamic sheet former and the rod coater, the size press is able to coat MFC on both sides of paper. For instance, no scientific papers deals with this process and only our conferences papers (Bardet, *et al.*, 2012, Lavoine, *et al.*, 2011) and one other conference (Luu T, *et al.*, 2011) mentioned it. In this latter study, they coated between 3 and 6 g/m^2 of MFC (at

3%wt) on paper pre-treated with AKD. They focused on the improvement of ink spreading and printing density, but did not carry out mechanical and barrier tests

Up to our knowledge, these studies are the only one relating to the MFC coating onto paper sheets. Our present work aims thus to compare and study the effect of two MFC coating processes, (i) the bar coating and (ii) the size press processes on the mechanical and barrier properties of coated samples (Figure 2-I.2). Compared to the other studies, a 2%wt MFC suspension has here been used. As the water content of the suspension is high (98%), similar analysis are presented on corresponding water-treated papers to highlight the possible effect of process on the different properties.

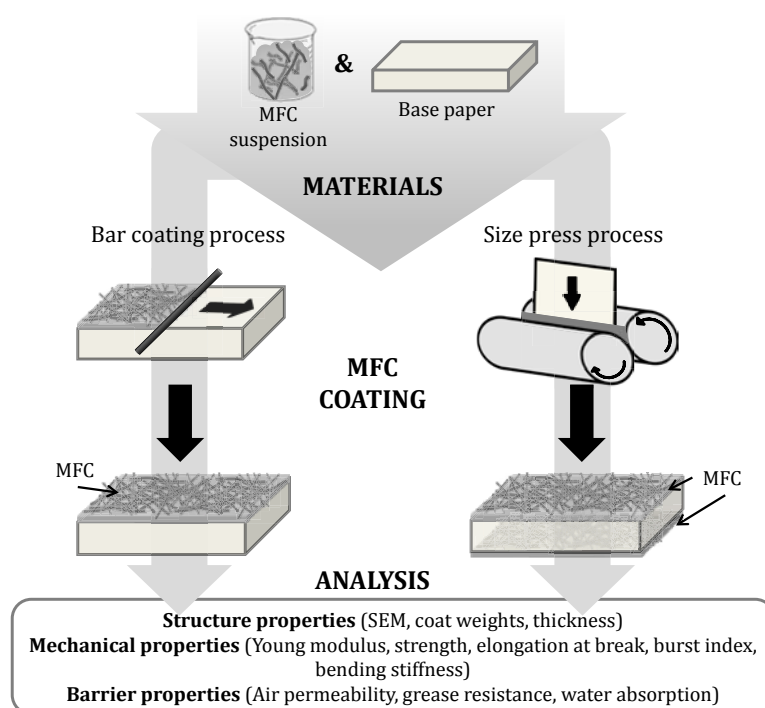


Figure 2-I.2. Impact of MFC coating processes on the mechanical and barrier properties of paper samples

I-2. Experimental Section

I-2.1. Materials

MFC suspension was kindly supplied by the FCBA (France). This suspension was produced from eucalyptus pulp, enzymatically pre-treated (during 2 h with an endoglucanase), and passed through a Microfluidizer® M-110EH-30 (5 passes through the 100 nm chamber, 4 passes through the 200 nm chamber). The concentration was 2% (w/w).

The base paper material was calendered paper with a basis weight of 41 g.m⁻² made with non-bleached pulp.

I-2.2. Methods

MFC suspension characterization

The suspension was observed using FE-SEM, (Field Emission Scanning Electron Microscopy, Zeiss® Ultra-55, USA). It was spread onto a metal substrate using carbon tape, let dried two nights at room temperature and finally coated with a thin layer of gold (162 nm). The working distance was 5.5 mm for an accelerating voltage of 2.00 kV at a magnification of $\times 20,000$. The viscosity of MFC suspension was measured with a rotational rheometer (Anton Paar Physica MCR 301, Benelux). A parallel plate geometry type was selected for measurements and a constant temperature of 20°C was established. The shear rates investigated were between 0 and 1000 s⁻¹. One measurement consisted in the gradual increase followed by the gradual decrease of shear rate. An average of at least three duplicates was plotted to have a mean value of the viscosity as function of the shear rate.

Coating processes

Two different coating processes were used to deposit MFC suspension onto paper samples, (i) bar coating and (ii) size press process.

(i) The MFC suspension at 2%wt was coated onto paper samples (A4 format) with a bar coating process (Endupap, France). A 0.9 mm Mayer bar was used with a coating speed of 5 cm.s⁻¹. The coated papers were then dried with a contact drying system under tension at 105°C for 3 min. These steps were repeated from one to ten times in order to deposit one to ten MFC layers onto paper samples.

Reference samples (water-treated papers) were obtained by impregnation of one side of the base paper in deionized water followed by drying under the same conditions described above. Reference samples were treated the same number of times as the MFC-coated samples.

(i) A "Labor Size Presse SP" (Mathis AG, Switzerland) was used and filled with the MFC suspension diluted at 1.6%wt. Paper samples (A4 format) were pressed between the two rolls at 8 bars at a speed of 50 m.min⁻¹. Papers were then coated onto both sides and dried with a contact drying system under tension at 105°C for 3 min. These steps were also repeated five and ten times to compare with the bar coating process. As reference samples, sized papers with deionized water were also prepared following the same conditions of pressure and speed.

Paper structure properties characterization

Each sample was maintained at a temperature of 23°C, and a relative humidity (RH) of 50% for at least 24 h before characterization.

The basis weight and MFC coat weights of samples were determined by weighting at least ten samples of 10×10 cm² with a balance of ±0.001 g of precision. The data given is an average value of ten measurements.

The thickness was measured with a Lhomargy micrometer (±0.01 mm) and expressed as an average of at least five measurements for each 10×10 cm² sample according to the standard ISO 534:2011. These values have also been checked with the Scanning Electron Microscopy (SEM) images of the cross-section of non-coated and coated papers by using a Quanta200® (The Netherlands). Cross-section and surface of base and coated papers were mounted onto a metal substrate covered with carbon tape to be analyzed with SEM. The back-scattered electron detector (BSE) was also used for coated papers surface. The working distance employed was 9.9 mm with a voltage of 12.5 kV at a magnification of ×100 for surfaces and ×1200 for cross-sections. Atomic Force Microscopy (AFM) was used to observe the organization of MFC after coating onto paper substrates. Smaller pieces (5×5 mm²) of MFC-coated papers were glued onto aluminium sample holders and scanned by tapping-mode AFM using a Dimension Icon with Scan Analyst (Bruker, USA). The images were recorded under ambient air conditions (temperature and relative humidity) with standard silica cantilever (Bruker, USA).

Paper mechanical properties characterization

Each sample was maintained and tested at a temperature of 23°C, and 50% RH for at least 24 h before characterization.

The Young's modulus of samples was determined following a method adapted from the standard ISO 1924-2/3. Using a Lorentzen & Wettre Tensile Tester, the speed was fixed at 100 mm.min⁻¹. The same device also allowed measurements of breaking length and elongation at break of each sample. At least five measurements were carried out to obtain an average value. In the same standard conditions of temperature and humidity, the bending stiffness was measured with a Kodak stiffness tester (Lhomargy, ISO 5629). The result is here again based on at least five specimens. The burst index (Adamel Lhomargy EC 0.5) was evaluated according to the standard ISO 2758/2759. Five measurements were done for each sample to obtain an average value.

Paper samples barrier properties characterization

Each sample was maintained at a temperature of 23°C, and 50% RH for at least 24 h before characterization.

The water absorption was measured following the Cobb 60 method (ISO 535). For each sample, at least five measurements were done to calculate an average value of the Cobb Index.

The air permeability tests were carried out with the system of Mariotte vases (ISO 5636) using a sample area of 2 cm² and a vacuum of 2.5 kPa. The results were expressed as an average of at least five measurements.

The grease resistance was measured according to the Kit test (T559 cm-02), which is based on twelve different grease solutions numbered from 1 to 12. The number 12 represents the highest grease resistance (or Kit number). A paper is considered grease resistant if its Kit number is at least of 8. Each analysis was made at least five times for each sort of samples. An additional internal test was also carried out to better conclude on the dispersion and penetration of grease into the sample. This test consists in the painting of coloured commercial oil onto the samples surface. The observation of the oil spreading over the sample was then possible by scanning the surface of the coloured grease reported onto the sample, and increasing the contrast. The software ImageJ® was helpful to quantify the percentage of area effectively covered by the oil. At least two samples were tested.

I-3. Results and Discussion

I-3.1. MFC characterization

Figure 2-I.3.a shows a FE-SEM observation of the MFC suspension. The mean value of their diameter is easily measurable by an image analysis using the ImageJ® software. They present a diameter of about 61 ± 16 nm. This is in accordance with values from literature (Pääkkö, *et al.*, 2007). Their length, however, cannot be measured since MFC forms a tight network not detectable at lower magnification. According to literature (Siqueira, *et al.*, 2009, Siró and Plackett, 2010), the length is estimated to be higher than 1 µm for MFC made from wood pulp with enzymatic pre-treatment. Unfortunately, this value is not enough accurate to check the quality of MFC and its homogeneity. In spite of several possibilities, no clear characterization is nowadays approved by scientific community. Usually optical microscopy, visual inspection or viscosity measurements can be performed.

In our case, a visual inspection of the MFC suspension (Figure 2-I.3.b) clearly shows homogeneous gel-like structure without any bigger fibers.

In comparison to classical paper coating slurry (Duan, *et al.*, 1999, Khwaldia, *et al.*, 2010, Rhim, *et al.*, 2006), the MFC suspension is only made of water and microfibrils without any additives or binders. The viscosity of this suspension has thus been considered in order to confirm its ability to be coated. In this study, the MFC suspension used to coat paper samples has a concentration of 2%wt. Compared to the previous studies on MFC coating (Aulin, *et al.*, 2010, Hamada, *et al.*, 2010, Nygård, *et al.*, 2011), this concentration is quite high for its use with the bar coating

process (generally the used concentration is lower than 1%wt), but low regarding its use with the size press process (3%wt) (Luu T, *et al.*, 2011). However, depending on the sources and the pre-treatment used to produce the MFC suspension, the viscosity is completely different whatever the concentration (Iotti, *et al.*, 2010). Besides, according to the study of Iotti, *et al.*, 2010, for viscosities higher than 100 Pa.s, the MFC suspension would not be suitable for coating applications. The rheological behavior of the MFC suspension is still not well-known. In spite of recent studies on this topic (Puisto, *et al.*, 2012), many different parameters could influence it such: temperature, concentration, pulp origin or nanonetwork stability under shearing. Herrick, *et al.*, 1983 were the first to study this rheology and for a 2%wt suspension, they concluded to a pseudoplastic behavior with viscosities of 17, 400 and 264 mPa.s for shear rates of 10 and 1000 s⁻¹ respectively. These values are quite closed to the values obtained for the MFC suspension used in this study (18, 400 and 121 mPa.s respectively, results not shown). This proves thus the shear-thinning effect of our MFC suspension, which makes it suitable for coating.

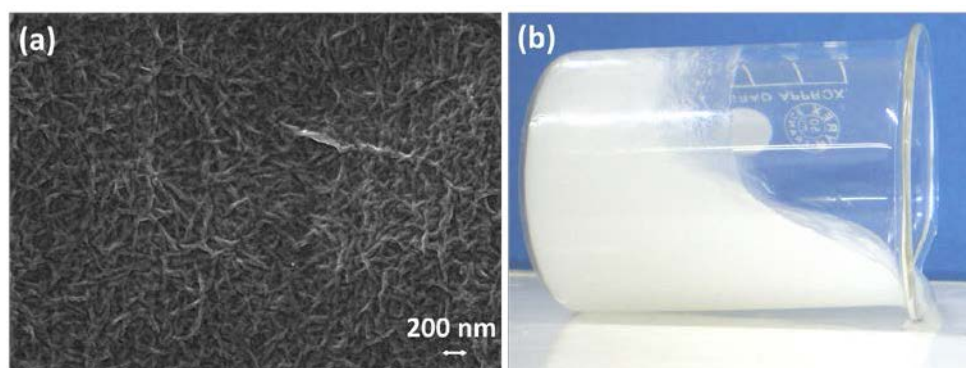


Figure 2-I.3. FE-SEM image (a - left) and picture (b - right) of the MFC suspension (2%wt) from eucalyptus. The MFC suspension looks like a gel and the microfibrils have diameter of about 61 ± 16 nm.

The shear thinning behavior was also concluded by Iotti, *et al.*, 2010 and Luu T, *et al.*, 2011 who studied the rheology of MFC suspension for coating it with size press.

The MFC suspension used in this study presents thus a closed rheological behavior to those of the previous works. It is thus able to be coated onto paper substrates.

1-3.2. Impact of two coating processes on samples structure properties

Two coating processes have been chosen to coat the MFC suspension onto paper samples: bar coating and size press. The mechanism of both processes is completely different as shown by Figure 2-I.2: (i) bar coating is used to coat onto only one side of the substrate compared to size press which gives the possibility to coat slurry onto both sides in once; (ii) a sized substrate is more compressed and subjected to higher pressure between the two rolls than a bar coated substrate. A clear difference is thus notified between each sample according to the

coating processes. The MFC coat weights present first of all high discrepancy. The Figure 2-I.4 shows the MFC coat weights measured for an increasing number of MFC layers for both coating processes. In the case of bar coating process, with one layer the sample only reached a MFC coat weight of about 2 g/m² and was not homogeneously coated.

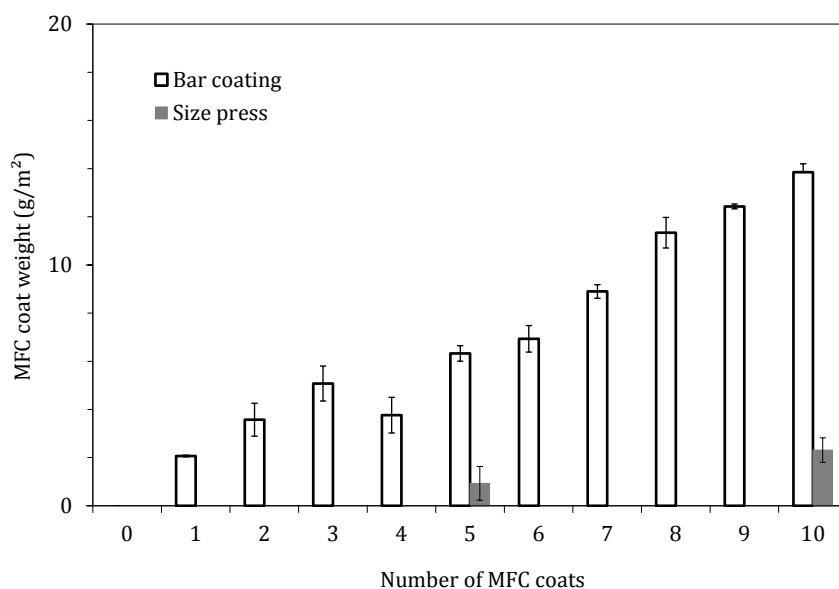


Figure 2-I.4. Evolution of MFC coat weight (g/m²) as a function of an increasing number of MFC coats deposited with bar coating and size press processes.

To increase the coat weight without increasing the concentration of the MFC suspension, several MFC layers were coated one after the other dried. From 5 MFC coats, a coat weight of almost 7 g/m² was reached and the surface of paper samples was entirely covered by MFC as shown Figure 2-I.5.

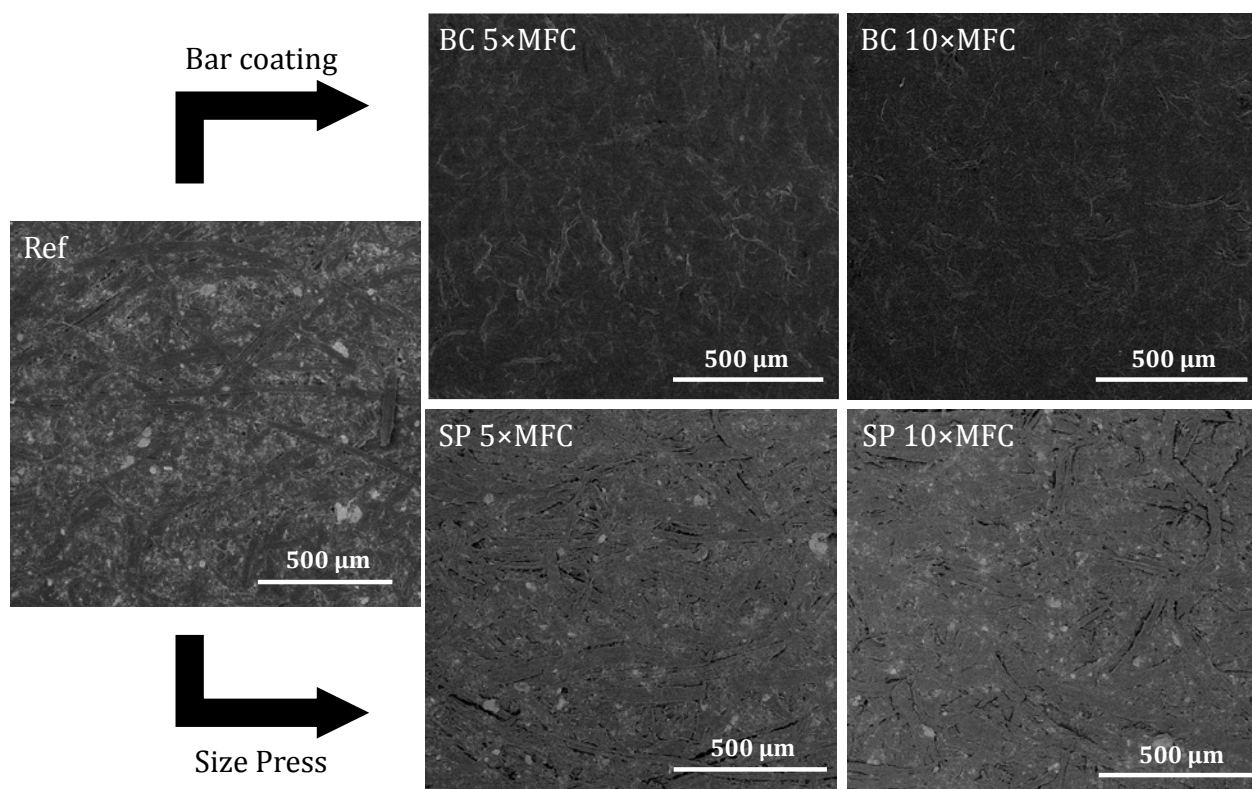


Figure 2-1.5. SEM images of the surface ($\times 100$) of MFC-coated samples using BSE mode: base paper (Ref), 5 \times and 10 \times bar coated (BC) paper samples (BC 5 \times MFC and BC 10 \times MFC respectively), 5 \times and 10 \times sized press (SP) paper samples (SP 5 \times MFC and SP 10 \times MFC respectively) Cellulosic parts are black and fillers of paper samples are represented by white spots.

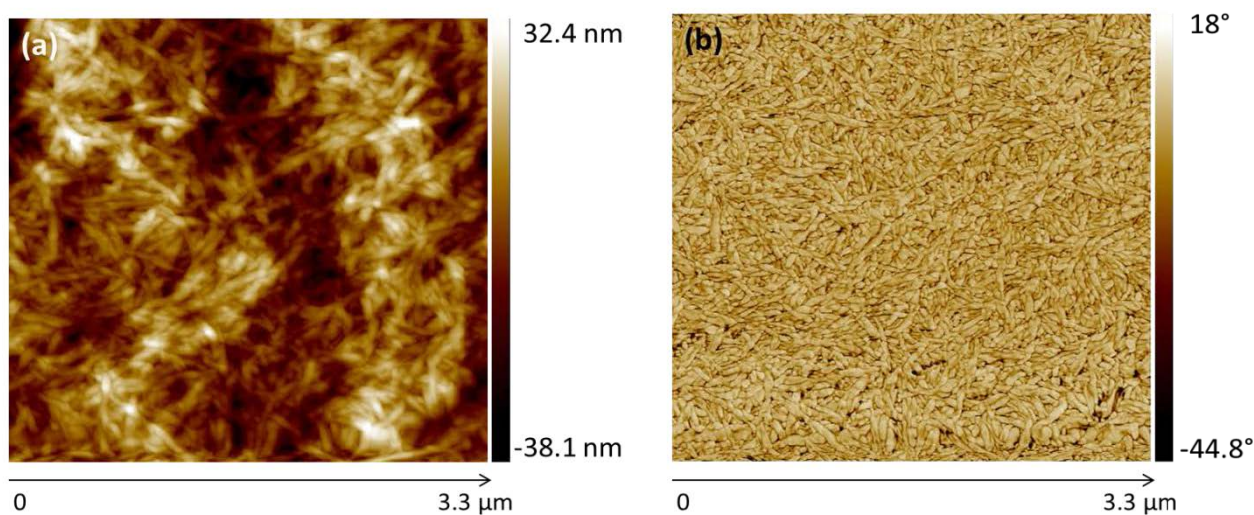


Figure 2-1.6. AFM images of paper substrates coated with five MFC layers (bar coating). The area studied is $3.3 \times 3.3 \mu\text{m}^2$. The AFM topography (*left*) shows the heterogeneity related to MFC coating and the AFM phase (*right*) highlights the non-specific orientation of MFC.

This figure highlights the coverage of paper surface with MFC by the means of BSE mode, which emphasizes the detection of fillers introduced into paper. With the microscopic tool, it is obvious that five and ten MFC layers deposited with bar coating completely covered the surface whereas it is not the case when size press is used (some fillers from papers are still detected). At smaller scale, some heterogeneity can indeed be perceived. AFM images of the 5× MFC bar coated samples are presented Figure 2-I.6. They show the difference of topography due to the non-homogeneous coating. Besides they also highlight the non-specific orientation of MFC during the coating (Phase image, Figure 2-I.6.b).

The same procedure was applied with the size press process. However, even with 5 MFC layers, the coat weight remains at 3 g/m² and increases very slowly with the number of layers (4 g/m² for 10 layers). One explanation resides in the size press mechanism: the two rolls apply pressure and the MFC suspension enters much deeper into the paper thickness. In order then to notify an evolution of the MFC coat weight, the inner structure of the substrate should be first entirely closed. Figure 2-I.7 highlights the MFC layers by showing the cross-section of each sample coated ten times.

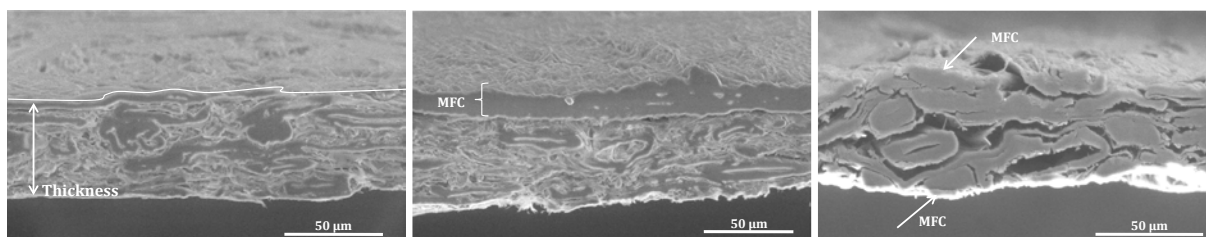


Figure 2-I.7. SEM images of the cross-section of base paper (1) and 10× MFC-coated paper samples with (2) bar coating and (3) size press process.

Ten MFC coats have been deposited on the samples (2) and (3).

The bar coated samples present a well-defined and clear MFC layer of $17 \pm 3 \mu\text{m}$. The sized samples however do not reveal clearly the MFC layer even if they still remain perceptible onto the surface. Besides, according to bulk values, these samples are less dense than the bar coated substrates (Table 2-I.1).

	MFC coat weights (g.m ⁻²)	Thickness (μm)		Bulk from Micrometer values	Air permeability (cm ³ /m ² .Pa.s)	Grease resistance Kit number	Young's modulus (GPa)		Breaking length (km)		Elongation at break (%)		Burst index (kPa.m ² /g)	
		Micrometer	SEM images				MD	CD	MD	CD	MD	CD		
BAR COATING	Base paper	0±0	54±1	39±1	1.31±0.01	268±14	0±0	7.3±0.5	2.3±0.09	7.2±0.6	2.3±0.1	1.2±0.1	1.7±0.3	2.4±0.1
	H ₂ O	0±0	70±1	-	1.61±0.01	644±80	-	4.7±0.2	1.7±0.02	6.3±0.3	2.1±0.07	1.5±0.1	1.7±0.1	2.2±0.1
	MFC	7±1	71±1	54±2	1.50±0.03	79±17	1±0.5	5.8±0.1	2.4±0.4	6.9±0.1	2.3±0.2	1.4±0.1	1.2±0.2	2.2±0.3
	5x													
SIZE PRESS	H ₂ O	0±0	60±1	-	1.47±0.02	421±66	-	5.1±0.2	1.1±0.5	6.2±1.3	1.9±0.4	1.3±0.1	1.7±0.4	2.5±0.1
	MFC	3±1	67±7	-	1.52±0.1	486±172	-	5.4±0.3	1.3±0.1	6.3±0.6	1.9±0.7	1.2±0.4	2.0±0.1	1.8±0.2
BAR COATING	H ₂ O	0±0	71±1	-	1.64±0.01	697±61	-	4.9±0.05	1.7±0.2	5.8±2.2	2.0±0.05	1.3±0.1	1.2±0.1	2.6±0.1
	MFC	14±0	69±3	60±4	1.26±0.03	26±15	5±0.5	5.2±0.05	2.6±0.5	6.0±0.2	2.0±0.5	1.5±0.1	1.0±0.1	1.8±0.4
SIZE PRESS	10x													
	H ₂ O	0±0	61±1	-	1.50±0.03	385±18	-	5.3±0.2	1.5±0.1	7.3±0.4	2.1±0.07	1.4±0.2	1.8±0.2	2.5±0.1
	MFC	4±1	65±1	48±4	1.43±0.02	271±34	-	5.8±0.4	1.2±0.1	6.7±0.7	2.0±0.1	1.3±0.0	2.5±0.3	2.6±0.2

Table 2-I.1. Comparison of the mechanical and barrier properties of the water-treated and MFC bar coated samples with those of the sized samples respectively. The base paper has a basis weight of 41 g/m².

The tests have been carried out at 23°C, 50% RH.

It confirms thus the previous explanation. The MFC suspension is made of 98%wt of water. As the size press helps penetrating the suspension into the substrate structure, a major part of water will be introduced into paper thickness and will thus open it by destruction of fibers network. As a matter of fact the MFC coat weights of sized papers are about three times lower than those of bar coated samples, whereas thicknesses are quite closed (Table 2-I.1). Nevertheless, the thickness is a disputed parameter which must be reconsidered. Depending on the test carried out for its evaluation, the values can differ of almost 20 μm as shown the Table 1 by comparison of thickness values measured with either micrometer or SEM. The use of micrometer is standardized and gives a mean thickness value for a specific pressure and a specific contact surface. However, it will either stop its measurement to the highest roughness or because of the pressure the values are then usually overestimated compared to those determined by SEM images analyses. However, these latter do not consider an enough sample surface, and are thus limited by the sample dimension. This matter of fact has already been studied in detail by Chinga-Carrasco, *et al.*, 2011. In this study, we will consider the thickness values measured with micrometer, according to paper and paperboard standards dealing with mechanical and barrier properties.

To emphasize the significant role of water during the coating of MFC suspension, similar tests have been carried out only with water. Table 2-I.1 presents also the thickness of the water-treated papers. The values are quite similar to the corresponding MFC-coated papers. Compared to base paper, each thickness is consequently increased (from +18% to +31%). This increase is thus mainly due to the effect of successive wetting and drying cycles. The water-treated papers will be consequently taken as references.

In conclusion, bar coating process confers a higher MFC coating than size press process (7 g/m^2 vs. 3 g/m^2 for five layers; see Figure 2-I.4). Usually, paper substrate is considered well-coated for barrier applications from coat weight of about 8 g/m^2 . Accounting for the standard deviations, the samples coated five and ten times only will be studied next, taking as reference the five and ten times water-treated paper.

I-3.3. Mechanical properties of MFC-coated paper samples

The study of MFC-coated papers properties highlights three main points: effects of (i) coating processes, (ii) wetting and (iii) MFC on coated papers mechanical properties. Up to our knowledge, no other studies have already discussed and compared these subjects together.

All the mechanical properties are summarized in Table 2-I.1.

The coating processes impact first the Young's modulus of paper samples. The values of water-treated papers are indeed lower than value of base paper. Although both coating processes weakened paper samples, the decrease is slightly higher regarding the bar coating process (-36% against -30% for size press process in machine direction). This result highlights differences between both processes: bar coating process is indeed able to coat a higher amount of slurry than size press process. Thus, the paper degradation is more consequent using bar coating process, but the effect of MFC will be also more significant.

An increase of Young's modulus is indeed observed with the addition of MFC. In machine direction, the MFC coating using bar coating process induces an increase by 23% with 5 MFC coats, whereas a 6% increase only is noticed using the size press process. In cross-direction, the enhancement of Young's modulus is more consequent, especially with bar coating process, which improves the modulus by 41% and 53% with five and ten MFC coats respectively. The E-modulus improvement is thus more significant for the MFC bar coated samples because of the highest MFC coat weight deposited with this process. Nevertheless, both coating processes have damaged this mechanical property, and the MFC coat weights reached are not sufficient to counterbalance this effect as values of Young's modulus remain lower than the base paper modulus.

Regarding now the effect of MFC on the breaking length of paper samples, a slight degradation of base paper property is also induced by coating processes using water. The decrease of breaking length is not as much consequent as the decrease of Young's modulus (maximum -20%). Nevertheless, the standard deviations are also more significant, and each value can be considered similar in both fibers direction, whatever the treatments applied.

Thus, the MFC coat weights reached do not improve the breaking length of samples.

According to previous observations as regards Young's modulus, the elongation at break is increased further to the coating of paper samples. Compared to base paper, values of water-treated samples are indeed slightly higher in machine direction (maximum +25%), whereas the values remain overall constant in cross-direction. The MFC coating does not induce a consequent improvement of this property. In cross-direction, in compliance with the Young's modulus variations, the elongation at break is decreased for bar coated samples and increased for sized paper samples (up to 40%). In machine direction, the variations are not so obvious: a slight increase is only noticed for 10× MFC bar coated paper samples, whereas values are kept constant for the others. These results were mostly expected since any specific treatment has been applied to improve the elongation of paper samples. And despite the numerous wetting/drying steps and drying conditions, the shrinkage was not significant for paper samples of 10×10 cm².

Whatever the coating process, MFC does not improve drastically the mechanical properties of paper samples. Compared to base paper, each property is indeed damaged by the coating process, and MFC does not counterbalance it. These first results can be explained by three main reasons. Firstly, the MFC coat weights are not very high, so maybe insufficient, to bring better mechanical properties to base paper. Secondly, when bar coating process is used, MFC is coated onto paper surface (Figure 2-1.7). The suspension does not penetrate into the paper structure and remains mainly onto surface. The MFC effect is thus barely noticeable during tensile tests. Concerning the sized samples, only water has penetrated into paper structure, that is why MFC-coated and water-treated paper samples have overall similar mechanical properties. Finally, the consequent standard deviations highlight also a non-completely homogeneous coating of the MFC suspension. These heterogeneities induce the presence of areas with few MFC, and are also a consequence of the non-effective mechanical reinforcement.

As MFC remains mostly onto paper surface, its effect has been evaluated and checked by the measurement of bending stiffness. Only the MFC bar coated paper samples have been studied since they own the highest MFC coat weight located onto paper surface.

The bending stiffness of water-treated and MFC-coated papers have been compared. Figure 2-1.8 shows the values obtained in cross-direction, which highlights the obtained enhancement. Compared to base paper, the bar coating process does not impact the bending stiffness. The value remains constant and equal to 0.03 mN.m. This result is quite surprising to the extent that thickness of water-treated paper samples is increased by about 30% compared to base paper. However, further to the conclusions drawn about mechanical properties, it is obvious that the bar coating degrades paper structure. Despite the increased thickness, paper

samples are too damaged to get an improved bending stiffness (Young's modulus decrease). The thickness of water-treated samples counterbalances thus this degradation, which induces a constant bending stiffness instead of a lower one. As MFC offsets the effect of coating process regarding the previous mechanical properties, it also impacts on the bending stiffness. The bending stiffness is indeed increased by 67% with 7 g/m² of MFC, and again by 40% with 7g/m² more.

These results attest effectively that MFC only remains onto paper surface during bar coating. The use of MFC shows thus its relevance as it enhances considerably the bending stiffness of both coated paper samples (as described above): with MFC, values indeed increase by about 50 to 83% (vs. 20 to 50% with H₂O) for machine direction and by about 67% - 133% (vs. 0% with H₂O) for cross direction (Figure 2.I.8). Thus, neither coating process nor water damaged this property. MFC can thus ensure a good bending stiffness of paper samples, which might be useful for future end use applications such as wrapping or printing.

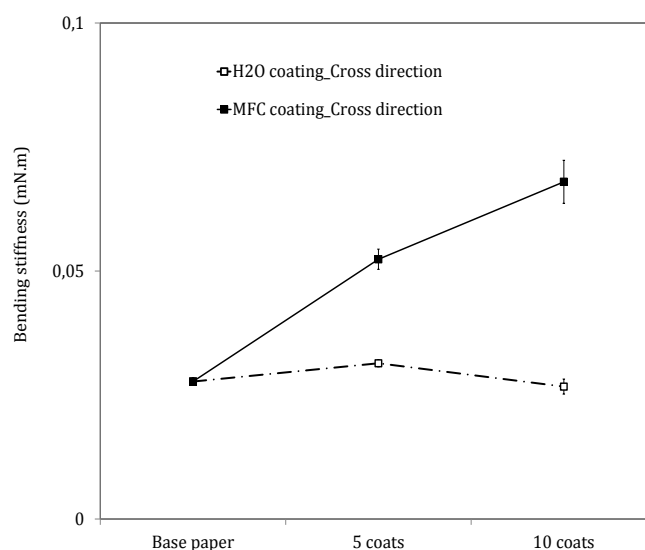


Figure 2-I.8. Bending stiffness of water-treated and MFC-coated paper samples using bar coating (in cross-direction) as a function of the number of treatments.

The last mechanical property carried out is the burst index (Table 2-I.1). Water-treated samples have overall an increased bursting strength compared to base paper, which is mainly due to the improvement of elongation. As described previously, the breaking lengths are indeed similar, and thus, do not influence the burst index values. The measurements are besides repeatable with a variation coefficient of about 4%. On the contrary, values of MFC-coated samples are very disparate with variation coefficients varying from 8% to 22%. The heterogeneity of the MFC-coating is the main explanation.

Nevertheless, in comparison with water-treated samples, it seems that the burst index is decreased or at best kept constant by the addition of MFC. Despite constant breaking lengths and

slight variations of elongation at break, the burst index values do not vary consequently. These results are not expected since the MFC network should reinforce the paper surface. The heterogeneity of MFC layers is emphasized with the bursting strength test since no fibres direction is favoured, and thus, explains these results.

According to the literature, Syverud and Stenius, 2009 were the first to study the elongation and the tensile strength of MFC-coated papers. They concluded on an improvement of mechanical properties from a MFC coat weight of 2 g/m² to 8 g/m², but accounting for their standard deviations, the values obtained were quite closed. Moreover, their base paper was a handsheet made with a dynamic handsheet former. Their base paper had low mechanical properties. The positive impact of MFC was thus more obvious as we also concluded with a poster presented at the TAPPI conference in 2011 (Lavoine, *et al.*, 2011) dealing with the effect of MFC onto handsheet papers. The other studies (Aulin, *et al.*, 2010, Hamada, *et al.*, 2010, Hamada and Bousfield W, 2010, Hult, *et al.*, 2010, Luu T, *et al.*, 2011) were more focused on barrier properties or printability and thus did not present any results on the mechanical properties of MFC-coated papers.

A new and significant point of this study is the comparison of all these properties with water-treated papers. These references have never been taken into account in the previous studies, and these first results show the relevance to consider these samples as references. Indeed, the repetition of the wetting/drying steps completely changes the paper structure and its mechanical properties.

1-3.4. MFC-coated samples barrier properties

According to research about MFC films (Chinga-Carrasco and Syverud, 2012, Syverud and Stenius, 2009), this new material presents very high barrier properties especially to gas. Although Aulin, *et al.*, 2010 associated MFC coat to MFC film, the barrier properties reached by MFC-coated papers are however completely different and much lower than those reached by a film. In this study, different barrier properties (water, air, and grease) have been carried out on the water-treated and MFC-coated papers. For each, the values have been compared to the corresponding values of the MFC films in order to firstly better distinguish the behaviour of MFC as films and as papers slurry, and secondly, to conclude on the effective use of MFC as coating slurry for cellulosic materials.

Regarding the water absorption, it is well known that cellulose is hydrophilic and thus the paper and the non-modified MFC suspension too. With the addition of MFC onto paper surface, the Cobb index measured was similar even with ten MFC layers: 61 ± 7 g/m² for the base paper compared to 66 ± 4 g/m² and 67 ± 10 g/m² for the 5× and 10× MFC bar coated

samples, respectively. As the MFC coat weights are higher for the bar coated samples, the Cobb index is slightly higher for bar coated samples than for sized samples: $60 \pm 8 \text{ g/m}^2$ and $62 \pm 2 \text{ g/m}^2$ for the 5× and 10× sized paper samples respectively. Nevertheless the values are quite closed and higher than a MFC film of about 44 g/m^2 whom the Cobb index is about 10 g/m^2 . This can be explained by the fact that the paper used in this study is already very porous and water absorbent. Besides, during the coating process, MFC does not make a network as tighter as the network formed during the film process. Some researchers have indeed made hypothesis that MFC films were continuous on their bulk (Kulachenko, *et al.*, 2012) and so cannot absorb water anymore. While coating onto paper samples, MFC tends thus to slightly increase the water absorption of the material instead of improving its water resistance.

The MFC coating had mainly a consequent effect on the air permeability. Indeed, whatever the coating processes used, water-treated paper samples have a consequent air permeability compared to base paper, whereas with MFC, the property is improved drastically (Figure 2-I.9). As matter of fact, the coating processes damage the paper structure, which induces a drastic increase of the air permeability: from 268 to $644 \text{ cm}^3 \cdot \text{m}^{-2} \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}$ for 5× bar coated samples, and to $421 \text{ cm}^3 \cdot \text{m}^{-2} \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}$ for 5× sized paper samples (Table 2-I.1).

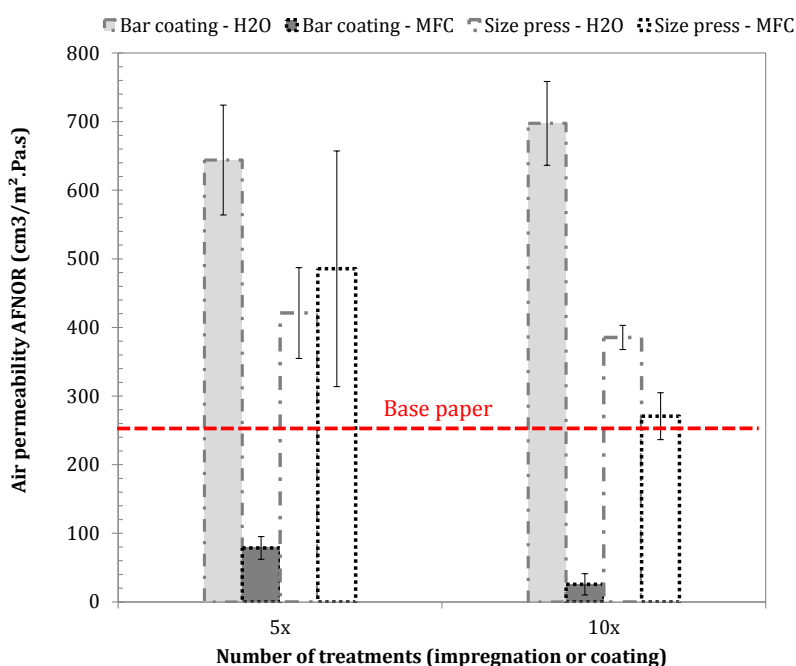


Figure 2-I.9. Air permeability of water-treated and MFC-coated paper samples as a function of the number of treatments. The full histograms are related to water-treated and MFC-coated samples using bar coating process, respectively. The empty histograms correspond to water-treated and MFC-coated samples using size press, respectively

With the addition of MFC, however, these values decrease by 90-97% in the case of MFC bar coated paper samples (Figure 2-I.9). Coat weight of 7 g/m² is already quite enough to improve drastically the air resistance of paper samples. MFC counterbalances thus the negative effect of coating processes.

On the contrary, the air permeability values obtained for sized papers show a different trend. With five MFC coats, unexpectedly, the value increases by 15% compared to water-treated samples. The high standard deviation (35%) does not permit to really conclude on the right effect of MFC. The permeability seems at this state more influenced by water, i.e. by the coating processes, than by MFC. However, with ten MFC coats (i.e. 4 g/m²), the air permeability decreases by 30% compared to the water reference. This decrease remains slight by comparison with the drastic decrease obtained with the bar coated samples. Nevertheless, it even suggests a significant positive effect of MFC on the air resistance.

Compared to previous studies using a rod coater, these values remain quite high. Aulin, *et al.*, 2010 as well as Nygård, *et al.*, 2011 found air permeability values closed to 0 for less than 2 g/m² and 6 g/m² of MFC respectively. In the first study, they used carboxymethylated MFC and different substrates (kraft and greaseproof papers). These both substrates had an initial air permeability value much lower than the base paper of our study and their closest structure eased the surface coating. This highest air resistance seems to be brought by the sort of MFC (Aulin, *et al.*, 2010). This conclusion was indeed confirmed by the work of Nygård, *et al.*, 2011, who compared the air permeability of cellulosic substrates coated with carboxymethylated MFC and enzymatically pre-treated MFC. For a MFC coat weight of 6 g/m², the air permeability reached 0.0006 $\mu\text{m.Pa}^{-1}.\text{s}^{-1}$ with the modified MFC, compared to 0.13 $\mu\text{m.Pa}^{-1}.\text{s}^{-1}$ with the enzymatically pre-treated one. This is due to the smaller length of modified MFC, their better homogeneity and the stronger hydrogen bonds formed by the COOH.

The nanoporous network formed by MFC during the coating process is tighter, and consequently, the air barrier of the cellulosic material is improved. In comparison to MFC films, however, this network still remains quite “open” and does not bring an effective oxygen barrier property. Indeed, the values measured were higher than 10,000 cm³.m⁻².d⁻¹.kPa⁻¹ for all MFC coated-samples (results not shown). As already said the coating presents heterogeneities and “large” nanopores and thus, is not able to block the oxygen molecule (diameter of 0.29 nm).

A similar conclusion can be drawn with the results of grease resistance. Aulin *et al.* (Aulin, 2009, Aulin, *et al.*, 2010) were the first to study the oil resistance of MFC-coated papers. Following the standard TAPPI T-454, they concluded on the ability of the MFC coating to provide oil barrier properties to cellulosic materials. The explanation is based on the ability of coating to seal pores of cellulosic substrate and to form a homogeneous and continuous film. Here again, the MFC studied was modified (carboxymethylated) and the MFC coat weight was lower than 2

g/m² with specific substrates. In our case, we do not assist to the formation of a film according to the AFM images. From a perspective of food-packaging application, the Kit test has been chosen to measure the grease resistance of the samples (Table 2-I.1). The samples coated with bar coating process only have been tested since they have the highest MFC coat weight. The increase of the MFC coat weight improves the grease barrier (from a Kit number of 0 to 5), but values remain insufficient compared to a polyethylene paper (Kit number of 12). This increase is mainly due to the nano-dimension of MFC. The biggest pores of the substrate are indeed sealed, but the nanopores, mainly added by coating, still remain and only slow down migration of oil through paper samples. This conclusion has been proved by the second oil test carried out (Figure 2-I.10).

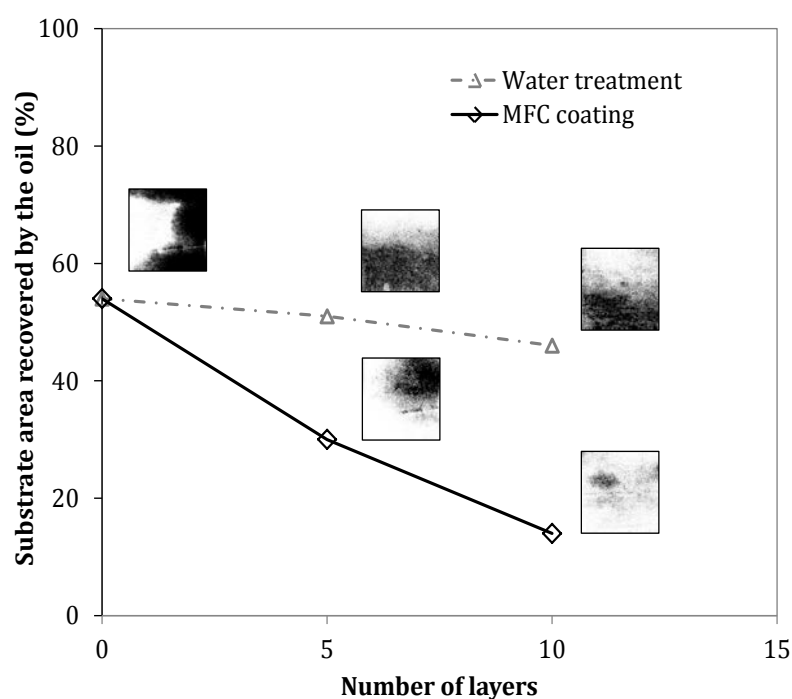


Figure 2-I.10. Percentage of the substrate area (%) penetrated by the oil after three minutes in contact with the samples as a function of the number of MFC layers (coated with bar coating) and corresponding water impregnations.

Compared to Kit test, commercial coloured oil was used to cover paper samples. After scanning of paper samples, each file has been analysed with the ImageJ® software to determine the percentage of the paper surface area covered by oil after three minutes in contact with. The results highlight a progressive migration of oil as well as a non-homogeneous MFC coating. More than the half area of base paper was covered by oil. But with five MFC coats, the covered area decreases to a third. With ten MFC layers, only 14% of the area was still greased. With the increasing number of MFC coats, the coating is more and more homogeneous and thus the grease barrier more efficient. Similar test has also been carried out on water-treated samples. Compared to base paper, the oil migration seems to be constant whatever the number of water

treatments. A drop of 10% only is noticeable from base paper to the ten times water-treated paper samples. In the present case, paper samples have been wet and dewatered at least five times with water. A tighter fibres network has thus been developed with smaller pores which have slowed down the oil migration. MFC coating shows thus here its relevance: grease barrier is more efficient due to the presence of nano-fibres sealing pores.

Among the barrier properties tested, only the air permeability has been drastically influenced by MFC coating. Due essentially to their nanometric scale, MFC coating brought indeed to paper substrates a non-negligible air resistance. It is however necessary to point out that this improvement cannot be done without a sufficient MFC coat weight. Depending on coating process and base paper, this quantity will differ, but in our study we can assume that from 4 g/m², the air permeability can be improved.

I-4. Conclusion

The effect of MFC coating on mechanical and barrier properties of paper substrates has been studied by comparing two different coating processes, the bar coating and size press. For a similar number of layers, bar coating process is able to coat a higher MFC coat weight than size press process. In comparison, the size press gives the possibility to coat the MFC suspension onto both sides of the substrate.

The effect of MFC on the mechanical properties was not significant, and only the analysis of the bending stiffness concluded on the interest in using MFC. The impact of the MFC coating was however more relevant concerning the air permeability. From 7 g/m² the permeability has been indeed decreased by 70% regarding the bar coated samples. The positive effect of MFC has also been highlighted with the consideration of specific references, water-treated papers. The study of these references has been thus necessary since, as regards mechanical properties, the decrease of properties was mainly induced by the coating process used with water.

Up to our knowledge, it is the first study in which a complete characterization of MFC-coated papers and a comparison between two coating processes was done. It is also the first time that the effect of coating processes with only water on these properties was clearly studied to better understand the action of MFC. In conclusion, the improvement of cellulosic substrate properties by the coating of MFC suspension will mainly depend on the substrate. In our case, MFC did not improve drastically the mechanical properties studied, but completely change the air barrier. Nevertheless, their use as coating slurry should be promoted for other kinds of applications such as drug delivery, for example.

This study allowed the implementation of a coating process using MFC. Paper-based substrates were successfully and homogeneously coated with a MFC coat weight of about 7 g/m² using the bar coating process.

Consequently, the objective is now to apply this similar process to a cardboard substrate. Indeed, the use of cardboard is a further step in the elaboration of a 3D-food-packaging.

Mechanical and barrier properties of cardboard and 3D packaging coated with microfibrillated cellulose

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Abstract

Microfibrillated cellulose (MFC) is increasingly used with cellulosic substrates and especially with paper materials. Its use with cardboard remains not reported and the study of mechanical and barrier properties of coated cardboard was investigated in this paper. The influence of coating process (successive wetting/drying cycles) and the effect of MFC were highlighted by comparing different coated cardboard samples with PE-coated cardboard samples. MFC was coated using bar coating process. Their distribution and homogeneity onto cardboard was observed using techniques such as SEM and FE-SEM. Tests such as oxygen and air permeability, bending stiffness and compressive strength were carried out.

The wetting/drying cycles impact significantly cardboard properties by two opposite ways: on one hand it damages the structure cohesion of cardboard decreasing its compressive strength; on the other hand it increases its bending stiffness by increasing considerably the samples thickness. The addition of MFC counterbalanced these negative effects: bending stiffness and compressive strength were indeed improved by 30% in machine direction. On the contrary, MFC did not enhance much cardboard barrier properties, although it considerably increases their water absorption. Within a framework of packaging application, MFC will rather have consequent effects on cardboard's properties as blend or as part of the multilayer structure. Other applications have to be considered for its use as top layer.

Inspired from Paper 3:

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II-1. Introduction

Fiber based materials used as packaging must both be able to protect the product from outer influences as well as withstand the influence of the product. Moreover it must respond to the current society's requirements eager to have more recyclable, biodegradable and lightweight food-packaging materials.

The most often used fiber-based packaging material is cardboard, i.e. fiber-based product with basis weight mostly between 200 and 400 g/m². Its production dates from the beginning of the nineteenth century and is nowadays evolving. With the increasing price of raw materials, the wastes reduction and the evolution of society's requirements, new challenges must be considered in the production of cardboard. On the one hand, industries are looking for reducing the price and the weight of the material without modifying its mechanical properties, especially its bending stiffness. On the other hand, to achieve a better protection of the packed product against the outer threats, cardboard needs more efficient barrier properties, for example against liquids, grease and/or gases.

Some solutions are already applied to offset these ongoing concerns, but remain insufficient. One way is to increase fillers content of the material and thus decrease the amount of fibers used (Axrup, *et al.*, 2012). The benefit is double: reduction of the amount of fibers and improvement of the opacity and printability of paperboard. However, it requires that the fillers cost is substantially lower than the fiber material one. Besides, a large amount of fillers decreases also the strength of paperboard. A compromise has thus to be found. Another way consists in using low density fibers such as thermo-mechanical cellulosic fibers. One drawback with those pulp fibers is their poor ability to form strong fiber-fiber bonds which results in insufficient strength properties. The improvement of these fiber-fiber bonds can be then performed by the addition, for example, of cationic starch. This addition rises however two main concerns: (i) cationic starch molecules tend to screen the anionic charges on cellulosic fibers. Thus, if an excess of starch is added, only one part will be retained in the sheet and the rest will circulate in the white water system causing runnability, microbiology or foaming problems; (ii) an excessive cationic starch addition can also prevent fibers from adsorbing other cationic additives which are commonly added (e.g. retention aids, sizing agents). Instead of using cationic starch, latex has for example also been used as binder in bulk layer to improve strength properties (Fredlund, *et al.*, 2000). However, retention problems appeared while adding latex to the wet end.

Concerning the improvement of barrier properties of cardboard, the most commonly used way consists in coating or laminating high barrier petroleum polymers on cardboard. Usually, polyethylene (PE) is used as efficient liquid and vapor barrier. Ethylene vinyl alcohol (EVOH) is rather used as oxygen barrier. The barrier properties then reached are very efficient. However a

thick layer is needed for, which induces a higher cost of production and the use of a significant amount of non-biodegradable polymers. Another method commonly used in order to produce high barrier cardboard is the addition of an aluminium layer. Aluminium offers an oxygen and light barrier with thickness of only around 7-9 μm . Nevertheless this layer increases strongly the carbon dioxide load of the material and decreases also the recycling ability of the multilayer package (Axrup, *et al.*, 2011). When considering the enhancement of the grease resistance, the current solution consists in treating or coating the cardboard with fluorinated hydrocarbons such as perfluorooctanoate (PFOA). These chemicals, however, have become the object of health and environmental concerns, mainly because of their persistence and tendency to bioaccumulate (Murphy, *et al.*, 2009).

There is thus still a need for an improved fiber-based material with high barrier properties and low density which could be both more economical and eco-friendly, intended for example to food-packaging materials and applications. The use of microfibrillated cellulose (MFC) is an emerging idea, attracting more and more researchers. Simply produced from cellulosic fibers with high shear mechanical treatment (Lavoine, *et al.*, 2012, Siró and Plackett, 2010), its manufacturing process was patented for the first time in 1985 by Turbak *et al.* (Turbak, *et al.*, 1985). Since then, improvements of this process have been carried out and published to reduce the high energy consumption induced by the high shear mechanical treatment of fibers (Spence, *et al.*, 2011). Two main pre-treatments are commonly used: enzymatic pre-treatment (Chen, *et al.*, 2009, Pääkkö, *et al.*, 2007) or TEMPO oxidation of cellulosic fibers (Isogai, *et al.*, 2011, Saito and Isogai, 2004, Saito, *et al.*, 2007). Depending on the cellulosic sources (Alila, *et al.*, 2012, Dinand, *et al.*, 2002, Zhang, *et al.*, 2010) and applied treatments, MFC will have different dimensions: diameters ranging from 10 to 60 nm (Klemm, *et al.*, 2011, Lavoine, *et al.*, 2012, Siró and Plackett, 2010) and lengths longer than 1 μm (Ishii, *et al.*, 2011). Its nanometer scale and high surface energy make MFC an ideal material for use in nanocomposites as mechanical reinforcement (Fujisawa, *et al.*, 2012, Shields, *et al.*, 2008, Siró and Plackett, 2010, Zimmermann, *et al.*, 2010). Its ability to form a nanoporous network (Arola, *et al.*, 2012, Nemoto, *et al.*, 2012) and, once dried, to make stiff and high barrier films (Chinga-Carrasco and Syverud, 2010, Fukuzumi, *et al.*, 2009, Syverud and Stenius, 2009, Yang, *et al.*, 2011) have besides broadened its application areas. The enhancement of mechanical (Siró and Plackett, 2010) and barrier properties (Lavoine, *et al.*, 2012) and their use as aqueous suspensions developed recently its application in cellulosic substrates such as papers (Aulin, *et al.*, 2010, Eriksen, *et al.*, 2008, Hamada, *et al.*, 2010, Ramos, *et al.*, 2011, Syverud and Stenius, 2009, Taipale, *et al.*, 2010). Nevertheless, the combination of MFC and cellulosic materials is quite recent and the first published study appeared in 2009 with the MFC coating of handsheet

papers (Syverud and Stenius, 2009). The number of studies in paper and paperboard applications has since slightly increased: at the end of 2012, only 31 journal articles, 18 patents and 11 international conferences proceedings have been published compared to the 2,300 scientific papers published in total about MFC (Bardet and Bras, 2013).

Regarding the use of MFC with cardboard, up to our knowledge, the number of scientific papers counts only one journal article (Hult, *et al.*, 2010) and five patents (Axrup, *et al.*, 2011, Axrup, *et al.*, 2012, Heiskanen, *et al.*, 2011a, Heiskanen, *et al.*, 2011b, Wildlock and Heijnesson-Hultén, 2008). Hult, *et al.*, 2010 used MFC mixed with shellac to decrease the air permeability of a 250 g/m² paperboard. They prepared first a blend of these two compounds. Then, they carried out a multilayer strategy, i.e. coating first MFC followed by a shellac layer. Depending on the paperboard surface coated, the air permeability decreased from 60 to 99% with coat weights ranging from 3 to 30 g/m² respectively (Table 2-II.1).

Coating type and description	Coat weight (g/m ²)	Coating thickness (μm)	Curing temperature/ time	Air permeance (nm/Pas) (25 °C/50% RH)	
				Base paper	Coated base paper
Blend:	2.7 ± 0.4	10.3 ± 0.3	23 °C/24 h	1994 ± 159	811 ± 49
Coating consisting of a MFC (0.75 wt%) and Shellac (21 wt%) blend	3.1 ± 0.2	10.7 ± 1.1	100 °C/1 h	1821 ± 87	765 ± 61
	3.2 ± 0.2	13.2 ± 0.7	23 °C/24 h	2271 ± 209	325 ± 52
dispersed in EtOH (78.25 wt%).	3.1 ± 0.3	12.1 ± 0.7	100 °C/1 h	1972 ± 131	550 ± 29
	9.4 ± 0.2	23.2 ± 1.1	23 °C/24 h	2018 ± 107	179 ± 46
Blend coating on the pre-treated side	6.8 ± 0.4	23.8 ± 1.4	100 °C/1 h	1526 ± 65	258 ± 14
	19.0 ± 0.5	32.0 ± 1.7	23 °C/24 h	1444 ± 136	64 ± 14
	18.6 ± 0.6	31.4 ± 1.1	100 °C/1 h	1800 ± 155	225 ± 46
Multilayer D1:	28.6 ± 0.5	43.3 ± 0.6	23 °C/24 h	1489 ± 204	561 ± 25
Multilayer coating on the untreated side	22.7 ± 0.2	39.8 ± 1.2	100 °C/1 h	1853 ± 179	1432 ± 126
Multilayer D2:	27.9 ± 0.1	43.2 ± 0.9	23 °C/24 h	1527 ± 94	52 ± 8
Multilayer coating on the pre-treated side	22.6 ± 0.5	38.7 ± 1.7	100 °C/1 h	2595 ± 102	281 ± 29
Shellac:	23.0 ± 0.7	8.2 ± 0.2	23 °C/24 h	1280 ± 120	801 ± 90
Monolayer coating with Shellac solution	17.4 ± 0.4	5.2 ± 0.1	100 °C/1 h	1882 ± 170	1995 ± 168

Table 2-I.1: Coating types and characterization (curing temperatures, coating thickness, coating weight) of cardboard sample (250 g/m² with a pre-and un-treated side) (from Hult, *et al.*, 2010)

This was compared to a maximal decrease of 40% obtained with the coating of shellac alone. The use of MFC was thus relevant for enhancing the air barrier of paperboard. However, its combination with shellac weakened also the paperboard structure and induced then a decrease of its tensile strength. Others also patented processes aiming to improve the barrier properties of cardboard using either coating dispersion (Heiskanen, *et al.*, 2011b) or multilayer process (Axrup, *et al.*, 2011). The purpose is to reach high barrier properties with an on-line process without using petroleum polymers. Besides, mechanical properties must not be impaired. The first idea consists in coating a dispersion including MFC (0.5 to 20 %wt) and colloidal particles

such as EVOH or latex either using roller coating, spray coating or immersion process (Heiskanen, *et al.*, 2011b). Samples coated with (latex/MFC) dispersion show for example a decrease of the water vapor transmission rate of 24% compared to samples coated with an aqueous latex dispersion only. The second solution aims to produce a multilayer material with a first layer of fibers, a second with MFC and a third one with polymer such as PE or PET mainly for giving heat-sealing property (Axrup, *et al.*, 2011). MFC confers density and smoothness, improving adhesion of the third layer and also decreases oxygen permeability of the multilayer material. However, the authors did not mention any analysis of mechanical properties.

Other patents are mainly focused on the price reduction by developing low density cardboard with good mechanical properties and more flexible structure. They either introduced MFC alone or with additives between plies of the base cardboard (Heiskanen, *et al.*, 2011a), producing a laminate with at least one layer of MFC (Wildlock and Heijnesson-Hultén, 2008), or mixed pulp with MFC, cationic and anionic polymers to make a final cardboard (Axrup, *et al.*, 2012).

None of these patents looked for coating only MFC without adding polymers, additives or fillers onto cardboard surface. Our study is thus focused on this perspective. MFC alone has been coated on cardboard with bar coating process. Mechanical and barrier properties of coated materials thus obtained have been analyzed to conclude on the effect of MFC, expecting mostly an enhancement of gases and grease barrier properties. The influence of the MFC on the cardboard properties was furthermore highlighted by a comparison with the properties of the base cardboard, a polyethylene-coated cardboard and a similar cardboard samples coated with only water.

II-2. Materials and methods

II-2.1. Materials

300g/m² cardboard sheets were produced and delivered by Cascades La Rochette (France). This folding cardboard, named Rochcoat®, is made of virgin pulp and mainly used in the food-packaging sector. One side is coated and ready for printing. The other side, non-coated, will be converted in this study.

The same cardboard sheets coated with Polyethylene (320 g/m² with a PE layer of about 30±3 µm) have also been produced and furnished by Cascades La Rochette (France) and will be used as reference in this study.

II-2.2. Methods

Preparation and characterization of MFC suspension

The MFC suspension has been produced and furnished by FCBA (Grenoble, France). An enzymatic pre-treatment has been applied on sulfite pulp (Domsjö®) using endoglucanase during 2 h. The pulp was then subjected to high-shear mechanical treatment using the homogenizer Ariete from GEA Niro Soavi® (4 passes, at 1,400 bars) in order to achieve a concentration of 2%wt.

The MFC suspension was characterized with a Field Emission Scanning Electron Microscopy (FE-SEM) Zeiss® Ultra-55 using a working distance of 5.5 mm and an accelerating voltage of 2.00 kV. The suspension was spread onto a metal substrate using carbon tape, allowed to dry one night at room temperature and coated with a thin layer of gold. The average value of the nanofibrils diameter has been also determined by at least 50 measurements performed with the ImageJ® software.

MFC coating onto cardboard

The MFC suspension has been coated on this back side with a bar coating process (Endupap, France), using a 0.9 mm Mayer bar, at a speed of 5 cm.s⁻¹. The coated samples were then dried with a contact drying system at 105°C, for 5 minutes. During this drying time, the cardboard sheets have been regularly turned over to limit the curl effect. These steps were repeated five and ten times in order to deposit five and ten MFC layers respectively. As reference samples, water-treated cardboard sheets were obtained by coating deionized water using the same procedure described above. Here, only the coating slurry was changed: deionized water without MFC was coated onto the cardboard surface to clearly underline the influence of the successive wetting/drying cycles induced by the five and ten successive coatings of the MFC suspension (containing 98%wt of water).

Structural characterization of cardboard samples

Coated samples have been characterized by Scanning Electron Microscopy (SEM), a Quanta200® (The Netherlands), using a working distance of 10.0 mm and a high voltage of 15.0 kV. For this technique, cardboard samples were precisely cut and mounted on a metal support covered with carbon tape for an observation of their surface and slice. Grammage and thickness (ISO 534:2011) of each sample were also measured using a Lhomargy balance (± 0.01 g) and micrometer (± 0.01 μ m) respectively. Average values of at least 10 measurements are given.

Characterization of transport phenomenon of cardboard samples

Before each test, samples were maintained at 23°C and 50% of relative humidity (RH) for at least 24h.

Air permeability: According to the standard ISO 5636, the air permeability was measured using Mariotte vases on samples area of 2 cm², under ambient air conditions, applying a vacuum of 2.5 kPa. The time measurement was fixed at 5 minutes for each sample. The average air permeability was then calculated from at least five measurements.

Oxygen permeability: Oxygen transmission rate (OTR) was determined using a MOCON OX-TRAN® 2/21 ML (ISO 15105-2:2003). Samples were before maintained at 23°C, at 50% RH, for 48 hours. Measurements have been carried out on an area of 1.1 cm². They have been repeated twice for each sample. Usually, cellulosic materials do not have a sufficient low OTR in order to be analyzed by this kind of equipment without being out-of-range. The study of our cardboard samples has been then investigated by thermo-sealing each sample with a polymer film (PE) using an office plastic sealer. The values have been compared to the OTR value of the polymer film alone to conclude on the effect of MFC coating.

Water absorption: Following the Cobb 60 method (ISO 535), the test consists in putting a sample's area of 3.5 cm of diameter in contact with 10 ml of deionized water during 45 s. The mass of the sample before and after wetting was determined with a Lhormargy balance (± 0.01 g). Cobb index represents the mass of water absorbed divided by the wet area (g/m²) and is an average value of at least five measurements.

Grease resistance: Kit Test method (T 559-cm 02) has been investigated to evaluate the grease resistance of cardboard samples. It consists in testing the sample with one droplet of 12 solutions constituted in different parts of castor oil, n-heptane and toluene. The solutions are numbered from 1 to 12 (Kit number), with 12 representing the highest grease resistance. From a Kit number of 8, a sample is considered grease resistant.

Characterization of the mechanical properties of non-coated and coated cardboard sheets

Before each test, the samples were maintained at 23°C and 50% RH for at least 24h. The mechanical tests were also carried out under same conditions.

Bending stiffness: According to the standard ISO 2493, the bending stiffness was measured using a Büchel Stiffness Tester (Büchel Van der Korput, the Netherlands) (Figure 2-II.1). Samples were previously cut into pieces of 38 × 70 mm². The bending stiffness was measured for an angle of 7.5° and the force was applied on the non-treated, water-treated or

MFC-coated side of cardboard sheets. At least 10 measurements were carried out to obtain an average value of the load needed to bend each kind of samples. The bending stiffness B ($mN \cdot m$) was then calculated as followed:

$$B = \frac{FL^2}{3a\theta}$$

with F (mN), the bending force, L (m), the length of bending, a (m), the width of the test piece and θ ($radian$), the angle of bending.

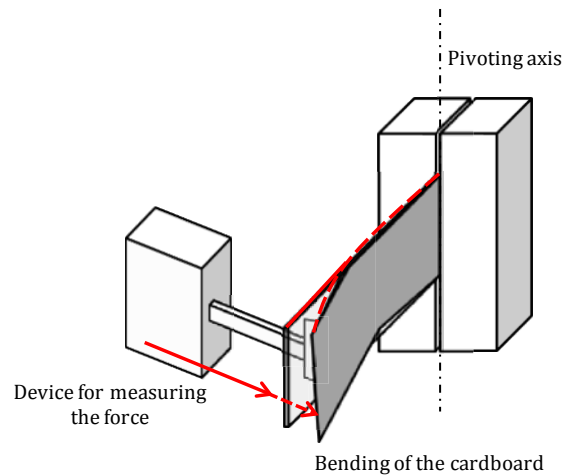


Figure 2-II.1: Sketch of the bending resistance cardboard testing

Similar measurements were also investigated on creased cardboard samples to evaluate their resistance to folding (mN). Each piece was previously creased with a laboratory cardboard creasing press (H.E. Messmer Ltd, UK). Depending on fibers' orientation and samples' thickness, the width of the groove has been adapted for a 0.7 mm width of creasing rule. For each sample in machine direction, the groove width was fixed at 1.524 mm. In cross direction, the width evolved between 1.397 and 1.651 mm depending on cardboard samples.

Five measurements were performed on creased cardboard samples.

Short-span compressive test: This test consists in determining the compressive strength in machine and cross-directions of cardboard using a short-span compressive tester (Büchel Van der Korput, The Netherlands) according to the standard ISO 9895. A test sample, 15 mm wide, is clamped between two clamps, spaced 0.70 mm apart, which are forced towards each other at a speed of $3 \pm 1 \text{ mm} \cdot \text{min}^{-1}$ until a compressive failure occurs. The average of maximum compressive strength is given in kN/m from at least ten measurements.

Box compression test: Using the software ArtiosCad®, template of final boxes ($10 \times 10 \times 5 \text{ cm}^3$) was designed on A4 cardboard sheet (Figure 2-II.2). The cardboard sheets were cut and creased by a laboratory converting pilot (Kongsberg, France). They were then folded and glued. Each package was designed so that the cross-direction of the material was submitted to the compressive force. As the material is less stiff in cross-direction, even a slight increase of the

compressive force of the box will be perceived. The compression test was carried out using a crush tester (Noviprofibre, France) with a maximal compressive load reaching 5 kN (TAPPI method 804). The box was maintained between two parallel plates. The upper plate went down at a speed of $12 \pm 2 \text{ mm}\cdot\text{min}^{-1}$. An average value of the compressive force was calculated from at least 10 measurements.

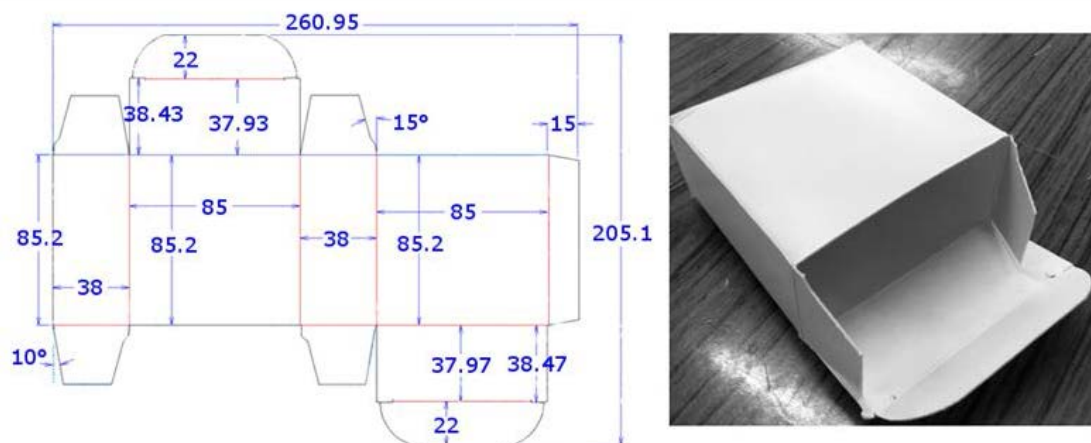


Figure 2-II.2. Box template designed with ArtiosCad® (left – dimensions in mm) and corresponding 3D-Box (right)

II-3. Results and discussion

II-3.1. Characterization of the MFC suspension

The MFC suspension produced from sulfite pulp at a concentration of 2%wt looks like a white gel (Figure 2-II.3a). This gel is made of microfibrils with diameters of about $33 \pm 8 \text{ nm}$ (Figure 2-II.3b). This value is consistent with the diameter values found in literature (Lavoine, *et al.*, 2012, Siró and Plackett, 2010). The microfibrils form an entangled and tight network, which makes the measurement of their length impossible at lower magnification. In literature, the length of MFC is estimated longer than $1 \mu\text{m}$. Very recently, Ishii *et al.* (Ishii, *et al.*, 2011) determined for the first time a more accurate value of the length of TEMPO oxidized MFC. Based on dynamic viscoelasticity measurements, they obtained a mean length of $2.2 \mu\text{m}$, which matches perfectly with previous estimations. Nevertheless, any other accurate value has been given regarding enzymatically pre-treated MFC.

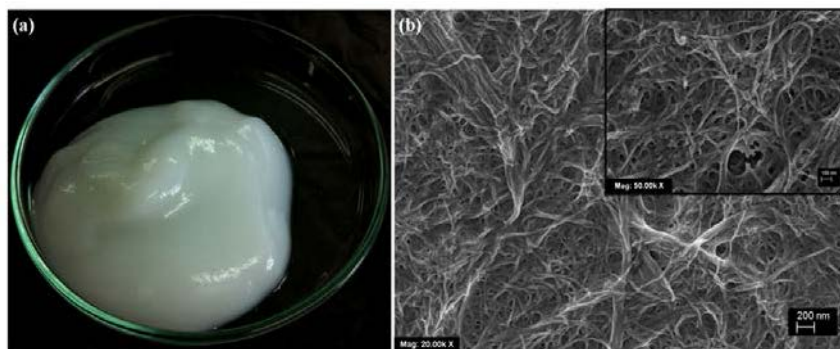


Figure 2-II.3. (a) MFC suspension (2wt%) produced from sulfite pulp (Domsjö®), enzymatically pre-treated and mechanically treated with a GEA Ariete® homogenizer.

(b) FE-SEM images (Mag: x20.00 k/50.00k; EHT: 3.00/2.00 kV; WD: 5.3/5.5 mm) of the MFC suspension

Thanks to these dimensions, the interest in using MFC is focused on the microfibers entanglement, which forms a specific network. Indeed, in previous works, this network has strongly contributed in the enhancement of mechanical and barrier properties of different materials (Fujisawa, *et al.*, 2012, Plackett, *et al.*, 2010, Shields, *et al.*, 2008, Syverud and Stenius, 2009, Zimmermann, *et al.*, 2010). From this perspective, the effect of this network formed while coating will be analyzed in this study on cardboard properties.

II-3.2. MFC-coated cardboards samples

A4 cardboard sheets were coated with the 2wt% MFC suspension using bar coating process. Due to the consequent viscosity of the MFC gel, the coating of only one layer of MFC was not enough to cover the whole surface of the cellulosic substrate. That is why the coating process has been repeated five and ten times in order to improve the homogeneity of coating but also to increase the MFC coat weight deposited (from 1 g/m² with one layer to 14 g/m² with 10 MFC layers). Indeed, the base cardboard already owns a basis weight of 300 g/m². The improvement of its properties will depend on the quantity, i.e. coat weight, deposited, but usually no more than 15-20 g/m² are coated onto the surface. It is worth keeping in mind that even our highest level of coating (14 g/m²) corresponds to only 5% of the total material basis weight. Table 2-II.2 summarizes the structure properties of the different samples. Figure 2-II.4 shows SEM images of the surface of cardboard samples coated with one, five and ten MFC layers. After one coat, compared to reference, the surface is clearly not entirely covered. From 5 MFC coats, the fibers of the base cardboard are not perceived and MFC covers the whole surface. Regarding the cross-section of these same samples (Figure 2-II.5), MFC is barely distinguished on the cardboard coated only once. Looking at the 5× and 10× MFC-coated cardboard, the MFC coat stands out clearly from thickness of the base cardboard (Figure 2-II.5). A MFC coat weight of about 6 and 14 g/m² is indeed deposited compared to 1 g/m² in the case of one coat.

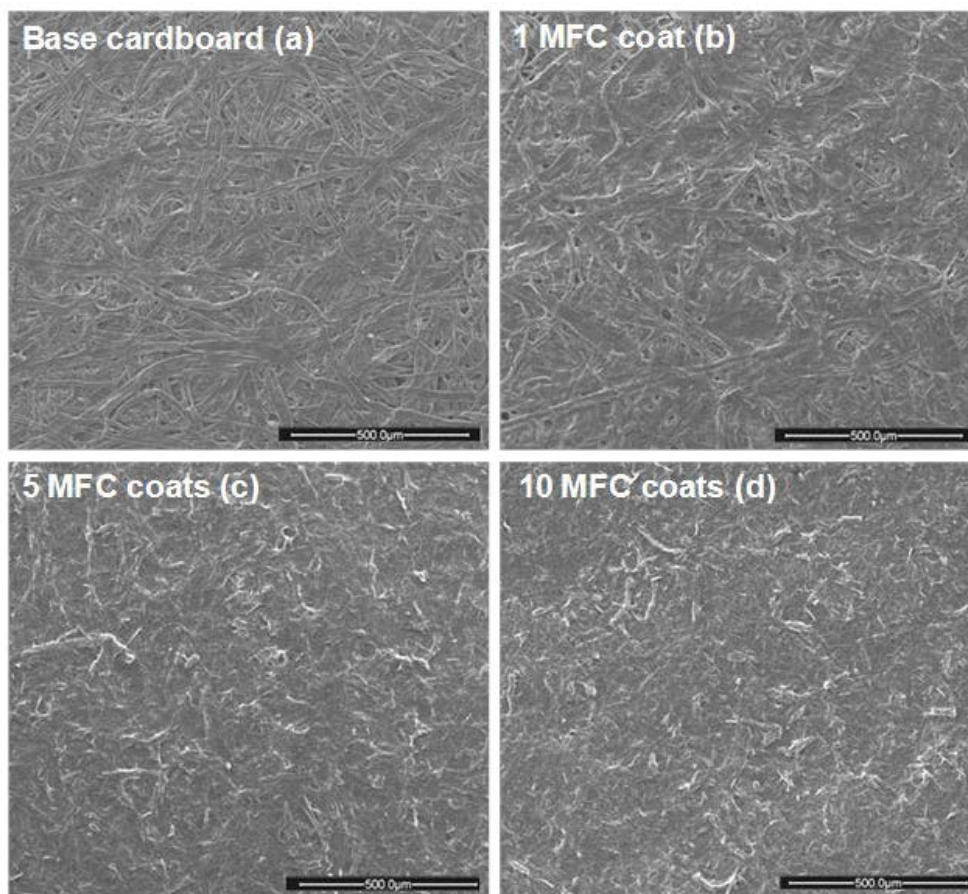


Figure 2-II.4. SEM images (Mag: $\times 100$; HV: 15 kV; WD: 10.0 mm) of the cardboard coated with MFC (b) once, (c) five and (d) ten times compared to the (a) base cardboard

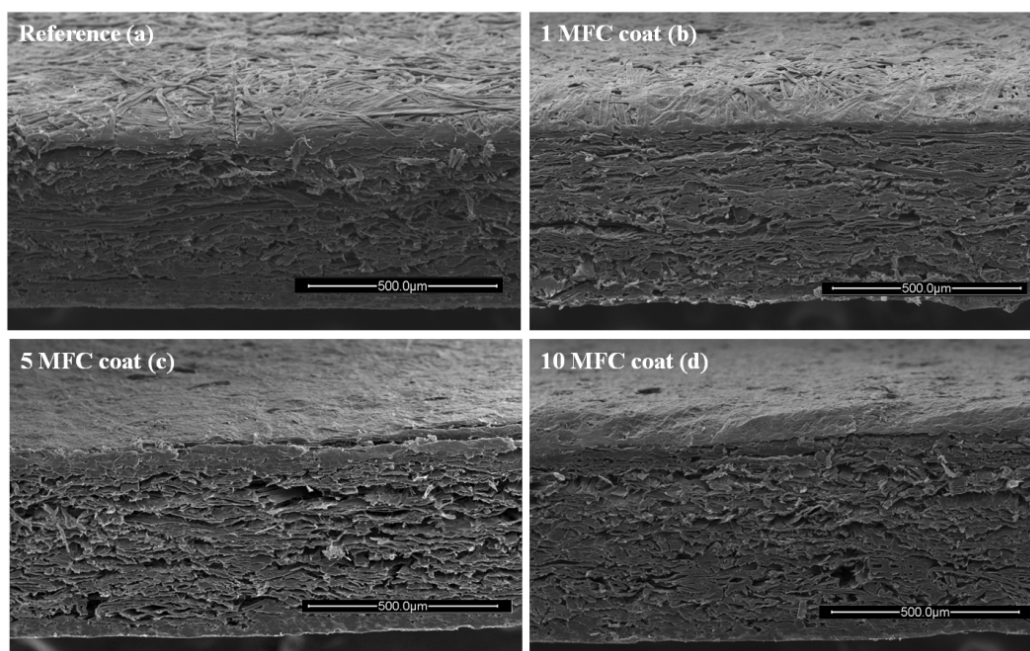


Figure 2-II.5. SEM pictures (Mag: $\times 100$; HV: 15 kV; WD: 10.0 mm) of the cross-sections of cardboard coated (b) once, (c) five and (d) ten times compared to cross-sections of (a) base cardboard.

Since the MFC suspension is mainly composed of water (98 wt% for 2 wt% of microfibers), water-treated cardboard sheets have been prepared as reference. The influence of water on cellulosic materials is a well-known phenomenon. It is thus necessary to analyze the effect of successive wetting and drying cycles on cardboard in order to rightly conclude on the effect of dried MFC. Compared to MFC coating, water tends to deconstruct cardboard samples by opening the fibers network onto surface (Figure 2-II.6).

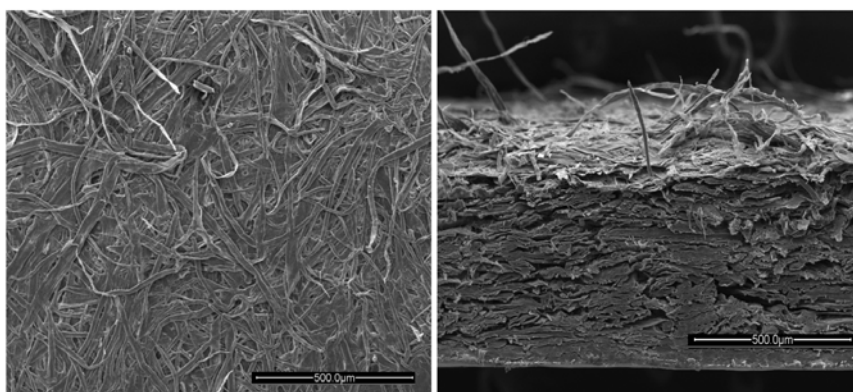


Figure 2-II.6. SEM images (Mag: $\times 100$; HV: 15 kV; WD: 10.0 mm) of surface and cross-sections of the 10 times water-treated cardboard sheets. Structure of the cardboard is damaged by this treatment.

Similar basis weight values are obtained, but the samples thicknesses were considerably modified by water. By opening the fibers network, cardboard samples roughness is indeed increased (by almost 36%, *results not shown*), which induces a thickness increase of about 20% (Table 2-II.2).

Samples		Structure properties			Barrier properties			
		Grammage (g/m ²)	Thickness (µm)	Bulk	Oxygen permeability (cm ³ /m ² .day)	Air permeability (cm ³ /m ² .Pa.s)	Water absorption (g/m ²)	Grease resistance (Kit Number)
Reference cardboard		303±2	501±8	1.65±0.01	12±0	0.17±0.01	43±7	0
PE cardboard		320±1	535±1	1.67±0.01	-	0.12±0.005	5±3	12
Water treatment	×1	301±2	579±17	1.92±0.05	-	0.16±0.01	51±4	0
	×5	304±4	602±11	1.98±0.02	-	0.16±0.01	89±8	0
	×10	304±2	619±9	2.04±0.01	-	0.15±0.01	84±11	0
MFC coating	×1	304±3	574±5	1.89±0.02	-	0.18±0.01	67±10	0
	×5	309±1	595±7	1.92±0.01	14±2	0.18±0.01	94±4	1.5±0.5
	×10	317±1	624±5	1.97±0.01	13±1	0.18±0.01	114±7	2.5±0.5

Table 2-II.2. Structure and barrier properties of the MFC-coated and water-treated cardboard samples as a function of the number of layers and water treatments. The sample named “PE cardboard” is the base cardboard coated with poly(ethylene) by the supplier. It is a reference as matter of barrier properties

This increase also induces a 20% increase of bulk values. The effect of successive wetting and drying cycles is besides highlighted by comparison with the MFC-coated cardboard samples. The bulk values of these latter are also increased but are slightly lower than those of water-treated cardboards. It is thus possible to bring matter without making denser the final material.

II-3.3. Barrier properties of MFC-coated cardboard

A common purpose in the production of cardboard package is the research of high barrier properties. The results are given Table 2-II.2.

The air permeability is affected neither by coating process nor by MFC coating. Water-treated cardboard samples present indeed similar permeability than reference, and MFC coating does not really improve this property, surely due to the insufficient MFC coat weight deposited compared to the starting material structure and basis weight (Table 2-II.2). Indeed, it is noteworthy to note that the reference cardboard has already a very low air resistance ($0.17 \text{ cm}^3 \cdot \text{m}^{-2} \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}$) thanks to its coated front side for printing. As the base material aims to be used as food-packaging material, it is thus coherent that very good air barrier is already achieved.

As oxygen is the main cause responsible of the degradation of food products, MFC-coated cardboard samples have been submitted to oxygen permeability tests and, similarly, very good oxygen barrier is expected. Considering the values measured on samples encapsulated with a polymer film ($27 \pm 0.5 \text{ cm}^3/\text{m}^2 \cdot \text{day}$), the reference cardboard owns effectively low oxygen permeability ($12 \text{ cm}^3 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$). The value is indeed of the same order than OTR values of materials such as PVC, amylopectin, chitosan or whey protein (Aulin, 2009, Aulin, *et al.*, 2010). As regards the MFC-coated samples, the OTR value does not decrease as expected (Table 2-II.2), and remains almost identical to reference. The MFC coat weight is probably not sufficient to improve this property, since the reference cardboard already has very good barrier properties. These results are not in accordance with literature showing a drastic improvement of OTR with only a thin MFC layers coated onto either plastic or paper (Aulin, *et al.*, 2010, Fukuzumi, *et al.*, 2009).

Despite the very good barrier properties of this reference and according to its supplier, this cardboard is meant to receive one treatment before using it with liquid or fatty food product. To achieve a liquid and grease resistance, a poly(ethylene) (PE) layer is usually added as final treatment. In this study, the PE layer has been replaced by MFC, and the water absorption as well as the grease barrier tests have been carried out by comparison to the commercial PE-coated cardboard (Table 2-II.2). As regards water absorption, compared to the reference, the addition of the PE layer decreases significantly (by 86%) the ability of cardboard to absorb water (from 43 to 5 g/m^2 respectively). The effect of the coating process used in this study impacts however significantly this property. Water-treated cardboard samples absorb much more water than reference, i.e. from 43 to 84 g/m^2 with 10× water-treatments. The quantity of water absorbed increases indeed with the increasing number of water treatments (Table 2-II.2). Nevertheless, from five treatments, this value begins to stabilize, which may be due to a structural balance of the fibers' network, achieved after successive wetting and drying cycles.

With the addition of MFC coats, the water absorption is increased even more strongly compared to water-treated samples. With only 1 g/m² of MFC, the water absorption increases by 31%. This increase is still observed with the increasing MFC coat weight (Table 2-II.2). MFC has thus a consequent impact on the cardboard capacity to absorb water, i.e. from 43 to 114 g/m². This is mostly due to the nanometer scale of MFC and its high specific area (×2 to ×55 higher than cellulosic fibers (Siqueira, *et al.*, 2010, Spence, *et al.*, 2010)). The hydrophilic nature of cellulose is thus amplified with the morphology of MFC, and as expected, the MFC-coated cardboard samples have a high affinity with water.

On the contrary, MFC tends to improve the grease barrier of cardboard samples (Table 2-II.2), which is in accordance with previous study on paper (Aulin, *et al.*, 2010). This improvement is however not enough to be considered in a food-packaging application. Compared to PE-coated cardboard samples, which have a Kit number of “12”, MFC-coated samples barely reached a Kit number of “2.5”. This value is nevertheless higher than the “0” Kit number of reference, but insufficient in comparison with highly grease-proof PE-coated cardboard.

II-3.4. Mechanical properties of MFC-coated cardboard samples

Cardboard is a packaging material generally used to carry and protect from damages every kind of product. Its mechanical properties are ensured thanks to the multilayer structure, which confers a significant basis weight and thickness. The addition of one top layer could thus have a consequent effect on the mechanical properties of final cardboard, if the increase of basis weight is significant compared to those of the initial material. Otherwise, this layer can improve the bending stiffness of the material, enhance its creasability or decrease its cracking tendency. Three kinds of mechanical tests have thus been carried out: bending stiffness, short-span compressive test and box compression test.

Bending stiffness of cardboard samples was firstly evaluated in machine and cross-direction (Figure 2-II.7a). Compared to reference, water-treated cardboard samples (opened squares and rounds) have an improved bending stiffness in both fibers direction (Machine direction in continuous line and Cross-direction in dotted line). This improvement is quite significant: the values are twice bigger than the bending stiffness of reference. The thickness increase (+15-25%) between reference and water-treated samples, and thus the damaged fiber's network induced by coating process, explain these results. Nevertheless, the bending stiffness of water-treated cardboard samples does not evolve with the number of treatments: from one water treatment, the values remain constant in both fibers direction (about 48 mN.m in machine direction and 24 mN.m in cross-direction). Thus, the coating process with highly diluted solution influences significantly the bending stiffness of cardboard, mainly because it deconstructs the

fibers network onto cardboard surface. However, the addition of MFC improves considerably this property in both fibers directions. Especially with 10 MFC layers, the increase is about 25-30% compared to water-treated samples. As the thickness values of water-treated and MFC-coated samples are similar, this enhancement is not due to the thickness increase. The mechanical reinforcement of MFC is thus clearly highlighted in spite of very small amount added. Indeed, similar tests with PE-coated cardboard samples gave lower bending stiffness values (46 ± 4 mN.m in MD and 19 ± 2 mN.m in CD) whereas a consequent coat weight of PE (17 g/m^2) was coated.

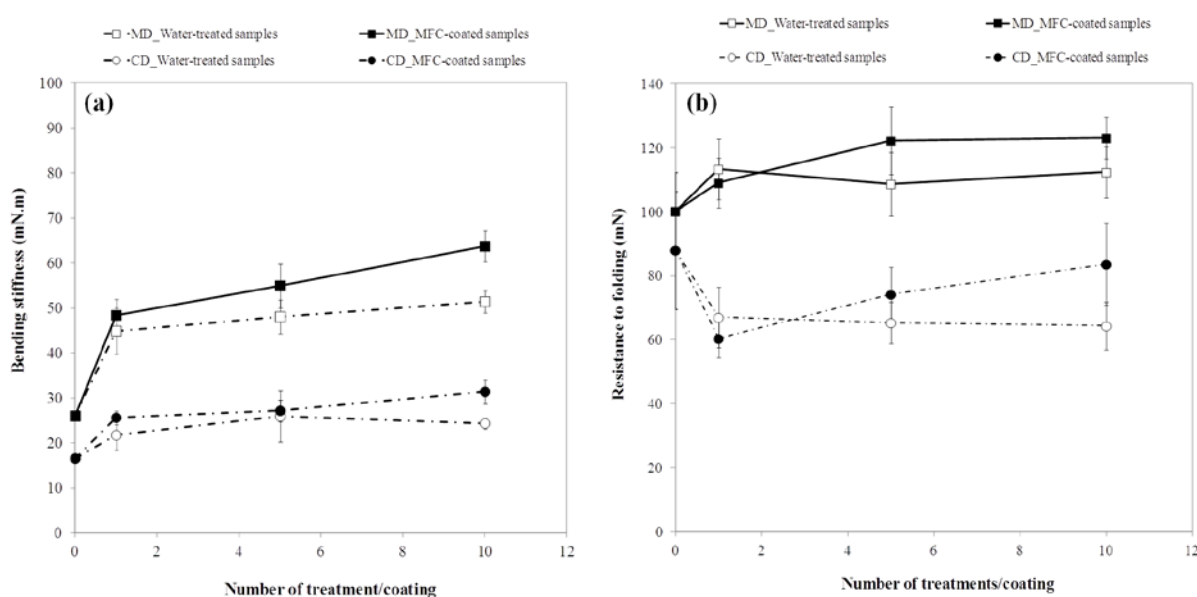


Figure 2-II.7. (a) Bending stiffness (mN.m) as a function of the number of water-treatments and MFC coats in machine (MD) and cross-directions (CD). (b) Resistance to folding (mN) of creased cardboard samples as a function of the number of treatments/coating.

Within a perspective of packaging application, the resistance to folding of cardboard samples was also measured (Figure 2-II.7b). Each sample has first been creased with a laboratory creasing press. The effect of the creasing process on both surfaces and internal structure of cardboard is complex. There are (1) tensile strains, which are greatest in the surface and reverse-side liner plies, (2) compression in the direction perpendicular to the surface and (3) shearing strains within the cardboard, parallel with the cardboard surfaces (Kirwan, 2005). Thus, a wrong crease is able to damage considerably the material. Consequently, a qualitative analysis has been done to evaluate our final creased cardboard. After creasing, all cardboard samples did not have stretching in the surface. The bulge was correctly made up with a partial internal delamination of the material. The samples were able to be folded without any stretching or break.

Figure 2-II.7b shows the resistance to folding (mN) of cardboard samples as a function of the treatment applied. Accounting the standard deviations, no difference is noticed for one, five and ten water-treatments in machine direction compared to reference. Resistance to folding is slightly increased by about 8-13%. However, in cross-direction, the resistance to folding is decreased by about 25% and remained at this value whatever the number of water-treatments. As regards the MFC-coated samples, in machine direction, a slight enhancement of this property is noticed for 5 and 10 MFC coats by comparison with water-treated samples (up to 10%). Nevertheless, whatever the treatments applied, each sample has a better resistance to folding than reference and values are increasing with the quantity of MFC. In cross-direction, the improvement of the resistance is a few better with MFC (up to 30%) compared to water-treated samples. Although an enhancement is noticed with the addition of MFC, the resistance to folding of MFC-coated cardboard remains either lower or equal to the resistance of reference in cross-direction.

MFC induces thus an improvement of the resistance to folding of cardboard samples. The effect is highlighted in machine direction, whereas in cross-direction, MFC counterbalances the loss of resistance induced by the coating process when a coat weight of 14 g/m² is achieved.

The second mechanical test carried out consists in evaluating the compressive strength of cardboard samples. This mechanical property depends mostly on the cohesion of cardboard structure and also, on its stiffness.

Figure 2-II.8 presents the results obtained for each cardboard sample. The coating process has here again a significant negative effect on this mechanical property. Indeed, as regards the short-span compressive strength of water-treated samples, the values are decreased by 10-20% compared to reference cardboard in both fibers directions. The coating process used with water has thus a negative effect on cardboard samples and damages their cohesion. Due to the successive wetting and drying cycles, layers of cardboard have indeed lost their cohesion throughout the thickness: by opening the fibers structure, some hydrogen bounds were broken. With the addition of MFC, the effect of coating process is counterbalanced. Indeed, the compressive strength is increased by 10% to 25% from one to ten MFC coats respectively in machine direction and by 2%, 11% and 6% for one, five and ten MFC coats respectively in cross-direction.

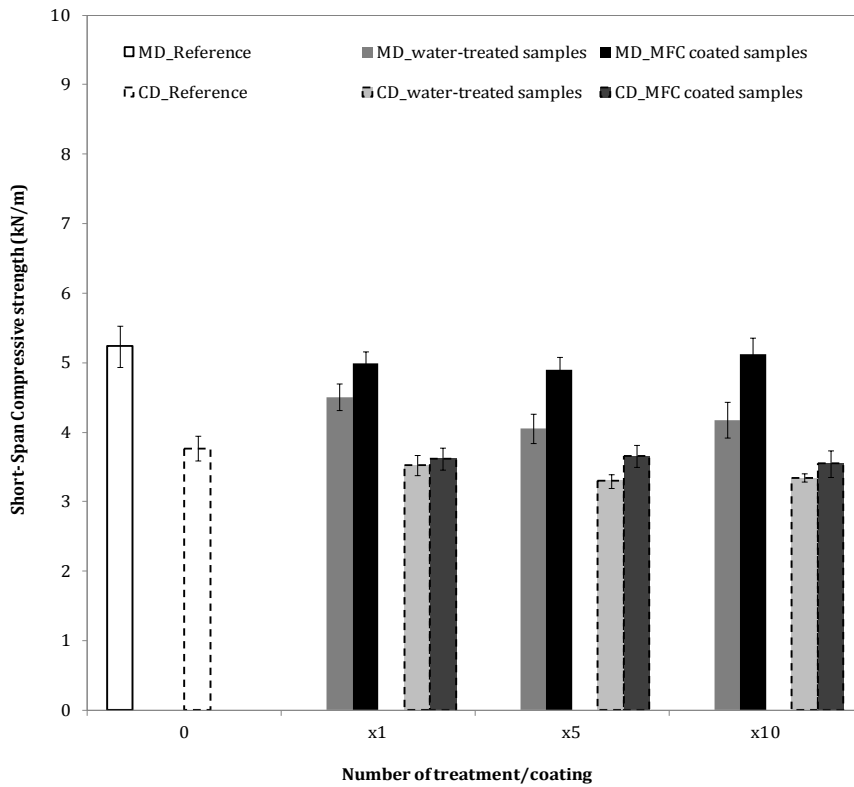


Figure 2-II.8. Short-Span Compressive strength (kN/m) as a function of the increasing number of water-treatments and MFC coats.

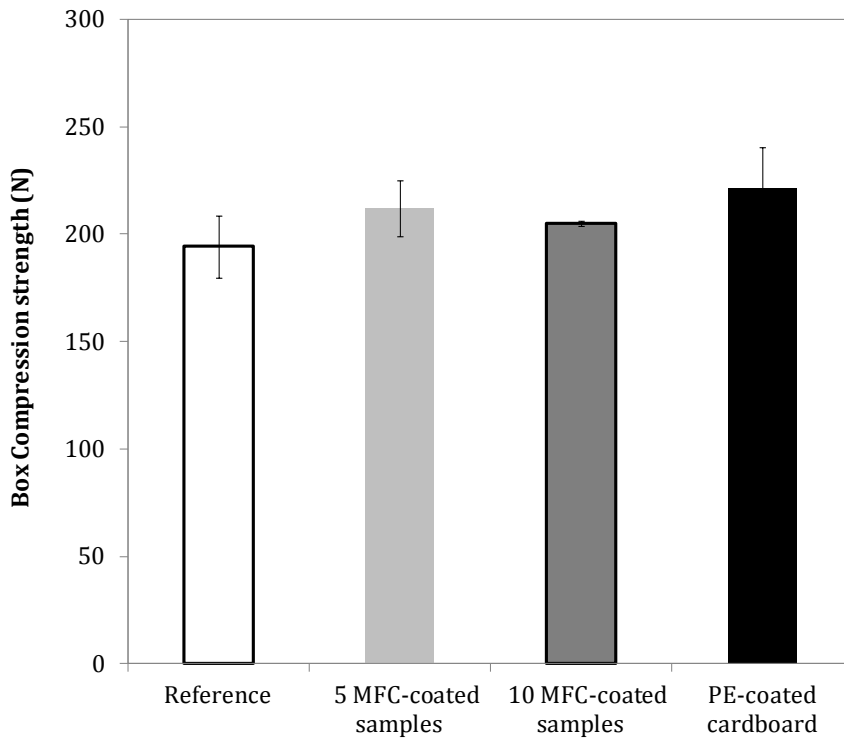


Figure 2-II.9. Strength (N) needed to crush cardboard boxes made with reference, ×5 and ×10 MFC-coated and PE-coated cardboard samples. Resistance of boxes was tested in cross-direction. These tests were carried out at 23°C and 50% RH.

The improvement is more consequent in machine direction than in cross-direction. Intuitively, this difference was not expected as usually improvements of mechanical properties are more significant in cross-direction. Nevertheless, MFC offsets the effect of water and allows a better cohesion of the material. One explanation is that MFC replace the hydrogen binding damaged by water by other and stronger MFC/MFC and MFC/cellulosic fibers bindings. Even if MFC does not penetrate into the cardboard structure, it prevents water to damage the cardboard surface and thus, to penetrate and deteriorate more its inner structure.

Finally, within a perspective of packaging application, the last mechanical property tested was carried out on a 3D packaging made from the different cardboard samples (Figure 2-II.9). The purpose here is to conclude on the resistance of a box submitted to a compressive force. Figure 2-II.9 shows the compressive strength (N) needed to crush the different boxes (made with reference, the PE- and MFC-coated cardboard).

The MFC-coated cardboard boxes have been compared with PE boxes. Compared to reference, PE boxes present a better compressive resistance. The PE layer improves indeed of 14% this mechanical property. The MFC-coated boxes have also an improved compressive resistance compared to reference (+5 and 10%), similarly than PE boxes. These results are logical and interrelate the previous one dealing with the bending stiffness and short-span compressive tests. Box compression strength (BCT) is indeed linked to both mechanical properties. As MFC improves the bending stiffness in cross-direction and does not damage the short-span compressive strength (SCT) in CD, these enhancements also impact on box compression strength in CD. Nevertheless, the differences are slight in comparison with base cardboard. Regarding the bending stiffness and SCT values of MFC-coated cardboard samples in MD, a more significant improvement of BCT values could be indeed expected.

These results are however very positive as MFC could replace PE without modifying mechanical properties and thus could be a good environmentally-friendly proposal. Furthermore, lower coat weights were applied with MFC inducing a more lightweight and biodegradable material for equal properties.

II-4. Conclusion

The study of mechanical and barrier properties of MFC-coated cardboard samples has been investigated. Due to the high water content of the MFC suspension, its coating without adding any additives or fillers could presume to a consequent effect of water on the cardboard's properties. Thus, water-treated cardboard samples have been considered as references to rightly distinguish the effects of coating process from those of MFC on cardboard's properties. The coating process has a major impact on the final properties of treated cardboard samples. Contrary to barrier properties, which are not significantly modified, mechanical properties are the most influenced by the successive wetting and drying cycles. They improved indeed significantly the bending stiffness of cardboard samples, whereas it damages its structure cohesion and weakens its compressive strength.

The addition of MFC does not enhance much the cardboard barrier properties surely due to the low MFC coat weight deposited. Only the water absorption is drastically modified: the MFC coating increases indeed this ability by 31% with 1 g/m² of MFC only. Considering the mechanical properties, the effect of MFC is clearly highlighted by the compressive strength results, especially in machine direction (+25%). Besides, from a MFC coat weight of 14 g/m², the bending stiffness is also significantly increased by 30% in machine direction. However, in cross-direction, the bending stiffness is not enough improved to achieve more than a 10% increase of the box compression strength. Only the water absorption is drastically modified, and a clear effect of the MFC is highlighted with the compressive strength and bending stiffness results.

Contrary to previous studies dealing with MFC coating onto paper substrates (Aulin, *et al.*, 2010, Lavoine, *et al.*, 2013), the same use considering cardboard substrate does not lead to similar improvements and conclusions.

This is mainly due to the high basis weight of cardboard compared to a paper substrate. The amount of MFC coated onto cardboard should be, in proportion, at least the same as the amount coated onto paper substrates. From this perspective, the quantity of MFC should be increased by either increasing the number of MFC coating, or making the MFC suspension more concentrated. Nevertheless, both solutions are for now not conceivable.

Within the framework of packaging application, MFC will rather have consequent effects on cardboard's properties as blend in the inner layers or as part of the multilayer structure than as top layer. Its use as top layer has indeed to be considered in other applications such as highly absorbent materials for box or food-basket. This MFC-coated cardboard could be also used for high-added value applications, such as bactericide or drug delivery systems (e.g. antibacterial packaging) and this, without damaging its mechanical properties.

Chapter 2-III Conclusions

The chapter 2 presented the first step of this PhD project: the elaboration of a bio-nano-material. Based on a paper or board substrate, it was possible to create a new material just as light and biodegradable than the reference, and with improved properties, owing to the coating of microfibrillated cellulose.

A specific protocol was implemented so that the coating of MFC was homogeneous onto the substrate surface and achieved a sufficient coat weight. This protocol was applied for each different substrate and will be considered as reference in the all next chapters.

The study of the end use properties of each cellulosic substrate coated with MFC proved that the higher the MFC coat weight, the better the properties. As expected and according to the literature, the mechanical properties were not so significantly improved by the coating of MFC, especially when the substrates already had a high basis weight and good properties. Nevertheless, despite the multiple successive wetting/drying cycles, the final materials still had equivalent or better properties than the base paper or cardboard.

The enhancement of the air resistance was drastic with the addition of MFC owing to its tight network. Whatever the substrates, the coating of MFC induced each time an exponential increase of the water absorption smoothing the path for new kinds of applications.

Although the results of this Chapter 2 were expected for the most of them, the optical analysis of the coated samples gave very promising results. We proved indeed that the MFC coated onto each cellulosic substrate kept its nanoporous structure and its tight entanglement; whatever the coating process used. It is worth noting that this nanoporous network is the main asset of the MFC paving the way for applications such as drug delivery systems; as concluded in the Chapter 1.

Consequently, the development of a new controlled release system based on these MFC coated cellulosic substrates is now possible and will be investigated in the Chapter 3.

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RESUME FRANÇAIS – FRENCH ABSTRACT

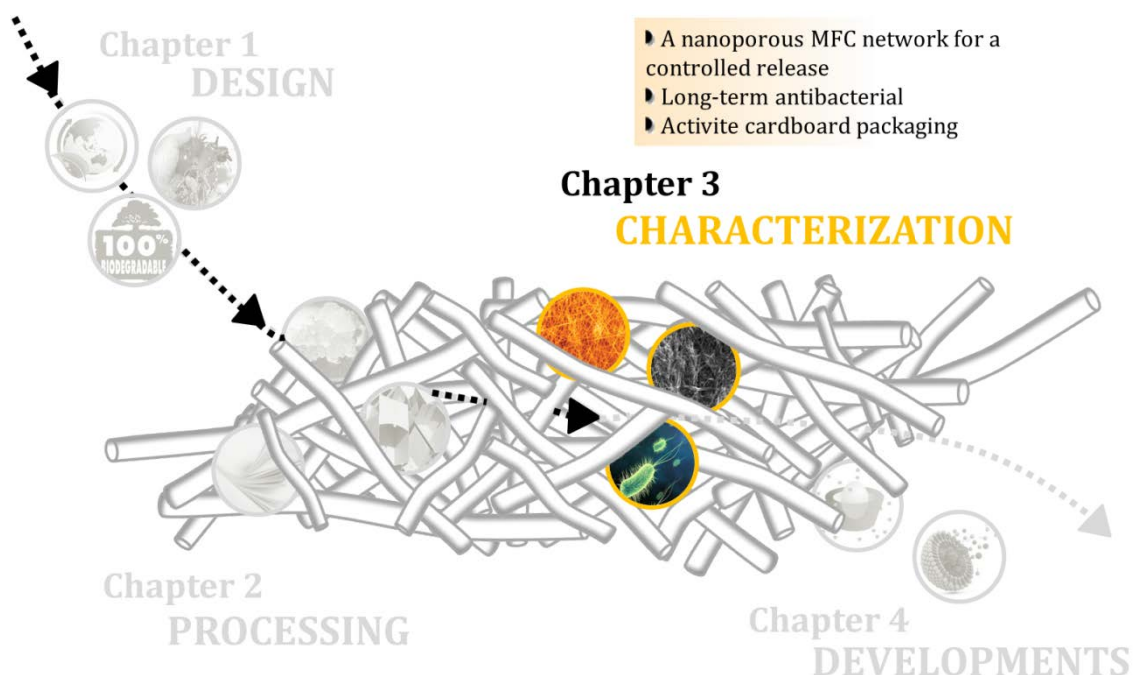


Figure Chapitre 3. Organisation générale du manuscrit

Le chapitre 2 a présenté la conception et mise en œuvre d'un nouveau bio-nano-matériau pour emballage alimentaire. Il s'agit d'un matériau biodégradable, recyclable et léger qui a été réalisé par le dépôt d'une couche de microfibrilles de cellulose (MFC) en surface d'un matériau papier ou carton.

Plusieurs procédés d'enduction de MFC ont été testés. Après caractérisations des matériaux couchés, le couchage à barre fut sélectionné comme procédé optimal d'enduction des MFC, et sera utilisé dans toute la suite de ce projet. Contrairement aux résultats évoqués dans la littérature, les propriétés mécaniques de nos supports cellulosiques n'ont été que peu améliorées suite à l'enduction de MFC. Seule la perméabilité à l'air fut fortement diminuée (+70%) et l'absorption à l'eau fortement augmentée.

Ces analyses nous ont toutefois permis de mettre en évidence le réseau nanoporeux des MFC qui s'est formé à la surface des matériaux papier/carton lors du procédé de couchage. Ce réseau est l'élément clé dans le développement d'un nouveau matériau fonctionnel pour emballage alimentaire.

Le Chapitre 3 démontre en effet que ce réseau de MFC peut être utilisé comme système de libération prolongée de molécules actives. Ce constat est très innovant car la première étude publiée relative à l'utilisation des MFC en tant que systèmes de libération contrôlée est apparue il y a quelques mois seulement (début 2013), et correspond à une étude lancée en parallèle à la nôtre. Au début de notre projet, aucune publication ne traitait de l'utilisation des MFC en tant que système de libération contrôlée. Nous avons été les premiers à proposer cette application par le biais de poster scientifique dans des congrès internationaux. Depuis, moins de 10 articles ont été recensés, utilisant les MFC sous toutes leurs formes (suspension, films, aerogels) comme système de libération de médicaments ou de molécules actives, principalement dans le domaine pharmaceutique.

La **première partie** de ce chapitre 3 (Article 4, soumis à *Carbohydrate Polymers*) est une preuve de concept. A l'aide d'une molécule modèle, la caféine, ce sous chapitre démontre que le réseau de MFC est en mesure de libérer plus progressivement et plus lentement les molécules de caféine dans un milieu aqueux. La caféine a été choisie pour plusieurs raisons : c'est une petite molécule hautement soluble dans l'eau, visible à l'U.V., peu chère, et inerte avec le matériau papier étudié. Si un effet (même minime) est observé, ce sera aussi le cas avec des molécules plus volumineuses et moins solubles, classiquement utilisées en tant qu'agent antimicrobien. Cette molécule est donc introduite dans le matériau papier (41 g/m², calandré) selon deux procédés : imprégnation et couchage. Trois échantillons ont été étudiés et comparés afin d'observer l'effet des MFC sur la libération de la caféine en milieu aqueux :

- (1) Un premier échantillon consiste en l'imprégnation du matériau papier dans une solution aqueuse de caféine concentrée à 21g/L (concentration de saturation : 21,7g/L).
- (2) Le deuxième échantillon est similaire au premier, à l'exception que cinq couches de MFC équivalentes à 7g/m² (cf. Chapitre 2) ont été déposées après imprégnation du matériau dans la solution de caféine.
- (3) Enfin, le dernier échantillon est couché cinq fois avec une suspension de MFC mélangée à de la caféine.

Ces trois méthodes de préparation d'échantillon seront répétées et réutilisées dans toute la suite de ce chapitre, avec d'autres molécules et d'autres matériaux celluloses.

Dans le cadre de cette preuve de concept, deux protocoles de libération de molécules en milieu aqueux ont été mis en place et testés plusieurs fois afin de les valider : un protocole de cinétique de libération (prélèvements au cours du temps du milieu de libération contenant au fur et à mesure de la caféine) et un protocole de libérations successives (prélèvement du milieu de libération à chaque Δt fixé, suivi d'un renouvellement du milieu). Ils seront appliqués tout au long de ce manuscrit.

Ces deux protocoles ont démontré que les MFC étaient en mesure de libérer des proportions moindres de caféine au cours du temps. Ainsi, 15 à 20% en moins sont libérées grâce aux 7g/m² de MFC. Par ailleurs, les échantillons couchés avec le mélange (caféine+MFC) se sont montrés les plus intéressants d'un point de vue système de libération contrôlée : la cinétique de libération fut en effet beaucoup plus lente (total libéré 10 h après les autres échantillons) et les proportions libérées furent beaucoup plus faibles et gardées constantes pendant 40 et 25 changements de milieu de plus par rapport, respectivement, aux échantillons imprégnés dans la solution de caféine et aux échantillons imprégnés puis couchés avec les MFC.

Suite à ces premiers résultats montrant un réel effet des MFC dans le contrôle de la libération de la caféine en milieu aqueux, une deuxième étude a été réalisée avec cette fois-ci une molécule antibactérienne. Cette **deuxième partie** (Article 5, soumis à *Applied Materials and Interface*) utilise une solution de digluconate de chlorhexidine (CHX) comme agent modèle antibactérien. Ce composé actif a principalement été choisi pour sa solubilité en milieu aqueux, sa visibilité en spectrophotométrie U.V., son traçage en microanalyse à rayons X et son action antibactérienne efficace contre une large gamme de bactéries.

Les études de libération en milieu aqueux ont confirmé les premiers résultats obtenus avec la caféine : les MFC ont libéré plus progressivement et plus lentement le CHX (cinétique de libération divisée par trois). Ces résultats sont d'autant plus prometteurs que des tests similaires ont été réalisés avec une couche modèle de latex. Contrairement aux MFC, le latex a empêché la libération du CHX en milieu aqueux et a ainsi limité toute action antibactérienne.

En effet, suite aux tests antibactériens réalisés avec une bactérie non pathogène, la *Bacillus subtilis*, les proportions libérées dans le cas de la couche de latex furent insuffisantes pour conférer une action antibactérienne aux matériaux papiers. *A contrario*, les échantillons couchés avec les MFC présentèrent une forte activité contrôlée dans le temps.

Ces tests antibactériens ont aussi été réalisés successivement jusqu'à épuisement de l'action antibactérienne du CHX. Là encore, les MFC ont montré un réel effet sur le contrôle de la libération du CHX : avec une couche de 7g/m² de MFC, l'action antibactérienne a été prolongée de 9 jours. Lorsque le CHX a directement été introduit dans la couche de MFC, l'action antibactérienne a duré 15 jours supplémentaires.

Cette deuxième partie a permis dans un premier temps de confirmer l'effet de la couche de MFC sur la libération de molécules en milieu aqueux. Dans un second temps, nous avons montré que ce nouveau système de libération prolongée était très prometteur pour une application antibactérienne sur le long terme. Par conséquent, afin de relier ce système au domaine de

l'emballage alimentaire, une **troisième partie** a été consacrée à l'élaboration d'un emballage carton 3D à effet antibactérien prolongé.

Une étude de libération a donc été réalisée comparant le matériau carton couché avec du CHX et le matériau couché avec la suspension de MFC contenant du CHX. Les MFC ont encore une fois libéré plus progressivement le CHX dans l'eau, bien que le carton, même sans MFC, ait libéré beaucoup plus longtemps le CHX que le matériau papier.

Tout comme dans le Chapitre 2, le matériau carton fut mis en 3D afin de réaliser des tests alimentaires. Ces tests ont été effectués sur le foie porc, viande très sensible dont la dégradation est rapidement et aisément appréciable. Le carton couché avec les MFC et le CHX prolongea l'état de la viande plus efficacement et plus longtemps que le carton de référence et le carton couché avec du polyéthylène. L'aspect final obtenu avec les MFC fut même très proche de celui du foie emballé avec de la pénicilline.

Le chapitre 3, cœur de ce projet de thèse, caractérise donc un nouveau bio-nano-matériau fonctionnel pour emballage alimentaire. L'utilisation du réseau nanoporeux de MFC formé à la surface d'un matériau cellulosique, après couchage, a été utilisé et considéré dans un nouveau type d'application : un système de libération contrôlée de molécules actives. C'est la première fois que des travaux portant sur l'utilisation du couchage de MFC pour contrôler la libération de molécules sont publiés. C'est aussi la première fois qu'une application alimentaire est envisagée dans ce contexte.

En effet, le domaine de l'emballage alimentaire se prête particulièrement bien à ce nouveau matériau actif grâce au côté biosourcé et biodégradable des MFC. Des domaines tels que le biomédical pourraient aussi être envisagés.

Chapter 3-I

Microfibrillated cellulose coatings as new release systems for active packaging

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Paper 4

Abstract

In this work, a new use of microfibrillated cellulose (MFC) is highlighted for high-added-value applications. For the first time, a nanoporous network formed by MFC coated on paper is used for a controlled release of molecules. The release study was carried out in water with caffeine as a model molecule. The release process was studied by means of (i) continuous, and (ii) intermittent diffusion experiments (with renewal of the medium every 10 min). The effect of the MFC was first observed for the samples impregnated in the caffeine solution. These samples, coated with MFC (coat weight of about 7 g/m²), released the caffeine over a longer period (29 washings compared with 16), even if the continuous diffusions were similar for both samples (without and with MFC coating). The slowest release of caffeine was observed for samples coated with the mixture (MFC + caffeine). Moreover, the caffeine was only fully released 9 h after the release from the other samples was completed.

This study compared two techniques for the introduction of model molecules in MFC-coated papers. The latter offers a more controlled and gradual release. This new approach creates many opportunities especially in the food-packaging field. A similar study could be carried out with an active species.

Inspired from Paper 4:

Nathalie Lavoine, Isabelle Desloges, and Julien Bras (2013) Microfibrillated cellulose coatings as new release systems for active packaging. Carbohydrate Polymers, Accepted.

Chapter 3: Controlled release system and active properties

I-1. Introduction

The development of new food packaging materials is being driven by increased consumer demand. Specifically, research is being focused on (i) the use of renewable resources, (ii) the improvement of barrier properties, and (iii) the introduction of new functionality to enhance the shelf-life of food products (Johansson, *et al.*, 2012). This study tries to address these three objectives.

Fiber-based materials such as paper seem to be the most promising packaging materials to address consumers' needs for renewable, biodegradable, lightweight, and recyclable packaging. However, the intrinsic properties of paper are not sufficient for some packaging applications, so that specific treatments are necessary by using polymers or multilayer materials. However, the general non-recyclability and non-biodegradability of many petroleum polymers limits interest in these current solutions. Thus, researchers are trying to avoid this issue by using new biopolymers or materials. Among the latter, the new family of nanocelluloses greatly interests such researchers, as proven by the recent increase in books (Dufresne, 2012) and reviews (Eichhorn, *et al.*, 2010, Klemm, *et al.*, 2011, Moon, *et al.*, 2011) on them. In particular, microfibrillated cellulose (MFC) is being increasingly studied (Lavoine, *et al.*, 2012, Siró and Plackett, 2010).

In 1985, Turbak, *et al.*, 1985 patented a manufacturing process for MFC. Due to its nanometer scale, its high surface energy and its ability to form a nanoporous network, MFC was studied for use in nanocomposites as a mechanical reinforcement (Siqueira, *et al.*, 2010, Siró and Plackett, 2010) and as a dispersion stabilizer (Andresen, *et al.*, 2006). Since then, research in this area has increased, and new applications are targeted: films (Ahola, *et al.*, 2008, Andresen, *et al.*, 2007, Aulin, *et al.*, 2010a, Fukuzumi, *et al.*, 2009), aerogels (Aulin, *et al.*, 2010b, Fischer, *et al.*, 2006), electronic devices (Shah and Malcolm Brown, 2004, Torvinen, *et al.*, 2012), etc. Increasingly, the combination of MFC and cellulosic materials such as papers and boards is being investigated (Lavoine, *et al.*, 2012). The first such study investigated the coating of MFC on papers to improve their barrier properties (Aulin, *et al.*, 2010a, Syverud and Stenius, 2009). Others have focused on MFC coatings with polymers or resins such as starch, wax or shellac (Hult, *et al.*, 2010, Spence, *et al.*, 2011b) to obtain a homogeneous MFC coat with a better barrier performance. The use of MFC suspensions as coating slurries is also advantageous for the printing industry (Ankerfors, *et al.*, 2009, Hamada, *et al.*, 2010, Ridgway, 2011) and, more recently, in the food packaging sector (Rodionova, *et al.*, 2010, Spence, *et al.*, 2010). The enhancement of barrier and mechanical properties is thus the main motivation for using MFC with paper. Its ability to form a nanoporous network (Fukuzumi, *et al.*, 2011, Nemoto, *et al.*, 2012) can be considered to be an advantage for other applications in comparison with classical

films. One further possibility, investigated in this study, is the use of MFC in drug release applications.

To our knowledge, very few papers have discussed the use of MFC as a drug release system, and even when it has been attempted, a different strategy has been employed in each study. The first such work in 2011 highlighted the ability of MFC to immobilize and protect well the drug nanoparticles in a suspension and in a freeze-dried MFC matrix (Valo, *et al.*, 2011). Thus, the nanoparticles could be stored for more than ten months without inducing major changes in their morphology. Dissolution tests were also carried out since there was an indication that the binding of the drug with the MFC could have controlled the release. Nevertheless, the authors did not establish any positive role of the MFC in slowing down release. Another recent study used MFC as a carrier for the encapsulation of drugs (Kolakovic, *et al.*, 2012a). Here, drug-loaded MFC microparticles were produced using a spray drying process. The objective of doing this was to use the tight, nanoporous network of the MFC around the drug to release it progressively. Long-term studies showed sustained drug release profiles over a period of two months. Thus, it was proven that the MFC had played a significant role in limiting the drug diffusion.

These both studies employed MFC as a new technology for drug dissolution. However, the present study focused rather on the release of active compounds from insoluble support, such as paper substrate. Such systems dealing with the release of active agents (e.g. drugs, flavors) from insoluble support (e.g. proteins, acrylic coatings) are already well-known (Chen *et al.*, 2006; Madene *et al.*, 2006). Technologies such as coating, extrusion or spray-drying (Gouin *et al.*, 2004; Guillard *et al.*, 2009) have been indeed used with different insoluble matrices (e.g. starch, carbohydrate, whey gluten, wax etc.) for the encapsulation and the controlled release of active compounds.

The use of MFC as insoluble matrix for controlled release system is, nevertheless, very new. It was firstly developed by Kolakovic *et al.*, in 2013. They published a study emphasizing the diffusion-control role of the nanoporous network of MFC (Kolakovic, *et al.*, 2012b). They produced MFC films that were impregnated with the drug, using a filtration process. They studied different drug release periods up to a maximum of three months and showed the utility of MFC for controlled-release applications as shown in the Figure 3-I.1. with the release of beclomethasone dipropionate (active pharmaceutical ingredient).

Nevertheless, they also noticed that the release rate was slow and that small quantities of the drug were released within 24 h. The latter observation limits the use of MFC for the sustained release of drugs over a long time.

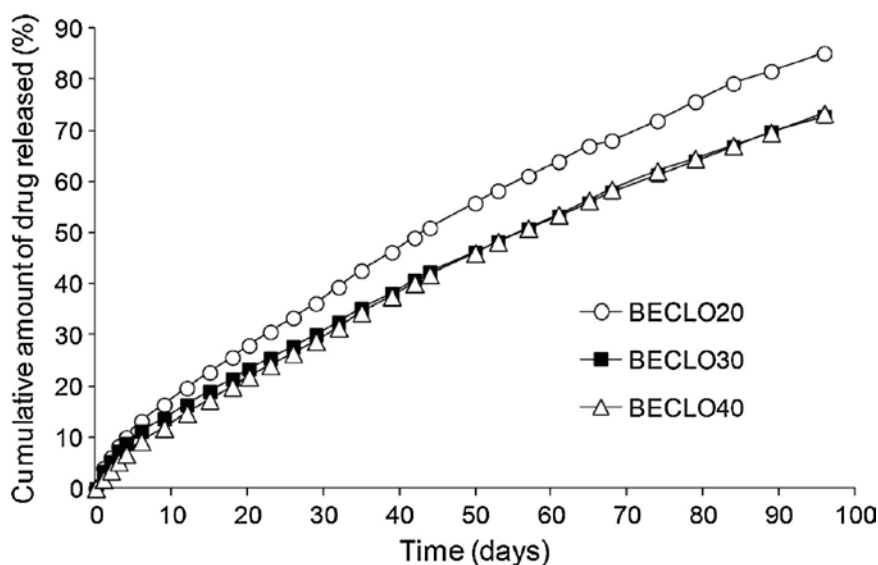


Figure 3-I.1. Drug release curves of MFC films loaded with different proportion of beclomethasone dipropionate (20, 30 and 40%) (from Kolakovic, *et al.*, 2013b)

In this study, we developed a new strategy. For the first time, we availed of the nanoporous network structure of MFC, which is formed during the paper coating process, to induce the controlled release of an active species. The study was carried out with a model species, caffeine, which was introduced using three different techniques in the paper. Caffeine was chosen as it is classically used as a simulator of hydrophilic drug release systems with a high solubility in water (Efentakis, *et al.*, 2007), UV detectable, and, in our case, has no chemical interactions with the cellulosic substrate. The effect of the MFC coats on caffeine release was then investigated to identify its positive impact.

I-2. Experimental

I-2.1. Materials

Two MFC suspensions (E-MFC-MIC and E-MFC-H) were supplied by the FCBA (France). The first suspension, E-MFC-MIC, was produced from eucalyptus pulp, enzymatically pre-treated (during 2 h with an endoglucanase) and passed through a Microfluidizer® M-110EH-30 (5 passes through the 100 nm chamber, 4 passes through the 200 nm chamber). The concentration was 2% (w/w).

The second suspension, E-MFC-H, was produced from sulfite pulp (Domsjö®), also enzymatically pre-treated as described above, and passed through a GEA Ariete® homogenizer (4 passes, at 1,400 bars). The concentration was 2% (w/w).

Both MFC suspensions are considered similar, since diameter dimensions are closed.

The base paper material was calendered paper with a basis weight of 41 g/m² made with non-bleached pulp. Caffeine (99%), C₈H₁₀N₄O₂, was purchased from Sigma-Aldrich (France) and used as received.

I-2.2. Methods

Paper coating process

The MFC suspensions were coated onto paper samples (A4 format) with a bar coating process (Endupap, France). A 0.9 mm Mayer bar was used with a coating speed of 5 cm/s. The coated papers were then dried with a contact drying system (Allimand, France) at 105°C for 3 min. These steps were repeated five times in order to deposit five MFC layers on the sample.

Reference samples (water-treated papers) were obtained by impregnation of one side of the base paper in deionized water followed by drying under the same conditions described above. They were treated the same number of times as the MFC-coated samples, i.e. five water impregnations, each one followed by a drying at 105°C for 3 min.

Determination of the caffeine absorption

Paper samples of 10 × 10 cm² were first dried at 105°C for 1 h. Their dried mass (M_i) was then determined. An aqueous caffeine solution of 21 g/L was then prepared at room temperature. Each paper sample was impregnated for different times (1, 5, 10, 20, 30, 40, and 60 min) in the solution. After removing the excess solution by manually squeezing, the samples were dried at 105°C for 1 h in a ventilated oven. The impregnated dried mass (M_f) was then determined.

The adsorbed amount of caffeine for each sample was calculated as follows:

$$C(\%) = \frac{(M_f - M_i)}{M_i} \times 100$$

Preparation of the samples for the release study

The tested samples were prepared using three different strategies as shown in Figure 3-I.2.

The **first strategy** (i) was the preparation of the reference samples by impregnation of paper samples in the caffeine solution (21 g/L) over the time determined. The full quantity of caffeine introduced was calculated using the mass difference between the dried impregnated samples after and before impregnation using a precision scale at $\pm 0.001\text{g}$.

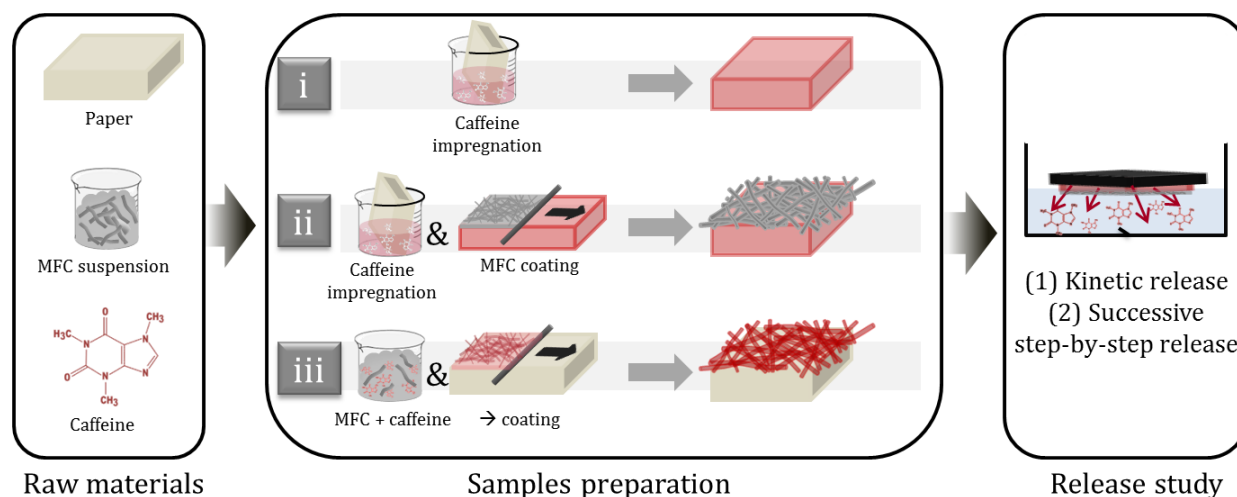


Figure 3-I.2. The caffeine release study - Samples preparation

Preparation of the tested samples using three different strategies: (i) impregnation of papers samples in the caffeine solution, (ii) impregnation of papers samples in the caffeine solution followed by coating with MFC and, (iii) papers samples coated with the mixture caffeine/MFC

The **second strategy** (ii) involved the impregnation of the paper samples according to strategy (i), followed by coating with five MFC layers. The full quantity of caffeine was determined as described previously, so that the authors could assess the hypothesis that little caffeine was released during the coating process.

The **final strategy** (iii) involved coating of the paper with the MFC suspension, in which the caffeine was previously introduced, to obtain a concentration of about 21 g of caffeine per liter of MFC suspension. The samples were coated five times with this slurry. In this case, the quantity of caffeine has been calculated from its initial quantity introduced in the slurry with MFC. The share of caffeine in the slurry caffeine/MFC is known and the coat mass of the slurry caffeine/MFC on paper samples is also measured. Supposing that the coating process is homogeneous and caffeine is homogeneously mixed with MFC, the share of caffeine in the slurry will be identical to the share in the coat on paper samples. The dried coat mass of each sample was determined by mass difference after drying each sample for at least 1 h at 105°C . The full

quantity of caffeine coated on the sample was then calculated from the percentage of caffeine introduced initially in the slurry and the dried coat mass of the sample.

Release protocols

Two protocols were developed for analyzing the caffeine release.

Each sample was adhered to a light expanded polystyrene support using scotch tape in order to study only the release of caffeine from the sample's surface. These experiments were repeated on three specimen of each sample, at room temperature and under sink conditions.

- *Continuous diffusion experiments*

The continuous diffusion was studied in 500 mL of deionized water that was continuously stirred with a magnetic stirring-bar. The samples were placed at the surface of the aqueous media. At successive intervals, 3 mL of the solution was taken for UV analysis at a wavelength of 272 nm. Using a calibration curve, the concentration of the caffeine released was then determined as a function of time.

- *Intermittent diffusion experiments*

This protocol was similar to the continuous diffusion protocol, except that between each 10 min sampling step, the aqueous media was renewed.

Analysis of the solution absorbance was carried out with a UV spectrophotometer SHIMADZU UV1800 at a wavelength of 272 nm.

Samples characterization

Before each test, the paper samples were maintained at a temperature of 23°C, and a relative humidity of 50% for at least 24 h.

Scanning Electron Microscopy (SEM) and Field Emission Gun Scanning Electron Microscopy (FE-SEM) images of the base or coated papers, and of the MFC suspensions, were taken using a Quanta200® and a Zeiss® Ultra-55, respectively. The MFC suspensions were spread onto a metal substrate using carbon tape, allowed to dry for two nights at room temperature, and coated with a thin layer of gold. The working distance used with the FE-SEM was 5.5 mm for an accelerating voltage of 2.00 kV at a magnitude of $\times 20.00K$. The respective surfaces of the base and coated papers were precisely cut, and mounted onto a similar support to be analyzed with SEM, using the back-scattered electron (BSE) detection mode. The working distance employed was 9.9 mm with a voltage of 12.5 kV at a magnitude of $\times 100$. The respective cross-sections of the base and coated papers were also analyzed with SEM at a working distance of 10.2 mm, a voltage of 15.0 kV and at a magnitude of $\times 600$.

The basis weight of the $10 \times 10 \text{ cm}^2$ samples was determined by weighing samples with a precision scale ($\pm 0.001 \text{ g}$). Each value was expressed as an average of at least five measurements.

Their thickness was measured by image analysis of the cross-sections analyzed with SEM. The ImageJ® software was used to obtain thickness average values of at least 50 measurements.

The Young's modulus of the samples was determined according to the standard ISO 1924 - 2/3 using a Lorentzen & Wettre Tensile Tester at a speed of 100 mm/min , at 23°C and $50\% \text{ RH}$. The same device also allowed the measurements of the strength-at-break and the elongation of each sample. At least five measurements were carried out to obtain the average value. At the same conditions of temperature and humidity, the bending stiffness was measured using a Kodak stiffness tester (ISO 5629). This measurement was also repeated on at least five specimens.

The air permeability tests were carried out using Mariotte vases (ISO 5636) with each sample having an area of 2 cm^2 , at a vacuum of 2.5 kPa . The results were expressed as an average of at least five measurements.

I-3. Results and Discussion

I-3.1. Characterization of MFC

Figure 3-I.3a shows a FE-SEM image of the E-MFC-GR suspension used to coat the paper samples. An average diameter of MFC of $60 \pm 16 \text{ nm}$ was determined after image analysis, using the ImageJ® software, after at least 50 measurements. This value agrees well with dimensions for similar MFC fibers found in the literature (Lavoine, *et al.*, 2012, Siqueira, *et al.*, 2009, Siró and Plackett, 2010).

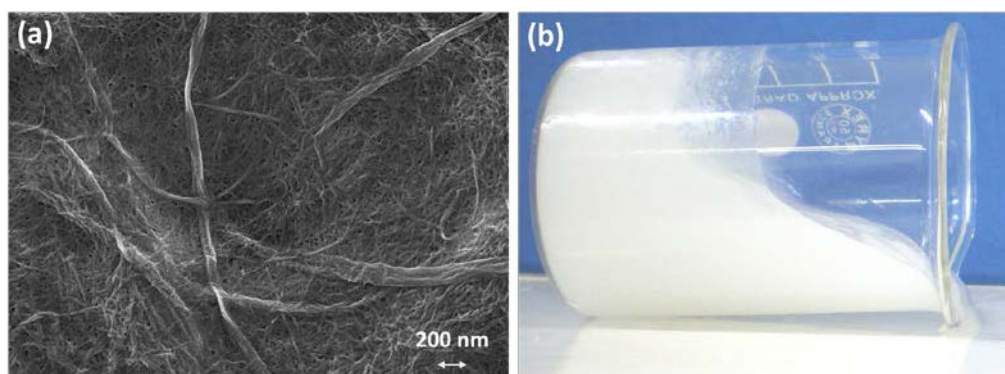


Figure 3-I.3. FE-SEM image of the E-MFC-GR suspension (dried) from eucalyptus pulp (a) and picture of E-MFC-GR (2% w/w) as gel (b)

Currently, different types of MFC are produced. However, depending on the MFC source and the treatment that is applied, the aspect ratio and dimensions of each type of MFC fiber will differ. Thus, FE-SEM was used to verify that the MFC suspensions were homogeneous and that it had no large fibers (with diameter higher than 100 μm). Moreover, the suspension used for coating clearly resembles a gel (Figure 3-I.3b) because of the high specific area and homogeneity observed in the suspension, both of which are characteristic of nanoscale features.

I-3.2. Characterization of the MFC-coated papers

As shown in Figure 3-I.2, three kinds of samples were prepared for investigating the influence of the nanoporous network (shown in Figure 3-I.3a). The impact of MFC coatings was first assessed by studying the mechanical and barrier properties of each sample: the base paper, the water-treated samples and the MFC-coated samples. As the MFC-coating contained about 98% of water and only 2% of dried MFC, wet reference samples (water-treated samples) were also prepared to compare and highlight the influence of the wetting and drying processes (during the coating steps). Indeed, it is well-known that wetting and drying steps influence paper structure (Thomson, *et al.*, 2008).

Due to the viscosity of the suspension, the coating was not homogeneous after one coat. Figure 3-I.4 presents SEM images of the surfaces of the coated papers between the first and fifth coats. In BSE detection mode, the fillers (calcium carbonate, kaolin) are represented by white and the cellulosic parts are represented by black. As the number of coats increases, more filler is progressively recovered. After five coats, the MFC coating was assumed to be homogeneous. Thus, for the release study, all the samples were coated with five MFC coats. This choice was further justified by the positive mechanical and barrier properties obtained in this case and detailed later in Table 3-I.1.

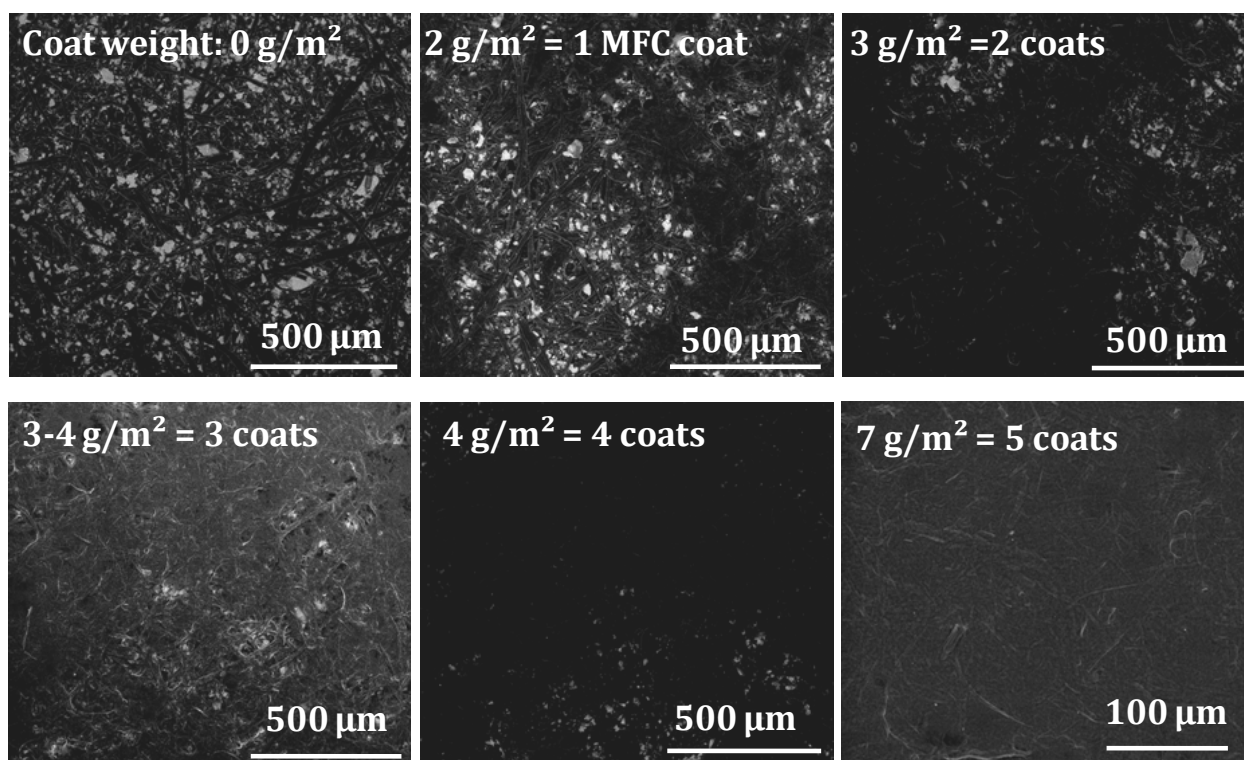


Figure 3-I.4. SEM images of the surfaces of MFC-coated papers between the first (coat mass of 2 g/m^2) and fifth passes (coat mass of 7 g/m^2 - bottom right corner) compared to the base paper (upper left corner) using the BSE detection mode at a magnitude of $\times 100$.

Figure 3-I.5 shows the differences between the base paper and the one with the 5-layer MFC coating. The MFC is well distinguished on the surface of the sample (Figures 3-I.5b and c) but do not penetrate through the whole thickness of the paper. A homogeneous thickness of about $7 \pm 2 \mu\text{m}$ is observed for the MFC layer on the surface.

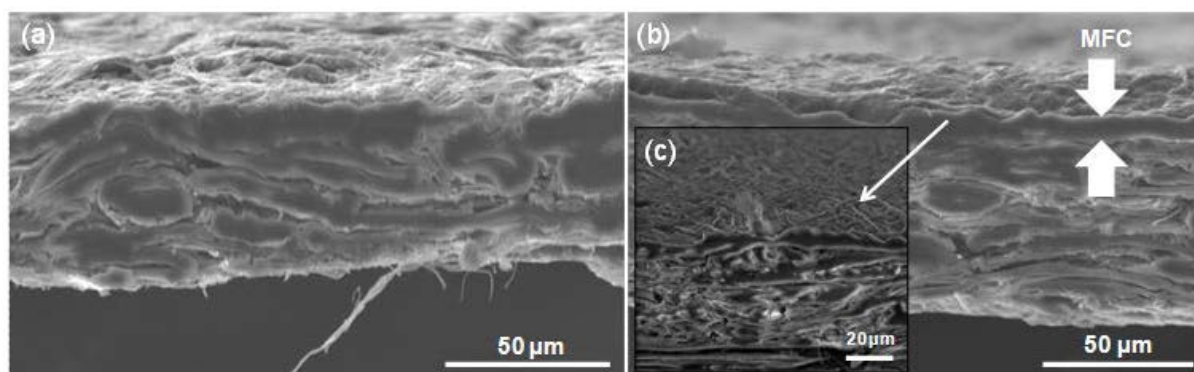


Figure 3-I.5. SEM images of the base paper (a) and paper with five MFC coats (b and c) slices (WD: 10.2 mm, HV: 15.0 kV, Mag: $\times 600$)

The picture (c) highlights the nanoporous MFC network formed at the surface of the paper surface (WD: 9.9 mm, HV: 12.5kV, Mag: $\times 1200$; BSE detector).

With five MFC coats, the coat mass obtained was 7 g/m^2 , which is the expected value for barrier coating. Table 3-I.1 summarizes the mechanical properties of the samples. The water-treated samples had a similar basis weight than the reference paper (from 41 ± 1 to $43 \pm 1 \text{ g/m}^2$) when taking into account the standard deviations.

However, the thickness values indicated a swelling of the paper samples after the water treatment, i.e. the successive wetting/drying cycles.

	MFC Coat weight (g/m^2)	Thickness (μm)	Young's modulus (Gpa)		Bending stiffness (mN.m)		Strength (N)		Elongation at break (%)		Air permeance $\mu\text{m/Pa.s}$
			MD	CD	MD	CD	MD	CD	MD	CD	
Base Paper	00±00	42±2	7.3±0.5	2.3±0.1	0.06±0.01	0.03±0.001	47±4	15±1	1.2±0.1	1.7±0.3	2679 ± 139
5 H2O treatments	00±00	52±7	4.7±0.2	1.7±0.1	0.10±0.002	0.03±0.001	39±2	13±1	1.5±0.1	1.7±0.1	6441 ± 801
5 MFC coats	6.8±1.3	72±4	5.8±0.1	2.4±0.4	0.11±0.01	0.05±0.0002	48±1	16±1	1.4±0.1	1.2±0.2	787 ± 166

Table 3-I.1. Mechanical properties and air permeability of the base paper and the MFC-coated papers compared to the mechanical properties and air permeability of the water-treated papers (at 23°C and 50% of relative humidity). Measurements in machine (MD) and cross-direction (CD) were carried out on at least five specimens.

Thickness values of water-treated samples measured by image analysis are indeed increased by about 25% demonstrating structural changes induced by the coating process. With the addition of MFC, this value increased again by 46%. As the thickness of MFC layers is about $7 \mu\text{m}$, this thickness increase is also explained by the water coating induced during MFC coating.

These structural changes have consequences on mechanical properties. Compared to base paper properties, values of Young's modulus and strength were indeed decreased after the water treatment (-36% and -17% in machine direction respectively). However, the addition of MFC counterbalanced these damages. MFC-coated paper samples have indeed similar mechanical properties than base paper. The use of MFC is here interesting as it reinforced the mechanical properties of base paper damaged during the coating process.

The mechanical reinforcement is also concluded with the bending stiffness values. Nevertheless, the enhancement is the same for water-treated and MFC-coated samples. Thus, this improvement is mainly due to the coating process, and especially to shrinkage, which confers a better elongation to paper samples. More details for this type of experiment have been reported recently (Lavoine, *et al.*, 2011).

The MFC coating has significant effect on air permeance. With five MFC coats, i.e., with about 7 g/m^2 of the MFC suspension, the air permeance decreases drastically to 70% of the uncoated value (Table 3-I.1). These results are significant, even when the MFC-coated samples are compared to water-treated samples, whose air permeance values are two to three times

higher than those of the base paper. These results are in agreement with those of a study published by Syverud and Stenius, 2009.

The characterization of the MFC-coated papers proved the positive effect of MFC-coatings (i.e., slightly better mechanical properties with a strong reduction in air permeability), as expected from recent papers (Aulin, 2009, Aulin, *et al.*, 2010a, Nygård, *et al.*, 2011). However, whether this interesting biobased, nanoporous network significantly affects the rate of molecule release and whether it can be used to control such a release still needs to be investigated.

I-3.3. Continuous diffusion

The caffeine release study comprises three steps: (1) the paper absorption, (2) the continuous diffusion experiments, and (3) the intermittent diffusion experiments of the species into an aqueous media. At the end, a comparison is made between (i) the base paper impregnated in caffeine, (ii) the same paper subsequently coated with MFC, and (iii) the base paper coated with the mixture of MFC and caffeine, as previously described in Figure 1.

Caffeine was selected as the release molecule because it is highly soluble in water, detectable by UV-Vis, and classically used as a simulator of drug release systems (Efentakis, *et al.*, 2007, Nicoli, *et al.*, 2005, Santander-Ortega, *et al.*, 2010, Sriamornsak and Kennedy, 2007).

The absorbed amount of caffeine was determined for different times of impregnation in the caffeine solution. After 10 min, no more absorption occurred with further increase in time. Accounting for standard deviation with a relative error between 5-15% (results not shown), the values were indeed similar and converged to an absorbed amount of caffeine of approximately 7%. Thus, in our next studies, each paper was impregnated for a minimum of 10 min in the caffeine solution.

After preparation, each sample surface was placed on the meniscus with an aqueous solution to study the release of the caffeine. It is important to note that caffeine is very soluble in water and should be released into aqueous solution very quickly compared with the release of classical drug medicines or antimicrobial molecules (which are usually less soluble) (Sriamornsak and Kennedy, 2007).

The continuous diffusion experiments for the three kinds of samples are plotted in Figure 3-I.6. The release rate of caffeine with time in the case of the paper impregnated in caffeine reaches a plateau after 40 min of release in water. A similar trend is observed for a similarly impregnated sample coated with five MFC layers. The MFC coats do not seem to prevent the caffeine from releasing or to slow this release. Even if the slope at the origin is slightly slower for the MFC-coating, the gradients of the curves for both samples are approximately identical (Figure 3-I.7b). Moreover, both curves do not indicate 100% caffeine release (they are 80% and 60%, respectively). This observation could be explained if little

caffeine were released during the coating process. The coating of the MFC suspension involves the coating of 98% of water for only 2% of dried MFC. Consequently, the caffeine previously introduced into the paper structure during impregnation already has an opportunity to be released. UV analysis (not presented here) of the support placed under the samples during the coating process proved the presence of caffeine that was released prematurely during the coating process. Unfortunately, the process has not allowed a precise measurement of this quantity despite several tentative efforts. This can lead to an inaccuracy when quoting the initial mass of caffeine. It is also important to note that the concentration of caffeine released into the solution is still very low and continuous diffusion into the aqueous media occurs. Indeed, the volume of the release media was chosen so that the equilibrium concentration of caffeine in water would never be reached (sink conditions Gibaldi and Feldman 1967).

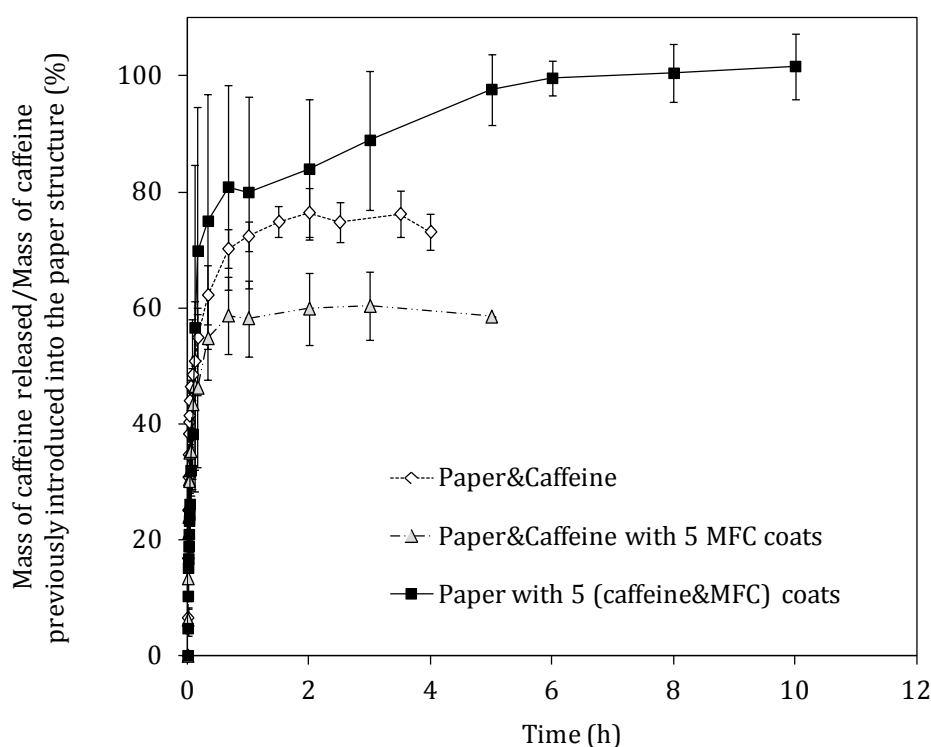


Figure 3-I.6. Continuous diffusion of caffeine in the case of three kinds of samples:

(1) paper impregnated in caffeine solution [Paper&Caffeine *white diamond*], (2) paper impregnated in caffeine solution and coated with five MFC coats [Paper&Caffeine with 5 MFC coats *grey triangle*] and, (3) paper coated five times with the slurry caffeine/MFC [Paper with 5(caffeine&MFC) coats *black square*]. The mass of caffeine released divided by the mass of caffeine previously introduced into the paper samples (%) is plotted as a function of the time of release (h). This experiment has been carried out on three specimens, at room temperature

Nevertheless, as no chemical grafts or hydrogen bonds are possible between paper or MFC, and caffeine, it is possible to test the hypothesis that all the caffeine within the samples is released

when the plateau is reached. For these two reasons, the results have also been expressed with the assumption that the maximal mass of caffeine released during the release process is equivalent to the whole mass introduced initially in the sample (Figure 3-1.8).

Indeed, considering the diffusion of the sample coated with the mixture (caffeine and MFC suspension), the release lasts longer and is slower. The curve indicates almost 100% caffeine release after 10 h compared to the previous 40 min for the two other samples (15 times longer duration). Its release is thus clearly more progressive without being blocked by the MFC. These results tend to prove that MFC forms a nanoporous network since (i) the caffeine can still be released and (ii) the release is much slower.

In other studies, however, this nanoporous MFC network was most of the time considered as a dense layer (Kolakovic, *et al.*, 2013; Cozzolino, *et al.*, 2013). In these previous works, indeed, a model based on the Fickian diffusion was established to understand the diffusion phenomenon of molecules released through a neat MFC films. In our case, when assuming a nanoporous MFC network, and consequently a swelling-controlled system (even for paper substrate), we can suppose that the molecule release is non-Fickian in nature. Frequently, the drug release in a swelling-controlled system is expressed by:

$$\frac{M_t}{M_\infty} = kt^n$$

Where $\frac{M_t}{M_\infty}$ is the fractional drug release, n is the diffusional exponent, and k is the pre-exponential factor (Huang, *et al.*, 2001).

Such representation of the caffeine release was performed so that a diffusion pattern can be deduced for each kind of our samples (graph not shown). For the first two paper samples impregnated in the caffeine solution, the molecule release seems to be dominated by the caffeine diffusion ($n \approx 0.5$). In the case of the paper samples coated with the caffeine/MFC mixture, however, the diffusional exponent was slightly higher than 0.5 presuming a possible anomalous diffusion, i.e. a process involving both the caffeine diffusion and the swelling of the MFC network. This last point still needs to be confirmed with further analyses to confirm the nanoporosity of the MFC network formed at the paper surface.

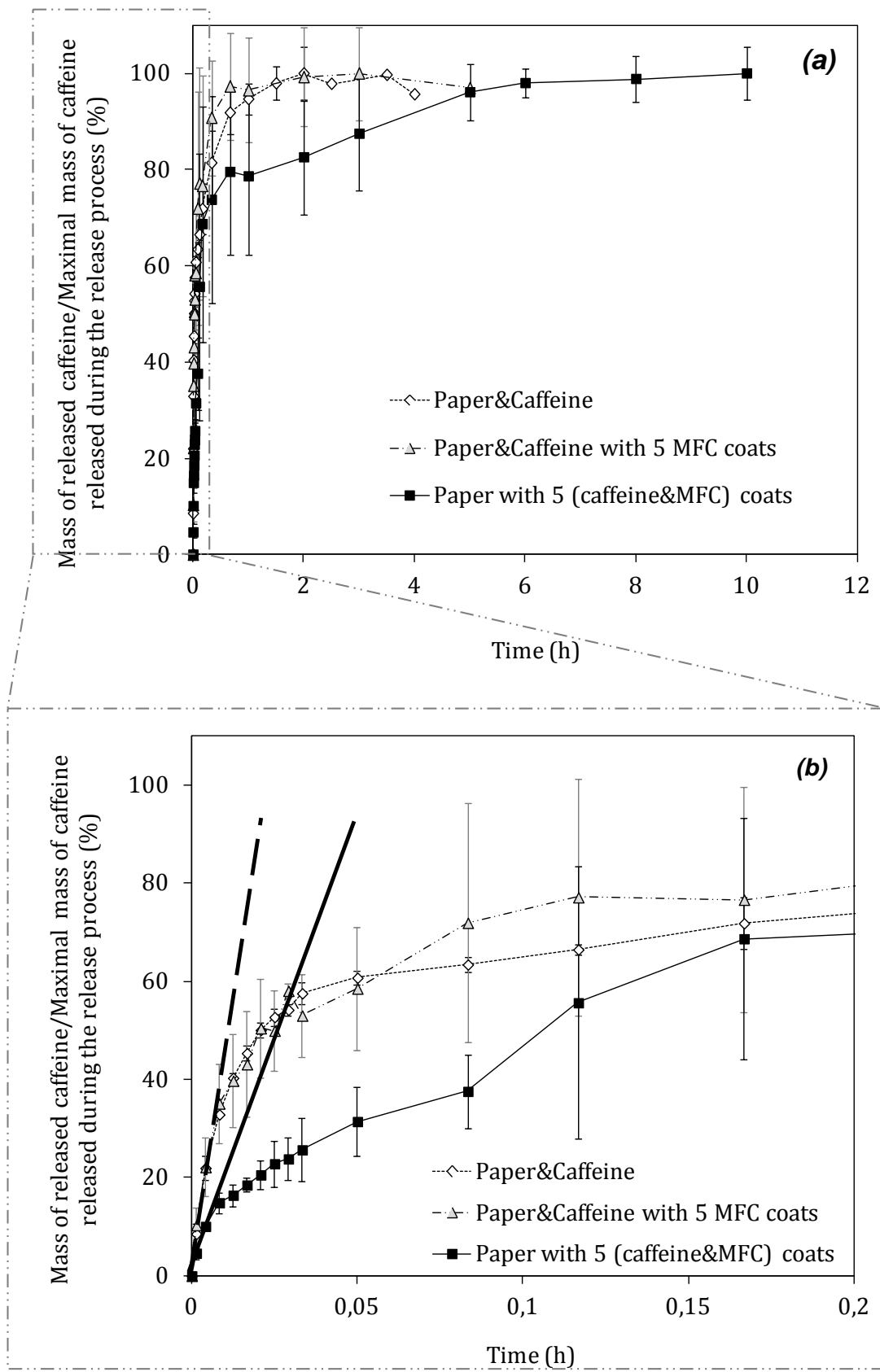


Figure 3-I.7. (a) Continuous diffusion of caffeine with the mass of caffeine released with time divided by the maximal quantity of caffeine released during the release process (plateau value) (%)
 (b) Zoom of the graph (a) on the gradient of each curve

It is, indeed, still difficult to exactly determine the diameter of the nanopores formed by the MFC-coating since the paper itself has its own specific porosity. However, considering the MFC layers alone without the paper substrate, we can compare them to a neat MFC film. The porosity of such MFC films has recently been studied and published for TEMPO-oxidized cellulose nanofibrils films (Fukuzumi, *et al.*, 2011). A diameter of 0.47 nm was determined using the PALS equipment. As already said, depending on MFC source and treatment, different types of MFC will have different properties and, consequently, will form different types of networks. In our study, we worked with enzymatically pre-treated MFC, which usually have larger fiber diameters than those of the TEMPO-oxidized MFC (ca. 40 nm vs. a few nm), and carry less negative charges. In an additional study about enzymatically pre-treated MFC films, we measured an average pore diameter of about 5 nm (results not shown). This value was determined by the mercury porosimetry method. A comparison of our value with that of (Fukuzumi, *et al.*, 2011) remains inconclusive since the method of determination is different, as well as the manufacturing process of the films. We can, however, consider these two values as an approximation of the range (maximum and minimum) of pore dimensions for comparison with the dimensions of the caffeine molecule. The size of the latter (0.376 nm (Rosenberg and Dan, 2010)) is lower than the porosity of the neat MFC films. However, the caffeine tends to crystallize, and according to Edwards G. M., *et al.*, 1997, the volume of one crystalline pattern of the caffeine hydrate is about 0.98 nm³. Considering that the pores of the nanoporous network are non-continuous, and approximating each pore to a sphere, the MFC pore volume ($V = 65 \text{ nm}^3$) is about 65 times bigger than the volume of the crystal lattice of caffeine in dried state. If caffeine can be released through dried pores, it is obvious that it will be released through wet – state materials, which have larger pores than in dry state. The release of caffeine is thus possible through the nanoporous network. Then, mixing the MFC and the caffeine jointly establish a tighter network due to both the entanglement of nanofibers and the caffeine that can then be trapped in these networks. Thus, the water can less easily open this network, which explains the longer and slower release of caffeine observed in the data. The gradient of the curve is indeed slightly lower than those of the two other samples, but there is also a two-phase phenomenon observed that can be associated with the two other curves. First, the caffeine that is not trapped by the entanglement is released at almost the same speed as that released from the reference sample (Burst effect (Raso *et al.*, 2010)). Then, the “trapped” caffeine is released. However, for the samples impregnated and then coated with MFC, no caffeine has been “trapped” by the entanglement of MFC, and a classical release due to nanoporosity is observed.

These continuous diffusion experiments highlight the ability of MFC to slow down the release of caffeine when it is directly introduced into the MFC network and then coated on

paper. This is in accordance with the results of a simultaneous study reported very recently (Kolakovic, *et al.*, 2012b), which deals with more concentrated 100% MFC films.

This study established for the first time a model describing the release behavior of drugs introduced into MFC films. They used the Higuchi model equation (1) supposing that diffusion is the only release mechanism:

$$(1) Q = A \sqrt{\frac{D\varepsilon}{\tau} (2\rho - \varepsilon C_s) C_s t}$$

where Q is the amount of drug released, A is the surface area of the film, D is the diffusion coefficient of the drug inside the film, ε is the porosity of the film, τ is the tortuosity of the film, ρ is the density of the drug material in the film, C_s is the saturated solubility of drug inside the film, and t is time.

This model fitted well to their experiments as they used hydrophobic drugs. Indeed, the Higuchi model is used for monolithic dispersions, i.e. when drug is homogeneously distributed within a matrix former at an initial concentration that exceeds drug solubility in the wetted system (Siepmann and Siepmann, 2011). Moreover, one other assumption of this model is that swelling or dissolution of the polymer carrier is negligible. The authors did not considered the possible swelling of MFC films in contact with water.

Although this approach could be used in our study, the two previous assumptions cannot be applied for our samples. Caffeine was indeed introduced at an initial concentration lower than its solubility. This assumption is the most important, because it provides the basis for the justification of the applied pseudo-steady state approach (basis of Higuchi model)(Siepmann and Peppas, 2012). Then, we cannot assume in our case that swelling of the matrix is negligible. Swelling of paper as well as MFC is a significant phenomenon, as it contributes the molecule release into aqueous medium.

It is also worth noting that this Higuchi model was applied considering the MFC films as a dense material. However, in the case of our study, the paper substrate as well as the MFC layer cannot be considered as dense materials (as explained previously). Indeed, the first studies on this topic demonstrated that the MFC film tends to be a porous substrate (Fukuzumi, *et al.*, 2011).

Nevertheless, this hypothesis can in a first step be taken into account to better understand the release mechanism. In this case, mathematical models based on the Fick diffusion can be applied. This was indeed done by a very recent study also dealing with the use of MFC films as carrier and delivery system of lysozyme (Cozzolino, *et al.*, 2013).

Although we could assume that the MFC coating is a MFC film in the present study, it seems however sensitive to only consider the Fick diffusion. We indeed tried previously to apply a classical law for swelling-controlled systems. Nevertheless, to our knowledge, this model was only applied for polymeric matrices, excluding cellulosic substrates.

Consequently, other models could be investigated and compared such as the Darcy/Poiseuille mechanisms or the Knudsen diffusion. These models are also developed for porous substrates, but usually consider diffusion of gases or vapors (Spence, *et al.*, 2011).

Many parameters have thus to be considered in the case of our substrates such as the presence of amorphous and crystalline parts in MFC, the porosity and the swelling of the MFC coating and of the paper substrates, which make it difficult to establish a fitted model. Modeling of molecules from delivery systems based on paper substrates and MFC is currently in progress and consider the all previous model introduced.

I-3.4. Intermittent diffusion experiments

The protocol of intermittent diffusion is generally used for the study of drug release in the pharmaceutical field (Cusola, *et al.*, 2012, Hoang Thi, *et al.*, 2010), It allows the determination of the quantity of drug released at the end of an exact period and measures the time taken for the release to occur. This protocol was also established to highlight the positive effect of MFC on the controlled release of caffeine. As described previously, the continuous diffusion was similar for paper samples impregnated in the caffeine solution and paper samples impregnated in caffeine and MFC-coated. Intermittent diffusion experiments will emphasize the prolonged release obtained using MFC.

In contrast to the usual period used (24 h), the protocol was adapted here because the release of the caffeine is more rapid. The measurement of the release was carried out every 10 min, and between each sampling point, the aqueous media was renewed to simulate saliva, or blood.

With the replacement of the aqueous media, the equilibrium of the system is shifting towards increased diffusion of caffeine in one direction and the molecules of caffeine are attracted by the new aqueous media, which accelerates their release. The intermittent diffusion is considered to be complete when the measured absorbance of the solution reaches the zero value. The released amount of caffeine is plotted as a function of the number of washing steps (Figure 3-I.8).

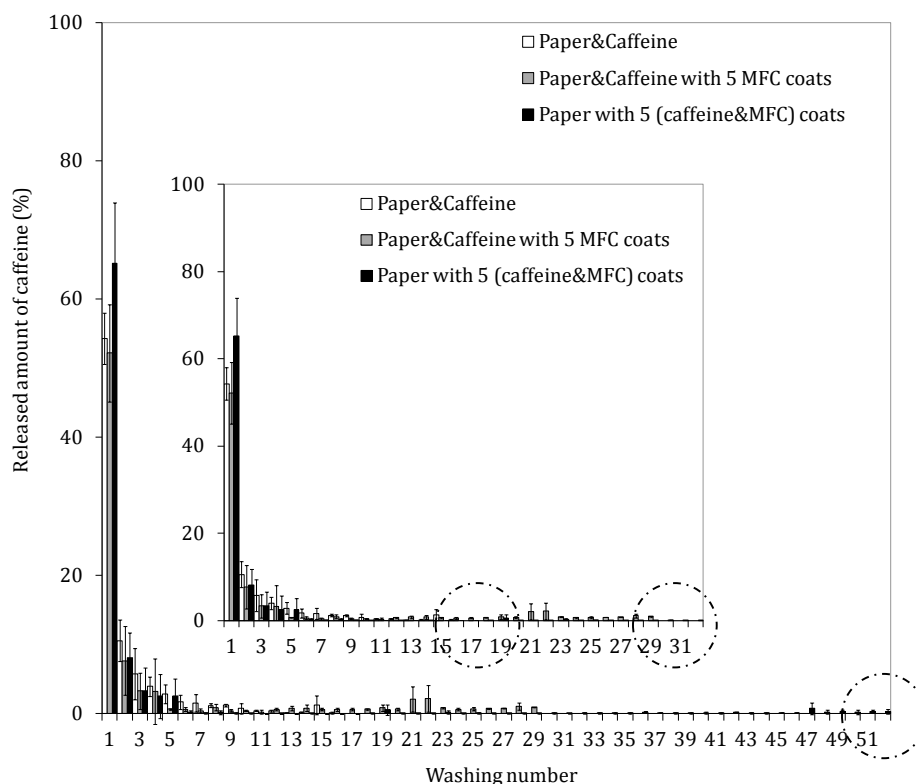


Figure 3-I.8. Intermittent diffusion of caffeine in the case of the three following samples: (1) Paper samples impregnated in the caffeine solution [Paper&Caffeine - *white*], (2) Paper samples impregnated in the caffeine solution and coated with 5 MFC coats [Paper&Caffeine with 5 MFC - *grey*] and, (3) Paper samples coated five times with the mixture caffeine/MFC [Paper with 5(caffeine&MFC) coats - *black*]. The graph plots the mass of caffeine released dividing by the total amount of caffeine introduced in paper samples.

In the paper impregnated in the caffeine solution, the release lasts 16 washings and about 90% of the caffeine was released after five washings (Figure 3-I.8). In the two other kinds of samples (papers impregnated in caffeine and coated with MFC and, papers coated with the mixture caffeine/MFC), the release lasts much longer. Paper samples impregnated in caffeine and MFC-coated released 87% of caffeine introduced during 29 washing steps. Contrary to continuous diffusion experiments, which showed a similar release kinetic for both samples, the intermittent diffusion experiments underline a more controlled and progressive caffeine release thanks to MFC.

The influence of the MFC coating was also highlighted by the Figure 3-I.9 representing the cumulative amount of caffeine released as a function of the number of washing step. In comparison with the paper impregnated in the caffeine solution, the samples coated with the MFC released more progressively the caffeine: the proportion of caffeine released between each washing step was indeed smaller than the proportion released by the samples without MFC. Consequently, the caffeine was more progressively released with a higher number of washing

steps owing to MFC, as confirmed in Figure 3-I.8. The Figure 3-I.9 also displayed the effect of the caffeine gradient. We concluded in the Figure 6 that the caffeine was released following a similar kinetic without and with the MFC coating. The graph (Fig. 3-I.9) confirmed this observation: when considering the gradient after the burst effect (i.e. after the first washing step), the gradients of the curves of the samples impregnated in the caffeine and then coated or not with MFC are similar.

In conclusion, the MFC coating did not slow down the release of caffeine, but controlled its release by releasing about 15 to 20% less than samples without MFC.

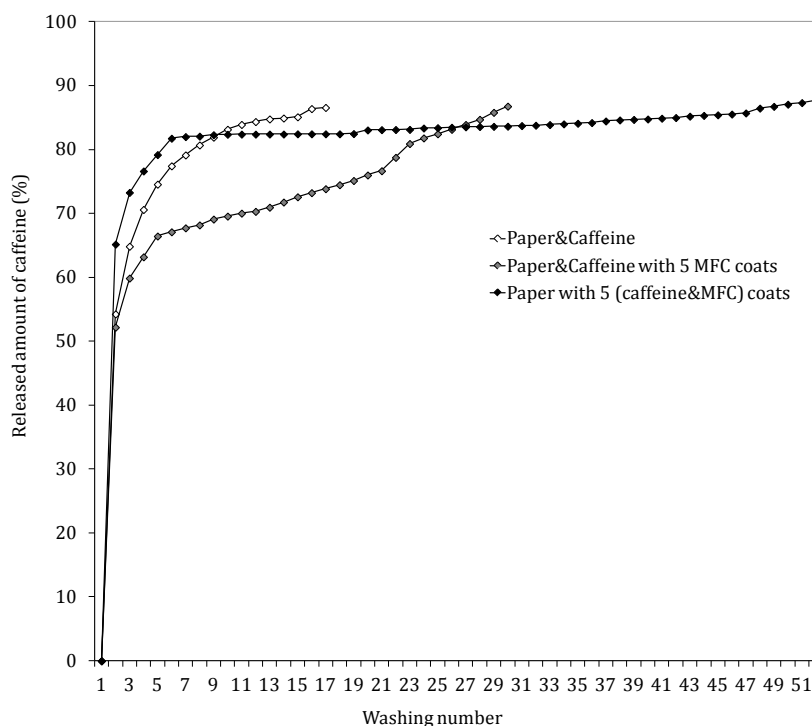


Figure 3-I.9. Cumulative amount of caffeine released (%) as a function of the number of washing step. In white, the paper impregnated in caffeine is represented and compared with the paper impregnated in the caffeine solution and then coated with the MFC (in grey) and with the paper coated with the caffeine/MFC mixture (in black).

Considering the release of samples coated with the mixture caffeine/MFC, the release lasts 52 washings, releasing 88% of caffeine (Figure 3-I.8). Here again, the method consisting in the coating of mixture caffeine/MFC is the most efficient as controlled release system. This confirms the continuous diffusion experiments and supports the hypothesis of a tighter network obtained with the caffeine/MFC mixture. The Figure 3-I.9 gathers the conclusions drawn from the Figures 6 and 7: the caffeine/MFC mixture slowed down the release of the caffeine and also released it more progressively. The gradient of the curves (Fig. 3-I.9) is indeed constant, and from a certain

number of washing steps (9 and 27 for the paper impregnated in the caffeine solution, and the same paper coated with MFC, respectively), the proportion of caffeine released is the smallest.

One explanation for the latter release behavior could be the effect of the coating technique used to apply the MFC suspension. The viscosity and fiber nanoscale dimensions of the MFC suspension have an effect on the quality of MFC coating achieved. However, during the bar-coating process, good homogeneity of the coating is not necessarily realized. Inspecting the SEM images of the coated papers' surfaces, which were obtained in the BSE mode (Figure 3-I.5), we suppose that systems with more than five MFC coats are perfectly coated. However, at a higher magnification, some minute areas that are not entirely covered by the MFC still exist. In general, when paper is impregnated in the caffeine solution and then coated with MFC, the caffeine can be released through (i) the MFC network but also more easily through (ii) these non-covered areas (Figure 3-I.10).

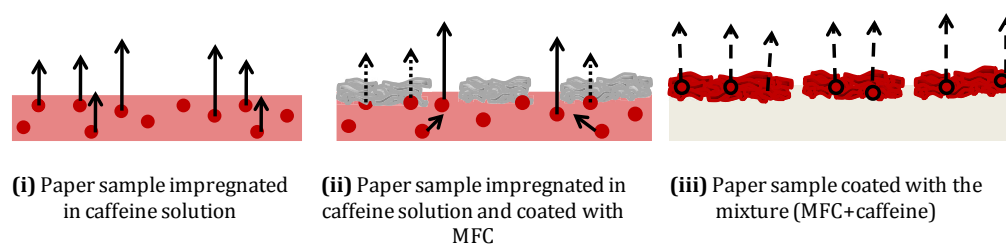
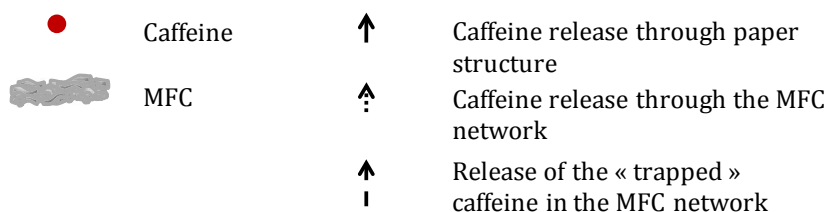


Figure 3-I.10. Scheme of diffusion of caffeine introduced in the three kinds of samples (i), (ii) and (iii). This scheme explains why the paper samples coated with the mixture caffeine/MFC release slower and longer caffeine than the two others.



By contrast, when the paper is coated with the mixture (caffeine and MFC), the caffeine has no other choice but to diffuse through the MFC network. Moreover, even if the coating is not homogeneous across the entire surface of a sample, the caffeine remains trapped in the MFC network and it will require a longer period to be released.

This study emphasizes the positive effect of employing MFC networks for the controlled release of caffeine. In particular, for paper impregnated with 5 MFC coats, the caffeine was released over a longer time (twice longer duration) than it was through paper without MFC.

Furthermore, by mixing MFC with caffeine, the release was prolonged of 23 washing steps, i.e. about 4 hours, and caffeine was entirely released over a longer time (15 times longer duration). These results clearly prove the concept that the nanoporous network formed by the MFC could be useful for the development of a more progressive and controlled release of active molecules when coated on cellulosic materials.

I-4. Conclusion

The use of MFC with cellulosic materials has recently begun to increase in importance. In this study, MFC is applied to obtain better mechanical properties. Specifically, they improve the air resistance of a base paper significantly, without using components other than the MFC suspension. This biobased material is already a high-performance material, but an added novelty of this study is in the idea of employing the nanoporous network, formed by the MFC during the coating process, to facilitate a controlled release of active molecules. Results obtained here demonstrate for the first time that MFC coatings effectively slow the release of active molecules.

Of the two different strategies studied for applying MFC, samples coated with the mixture (caffeine and MFC) showed better results than the samples that were firstly impregnated in the caffeine solution and then coated with MFC. This was due to the entanglement of nanofibers that clearly trapped diffusing species with molecular sizes as small as that of the model compound caffeine. It will be interesting to apply this solution to a final application.

In general, these initial results clearly create further research opportunities. For example, other tests could be performed with an active molecule, to demonstrate a controlled and progressive antimicrobial action for application in the food-packaging field.

Owing to a model molecule, we proved in this chapter that the nanoporous MFC network was able to retain and release progressively and slowly these molecules into water. Two different and complementary protocols were implemented for that purpose.

In the next chapter, the objective will be to test this concept with an antibacterial molecule, the chlorhexidine digluconate. Contrary to caffeine, these molecules have strong chemical interactions with cellulose, which will influence their release.

Controlled release and long-term antibacterial activity of chlorhexidine digluconate through the nanoporous network of microfibrillated cellulose

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Abstract

The recent study focusing on paper coated with microfibrillated cellulose (MFC) revealed the ability of such a structure to achieve a controlled release of molecules introduced into its nanoporous network. The present study examines this concept using a chlorhexidine digluconate-based (CHX) antibacterial solution. Various analyses were performed, optical microscopy, FE-SEM and AFM to underline the structure of the nanoporous MFC network. Release studies were conducted in an aqueous medium following two different protocols and antibacterial tests were done to evaluate the efficiency of the final materials obtained.

MFC coating provided a slower and more progressive release of CHX. Indeed, papers impregnated with CHX were active for 18 days, whereas papers coated with CHX/MFC retained their antibacterial activity for 45 days. In parallel, similar tests were carried using a model coating slurry, and although the rate of release of CHX was also slowed down, the quantities released were insufficient to confer any antibacterial activity. In conclusion, this study suggests that the use of MFC as a coating could be very promising since it allows a controlled and progressive release of molecules preserving long-term antibacterial activity.

Inspired from Paper 5:

Nathalie Lavoine, Isabelle Desloge, Cécile Sillard and Julien Bras (2013) Controlled release and long-term antibacterial activity of chlorhexidine digluconate through the nanoporous network of microfibrillated cellulose. Submitted to Applied Materials & Interfaces.

II-1. Introduction

Cellulose is considered to be the most abundant organic compound produced by biomass. Cellulose molecules are brought together into larger units known as elementary fibrils (Habibi, *et al.*, 2010) or microfibrils, which are joined to create larger units called microfibrillated cellulose. The diameter of microfibrillated cellulose, also named nanofibrillated cellulose (Siqueira, *et al.*, 2010), ranges from 20 to 50 nm, and length generally exceeds than 1 μm ; these measurements depend mainly on the origin of the MFC and on the production process.

Different processes for producing MFC currently exist, based on the first patents published in 1983 and 1985 by Herrick, *et al.*, 1983 and Turbak, *et al.*, 1985 respectively. Their approach consisted in degrading the cellulosic pulp several times in a high-pressure homogenizer to obtain a viscous and shear-thinning aqueous gel at a low concentration (between 2 and 7% w/w). Since then, the process has improved and primarily to reduce the energetic consumption linked to the mechanical treatment. Enzymatic pre-treatment (Pääkkö, *et al.*, 2007) or TEMPO oxidation (Isogai, *et al.*, 2011) of cellulosic fibers have thus been implemented. New tools for applying mechanical treatments have also been developed, such as the Microfluidizer® or the Masuko® grinder. The production of microfibrillated cellulose (MFC) has increased over the last fifteen years and its mass production is now possible leading to its commercialization; first reported in 2012 (Chauve and Bras, 2013).

The interest in using MFC dates back to its discovery, and has grown dramatically in the last five years as seen by the growing number of conferences, books (Dufresne, 2012) and reviews (Eichhorn, *et al.*, 2010, Lavoine, *et al.*, 2012, Missoum, *et al.*, 2013, Moon, *et al.*, 2011, Siró and Plackett, 2010). MFC has nanometric dimensions and can tightly entangle each other owing to their hydrogen bonds and high specific area. This entanglement can change, but is preserved no matter what physical or chemical modifications are applied (Aulin, *et al.*, 2010b, Fukuzumi, *et al.*, 2011, Minelli, *et al.*, 2010, Sehaqui, *et al.*, 2011a, Sehaqui, *et al.*, 2011b). Once dried, the MFC suspension produces a MFC “film”, i.e. a nanoporous network. These films display very good barrier properties (Aulin, *et al.*, 2010a), strong mechanical properties (Syverud and Stenius, 2009), but also a specific nanoporosity (Chinga-Carrasco and Syverud, 2010). MFC is, therefore, used in various applications: in nanocomposites for its mechanical reinforcement (Fujisawa, *et al.*, 2012, Shields, *et al.*, 2008, Zimmermann, *et al.*, 2010), as porous and light aerogels (Aulin, *et al.*, 2010b, Sehaqui, *et al.*, 2011a, Silva, *et al.*, 2012), as neat MFC films (Fukuzumi, *et al.*, 2009), and more recently, as coating for cellulosic materials (Aulin, *et al.*, 2010a, Hamada, *et al.*, 2010, Lavoine, *et al.*, 2013b, Ridgway and Gane, 2012).

The use of MFC with paper/cardboard is relatively recent and was the subject of many investigations since the study by Syverud and Stenius, 2009, which underlined the positive effect of MFC on the mechanical and barrier properties of handsheet paper. Another positive effect of

MFC coating of paper is the improvement of the printing quality (Hamada, *et al.*, 2010, Luu T, *et al.*, 2011). Moreover, the addition of MFC layer to other typical slurry onto paper helps to decrease the weight of the final material, improve its properties, especially its smoothness and uniformity (Ridgway and Gane, 2012). Until recently, however, the use of MFC with paper/cardboard essentially targeted printing applications (Ankerfors, *et al.*, 2009, Hamada, *et al.*, 2010, Hamada and Bousfield W, 2010, Luu T, *et al.*, 2011) and barrier materials (Aulin, *et al.*, 2010a, Hult, *et al.*, 2010, Lavoine, *et al.*, 2012, Syverud and Stenius, 2009), and novel application such as functional substrates (Martins, *et al.*, 2012) and high-added value uses, are now being targeted.

Indeed, the use of MFC as a delivery system or a drug carrier is at preliminary stage and since 2011, less than 10 scientific journals and conferences were published dealing with this thematic. The first research group used MFC either as a stabilizer of nanoparticles (Valo, *et al.*, 2011) or as drug carrier in aerogel (Valo, *et al.*, 2013). In their first paper (Valo, *et al.*, 2011), two drug nanoparticles were immobilized to MFC by mixing in water. The authors highlighted the ability of MFC to stabilize nanoparticles properly and prevent them from aggregating. However, the MFC did not prevent the drug release from bound nanoparticles in spite of stronger binding to cellulose microfibrils. In their second paper, Valo, *et al.*, 2013 prepared MFC aerogels from various sources. They introduced and studied the release of a model drug. They observed that aerogels made from cellulose powder released the drug immediately, whereas the aerogels made from nanocelluloses (TEMPO-oxidized nanocellulose) showed sustained drug release (Figure 3-II.1).

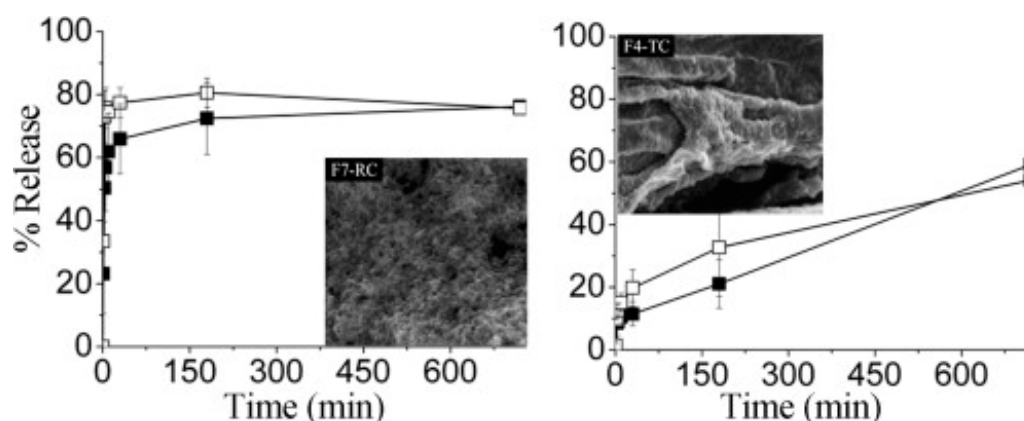


Figure 3-II.1. Comparison of drugs release loaded into aerogels made from red pepper (RC - left) and from TEMPO-oxidized MFC (TC - right) (from Valo, *et al.*, 2013)

The second research group (Kolakovic, 2013) very recently evaluated the potential of MFC to be used as an excipient in the production of pharmaceutical dosage forms, especially as an excipient for immediate release tablets and compressions (Kolakovic, *et al.*, 2011), and for

providing sustained drug release profiles from microparticles (Kolakovic, *et al.*, 2012a) and film-type controlled release systems (Kolakovic, *et al.*, 2012b). The drugs (indomethacin, nadolol, atenolol, metoprolol tartrate, verapamil hydrochloride and ibuprofen) were initially entrapped in the nanoporous network formed by the MFC using a spray-drying process. This tight MFC network sustained the release of the drugs for up to two months. Nevertheless, the spray-drying process includes several drawbacks with regards to the elaboration of drug delivery systems: (i) it is limited to water soluble compounds and (ii) it has low loadings due to the different drying kinetics of drugs and MFC. Taking these results into consideration, Kolakovic, *et al.*, 2012b produced MFC films loaded with indomethacin (IND), itraconazole (ITR) or beclomethasone dipropionate (BE) using a filtration method. Interestingly, the drugs did not affect the morphology of the films and their structure remained unchanged once the drugs had been released (Figure 3-II.2).

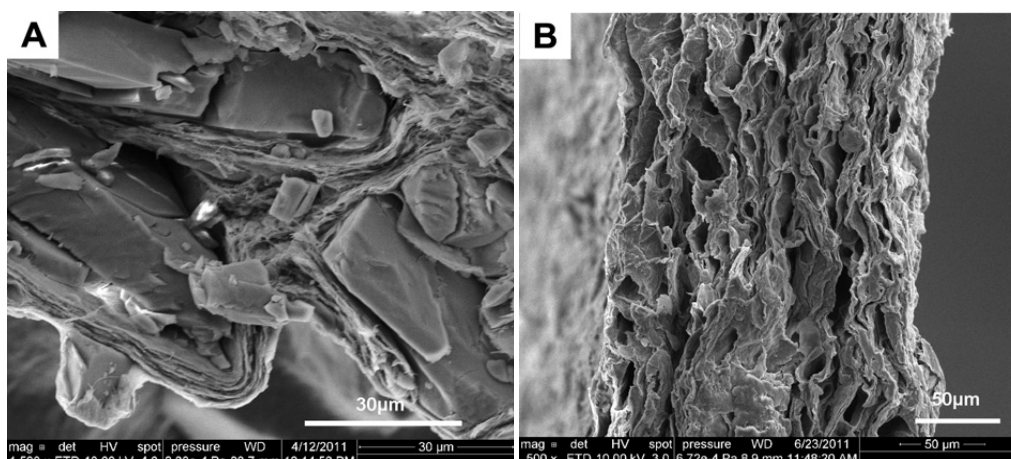


Figure 3-II.2. SEM Images of a cross-section of a matrix containing 40% of indomethacin before (A) and after (B) the drug release test. (from Kolakovic, *et al.*, 2013b)

Each drug showed a different release, either a diffusion limited release (IND) or a zero-order release kinetics (ITR and BE). This was a consequence of the different level of drug solubility in the dissolution medium, as well as the different level of drug binding to MFC. Nevertheless, MFC films provided a sustained drug release, for each drug, even up to 90 days.

These previous studies published only months ago demonstrated for the first time that MFC is a highly interesting new material for controlled release applications. They also highlighted the fact that it necessary to understand the interactions between drugs and the MFC matrix since they influenced the release of the drug concerned. However, both research groups conducted their release studies on MFC aerogels, particles or films loaded with drugs. Either group used the thin MFC nanoporous network formed during its coating onto papers. A preliminary study submitted by our group focused on this aspect and proved that the MFC

coating induced a slower and longer release period of a model molecule in an aqueous medium (Lavoine, *et al.*, 2013a). The studied molecule was caffeine, since it is often used as drug simulator (Efentakis, *et al.*, 2007, Nicoli, *et al.*, 2005, Sriamornsak and Kennedy, 2007). Besides, the fact that this molecule is highly water soluble and that it does not chemically interact with MFC made it an ideal model for implementing the protocols investigated in this study and for demonstrating the positive effect of the MFC network on caffeine release. The duration of the molecule release was, indeed, extended to 10 h compared to 40 min for samples devoid of MFC (Lavoine, *et al.*, 2013a).

The following study is based on a similar principle to that described previously (Lavoine, *et al.*, 2013a), but aims to apply that approach to an antibacterial molecule. The molecule tested herein is the chlorhexidine digluconate aqueous solution (CHX), which is often used as antibacterial agent for medical applications (Houston, *et al.*, 2002, Jeansonne and White, 1994, Şenel, *et al.*, 2000). The efficacy of CHX as antibacterial agent is mainly due to its cationic quaternary azote. The mechanism of action of CHX is as follows: two symmetrically positioned p-chlorophenyl guanide groups can penetrate through the cellular wall of bacteria and irreversibly disrupt the bacterial membrane, thus killing the microorganism (Giménez-Martín, *et al.*, 2009). Furthermore, contrary to caffeine, this molecule should interact with the nanocelluloses, and this might alter its release behavior.

The release study of this molecule included in paper substrates without coating or coated with MFC was analyzed following two protocols: (i) continuous diffusion, which is a release kinetic in an aqueous medium and (ii) intermittent diffusion, during which the aqueous release medium is renewed after every sampling. The effect of the MFC on the molecule release-pattern behavior was thus studied in a liquid aqueous medium and compared with the effect of a model coating slurry. Then, the sustained antibacterial activity of each sample was proved using successive diffusion disc tests into agar.

II-2. Materials and methods

II-2.1. Materials

The microfibrillated cellulose (MFC) was supplied and produced by FCBA (France). Domsjö pulp underwent an enzymatic pre-treatment with an endoglucanase for 2 h, and subsequently underwent a mechanical treatment using an Ariete® homogenizer (GEA Nivo Soavi, Italy) (4 passes at 1,400 bars). The final concentration of the MFC suspension was 2% w/w.

Styrene-butadiene latex (50% w/w) was used as model slurry for comparing with MFC coating.

The base paper material used had a basis weight of 41 g/m². It was calendered and made from non-bleached pulp.

Chlorhexidine digluconate (CHX) (CAS N°18472-51-0) was purchased from Sigma-Aldrich in a 20% aqueous solution. The solution was diluted 10 times with deionized water in order to be used as an antibacterial model agent. It is detectable by UV-Vis at 254 nm.

A suspension of *Bacillus Subtilis* (10⁷ spores/mL) was purchased from Humeau (France) and used as test microorganism to evaluate the antibacterial activity of chlorhexidine digluconate. A nutrient agar adapted to the development of the spores was also purchased from Humeau (France). The agar powder was dissolved in deionized water to reach a final concentration of 28 g/L and boiled to obtain a clear nutrient agar solution. Petri dishes with a diameter of 90 mm were purchased from Humeau (France) and used for the antibacterial tests.

II-2.2. Methods

Characterization of the MFC nanoporous network

Field Emission Gun Scanning Electron Microscopy (FE-SEM) images of the MFC suspension were taken using a Zeiss® Ultra-55 (France). The MFC suspension was spread onto a metal substrate using a carbon tape, allowed to dry for 48 h at room temperature, and was coated with a thin layer of gold. The working distance used with the FE-SEM was 5.5 mm for an accelerating voltage of 2.00 kV at a magnification of ×20.00K. The diameter of the MFC was then determined by image analysis using the ImageJ® software, based on at least 50 measurements.

The homogeneity of the suspension was confirmed using an optical microscope (Axio Imager M1m Zeiss, France) linked to an AxioCam MRc5 camera. The MFC suspension was diluted to 0.1% in deionized water and mechanically stirred with an Ultra-Turax (France). It was observed at a ×5 magnification applying a multidimensional acquisition with the AxioVision software.

The MFC-coated sample surfaces were observed using a Dimension Icon with Scan Analyst (Bruker, USA) Atomic Force Microscope (AFM). Smaller pieces (5×5 mm²) of MFC-coated papers were glued onto aluminium sample holders and scanned by tapping-mode using a standard silica cantilever (Bruker, USA).

The preparation of paper samples for release studies

The tested samples were prepared following three different methods (Figure 3-II.3a):

The **first method** (i) was the elaboration of the reference sample by impregnation of paper samples in the 2% CHX solution over 20 minutes. The final concentration of CHX

introduced was calculated using the difference in mass between the dried samples before and after the impregnation. High accurate scales and optimal sample dimensions ($10 \times 10 \text{ cm}^2$) were used to limit standard deviation values. The samples were dried between two aluminium foils in an oven for 1 h at 105°C , and weighed with the same Lhomargy scale ($\pm 0.001 \text{ g}$).

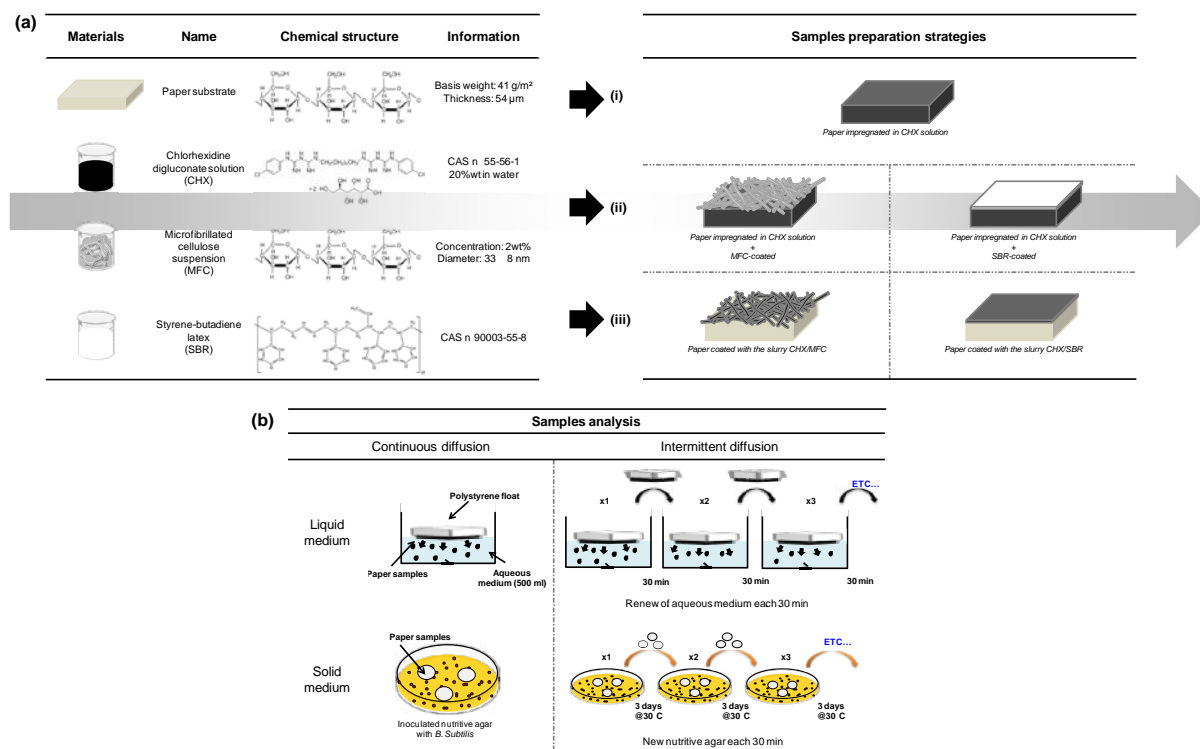


Figure 3-II.3. Methods for studying the controlled release and long-term antibacterial activity of CHX through the nanoporous network of MFC.

(a) Details of the materials used to prepare each sample (*left*) and description of the different samples prepared following three strategies (i, ii and iii) (*right*).

(b) Analysis of the samples prepared: continuous and intermittent diffusions in liquid (deionized water) and solid (agar) media

The **second method** (ii) involved the impregnation of reference samples according to the method described above (i), followed by the successive coating with five layers of MFC. The MFC suspension was coated onto the dried impregnated paper samples using a bar coating process. A 0.9 Mayer bar was used with a coating speed of 5 cm/s . The coated paper substrates were then dried using a contact drying system at 105°C for 3 min. To ensure a homogeneous coating and a total coverage of the surface of the paper, five successive MFC layers were deposited onto paper samples as previously described in Lavoine, *et al.*, 2013a. The coat weight of the MFC reached 7 g/m^2 . The final concentration of CHX was determined as described previously (i).

The **final method** (iii) (Figure 3-II.3a) was based on the coating of paper samples with the MFC suspension, in which the CHX solution was previously introduced. The slurry was composed of 10% of the 20% CHX solution and 90% of the 2% MFC suspension. It was homogeneously stirred with a mechanical stirrer for 15 min. The samples were coated five times with this slurry. The coat weight of the coated samples was also determined using the difference in mass between the dried samples before and after coating. The exact quantity of CHX coated onto paper samples was calculated based on the initial quantity included in the slurry. Indeed, the relative proportion of the CHX solution and the MFC suspension in the slurry before coating was known and we assumed that the coating slurry remained homogeneous during the coating process. The percentage of CHX in the slurry before coating was, thus, the same that the percentage of CHX in the coated layers. The final concentration of CHX coated onto the samples was then calculated from the percentage of CHX solution initially introduced into the slurry and from the dried coat weight of the samples. The determination of the exact quantity of CHX introduced or coated onto paper samples was also confirmed by conducting an elemental analysis test detailed later.

The methods (ii) and (iii) were also repeated with the model slurry. Styrene-butadiene latex (50% w/w) was coated onto paper samples to reach a latex coat weight of about 7 g/m². Two coats were necessary to reach this quantity. For the method (iii), the CHX solution was introduced into the latex following the same proportions as described previously with the MFC suspension.

Molecule release protocols

Two release protocols were implemented in order to study the release of CHX in an aqueous medium (Lavoine, *et al.*, 2013a) (Figure 3-II.3b).

These tests were carried out at least three times for each kind of sample (preparation described above) and under sink conditions. Samples of 10 × 10 cm² were stuck to a light expanded polystyrene (PS) support using scotch tape so as to study only the release of CHX from the samples surface. The whole perimeter of the samples was taped.

Two methods were followed as described in Figure 1b:

- *Continuous diffusion experiments*

The release pattern was studied in an aqueous bath of 500 mL that was continuously stirred with a magnetic stirring-bar. The samples were placed at the surface of the aqueous medium. At successive intervals, 3 mL of the solution were taken by conserving sink conditions. The absorbance of the solution absorbance was analyzed using a UV spectrophotometer SHIMADZU

UV1800 at a wavelength of 254 nm. Using a calibration curve, the concentration of CHX released was then determined as a function of time.

- *Intermittent diffusion experiments*

This protocol was similar to the continuous diffusion protocol, except that between each 30 min sampling step, the aqueous medium was renewed.

Antibacterial tests

The antibacterial tests were conducted following the standard EN 1104 procedure (AFNOR, 2005). The agar diffusion test consisted in placing the samples to be tested (previously cut into circles of 16 mm in diameter) onto a nutrient agar inoculated with bacteria. The nutrient agar was previously inoculated with *Bacillus Subtilis* to reach a final concentration of 10^4 spores/mL. This solution was poured into Petri dishes and the antibacterial paper samples were placed onto it before cooling. Each Petri dish was maintained in the fridge for 2 h and was, subsequently stored at 30°C for an incubation period of 3 days. Each test was repeated three times.

Three types of control Petri dishes were also prepared to evaluate and confirm the results namely using: (i) non-inoculated agar, (ii) inoculated agar and (iii) inoculated agar with Penicillin discs, which is a well-known *Bacillus Subtilis*-specific antibiotic.

In the event of any antibacterial activity, the Petri dish will present inhibition zones around each sample; the larger the diameter of the inhibition zone, the more efficient the antibacterial activity. For each test, the diameter of the inhibition zone was, thus, measured with a ruler (± 0.5 mm). At least five diameter measurements were performed for each one of the three specimens per Petri dish. An average value was then calculated.

Intermittent antibacterial tests

This protocol was similar to the previous one, except that at the end of each antibacterial test (according to the standard EN 1104), the paper samples were taken out and placed onto new Petri dishes with freshly inoculated agar for a new antibacterial test (Figure 3-II.3b).

This protocol was implemented in order to conclude on a long-term antibacterial activity of paper samples containing CHX, and either without coating or coated with MFC. The antibacterial activity of paper samples was, thus, followed every three days until the inhibition zones could no longer be measured.

Presence and characterization of CHX

A paper sample impregnated in the CHX solution was successively washed with condensed water using a soxhlet extractor system for 24 h. This sample was then dried (in an oven, at 105°C, for 1 h) and analyzed using an elemental analysis, which gave the percentage of Cl-atoms remaining. The elemental analysis was performed by the Service Central d'Analyse of the CNRS (Solaize, France). The percentage of Cl-atoms detected was then linked to the CHX molecule in order to calculate the percentage of CHX remaining into paper samples.

II-3. Results and discussion

II-3.1. The nanoporous MFC network

The MFC suspension used in this study looks like a white and homogeneous gel at a concentration of 2% w/w. Figure 3-II.4a shows an optical microscopy image of the diluted suspension. The size distribution of microfibrils is quite homogeneous. Nevertheless, even diluted, the MFC tends to form agglomerates.

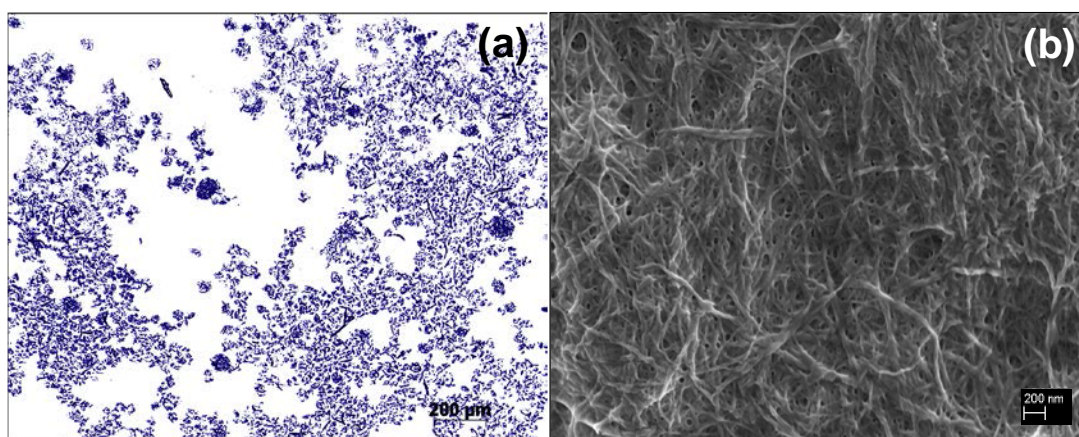


Figure 3-II.4. (a) Optical microscopy image of the MFC suspension diluted to 0.1%wt (Multidimensional acquisition - Mag. $\times 5$). (b) FE-SEM image of the dried 2%wt MFC suspension (WD: 5.3 mm, Mag.: $\times 20.00$ k)

The FE-SEM image (Figure 3-II.4b) of this dried suspension highlights the tight entanglement of microfibrils, which makes it impossible to measure their length accurately. According to the literature (Ishii, *et al.*, 2011, Siró and Plackett, 2010), the estimated length of MFC extracted from wood exceeds 1 μm , but is not measurable at low a magnification. An estimation of the diameter was nevertheless considered using image analysis. A mean diameter of 33 ± 8 nm was determined after analyzing the FE-SEM image of this MFC suspension. This value corroborates those described in the literature (Henriksson, *et al.*, 2007, Pääkkö, *et al.*, 2007).

The MFC suspension could directly be coated onto paper substrates without any prior dilution. However, five MFC coats were needed to achieve a homogeneous coverage of the surface of the paper. This was also noted by a previous study, which detailed on the impact of such a MFC coating on the properties of the end-use paper (Lavoine, *et al.*, 2013b). Indeed, MFC coating limited the decrease of mechanical properties due to successive wetting/drying steps, and strongly decreased the air permeability. These improvements in the mechanical and barrier properties are in accordance with the literature and, highlight the favorable application of MFC in the food-packaging industry.

The use of MFC as a molecule delivery system was highlighted very recently (Cozzolino, *et al.*, 2013, Kolakovic, 2013). These results were probably due to the nanoporous MFC network. In the current study, the nanoporous network formed by the MFC during its coating was underlined. Indeed, AFM images of the MFC-coated paper surface were performed to point out the nanoporous network of MFC formed during the coating process. Figure 3-II.5 shows the phase image of the coated paper surface with five MFC layers. The microfibrils are clearly distinguished as well as their entanglement. They do not follow any specific direction, which could have been induced by the coating process.

MFC coated onto paper thus forms a nanoporous network, which was subsequently tested as a controlled delivery system.

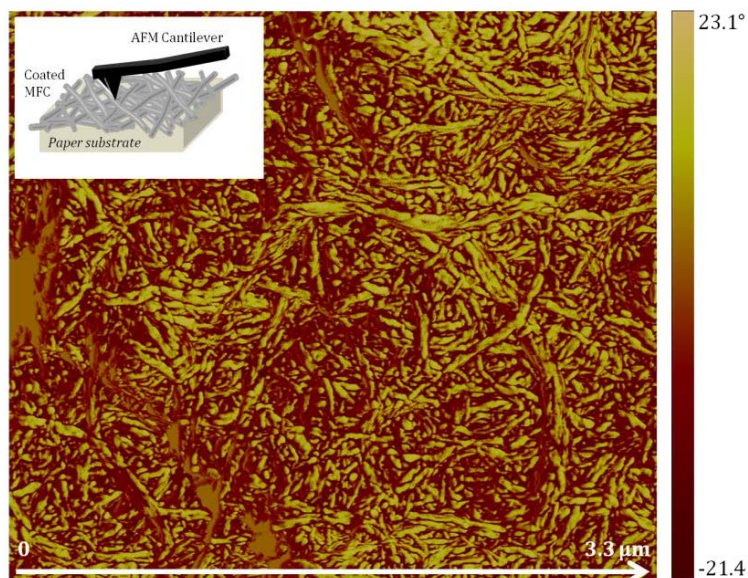


Figure 3-II.5. AFM phase image of the MFC-coated paper samples (Scheme of principle in the upper-left corner). The analysis was performed using a $3.3 \mu\text{m}^2$ sample surface.

II-3.2. Release study of CHX through MFC coating

Effect of MFC coating – Continuous diffusion

As illustrated in Figure 3-II.3b, the continuous diffusion was performed to compare (i) paper samples impregnated in the CHX solution and (ii) paper samples impregnated in the CHX solution and coated with MFC.

Figure 3-II.6a shows the amount (%) of CHX released as a function of time (h). The amount of CHX released is the quantity of CHX released (g) divided by the exact quantity of CHX introduced into paper samples before performing the release tests (g). The exact quantity of CHX was measured following two methods: (i) weighing and (ii) elemental analysis.

For each sample, a mean Cl-atom amount of 1% was determined (1.19% and 0.80% ($\pm 0.1\%$) for samples (i) and (ii), respectively) using the elemental analysis. Taking into account the sample masses and the molar mass of CHX, the exact quantity of CHX introduced into samples was calculated. Compared to weighed mass, values only differ from about 15%, which is convenient considering the standard deviations of release study. The weighing method was thus supported by the elemental analysis. Further results will be expressed as a function of the amount of CHX weighed.

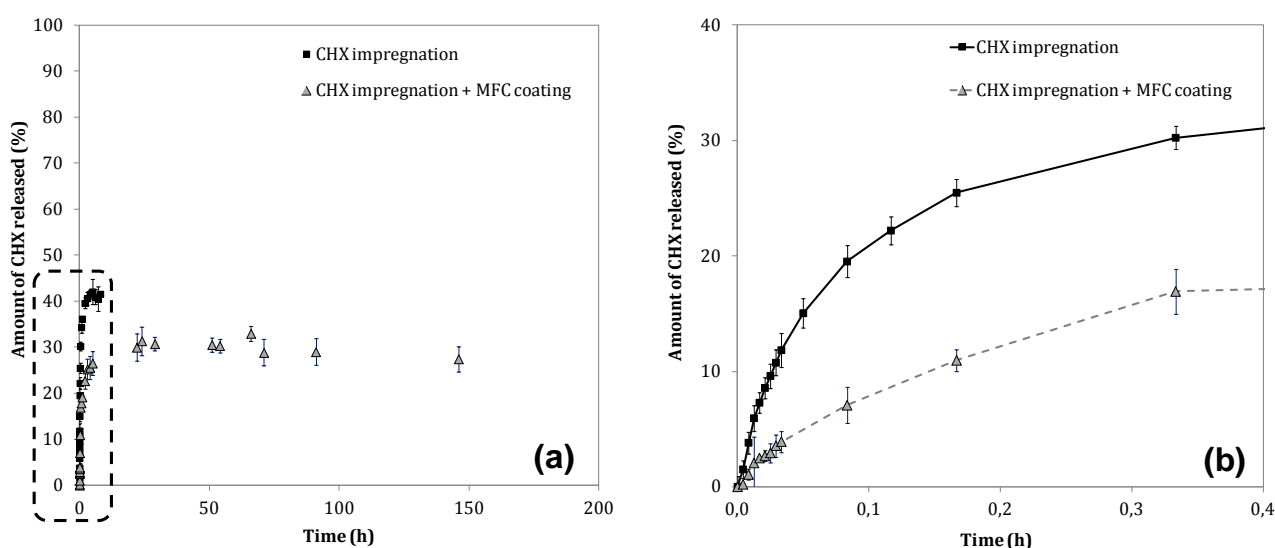


Figure 3-II.6. Continuous diffusion of (i) paper samples impregnated in the CHX solution (black squares), (ii) paper impregnated in the CHX solution and coated with MFC (gray triangles)

The graph (a) shows the amount of CHX (%) released as a function of time (h). The graph (b) represents the continuous diffusion of CHX the first 30 minutes for highlighting the difference between each gradient of curves.

When considering Figure 3-II.6a, each sample reached a plateau value at a specific time, which is the maximum quantity of CHX released during the diffusion process. Paper samples impregnated in the CHX solution reached this value after 3 h, whereas the samples coated with

MFC required 24 h to release their maximum quantity of CHX. The presence of MFC thus induced a more progressive release of CHX. Moreover, considering the beginning of the curves (Figure 3-II.6b), the gradient of each curve clearly showed a slower release of CHX in presence of MFC. Indeed, a release kinetic of 0.11 g/s was calculated for the paper samples impregnated in the CHX whereas a kinetic release value of 0.036 g/s was obtained for samples impregnated in the CHX and coated with MFC. The MFC coating increases the CHX release time, threefold. This result is very promising, since the MFC network decreases the release kinetic of CHX, but does not prevent it from being released contrary to latex coating (detailed later).

According to the quantification of the CHX released, however, the plateau values did not reach 100%. Indeed, paper samples impregnated in the CHX solution only released 40% of CHX and similar samples coated with MFC reached only 30% of CHX. This could be due to the potential interactions between the CHX and the MFC network. In two previous studies (Blackburn, *et al.*, 2007, Giménez-Martín, *et al.*, 2009), the authors proved the possible adsorption of CHX onto cellulosic fibers. At a low concentration, the mechanism seems to be governed primarily by electrostatic forces between the cationic groups of the CHX and the carboxyl acid groups of cellulose fibers. Hydrogen bonding between the biguanide group and the *p*-chlorophenol of the CHX with hydroxyl groups of cellulose could also be responsible for increasing the adsorption. Due to the high specific area (at least 10× higher than cellulosic fibers) and the nanometric scale of the MFC, the number of hydrogen bonds between CHX and MFC will be more abundant than the number of bonds occurring with the paper samples. Moreover, in the case of our study, paper samples were impregnated in the CHX solution and then dried for a long period of time. The drying process could also lead to the covalent grafting of CHX molecules onto cellulose.

These interactions were highlighted by two experiments. A paper sample impregnated in the CHX solution was washed using a soxhlet extractor for 24 h. The results of the elemental analysis conducted on this sample proved that 0.46% of Cl-atoms were still present in the washed paper samples (against 1.19% before washing).

Thus, in comparison with the release study of caffeine⁴¹, the plateau values cannot be linked to the total amount of CHX introduced into the paper samples. It would be inaccurate to normalize each release value to this plateau value.

Another type of sample was also studied: paper samples coated with a slurry containing CHX/MFC (strategy (iii) Figure 3-II.3a). Similar trends were observed as seen previously, and the main results are gathered in Table 3-II.1.

Surprisingly, the paper samples coated with the slurry containing CHX/MFC released the CHX at almost the same rate as paper samples impregnated in CHX. A release kinetic of 0.15 g/s was indeed calculated. This observation differs from our first experiments carried out with a model molecule. In our previous study (Lavoine, *et al.*, 2013a), the paper samples coated with the slurry containing caffeine/MFC were indeed the most efficient in terms of controlled delivery system. Here, the continuous diffusion of paper samples coated with the slurry is identical to the diffusion of paper samples impregnated in the CHX solution only. However, CHX/MFC-coated samples reached the plateau value much later, i.e. 22 hours later. This could be due to the quantity of CHX molecules introduced in the MFC/CHX suspension. This latter was indeed more than twice the quantity of dried MFC in the slurry. Moreover, the fast preliminary release could be considered as a kind of burst effect observed in some prolonged release systems⁵⁴. Since the links between CHX and MFC are numerous, we can presume that the MFC network is less tight, since the MFC/MFC links have been replaced by the CHX/MFC links. During the continuous diffusion experiments, the excess of CHX molecules was released first. The molecules trapped in the MFC network and not grafted were then released progressively, which might explained why the plateau value was reached later.

Samples	Continuous diffusion			Intermittent diffusion		
	Kinetic (g/s)	Time to achieve plateau value (h)	Total number of washing steps	Released amount of CHX during 1 st washing (%)	Released amount of CHX during 2 nd washing (%)	Total released amount (%)
Impregnated in CHX	0.11	2 ± 0.6	18 ± 2	35 ± 5	5 ± 1	50
Impregnated in CHX and MFC-coated	0.036	19 ± 3	28 ± 2	25 ± 5	4 ± 2	65
Coated with (CHX/MFC)	0.15	24 ± 0.5	23 ± 2	34 ± 3	8 ± 2	53
Impregnated in CHX and Latex-coated	-	288 ± 0	> 35	2 ± 0	2 ± 0	15
Coated with (CHX/Latex)	-	336 ± 0	> 35	5 ± 0	4 ± 0	81

Table3-II.1. Release study of paper samples with CHX coated either with MFC or latex.

Data include a comparison between their kinetics (g/s), the required time to release the whole amount of CHX (h) during the continuous diffusion experiments, the total number of washing steps required to achieve a complete release of CHX during intermittent diffusion experiments, the quantity of CHX released during the 1st and 2nd washing steps and the total amount of CHX released at the end of the intermittent diffusion experiments

Intermittent diffusion

The continuous diffusion experiments revealed a positive correlation between the MFC coating of substrates and the CHX released. This effect was also highlighted with the results of the intermittent diffusion. Table 3-II.1 presents the main results obtained for each sample following these experiments: the total number of washing steps (i.e. water renewal every 30 min) required to release the whole quantity of CHX, the released amount of CHX after the first and second washing steps and the whole quantity of CHX released at the end of the diffusion experiments.

Paper samples impregnated in CHX and coated with MFC released CHX over 28 washes, whereas paper samples without MFC no longer released CHX after 18 washes. The MFC coating, thus, induced a slower and more progressive release of CHX in water. Paper samples coated with the slurry CHX/MFC released CHX over 23 washings, and therefore released CHX over a longer period than paper samples without MFC. This also clearly proves a positive correlation between the MFC coating and the CHX released. These results of the intermittent diffusion experiments confirm the previous hypothesis: an initial release of the CHX molecules present in excess, followed by the release of CHX trapped in the MFC network and not grafted.

It confirms the previous conclusions drawn with the continuous diffusion experiments using caffeine (Lavoine, *et al.*, 2013a). This is also consistent with the only scientific paper on the topic (Kolakovic, *et al.*, 2012b), studying MFC films as drug delivery system. In their case, they used thick MFC films in the range of 150-200 μm .

Nevertheless, in spite of a positive effect of MFC on the release of CHX, the first washing step leads to a significant reduction in CHX release, since about 35% of CHX is lost. This is also supported by the continuous diffusion experiments. A phenomenon of burst effect is clearly underlined, since the release of CHX is more rapid at early stage.

The role of MFC on the release of CHX is well highlighted: the presence of MFC, as coating slurry or mixed with CHX, allows for a slower and longer release of the molecule. Due to the interactions observed between CHX and cellulose, the best solution for a producing controlled delivery system is paper samples impregnated in CHX and coated with MFC.

The use of MFC as controlled delivery system shows its relevance compared to classical coating slurry. Its environmentally-friendly aspect, its biodegradability, as well as its lightweight are numerous advantages which are added to its ability to release a molecule slowly and more progressively. A further understanding of the release process is under investigation to establish a diffusion model following the Fick diffusion.

Based on different hypotheses, models of Higuchi (Kolakovic, *et al.*, 2012b) or adapted to monolithic systems (Siepmann and Siepmann, 2011) can be investigated.

Release study comparison with classical coating slurry

To verify the positive impact of the MFC network on the release of CHX, similar tests were carried out using a model coating slurry. The standard latex (styrene-butadiene) used in papermaking industries was used as model and coated onto paper samples following the same methods implemented for MFC coating.

Continuous and intermittent diffusion results of paper samples impregnated in CHX and coated with latex as well as samples coated with the slurry CHX/latex were compared with the release study results obtained using the previous paper samples coated with MFC (Table 3-II1 and Figure 3-II.7).

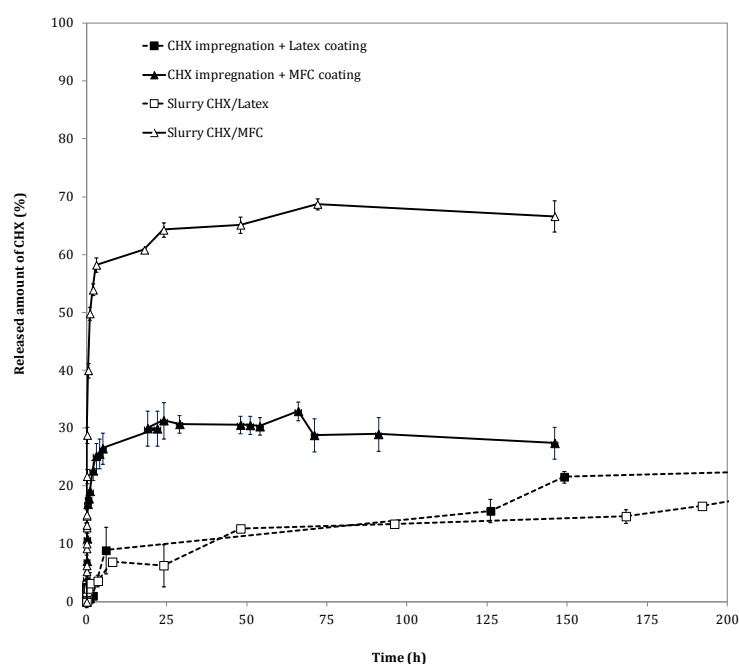


Figure 3-II.7. Continuous diffusion of paper samples coated with MFC vs. paper samples coated with latex

With latex (represented by white/black squares), the release of CHX lasted longer and reached the plateau value after 15 days (Table 3-II.1). Nevertheless, the quantity of CHX released was around 25% for both kinds of samples, compared to the 30% (with coating) and 65% (with slurry) reached using MFC (triangles).

A similar conclusion could be drawn with the intermittent diffusion experiments. Although the release period lasted longer than with MFC (more than 35 washing steps against 23 and 28), the released amount of CHX remained quite low. A mean value of only 2% was, indeed, released every 30 min for paper samples coated with the slurry containing CHX/latex. Nevertheless, these samples released about 80% of the full quantity of CHX initially introduced. Compared to MFC, CHX has no chemical interactions with latex and was totally free to be released. Regarding samples impregnated in CHX and then coated with latex, a total of only 15%

of CHX was released after 35 washing steps, with less than 1% released each 30 min. These results proved that latex is a continuous and closed layer preventing the CHX from being released.

Compared to paper samples with MFC, the diffusion mechanism of samples with latex is, thus, completely different, possibly due to the closed and non-porous structure of latex, which prevents CHX from being released, leading only to the release of very small amount of CHX. Furthermore, these released quantities were not able to ensure an effective and long-term antibacterial activity (see below), and owing to the petroleum and non-biodegradable nature of latex, the use of MFC appears to be the best solution.

II-3.3. Evaluation of the antibacterial activity

Chlorhexidine digluconate solution is a strong antibacterial solution. To determine the minimal CHX concentration for which an antibacterial activity is still perceived and measurable, antibacterial tests were carried out using diffusion discs impregnated with CHX solutions at various concentrations. Figure 3-II.8 presents the evolution of the inhibition zone areas measured as a function of the concentration of aqueous CHX solution (in which the discs were impregnated). Decreasing concentrations of CHX induce a decrease of the inhibition zone area suggesting that the antibacterial activity was less and less efficient. A clear decrease was observed under a concentration of 0.01% of CHX in water. Below that level of dilution, no inhibition zone appeared.

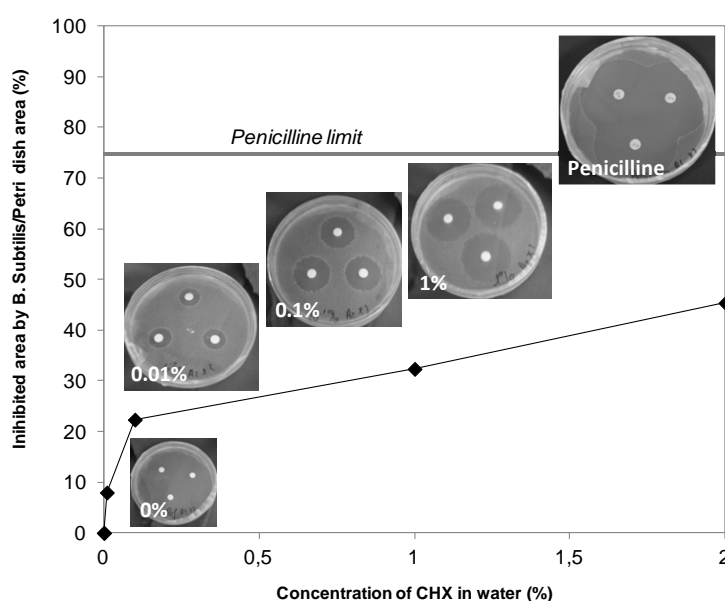


Figure 3-II.8. Minimal inhibition concentration of chlorhexidine digluconate solution against *Bacillus Subtilis*. Evolution of the inhibited areas (%) as a function of the CHX concentration (%)

The evaluation of the antibacterial activity of each sample in terms of food-packaging application was conducted following the standard EN 1104 procedure (AFNOR, 2005), except that at the end of every three days, the samples were removed and placed onto new Petri dishes with inoculated agar. This approach was similar to the intermittent diffusion experiments, except that the release study was carried out with a solid medium and evaluated every three days. These tests provide a better model for evaluating real contacts between food and its antibacterial packaging, since agar could be considered as a model food. Nevertheless, this model still remains very basic for food-contact studies, since many parameters were not considered (water and humidity contents, nutrients, salts, etc.). The results of these tests are plotted in Figure 3-II.9.

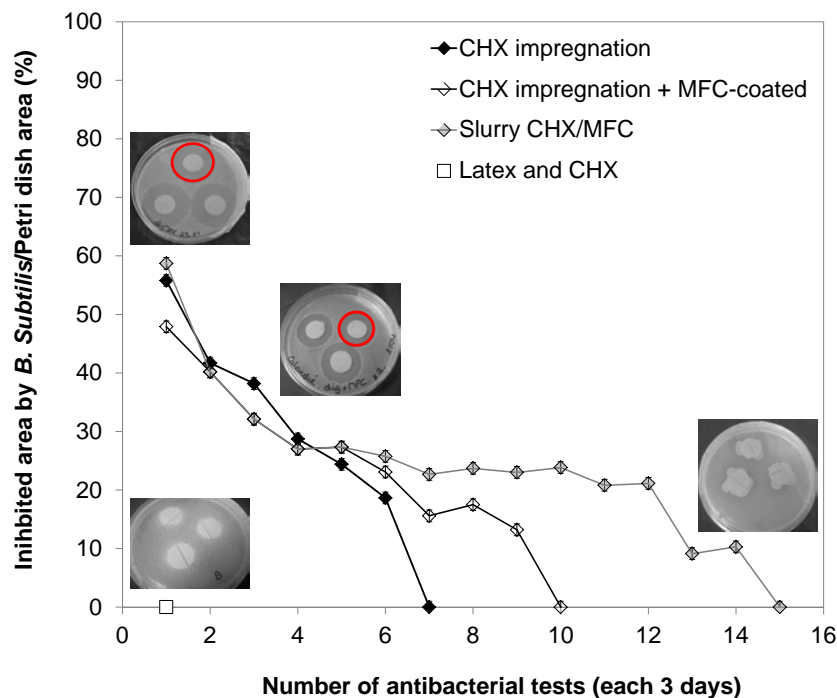


Figure3-II.9. Successive antibacterial tests.

Inhibited areas (%) as a function of the number of antibacterial tests carried out for each sample, every three days. Paper samples impregnated with CHX (black diamonds) were compared with paper samples impregnated with CHX and coated with MFC (white diamonds) and with paper samples coated with the slurry containing CHX/MFC (grey diamonds).

The rate of CHX released in a solid medium (agar) is obviously slower than in an aqueous liquid medium (e.g. end of release after 3 weeks against 6h30 for CHX impregnated samples), but more efficient since a complete inhibition of the antibacterial activity was reached (0% of inhibited area). The difference between both diffusions in a liquid and in a solid medium was possibly due to the types of molecule motion and to the hydrophilic nature of the samples. In a liquid aqueous medium, water induces CHX diffusion by opening and swelling both fiber

networks (MFC and paper). Besides, the movement of molecules is more important in liquid than solid, thus, favoring, the release of CHX molecules.

These intermittent diffusions results in a solid medium corroborate the positive correlation observed between MFC and the prolonged release of CHX. Compared to CHX impregnated paper samples, samples with MFC still displayed an antibacterial activity after six, three-day periods, i.e. after 18 days. With the MFC coating, the activity lasted around 1 month (30 days), and with the slurry containing CHX/MFC, samples were still active after 45 days. These results are in accordance with the previous results obtained by intermittent diffusion in a liquid medium. Samples with MFC released CHX over longer periods of time in agar, and those coated with the slurry containing CHX/MFC were significantly more efficient. These results are very promising for a future food-packaging application.

MFC coating is, thus, able to control the release of CHX and a long-term antibacterial activity has to be considered whatever the release medium (liquid or solid). Final antibacterial tests were also carried out with paper samples coated with latex. Despite the slow release of CHX in an aqueous medium induced by latex coating, no antibacterial activity was noticed. The use of MFC, thus, highlights its relevance for the food-packaging industry, since the classical coating slurry prevents CHX from being released and inhibiting bacterial growth.

II-4. Conclusion

Following on from our previous study, which revealed a new use for MFC coating as a controlled delivery system, the present study confirmed the previous observations obtained using caffeine, this time with an antibacterial agent, chlorhexidine digluconate.

In a liquid aqueous medium, the addition of MFC induces a slower and more progressive release of CHX, indeed, the time required to release the molecule completely increased threefold.

In a solid aqueous medium, the impact of MFC on the release of CHX was even more significant, since the samples coated with MFC released CHX molecules over one month, compared to 18 days without MFC, and maintained their antibacterial activity for the duration of the experiments.

To confirm the positive effect of MFC on the release of CHX, a comparison with a classical latex-based coating slurry was also performed. Even if the continuous diffusion into a liquid aqueous medium was slower than with MFC-coated samples, the full amount of CHX released was not sufficient to confer an antibacterial activity to paper samples. In comparison, MFC was able to control and slow down the release of CHX without preventing it from being released and maintaining an efficient antibacterial activity.

These results are very promising with regards to future food-packaging applications. A controlled and progressive release of CHX with long-term antibacterial activity was, indeed, achieved owing to the nanoporous network of MFC. This study encourages the elaboration of a new antibacterial bio-material for the food-packaging industry, but also paves the way for a variety of future applications, such as medical patches.

This subchapter detailed the elaboration of a new functional bio-nano-material with a long-term antibacterial activity. A food application can thus be investigated. For that purpose, a similar study was carried out in the next subchapter using a cardboard substrate as functional food-packaging.

Cardboard packaging with long-term antibacterial properties using microfibrillated cellulose

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Abstract

Industrials and consumers are focusing more and more on the development of biodegradable and lightweight food-packaging materials, which could offer a better protection for food.

In an attempt to meet these requirements, this study presents a novel substrate containing active bio-materials for food-packaging applications. Based on a cardboard substrate, the development of an antibacterial bio-packaging material is, therein, achieved using a chlorhexidine digluconate (CHX) solution as an efficient antibacterial agent. A long-term antibacterial activity was achieved using microfibrillated cellulose (MFC) mixed with CHX and coated onto cardboard samples. AFM and FE-SEM analysis were performed to underline the MFC nanoporous network, which helped for prolonging the release of the active molecule. A kinetic study in an aqueous medium was, thus, performed showing a significant increase in the release time of CHX when using MFC.

Finally, antibacterial and food tests were carried out in order to conclude on an effective antibacterial activity and an improvement in the shelf-life of food. Indeed, cardboard samples were extremely active against *Bacillus subtilis*, with better results being obtained when MFC was used, and the shelf-life of food increased compared to reference packaging boards.

Inspired from Paper 6:

Nathalie Lavoine, Isabelle Desloge, Brigitte Manship and Julien Bras (2013) Cardboard packaging with long-term antibacterial properties using microfibrillated cellulose. Submitted to Food Control.

III-1. Introduction

In response to the society's growing expectations, the domain of active bio-packaging is increasing significantly (Pereira de Abreu, *et al.*, 2012). Indeed, the development of more biodegradable and lightweight food-packaging materials (Johansson, *et al.*, 2012), which could offer a better protection of the product, is expected. Active packaging is one way of meeting these requirements, as it aims to improve the shelf-life of the packaged product (Council, 2004). Its market is already well developed in Japan and in the USA, and is now expanding in Europe.

Active packaging can be classified in two main categories: (i) scavengers and (ii) antimicrobial packaging. The first family aims to absorb specific elements responsible for the degradation of the packaged food, such as oxygen or ethylene (Brody, *et al.*, 2001, Pereira de Abreu, *et al.*, 2012, Vermeiren, *et al.*, 1999). The second family aims to inhibit or kill the growth of bacteria or fungi (Figure 3-III.1), which develop under specific temperature or humidity conditions with an appropriate medium providing them with nutrients (Appendini and Hotchkiss, 2002, Quintavalla and Vicini, 2002, Suppakul, *et al.*, 2003).

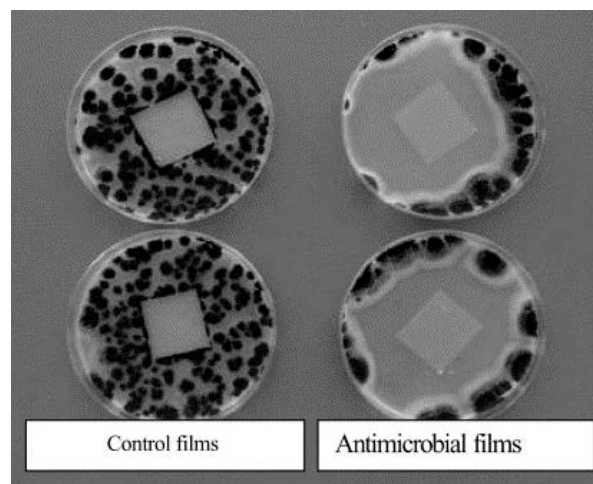


Figure 3-III.1. Effect of antimicrobial plastic film on *Aspergillus Niger*. Agar diffusion method (from Appendini and Hotchkiss, 2002)

To our knowledge, this second family is still not commercialized in Europe (contrary to scavengers packaging), despite the increasing interest of researchers (Gontard, 2000).

Given the various kinds of antibacterial materials (microcapsules (Gibbs, *et al.*, 1999, Madene, *et al.*, 2006), antibacterial polymeric films (Cerisuelo, *et al.*, 2012) or coated foils (Dogan, *et al.*, 2009), the combination of active agent and cellulosic substrates creates a number of attractive opportunities. Indeed, cellulosic substrates such as paper or cardboard are usually used as food-packaging materials. Their price remains lower than biopolymers and from a consumers' point of view, they are immediately linked to biodegradability and recyclability.

Nevertheless, when taking into account the few studies published in the subject, the development of active packaging combined with cellulosic substrates remains less intense than their development as films (Guillard, *et al.*, 2009, Ramos, *et al.*, 2012, Sanla-Ead, *et al.*, 2012, Suppakul, *et al.*, 2011), mainly because the focus is turned to the incorporation of the active component directly into the polymeric matrix itself.

Indeed, the main studies about active cellulosic substrate focused on the incorporation of active agents, such as essential oil, in wax coating for paper packaging. (Rodríguez, *et al.*, 2007) conducted for example a complete study on paraffin-based “active coatings”. Essential oils such as cinnamaldehyde and carvacrol were also introduced in other paper coating slurries such as soy protein isolates (SPI) and octenyl-succinate modified starch (Ben Arfa, *et al.*, 2007). In spite of the positive results obtained in each case, the main issue was the organoleptic modification of foods depending on the essential oils used.

Organic acids, which are the most classical preservative agents, were also introduced into coating slurries for paper-based materials as they inhibit the outgrowth of bacteria and fungi (Khwaldia, *et al.*, 2010). Sorbic acid was then introduced into wax-based coating on papers and used as sausage or cheese packaging materials (Labuza and Breene, 1989). Lactic, propionic and acetic acids were also tested as antibacterial compounds and introduced into chitosan coating for papers (Vartiainen, *et al.*, 2004). The lactic acid/chitosan slurry was the only one that showed antibacterial activity against *Bacillus subtilis*. Other antibacterial compounds were also directly mixed with coating formulations for paper packaging such as nisin (Lee, *et al.*, 2003, Vartiainen, *et al.*, 2004) or silver nanoparticles (Nassar and Youssef, 2012), yet no viable solution has so far been developed for future commercialization.

Further research is, thus, needed to overcome some unresolved issues still prevailing like: (i) the impact of the active agents on the organoleptic properties of the packaged food, (ii) the controlled release of the active agents for a long-term antibacterial activity, and (iii) the biodegradability, recyclability and inertness of the active packaging materials.

Considering all the previous studies and challenges, we suggest, in the present study, a new kind of biodegradable active packaging material able to release the antibacterial compound over a longer period of time and so to improve the shelf-life of the packaged product.

Chlorhexidine digluconate solution (CHX) was used as an antibacterial model agent. Chlorhexidine digluconate is the most efficient chemical agent in plaque control and is mainly used for oral hygiene (Houston, *et al.*, 2002, Şenel, *et al.*, 2000). Due to its cationic nature (Giménez-Martín, *et al.*, 2009), CHX is efficient against a wide range of bacteria. Besides, owing to the presence of its two Cl-atoms, CHX is easily detected by elemental analysis so that its presence in the substrate can be evaluated.

Based on our previous studies dealing with the release of caffeine (Lavoine, *et al.*, 2013a) and CHX introduced in the paper coating (Lavoine, *et al.*, 2013c), CHX was coated onto the surface of cardboard using a microfibrillated cellulose (MFC)-based coat.

MFC is produced from cellulosic fibers by applying a strong mechanical treatment. In 1985, the first MFC manufacturing process was patented by Turbak, *et al.*, 1985. It consisted in the defibrillation of wood pulp under high pressure using a homogenizer. Nowadays, new kinds of mechanical treatments (Siró and Plackett, 2010) exist, and pre-treatments (Isogai, *et al.*, 2011, Pääkkö, *et al.*, 2007) are also applied to reduce the high energy cost resulting from the use of the mechanical treatments (Spence, *et al.*, 2011a). Thanks to its nanometric dimensions (diameters in the range of 10-50 nm and lengths higher than 1 μm (Lavoine, *et al.*, 2012)), to the entanglement of the microfibers and to the ability to form strong and high barrier neat MFC films, the appeal of using MFC is increasing considerably with several book or conferences and almost one scientific paper per day. Indeed, MFC is extensively used in nanocomposites (Siró and Plackett, 2010) due to its mechanical reinforcing properties, as a film essentially for its barrier properties (Belbekhouche, *et al.*, 2011, Syverud and Stenius, 2009), as an aerogel for its nanoporous network and lightweight (Aulin, *et al.*, 2010b, Korhonen, *et al.*, 2011), as a coating slurry for its barrier properties and its ability to improve the printing quality (Hamada, *et al.*, 2010, Hamada and Bousfield W, 2010, Lavoine, *et al.*, 2011, Ridgway and Gane, 2012), and more recently as a drug delivery system (DDS) (Kolakovic, *et al.*, 2012a, Kolakovic, *et al.*, 2011, Kolakovic, *et al.*, 2012b, Valo, *et al.*, 2013, Valo, *et al.*, 2011).

The elaboration of DDS was investigated by taking into account the different kinds of materials obtained with MFC. For example, Valo, *et al.*, 2011 used it as a suspension to stabilize drug nanoparticles and increase their storage ability. In another study, Valo, *et al.*, 2013 developed MFC aerogels containing drugs and studied the influence of the sources of MFC on the release of the drug. Kolakovic, *et al.*, 2011 worked with spray-dried MFC as a tablet excipient, as a sustained drug release system (Kolakovic, *et al.*, 2012a), and also as a drug loaded MFC film (Kolakovic, *et al.*, 2012b). These films displayed promising results, since the release of drugs was sustained for 90 days. It was also confirmed by a more recent study focusing on food-packaging applications, which proposed the addition of lysozymes (antibacterial proteins) into MFC films (Cozzolino, *et al.*, 2013). Following these previous studies, we demonstrated this year that MFC coated onto paper substrates can also be used as a delivery system for different kinds of molecules (Lavoine, *et al.*, 2013a, Lavoine, *et al.*, 2013c). These innovative studies were the first to investigate an active packaging application using a MFC coating process. Thanks to its nanoporous network preserved during the process, the MFC substrate ensured the slow and progressive release of molecules into an aqueous medium (like water or agar). However, no

tests were conducted using food and the elaboration of a 3D-packaging was not feasible with a coated paper substrate.

In the present study, we thus applied this MFC/active molecules coating strategy onto a cardboard packaging material for 3D applications. The release of CHX into an aqueous medium was analyzed by UV-Vis spectrophotometry. Antibacterial tests were performed to evaluate the efficiency of the coated cardboard against *Bacillus subtilis*. Food tests were also carried out to complete the study and conclude on an improved shelf-life of the packaged food compared to classical cardboard packaging.

III-2. Materials and methods

III-2.1. MFC suspension preparation and characterization

MFC was produced and supplied by the FCBA (France). The diluted sulfite pulp (Domsjö®) was initially enzymatically pre-treated with an endoglucanase for 1 h (at 50°C). A subsequent mechanical treatment was applied using a GEA Ariete® homogenizer at 1,000 bars for 1 pass followed by 4 passes at 1,450 bars. The final concentration of the MFC suspension in water was 2% wt.

Optical light microscopy (Axio Image M1m Zeiss, France) was firstly used to observe the suspension. The MFC suspension was diluted at 0.1%wt in deionized water and mechanically stirred using an Ultra-Turax (France). A multidimensional acquisition (AxioVision software) was performed at a $\times 5$ magnitude. Approximately $N=10$ pictures were taken of the suspension, and a representative image was selected.

Finally, the MFC suspension was analyzed using FE-SEM (Field Electron Scanning Electron Microscope, Zeiss® Ultra-55, USA). The 2%wt suspension was spread onto a metal substrate using carbon tape, dried over two nights at room temperature and finally coated with a thin layer of gold (162 nm). The observation was performed using a working distance of 5.5 mm for an accelerating voltage of 2.00kV at a magnitude of $\times 50.00k$. The mean diameter of MFC was determined using an image analysis system (ImageJ® software) after at least 50 measurements.

III-2.2. Sample preparation and characterization

A4 cardboard sheets were produced and supplied by Cascades La Rochette (France). With a basis weight of 300 g/m², this cardboard was made of virgin pulp and coated on one side to facilitate printing. The other side was left uncoated in order to follow the coating process we developed, as described below.

Three different slurries were coated onto the cardboard surface:

- (1) The chlorhexidine digluconate (CHX) solution was purchased from Sigma-Aldrich (France). Its concentration was 20wt% in water. This solution was diluted to 2wt% in deionized water and coated as such onto the surface of the cardboard.
- (2) The MFC suspension (previously described) was coated as such: at 2wt%, following a homogenization using an Ultra-Turax (France) for 5 min.
- (3) A slurry containing CHX/MFC was prepared by 10/90% of mixing CHX at 20wt% and MFC at 2wt%, respectively. As a result, 100 ml of this slurry contained about 2 g of dried CHX and 1.8 g of dried MFC. The slurry was stirred and homogenized using an Ultra-Turax (France) for 5 min, and then coated onto the surface of the cardboard.

The coating of these solution and suspensions was performed using a bar coating process (Endupap, France). A 0.9 Mayer bar was used at a speed of 5 cm.s⁻¹. Each slurry was deposited onto the surface of the cardboard in a similar and homogeneous way to ensure a homogeneous coating onto the whole sample surface. Using a contact drying system at 105°C, each cardboard sheet was dried under tension for 3 min on each side.

These steps were repeated five times ensuring a complete recovering of the surface of the cardboard.

SEM images of the surfaces of the coated cardboard were obtained using an Environmental Scanning Electron Microscopy (E-SEM, Quanta200®, The Netherlands). The samples were mounted onto a metal stub with a double sided carbon tape, and observed at a working distance of 10.0 mm, under high pressure at 15 kV. The Back-Scattered Electron Detector (BSE) was also used for obtaining high-resolution compositional maps of the surfaces of the samples.

The nanoporous MFC network formed during the coating was identified and studied by Atomic Force Microscopy (AFM). Small pieces of 5 × 5 cm² of MFC-coated cardboard were glued onto aluminium sample holders and scanned by tapping-mode using a Dimension Icon with a Scan Analyst (Bruker, USA). The images were recorded at room temperature with a standard silica cantilever (Bruker, USA).

Several AFM and E-SEM analyses were performed and the most representative pictures were selected.

III-2.3. The distribution and quantification of CHX

The distribution of the CHX molecules in the cardboard samples was observed by X-Ray microanalysis using an elemental mapping mode of the distribution of the chlorine atoms as recorded at the surfaces and in the cross-section of each sample.

The exact amount of CHX introduced into the cardboard samples was measured according to two different methods: (1) calculation of the difference in mass and (2) an elemental analysis. The differences in mass between the dried coated samples and dried cardboard reference samples were calculated to evaluate the dried coat weight, and thus, to determine the quantity of CHX introduced into each slurry. Measurements were performed after drying each $10 \times 10 \text{ cm}^2$ sample for 1 h, at 105°C using a contact drying system and a Lhormargy high resolution scale ($\pm 0.001 \text{ g}$). These measurements were repeated up to three times for each sample.

The weighed values were then compared with those calculated from the percentage of Cl-atoms obtained after an elemental analysis. The elemental analysis was performed by the Service Central d'Analyse of the CNRS (France). Two measurements per sample were carried out.

Table 3-III.1 summarizes the different quantities of CHX and MFC, respectively, coated onto the surface of the cardboard samples, and measured either using the difference in mass or after an elemental analysis.

Samples	Weighed compounds	Methods of measurement	
		<i>Difference in mass</i>	<i>Elemental analysis</i>
CHX-coated cardboard			
	CHX (/g of dried cardboard)	0.010 ± 0.003	0.0055 ± 0.0002
CHX/MFC coated cardboard			
	CHX (/g of dried cardboard)	0.056 ± 0.009	0.021 ± 0.002
	MFC (/g of dried cardboard)	0.036 ± 0.013	-

Table 3-III.1. Amount of dried CHX and MFC per gram of dried cardboard according to the kind of sample. Two different methods were compared: (i) difference in mass and (ii) elemental analysis

III-2.3. Release study of CHX in an aqueous medium

The release of CHX was studied following two protocols: (i) continuous and (ii) intermittent diffusions. Both protocols were carried out under sink conditions (conditions limiting the saturation of the medium by the released molecules), by applying continuous and homogenous stirring, and at room temperature.

The coated cardboard samples ($10 \times 10 \text{ cm}^2$) were initially taped onto an expanded polystyrene support and brought in contact with a 500 ml bath of deionized water in order to study the release of the molecules specifically through the surface of the sample.

The continuous diffusion method consisted in sampling the medium containing the released molecules at different times. The released amount of CHX (%) was then plotted as a function of time. The released amount of CHX was equal to quantity of CHX released divided by the quantity of CHX introduced into the cardboard samples before testing (Table 3-III.1).

The intermittent diffusion protocol was similar to the previous one, except that between each sampling step, i.e. each 30 min, the aqueous release medium was renewed. The released amount of CHX (%) was plotted as a function of the cumulative number of washing steps, i.e. the number of medium renewal steps.

The complete release study was repeated using three replicates of each specimen.

III-2.4. Antibacterial activity of coated-cardboard samples

The antibacterial efficiency of the coated-cardboard samples was evaluated against *Bacillus subtilis* according to the standard EN 1104 (AFNOR, 2005).

Spore suspensions of *Bacillus subtilis* at a concentration of 10^7 spores/ml were purchased from Humeau Laboratories (France). Nutrient agar (Humeau, France) was dissolved with deionized water, sterilized and cooled to 60°C or under, so that it could be inoculated with the spore suspension (final concentration of 10^4 spores/ml). After cooling, the inoculated agar was poured into Petri dishes of 90 mm in diameter (Humeau, France). The antibacterial cardboard samples were placed onto the inoculated agar and stored at $4\text{-}5^\circ\text{C}$ for 2 h. The Petri dishes were then kept at 30°C for 3 days. Three Petri dishes were prepared for each sample, and three antibacterial discs of 16 mm in diameter were placed onto the Petri dishes.

Reference Petri dishes were also prepared and experiments were repeated three times with: (i) neat agar (ii) inoculated agar and (iii) inoculated agar with three Penicillin discs, which is a well-known antibacterial agent against *Bacillus subtilis*.

The antibacterial activity was determined by measuring the inhibition zone formed around each sample using a ruler ($\pm 0.1 \text{ mm}$). The larger the diameter, the more efficient the antibacterial activity of the samples. The inhibited area was calculated in triplicate by considering only the surface of the diffusion, and not the surface of the sample and diffusion zone.

III-2.5. Food tests

Using the ArtiosCad® software, the final template of the boxes ($10 \times 10 \times 5 \text{ cm}^3$) was designed on A4 cardboard sheets. These latter were cut, creased and folded into 3-D cardboard packaging using a cutting and creasing laboratory converting pilot (Kongsberg, Esko, France). Three boxes of each coated cardboard sample were made.

Pork liver was chosen as a model food since its putrefaction is achieved within 3 days and is clearly measurable by observing the change in color. Pieces of pork liver measuring about $5 \times 5 \text{ cm}^2$ were cut using a sterilized blade. One piece was placed inside each cardboard box and brought into contact with the active coated cardboard surface. The boxes were closed, wrapped and sealed in PE plastic bags using a heated seal press, and were then stored in a fridge at $4\text{-}5^\circ\text{C}$ for 5 days, i.e. more than two days after the use-by date.

After the storage period, each sample was photographed. The images were submitted to 20 witnesses responsible for judging the degradation of the liver. Each one classified the liver samples from the least fresh to the freshest by giving them a grade ranging from 1 to 5; with 5 corresponding to the freshest liver. The highest grade which could be reached was thus, 100 (i.e. 20×5) corresponding to the pork liver analyzed just after its purchase.

An image analysis was also carried out using the ImageJ® software to compare the brightness of the red pixels making up the images. The cumulative number of red pixels (a total of 300 pixels) was plotted as a function of their brightness. The median value of the brightness was then determined so that the number of red pixels with a lower value was equal to the number of red pixels with a higher brightness value. The maximum of brightness detected was 100 corresponding to the pork liver analyzed just after its purchase (value corresponding to the brightness of 150 pixels).

III-3. Results and discussion

III-3.1. Observation and characterization of the MFC suspension

The MFC suspension is a white aqueous gel (Figure 3-III.2a) made of an entanglement of microfibrillated cellulose. Even after a consequent dilution of the suspension, it was quite difficult to isolate only one microfiber. It is, indeed, well-known that an accurate measurement of their length is difficult (Siró and Plackett, 2010) despite two recent scientific studies (Ishii, *et al.*, 2011, Shinoda, *et al.*, 2012).

An estimation of their diameter was nevertheless achievable from a FE-SEM image (Figure 3-III.2c). After an analysis with ImageJ®, a mean value of $33 \pm 8 \text{ nm}$ was obtained. This value corroborates with other measurements found in the literature (Klemm, *et al.*, 2011,

Lavoine, *et al.*, 2012). Generally, the diameter of MFC ranges between 10-50 nm, no matter what treatment is used to produce it. Images obtained by light microscopy (Figure 3-III.2b), and the high viscosity of the gel proved the homogeneity of the fibrillation process. It is worth verifying this point because to date no standard about MFC exists in spite of the development of ISO working groups (Marrapese, 2013). As a result, some researchers call MFC microfibrils with smaller diameters, or fibers with larger diameters. In our case, we confirmed that a good quality and a homogeneous MFC suspension was produced and further used as a coating.

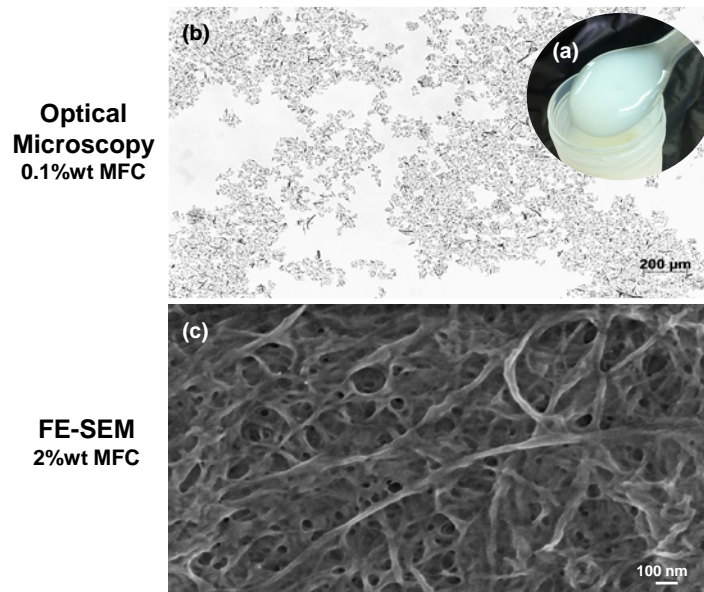


Figure 3-III.2. Observation and characterization of the MFC suspension

(a) Picture of the white 2%wt MFC gel, (b) light microscopy image of the 0.1%wt MFC suspension at a $\times 5$ magnification and (c) FE-SEM image of the 2%wt MFC suspension at $\times 50.00k$

III-3.2. MFC-coated cardboard

A4 cardboard sheets were coated with the MFC using a bar coating process. Five MFC layers were successively deposited onto the surface of the cardboard to ensure a homogeneous coat. A complete characterization of these samples (mechanical and barrier properties) was already published in one of our previous works (Lavoine, *et al.*, 2014). With five MFC layers, a coat weight of about 7 g/m^2 was achieved. Our previous study proved that the MFC layers counterbalanced the loss of mechanical properties induced by the successive wetting/drying cycles of the aqueous coating process. The MFC even improved some mechanical properties, such as the bending stiffness. It was, thus, possible to produce a cardboard box coated with a thin MFC layer (7 g/m^2) having similar mechanical properties as a box coated with 17 g/m^2 of polyethylene. The use of this MFC layer as a prolonged release system could then be investigated in the present study.

The observation of the nanoporous MFC network is a key aspect for this application. Indeed, this network plays a major role in the controlled release of CHX. The sample surfaces were, therefore, analyzed by SEM and AFM. Figure 3-III.3 shows SEM images of the surface of the cardboard sample taken with SE and BSE detectors.

When comparing the images of the reference cardboard and the CHX-coated cardboard, no difference could be observed, which was as expected. On the contrary, MFC-coated cardboard samples had a surface entirely covered by the MFC: no more pores were perceived. However, this homogeneity was not maintained overall following the coating process with the CHX/MFC mixture: some areas were, indeed, not entirely covered. Charge interactions between CHX and MFC might induce the formation of aggregates, and result in a less homogeneous surface coverage. BSE images clearly revealed the presence of non-coated zones at the surface of CHX/MFC coated samples, contrary to MFC-coated samples. Nevertheless, these heterogeneities did not drastically influence the release pattern of CHX as will be demonstrated further. Figure 3-III.4 shows the AFM images of the MFC-coated cardboard samples.

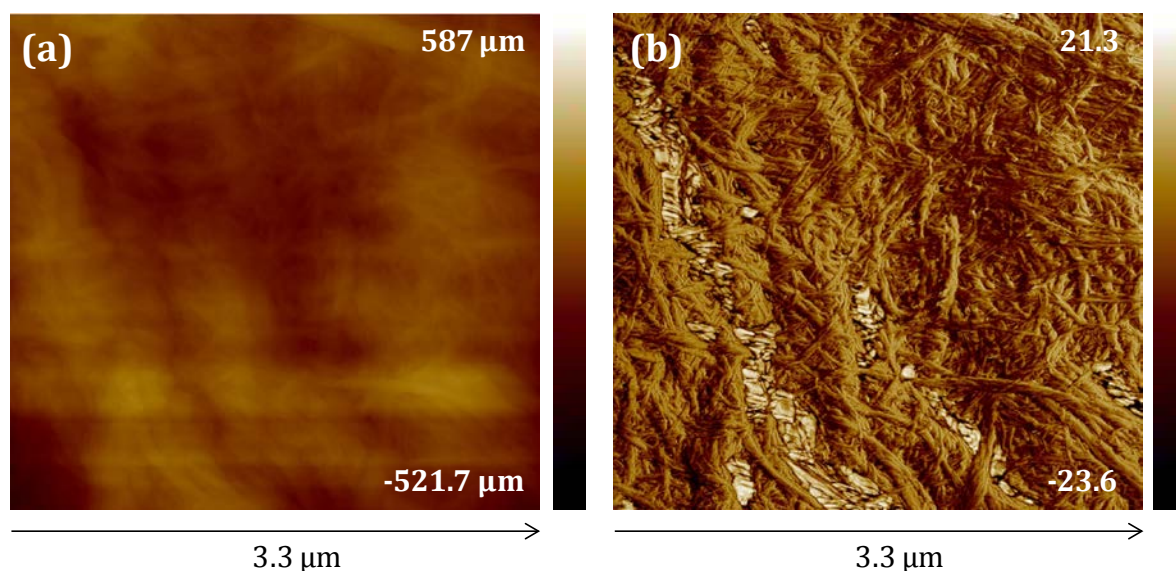


Figure 3-III.4. AFM images of the surface of the MFC-coated cardboard sample: (a) Topography and (b) Phase

This microscopy technique is the only one, which accurately underlined the nanoporous MFC network at the surface of the samples. These pictures, of the surface of the MFC-coated cardboard, are quite rare and rather complicated to obtain, however they clearly prove that even after the coating, microfibrils were tightly entangled, as they were in the original suspension (Figure 3-III.2).

The effect of this network on the release of CHX will therefore be studied more extensively herein.

SE mode

BSE mode

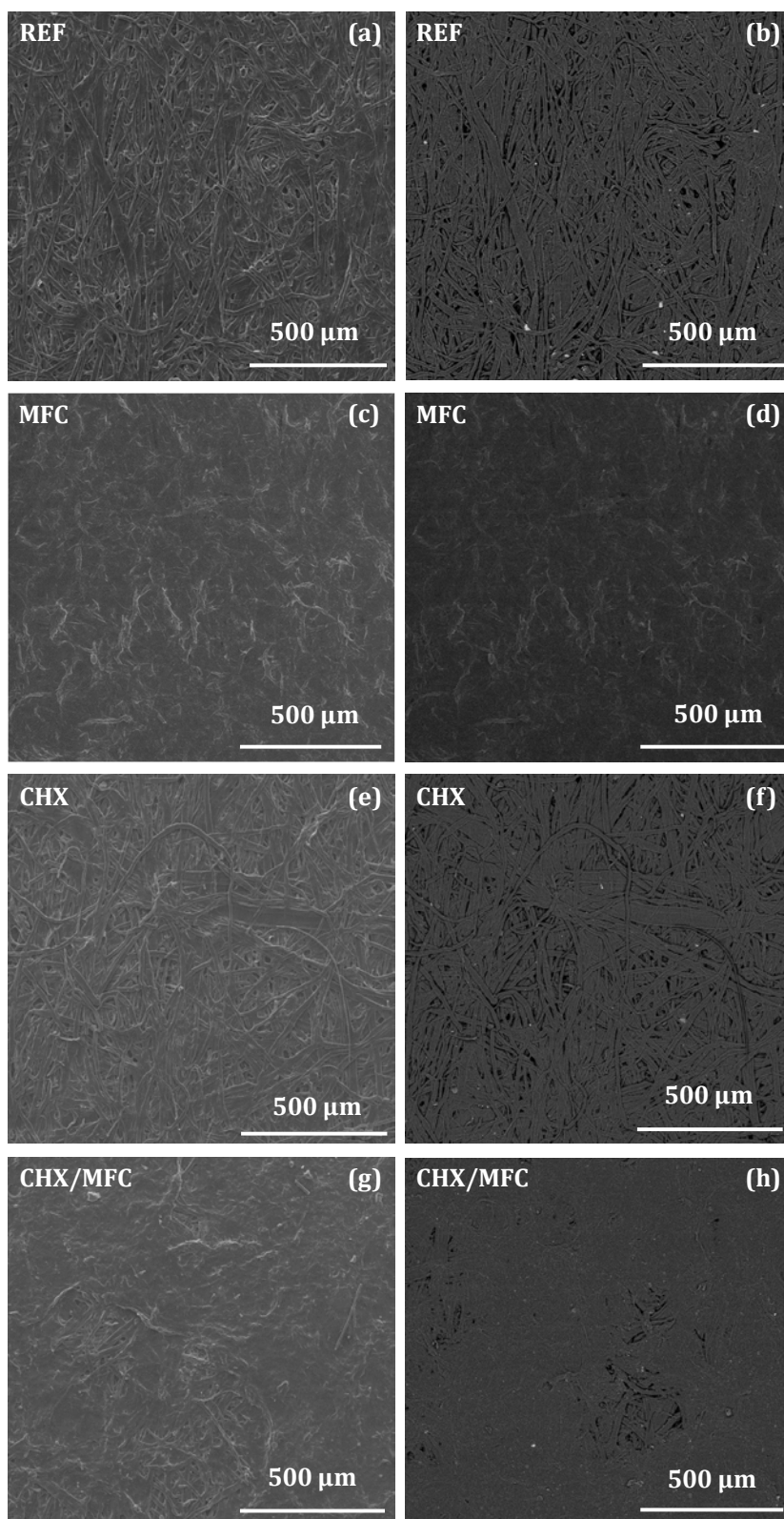


Figure 3-III.3. SEM images ($\times 100$) of each sample surface using the classic Secondary Electron (SE - (a), (c), (e), (g)) and Back-Scattered Electron (BSE - (b), (d), (f), (h)) detectors. Images (a) and (b) are the cardboard reference; (c) and (d), the MFC-coated cardboard; (e) and (f), the CHX-coated cardboard; (g) and (h) display the CHX/MFC coated cardboard samples.

III.3.3. Release study of CHX into an aqueous medium

Two kinds of release protocols were implemented to study the diffusion of CHX into an aqueous medium. CHX-coated cardboard samples were compared with CHX/MFC-coated cardboard samples. Indeed, in our previous study (Lavoine, *et al.*, 2013c), we proved that the most efficient release of CHX was achieved from papers coated with the CHX/MFC mixture, instead of papers impregnated in CHX and then coated with MFC. Consequently, in the present study, we limited our analysis to cardboard samples directly coated with the CHX/MFC mixture. To highlight the relevance of MFC, CHX-coated cardboard samples were prepared as a control. Nevertheless the amount of CHX introduced into both CHX- and CHX/MFC coated cardboard differed (Table 3-III.1). Indeed, it was quite complicated to achieve a similar quantity using the bar coating process, especially with the CHX solution. Even after applying five successive layers, the total quantity of CHX coated onto the cardboard was very low compared to the quantity introduced into the MFC suspension.

The results of the continuous diffusion assays are presented in Figure 3-III.5. The graph represents the amount of CHX (%) released as a function of time. The amount of CHX released was determined by the quantity of CHX released at the time t divided by the total amount of CHX introduced into the samples before the releasing assays. This latter was measured using two different methods, as previously described in the experimental part: (i) differences in mass and (ii) elemental analysis.

Results of both methods were plotted in Figure 3-III.5a and Figure 3-III.5b. Similar trends were observed in both cases: CHX-coated cardboard samples released a higher proportion of CHX than CHX/MFC-coated cardboard samples (e.g. 80% vs. 10% in 1 h; Figure 3-III.5a). Furthermore, the plateau values were reached at the same time for both kinds of samples, i.e. in 1 h. Nevertheless, these plateau values inform us only about the highest proportion of CHX released for each kind of sample independently of the initial amount of CHX. When considering the curves representing the quantity of CHX released (g) as a function of the time (h) (results not shown), the slopes of both curves were almost equal (about 0.2 g/h), proving that the release kinetic was similar for CHX and CHX/MFC-coated cardboard samples.

As a result, the nanoporous MFC network did not release the CHX molecules more slowly, but released them more progressively, i.e. at a lower rate. The use of MFC as a delivery system thus tends to be promising, owing to the achievement of a controlled molecules release system. This will be further confirmed with the intermittent diffusion experiments.

A major concern in the comparison of both samples was the determination of the exact amount of CHX introduced into both samples. As said previously, two quantification methods were performed (difference in mass and elemental analysis) and compared. Considering the Figures 3-III.5a and b, the total quantities of CHX reached were completely different. CHX-coated cardboard samples released 80% of CHX in 1 h according to the quantification based on a difference in mass, whereas only 20% of CHX was released in 1 h when considering the results obtained by elemental analysis. The same conclusion was drawn with the CHX/MFC-coated cardboard samples. Only 8% of CHX was released according to the difference in mass method, against 20% using the elemental analysis.

Differences between both methods are quite significant. This proves that the results obtained by difference in mass, generally used in this field cannot be used in the case of cardboard samples. The quantity of CHX was, indeed, too low to be accurately quantified with a scale: for cardboard samples of about 3 g, a mean value of only 0.015 g of CHX was quantified by elemental analysis just after coating. Thus, in the following, only the results determined by elemental analysis will be considered. Consequently, only 20% of CHX was released in 1 h when MFC-coating was used, against 40% for the CHX-coated cardboard samples, highlighting the impact of MFC, which enables the release of smaller quantities. This might be due to the higher surface area of the MFC increasing the number of interactions with CHX.

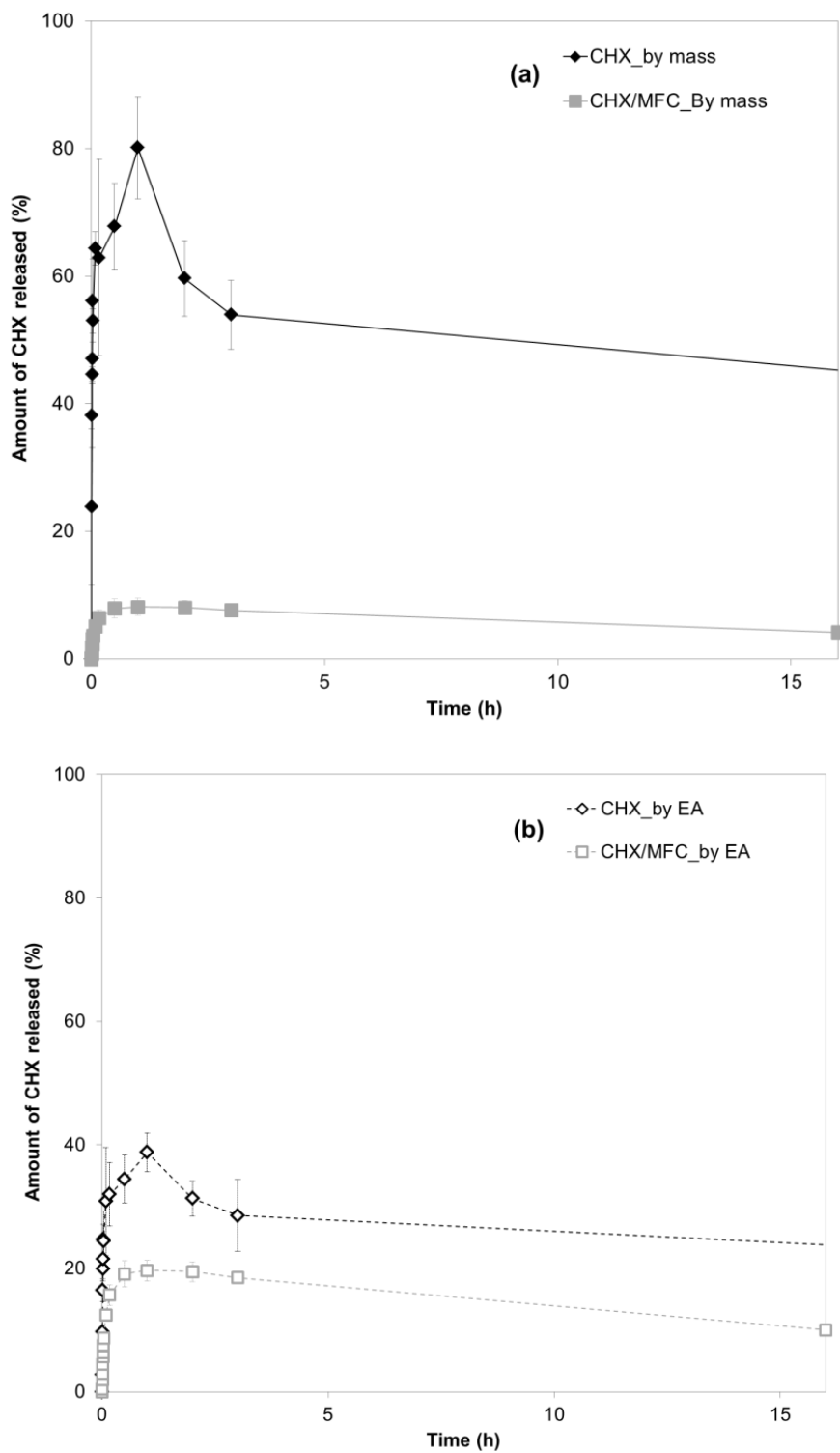


Figure 3-III.5. Continuous diffusion of CHX into an aqueous medium.

CHX-coated cardboard samples (black diamond) are compared with CHX/MFC coated cardboard samples (grey square).

The amount of CHX released is equal to the quantity of CHX released at t divided by the whole quantity of CHX introduced into the samples. This quantity was measured using two different methods: (i) differences in mass (graph a) and (ii) elemental analysis (EA) (graph b).

Indeed, for a similar reason, the whole quantity of CHX was not released. This could be expected due to the interactions between CHX and cellulose. Adsorption and covalent bonding of CHX onto cellulosic fibers were already studied and confirmed in two previous studies (Blackburn, *et al.*, 2007, Giménez-Martín, *et al.*, 2009). The mapping of the Cl-atom (from CHX) of our samples before and after the releasing assays were conducted, and confirmed these interactions between cellulose and CHX as well (Figure 3-III.6).

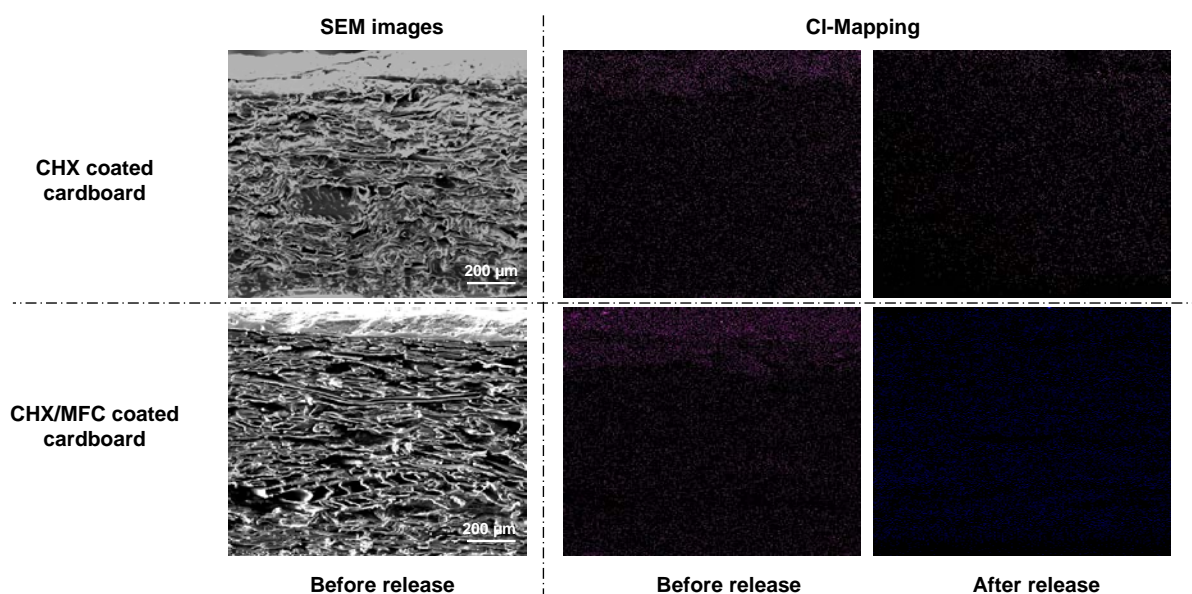


Figure 3-III.6. Maps of the Cl-atoms present into the cardboard samples before and after being released into the aqueous medium. CHX coated cardboard (*up*) are compared with the CHX/MFC coated cardboard (*down*).

The mapping was conducted on the whole cardboard thickness represented by the SEM images. The distribution of the Cl-atoms is represented by the distribution and intensity of the colored dots.

Indeed, the distribution of the Cl-atoms throughout the thickness of the samples demonstrated the presence of CHX in samples even after release tests were conducted (Figure 3-III.7). It is worth noting that before the releasing assays were conducted, the whole quantity of CHX was mainly located on the surface of the cardboard. After the releasing assays, CHX mainly migrated throughout the thickness of the cardboard. This can possibly explain why a large quantity of CHX was still not released. When a thick and porous material (like cardboard) is used, CHX is able to diffuse in two directions: into the release medium and into the thickness of the material. With thinner materials like paper (Lavoine, *et al.*, 2013c), the diffusion also happens in both “directions”, but due to the thin thickness of paper rapidly soaked by the molecules, CHX mainly diffuses into the release medium.

Besides, according to the distribution profiles (Figure 3-III.7), very few CHX molecules were released during the continuous diffusion experiments. On the contrary, during intermittent diffusion experiments, a larger quantity of CHX was released, as the medium was renewed every

30 min after each sampling. Indeed, maps and profiles of the distribution of Cl-atoms throughout the cardboard thickness showed that CHX was still present in samples, but only in very low amount (Figures 3-III.6 and 7).

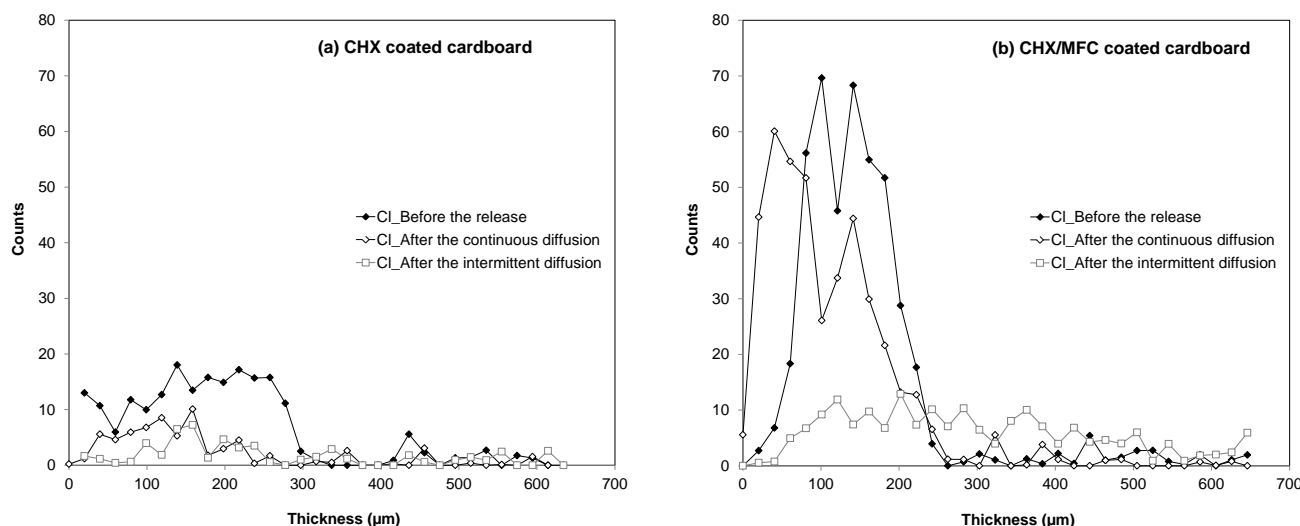


Figure 3-III.7. Distribution profiles of the Cl-atoms throughout the thickness of the cardboard.

These graphs represent the number of counts corresponding to the Chlorine atoms measured by X-ray microanalysis as a function of the sample thickness. The graphic (a - left) presents the distribution throughout the thickness of CHX coated cardboard before and after the release of CHX. The graphic (b - right) shows similar results for the CHX/MFC coated cardboard.

Figure 3-III.8 presents the results of the intermittent diffusion experiments. In this case, the abscissa does not represent the time, but the number of washing steps and the cumulative amount of CHX released into the aqueous medium is plotted as a function of the number of washing steps, i.e. the number of times the medium was renewed every 30 min. CHX-coated samples continuously released significant amounts of CHX after each medium renewal until a maximum value of about 100% of released CHX was reached. On the contrary, very small quantities of CHX were released from cardboard samples coated with CHX/MFC after the first medium renewal. Indeed, during the first 30 minutes, the excess of CHX was entirely released (about 20%). This corroborates the concept of burst effect proposed by (Raso, *et al.*, 2010). After this first washing step, the proportion of CHX released was very similar between all of the medium renewals and did not exceed the 5%.

After 84 washing steps, the entire quantity of CHX initially introduced in CHX-coated samples was released (according to the elemental analysis). CHX-MFC coated samples, however, released CHX during 51 washing steps. The quantity released after these 51 steps was only 25%. It, thus, means that CHX was still released from these samples, but only in very small amounts.

The experiments were stopped when three consecutive samples displayed a zero absorbance, although some samples continued to release CHX even. It was, therefore, difficult to complete the CHX release experiments due possibly to the fact that CHX was trapped in the MFC network.

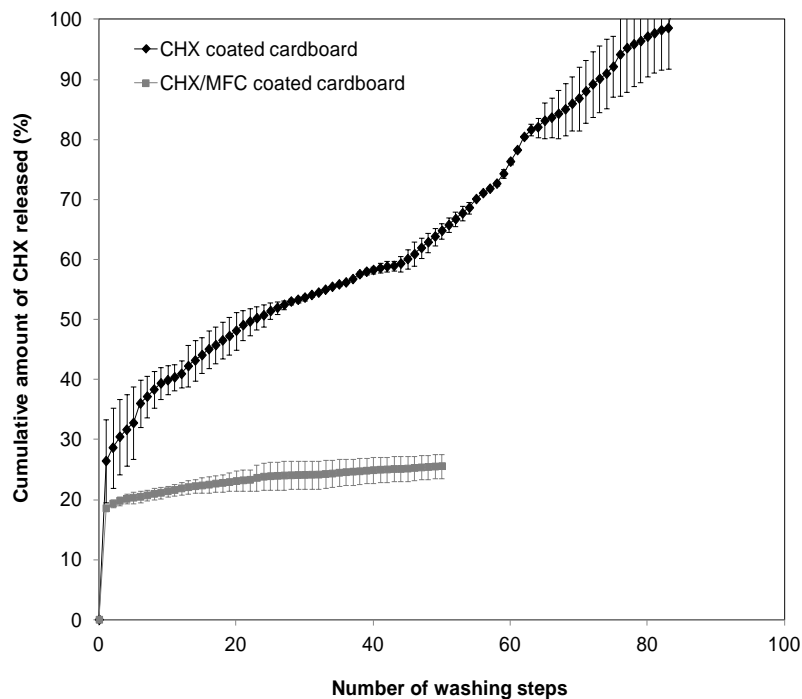


Figure 3-III.8. Cumulative amount of CHX released (%) according to the number of washing steps. CHX-coated cardboard (black diamond) are compared with the CHX/MFC coated cardboard (grey squares).

However, it was quite surprising that the CHX was released from cardboard samples coated with CHX into the aqueous medium during 84 washing steps. Into our previous study (Lavoine, *et al.*, 2013c), the release of CHX introduced into paper samples, was faster and lasted overall only 18 washing steps. The reason for this discrepancy could mainly be due to the multilayer structure of cardboard since the quantity of CHX introduced into the cardboard samples was ten-times lower than the quantity introduced into the paper samples. As CHX was diluted in an aqueous solution, it had the opportunity to penetrate into the whole structure of the cardboard. CHX was, thus, able to interact chemically with each layer of cardboard, which drastically slowed down its release. The structure of cardboard, therefore, allows for a slower release of CHX compared to paper.

Nevertheless, even if the CHX molecules were released over a longer period of time and more continuously by CHX-coated samples, the addition of MFC allowed a more progressive release of CHX since only 25% of CHX was released after 50 washing steps, against 65% for the CHX-coated samples (Figure 3-III.8).

Finally these results corroborate those obtained by continuous diffusion: the nanoporous MFC network allowed for a more progressive release of the CHX molecules into water, but after reaching a certain level of CHX, the MFC network seemed to prevent the release of CHX.

Antibacterial tests were, thus, be carried out in order to verify whether an antibacterial activity was released in spite of this interrupted diffusion.

III-3.4. Antibacterial activity against *Bacillus subtilis*

Antibacterial activity of coated-cardboard samples was conducted according to the standard EN 1104 procedure, before and after CHX releasing assays. The bacterium targeted in our tests is a non-pathogenic organism, named *Bacillus subtilis*. These tests were qualitative and informed us if the samples were antibacterial or not, before and after the intermittent diffusion experiments. In our case, the antibacterial activity of CHX/MFC-coated samples after releasing was expected as the whole quantity of CHX was not released after 51 washing steps, whereas in the case of the CHX-coated samples, no activity was expected since 100% of the CHX introduced was released after 84 washing steps.

The results of the antibacterial tests are presented in Figure 3-III.9.

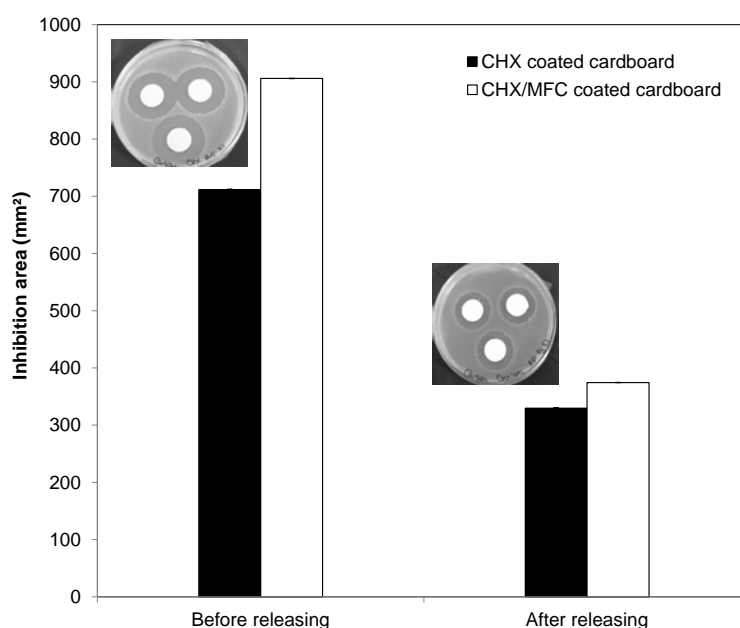


Figure 3-III.9. Inhibition areas obtained by each antibacterial sample before and after the intermittent release of CHX into an aqueous medium. These areas were calculated from the diameter of the inhibition zones produced by the active samples, which had an initial diameter of 16 mm. Standard deviations were added in this graph, but are so low that they are almost not visible.

Before the release study, the samples were quite active against *Bacillus subtilis*: inhibition areas of 712 and 905 mm² for CHX and CHX/MFC-coated samples, respectively, were measured. Compared to the Penicillin reference samples, which had an inhibited area of approximately 1560 mm², CHX was quite efficient against these bacteria, and diffused perfectly into agar.

When we consider the results obtained after the intermittent diffusion experiments, both samples still exhibited an antibacterial zone (Figure 3-III.9).

The CHX-coated cardboard samples still had an inhibition area of approximately 330 mm², i.e. a decrease of 54% compared to the samples before the release study, whereas their whole quantity of CHX was released during the intermittent diffusion experiments according to Figure 3-III.8. The low quantity of CHX remaining (not measurable with the U.V. Vis spectrophotometer but detectable with the profile curves Figure 3-III.7) did not prevent the samples from being active against *Bacillus subtilis*.

The same pattern was observed with MFC/CHX samples: after the diffusion experiments, the inhibition areas were reduced by approximately 60%. Thus, even after a prolonged diffusion into an aqueous medium, renewed several times, the samples were still highly active. This was expected since only 25% of the CHX was released according to the Figure 3-III.7. Nevertheless, the MFC did not prevent the remaining CHX from being released into the agar (whereas it was quite complicated to detect it into water with the U.V. Vis spectrophotometer) and preserved in parallel the antibacterial activity of the samples.

In spite of previous results proving the positive impact of MFC limiting the release of CHX, no clear difference between both samples was observed after the releasing assays when we consider these antibacterial tests. Indeed, following the releasing tests, each cardboard sample remained active against *Bacillus subtilis* and displayed a similar inhibition zone. This was expected since CHX is a strong antibacterial agent with a very low minimum inhibitory concentration (MIC) against the bacteria studied. Other kinds of bacteria with a stronger metabolism should nevertheless be tested. The low quantity of CHX remaining in the samples after the releasing assays were conducted may not be sufficient against more virulent bacteria.

We also have to keep in mind that, even if the CHX-coated cardboard samples seemed as efficient as the CHX/MFC-coated cardboard samples, their mechanical properties were completely different. Indeed, this was shown in our previous study (Lavoine, *et al.*, 2014), CHX-coated cardboards have much weaker mechanical properties than MFC-coated cardboards. It is worth noting that the CHX solution is composed of 98 wt% of water and, thus, has a similar impact on cardboard as water once coated.

Consequently, food tests were carried out using standard conditions (4-6°C) with the CHX/MFC-coated cardboard samples.

III-3.5. Food test: Shelf-life of pork liver

Pork liver was chosen to carry out the food tests and test the antibacterial activity of the cardboard samples. Pork liver is a sensitive meat with a short shelf-life, even under standard storage conditions (fridge at 4-6°C). Its degradation is thus very rapid and clearly noticeable without any specific measurements. Furthermore, meat has a high water content preventing it from drying during usual storage periods. This food model was, thus, ideal to test the antibacterial efficiency of the cardboard samples, described previously.

Meat is not generally preserved directly in contact with the cardboard packaging. But a novel application could be investigated with this new bio-material. Indeed, we recently proved that the MFC coating significantly improved water absorption properties of the cardboard (Lavoine, *et al.*, 2014). Thus, within the context of a meat-packaging application, the antibacterial cardboard samples could be added into plastic baskets, instead of the current tissues used to absorb the excess of blood. Furthermore, CHX is only active by direct contact between the packaging material and the food, contrary to volatile molecules. The food, therefore, needs to be directly into contact with the antibacterial cardboard to be protected.

3D-boxes were built with A4-cardboard sheets coated or not with antibacterial molecules. CHX/MFC-coated samples were tested and compared with the reference cardboard (none coated) and the polyethylene-coated (PE) cardboard (which displayed similar mechanical properties to MFC-coated cardboard (Lavoine, *et al.*, 2014).

Similar pieces of pork liver were placed in contact with the coated side of the cardboard box. Each box was then wrapped into a plastic bag and placed in the fridge at 4°C. Various references were also added: pork liver directly after purchase (called reference in Figure 3-III.9 and 10), pork liver wrapped in a plastic bag alone and pork liver in contact with Penicillin discs wrapped in a plastic bag.

The degradation of the liver samples was judged according to two different methods: (i) an image analysis of the brightness of the red pixels and (ii) the consumers' point of view.

The first method consisted in performing image analysis of the picture of each pork liver after their storage in fridge. The analysis gave the number and the brightness of the red, blue and green pixels making up the picture. Considering the red pixels only, their cumulative number was plotted as a function of their brightness (from 0 to 255, with 255 the brightener red). The median value of the red pixel brightness was determined from this graph so that the number of red pixels with a lower value was equal to the number of red pixels with a higher value than the median value. Figure 3-III.10, thus, gives the median value of the brightness of the red pixels obtained for each kind of sample. The pork liver directly after the purchase had a brightness of 100, which was the highest value in this study. Considering this optimum, the pork

liver wrapped into the active packaging (CHX/MFC) had the second highest value, proving thus that it was kept fresh longer than the other samples. Nevertheless, its brightness was only slightly higher than the others.

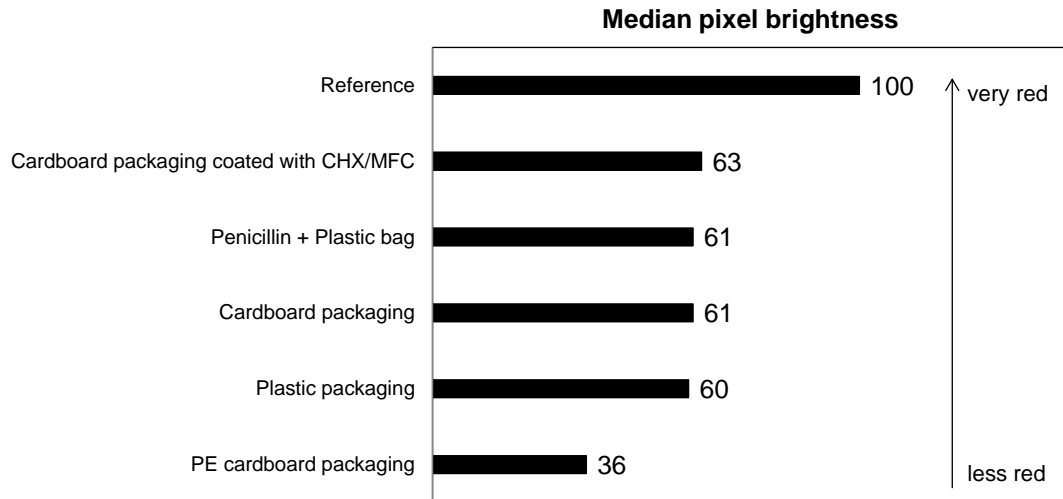


Figure 3-III.10. Median value of the red pixel brightness obtained by an image analysis of the pictures of the pork liver.

Each analysis gave the number and the brightness of the red, blue and green pixels of each picture. The cumulative number of red pixels was plotted as a function of the brightness of the pixel (from 0 to 255, with 255 the brightener red). From this graph, the median value for the brightness of the pixels was determined so that the number of pixels with a higher brightness was equal to the number of pixels with a lower brightness.

This test, thus, highlighted clearly that the pork liver analyzed just after its purchase had the best appearance, whereas the pork liver wrapped in the PE-coated cardboard was the worst packaging solution. The differences between the four other samples were not very significant.

Another test was subsequently carried out to improve the distinction made between the samples. Pictures of the pork liver were submitted to 18 different consumers. They had to classify each picture and give it a grade between 1-5. The highest grade was assigned to the freshest pork liver. Thus, according to this evaluation, the pork liver analyzed directly after its purchase got a maximum of 100 points (=20*5). Figure 3-III.11 presents the pictures of each pork liver after their storage and the number of points assigned to each according to the consumers' point of view. According to the results of the image analysis, the PE-coated cardboard got the lowest grade. Consumers were then able to distinguish the other samples: the pork liver packaged in the active cardboard samples was better graded than those packaged in the reference cardboard and the plastic bag, but appeared less fresh than the pork liver with the Penicillin. According to these results, the CHX/MFC cardboard samples were able to preserve the pork liver much better than the other cardboard samples devoid of any antibacterial agent. Thus,

CHX has quite a strong antibacterial activity, though, according to the antibacterial test results, this activity was less efficient than Penicillin.

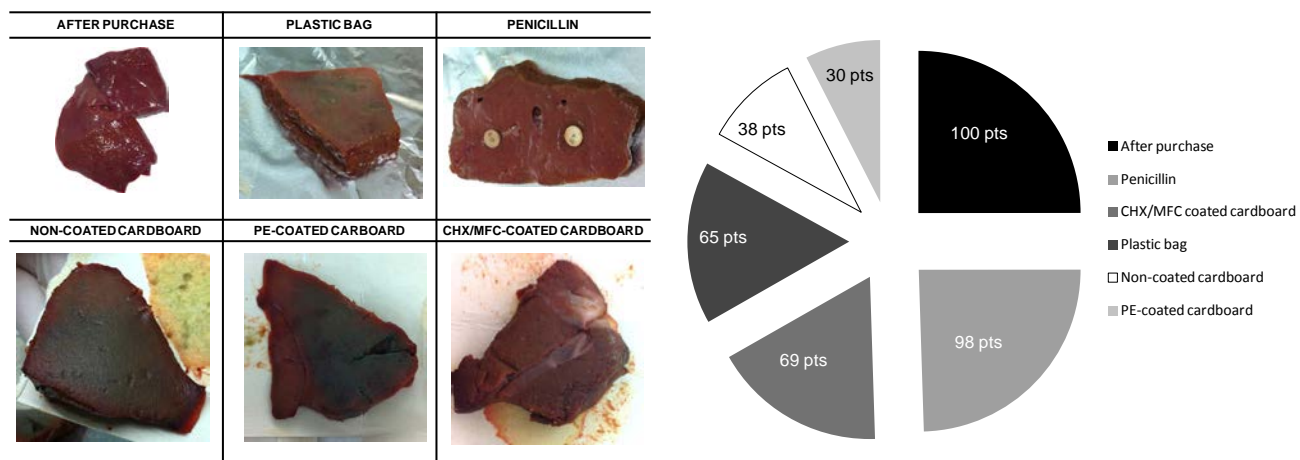


Figure 3-III.11. Classification by the consumers of pork liver samples

The pie chart represents the total number of points assigned to each pork liver sample by the consumer. Between five and one points were assigned respectively to the freshest and less fresh pork liver. The reference 1 had the highest number of points, i.e. 100 points.

Further tests should be conducted following this study and the perspectives it has unveiled for the food-packaging industry. Food tests were quite sensitive since many parameters come into play (nutrient, water content, salt, etc.). Consequently, others tests have to be performed to analyze the migration and impact of the antibacterial agent on the organoleptic properties of food. Toxicity is also an additional parameter to be considered since CHX can be ingested by humans until a certain limit assuring his health. Other antibacterial molecules could also be tested.

III-4. Conclusion

The elaboration of a new antibacterial packaging material was achieved within this study. In order to meet the needs of the current society, a bio-material with antibacterial properties was developed and showed a long-term antibacterial activity improving the shelf-life of food.

A recyclable and biodegradable material was firstly obtained using a cardboard substrate and microfibrillated cellulose (MFC). A model antibacterial agent was introduced into the nanoporous MFC network, preserved during the coating process. This network allowed a more progressive and longer release of the antibacterial agent into the aqueous medium: the amount of CHX released was reduced by 20% owing to MFC.

These results confirmed our team findings on the coating of antibacterial agents in combination with MFC. This combination is besides more adapted to the development of active cardboard materials. The MFC suspension is a biodegradable, lightweight and porous layer able to carry active compounds in a sufficient quantity to bring antibacterial properties.

Indeed, the antibacterial activity of the cardboard samples against *Bacillus subtilis* was preserved, even after the release study was conducted. Furthermore, owing to the cardboard ability to be creased and folded, active boxes were made and their antibacterial activity was tested with pork liver. The CHX/MFC coated cardboard samples were able to preserve the pork liver much better than the other samples, in particular the polyethylene-coated cardboard samples.

The use of MFC, thus, showed its relevant interest in the elaboration of a lightweight antibacterial bio-material with a long-term activity, improving consequently the shelf-life of food. Further food tests and other antibacterial compounds should be tested following this study and the perspective it has unveiled for the food-packaging industry.

Chapter 3-IV Conclusions

In the chapter 3, a new use of the MFC-coated cellulosic materials was investigated. Thanks to the nanoporous MFC network characterized and highlighted in the Chapter 2, the elaboration of a controlled release system of active compounds was achieved in this chapter.

A first molecule was tested so that a complete release study was implemented and performed. The caffeine was an ideal model since it has no chemical interactions with the cellulose, is highly soluble in water and U.V. detectable. Consequently, two main experiments were conducted to study in details the release of caffeine into an aqueous medium: continuous and intermittent diffusion experiments.

Both protocols gave complementary information on the release of caffeine and was either adapted to a food-packaging or biomedical applications.

After comparison of three different samples containing the caffeine with or without MFC, we proved that the MFC layers were able to prolong the release of the caffeine. The amount of caffeine released was indeed smaller when MFC was coated onto the paper surface, and the release of the whole quantity of caffeine lasted, therefore, much longer.

Further to these promising results, similar experiments were carried out with an antibacterial solution of chlorhexidine digluconate (CHX). The previous results were confirmed with these active molecules, although they had strong chemical interactions with the cellulose. In addition to a prolong release of the CHX, the MFC coating preserved the antibacterial activity of the molecules contrary to latex coated samples. Consequently it maintained this activity over a longer period than the samples without any MFC coating (+ 10 and 15 days)..

Following these two studies dealing with the release of either caffeine or CHX into an aqueous medium, the elaboration of an optimal active sample was achieved. Indeed, the most efficient samples in terms of controlled release system were the cellulosic materials coated with the slurry containing the MFC suspension and the active molecules.

Consequently, we applied this strategy to a cardboard material for 3D packaging applications. As expected, the MFC layers released more progressively the CHX molecules, and also preserved the antibacterial activity during the food tests. This strategy was the most adapted to this material of high basis weight. Indeed, the MFC layer did not increase the bulk of the final material and was able to carry more active molecules than the cardboard itself.

The elaboration of a functional bio-nano-material with long-term antibacterial properties was, thus, attained within the context of this Chapter 3. The food-packaging sector was firstly targeted within the framework of this PhD, but other fields could also be investigated such as biomedicine.

Consequently, in this optic, new kinds of material were also developed. The Chapter 4 proposes thus the elaboration of a new functional bio-nano-material based on the synergy of two controlled delivery techniques, the MFC (as proved in this chapter) and the cyclodextrin (as well-known system).

Chapter 3-V References

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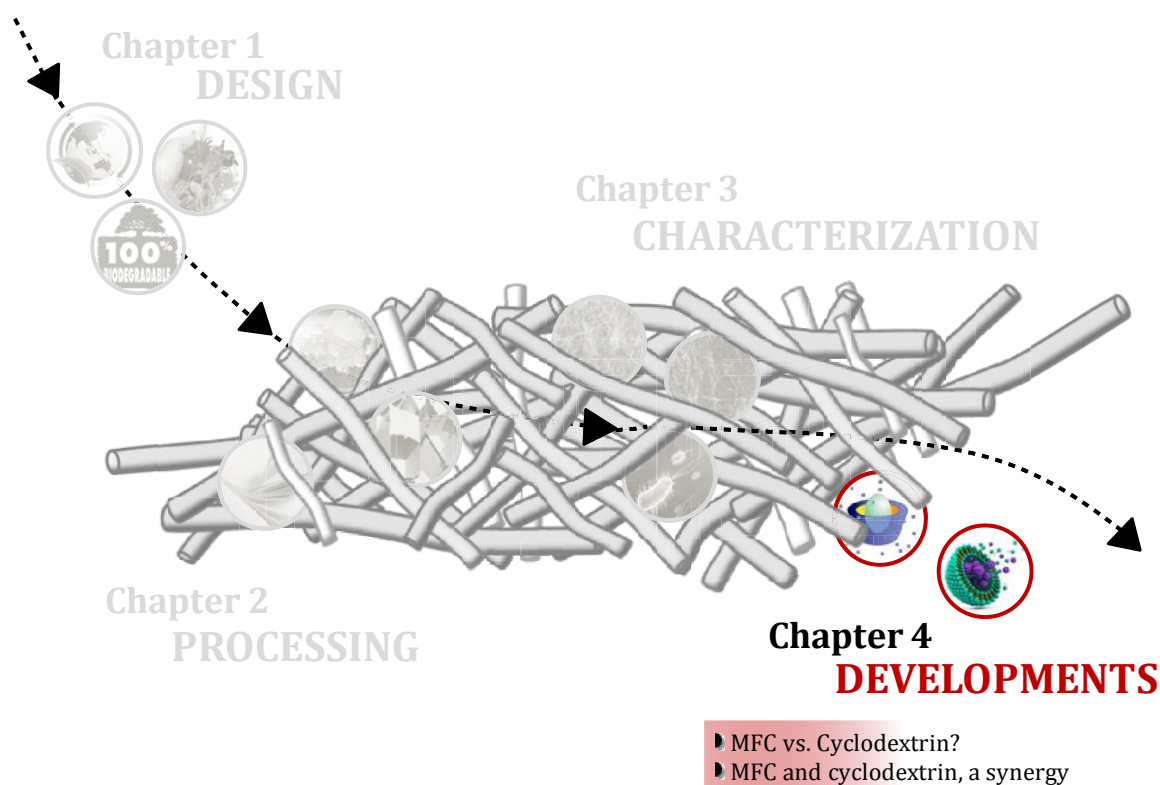


Figure Chapitre 4. Représentation schématique de l'organisation du manuscrit

A la suite du chapitre 3, nous avons démontré que le réseau nanoporeux des MFC couchées sur un matériau cellulosique était à même d'être utilisé comme système de libération contrôlée de molécules actives.

Une molécule antibactérienne en particulier a été testée tout au long de ce chapitre : le digluconate de chlorhexidine (CHX). Cette molécule a été introduite dans le matériau cellulosique, papier ou carton, selon deux stratégies : par imprégnation ou par couchage. La méthode par couchage s'est révélée la plus prometteuse : la molécule active, directement introduite dans la suspension de MFC, a été libérée plus progressivement et sur une plus longue période que la molécule simplement imprégnée dans le matériau cellulosique puis recouverte de MFC. Avec le matériau carton, cette stratégie se montre d'autant plus intéressante qu'il a été possible d'incorporer une quantité plus importante d'agent actif dans le matériau, sans pour autant modifier ses propriétés. Le matériau actif final, couché avec des MFC, se montra tout aussi résistant et barrière qu'un matériau carton couché avec du polyéthylène, et conserva l'état de fraîcheur d'un morceau de foie de porc sur une période plus longue.

Suite à ces résultats prometteurs, nous avons souhaité, dans une dernière partie, améliorer la propriété de libération contrôlée de ce nouveau matériau afin d'élargir son champ d'application à d'autres secteurs tels que le biomédical.

Dans cette perspective, nous avons développé une collaboration avec le laboratoire UMET (Unité Matériaux et Transformation, UMR CNRS 8207), département d'Ingénierie des Systèmes Polymères, spécialisé dans l'étude et l'utilisation de cyclodextrine.

Les cyclodextrines sont des molécules cages ayant la forme d'un cône tronqué dont la cavité est hydrophobe et la membrane extérieure hydrophile. Sa structure conique est formée d'un cycle d'oligosaccharides d'unités glucosidiques liées par liaison α -1,4. En fonction du nombre d'unités de glucose, les dimensions de la molécule ainsi que ses propriétés diffèrent. Dans le cadre de ce Chapitre 4, nous avons utilisé la β -cyclodextrine, composée de sept chaînes de glucose.

La configuration particulière de la cyclodextrine lui permet de se complexer avec des molécules hôtes par interactions chimiques, afin de former ce qu'on appelle un « complexe d'inclusion ». La cyclodextrine est donc en mesure d'encapsuler une molécule, avec qui elle présente des affinités chimiques, mais aussi de la libérer, progressivement, en fonction des conditions extérieures (milieu de libération, température, pH, etc.). Ainsi, cette molécule est très recherchée et exploitée dans le domaine médical pour l'élaboration de système de libération contrôlée de médicaments.

Généralement utilisée comme additif ou greffées sur des matrices polymériques ou des matériaux textiles, la cyclodextrine a fait ses preuves dans le domaine de la libération contrôlée de médicaments (ex : application pour des prothèses), d'agents antibactériens (ex : textiles médicaux) ou d'insecticides (ex : moustiquaires plastiques). Le laboratoire UMET a, par ailleurs, grandement contribué à son développement puisqu'il est l'auteur d'une méthode brevetée d'un greffage chimique de cyclodextrines sur tout type de biomatériaux.

Ce protocole a été pour la première fois appliqué sur des substrats celluloseux par notre laboratoire avec l'étude de Cusola, *et al.* en 2013. Cette étude a montré que le matériau papier pouvait lui aussi être considéré comme système de libération prolongée de molécules actives.

Ces premiers résultats, ainsi que ceux développées dans les chapitres précédents, ont donc centré notre collaboration sur deux stratégies principales pour le développement de nouveaux systèmes de libération contrôlée : (1) la réalisation d'un papier couché avec à la fois des MFC et de la cyclodextrine et (2) l'élaboration d'un papier préalablement greffé avec des cyclodextrines, puis couché avec des MFC.

Ce chapitre 4 présente donc des résultats innovants, en ce sens que, pour la première fois, l'association de la cyclodextrine et des MFC est envisagée avec le matériau papier pour l'élaboration de systèmes de libération contrôlée.

Ainsi, dans une **première partie**, la stratégie (1) a été mise en application. Le chapitre 3 nous a en effet montré que le réseau nanoporeux de MFC était en mesure de libérer plus progressivement les molécules actives directement incorporées dans la suspension. Partant d'un principe similaire, nous avons souhaité introduire dans ce réseau le complexe d'inclusion comprenant la cyclodextrine et la molécule active. La solution de digluconate de chlorhexidine (CHX) a, ici encore, été utilisée. L'inclusion de ce composé actif dans la cyclodextrine a en effet été prouvée dans plusieurs études précédentes.

Des études de libération, basées sur les protocoles exposés au chapitre 3.1., ont été réalisées analysant l'influence des MFC, de la cyclodextrine et du mélange sur la libération du CHX dans l'eau.

Les MFC et la cyclodextrine seules ont, en accord avec la littérature, libéré les molécules de CHX plus progressivement que le matériau ne comprenant aucun des deux composés (palier de libération atteint en 24 h au lieu de 3 h).

Cependant, la cyclodextrine s'est montrée plus efficace que les MFC : sa libération a perduré pendant un total de 36 lavages alors que les MFC ont libéré l'ensemble du CHX au bout de 26 lavages.

Contrairement à l'objectif premier visé, le mélange des MFC avec la cyclodextrine n'a pas donné lieu à une synergie de libération : la quantité totale de CHX a aussi été libérée en 24 h, et le nombre de lavages obtenu a été légèrement plus élevé que celui de l'échantillon couché avec les MFC (28 vs. 26), mais plus faible que le total obtenu avec l'échantillon enduit de cyclodextrine (28 vs. 36).

Ce dernier échantillon présenta un résultat tout à fait surprenant et a orienté l'objectif premier de cette partie vers une perspective d'application très prometteuse. En effet, contre toute attente, l'échantillon couché avec le mélange (cyclodextrine + CHX) s'est révélé le plus efficace en termes de systèmes de libération contrôlée, alors qu'aucun greffage de la cyclodextrine n'a été réalisé. Ceci est dû au complexe (cyclodextrine : -CHX) qui bénéficie de beaucoup plus d'interactions avec la cellulose que les molécules seules de CHX ou de cyclodextrine.

En effet, il est important de préciser que cette application n'aurait pu être possible sans la présence d'une molécule telle que le CHX, qui présente de fortes interactions avec la cellulose (hydrogène et ionique). Par ailleurs, dans ce cas particulier, la libération du CHX implique la libération de tout le complexe. Par conséquent, une fois la libération de l'agent actif terminée, le matériau papier devient complètement obsolète et ne peut, éventuellement, être ré-utilisé.

Ainsi, cette première partie a montré qu'il était possible de coucher simplement la cyclodextrine avec le CHX sur le matériau papier afin d'obtenir un système de libération contrôlée sur le long terme mais pour une seule utilisation. L'activité antimicrobienne du

complexe cyclodextrine :-CHX peut aussi être remise en question pour des bactéries plus résistantes que la *Bacillus subtilis* testée dans ce projet.

Afin de pallier à cette libération de la cyclodextrine en milieu aqueux, la **deuxième partie** de ce chapitre présente la stratégie (ii) envisagée dans le cadre de la collaboration: le greffage du papier avec de la cyclodextrine, suivi de son couchage avec les MFC.

Le greffage du matériau papier avec la cyclodextrine a été réalisé selon le protocole développé par le laboratoire UMET, qui consiste en l'imprégnation du matériau papier dans une solution de cyclodextrine, d'acide citrique et d'un catalyseur. Le greffage de la cyclodextrine est obtenu après séchage à 160°C, pendant 30 minutes du matériau imprégné. Ce protocole est effectif puisqu'il permet de greffer environ 10% de cyclodextrines sur un substrat papier d'environ 40 g/m². Cependant, ce protocole entraîne aussi le greffage de l'acide citrique à un taux de 4%, qui induit, en parallèle, une perte considérable des propriétés mécaniques du papier.

Cette perte mécanique provient de la détérioration des fibres de cellulose, et par conséquent le couchage des MFC réalisé par après n'a pu que légèrement contrebalancer l'impact négatif du procédé de greffage sur les propriétés mécaniques du réseau fibreux. Malgré cela, qualitativement, le papier non couché sembla plus friable et beaucoup plus jaune que le papier couché avec des MFC. La présence des MFC, en synergie avec la cyclodextrine, a toutefois contribué à une libération à plus long terme de la molécule active.

Dans le cadre de cette deuxième partie, une autre molécule antibactérienne a été testée, le carvacrol. Extraite des huiles essentielles d'origan ou de thym, cette molécule est hydrophobe et volatile. Son association avec des matériaux hydrophiles ainsi que la conservation de son activité antibactérienne, fortement liée à sa nature volatile, sont par conséquent très délicats. Cette molécule est cependant particulièrement bien adaptée au principe de la cyclodextrine : par son aptitude à être encapsuler par la cyclodextrine, sa diffusion est limitée et ses propriétés antibactériennes sont préservées.

Le carvacrol a donc été introduit dans le matériau papier greffé avec de la cyclodextrine suivant deux stratégies : par (i) diffusion et par (ii) imprégnation.

La première stratégie (i) fut inefficace : en effet, la quantité de carvacrol diffusée dans le matériau papier fut si minime qu'aucune action antibactérienne ne fut détectée.

La deuxième stratégie (ii) a donc été privilégiée, et chaque papier greffé a été imprégné dans une solution de carvacrol mélangée à de l'éthanol afin de limiter l'aspect « huileux » du papier final.

Suite à l'étude de la libération du carvacrol dans l'agar (considéré comme aliment modèle), le papier greffé avec de la cyclodextrine puis couché avec des MFC libéra le carvacrol sur la plus longue période en comparaison avec les autres échantillons.

Si le papier simplement greffé avec de la cyclodextrine fut actif pendant près de 14 h, l'ajout de MFC prolongea son activité de 2 h de manière reproductible. Une légère synergie de libération a donc été observée entre les MFC et la cyclodextrine. Cette dernière a permis le contrôle de la libération du carvacrol dans toute l'épaisseur du papier, et les MFC ont agi par la suite exclusivement en surface, prolongeant davantage l'action antibactérienne de l'échantillon.

Cette synergie aurait été d'autant plus efficace si la couche de MFC avait été réalisée dans des conditions optimales. En raison de la présence de carvacrol, les couches de MFC n'ont pu être séchées à 105°C (température prévue dans le protocole développé dans le chapitre 2), afin d'éviter la perte massive d'agent antibactérien. Par conséquent, le réseau nanoporeux des MFC fut beaucoup moins lié, ce qui s'est confirmé lors des études de libération en milieu aqueux : la couche de MFC a commencé à se déliter.

Ce chapitre 4 propose un nouveau modèle de système de libération contrôlée fondé sur l'utilisation en parallèle des MFC et de la cyclodextrine. Deux stratégies ont été développées : (1) le couchage de la cyclodextrine avec le MFC et (2) le greffage de la cyclodextrine suivi du couchage des MFC.

Si la stratégie (1) n'a pas donné lieu à une synergie de libération entre les MFC et la cyclodextrine, la stratégie (2), bien qu'efficace, a considérablement endommagé les propriétés mécaniques du matériau final.

Un compromis est donc à trouver entre ces deux stratégies pour a) développer un système de libération prolongée reposant sur l'action complémentaire des MFC et de la cyclodextrine et pour b) obtenir un matériau final aux propriétés mécaniques et barrières nécessaires et suffisantes pour une application soit dans l'emballage alimentaire soit dans le biomédicale. Plusieurs perspectives peuvent donc envisagées dans cette optique :

- Le couchage successif de cyclodextrine puis de MFC en se basant sur un concept de « Layer-by-Layer » ;
- Le greffage direct des MFC avec la molécule de cyclodextrine, en préalable de la fonctionnalisation et du couchage de cette suspension greffée sur le matériau papier ;
- L'utilisation d'un polymère de cyclodextrine qui pourra directement être introduit dans la suspension de MFC, évitant ainsi le procédé de greffage à l'acide citrique et la libération de toute la cyclodextrine dans le milieu de libération.
- Enfin, d'autres MFC pourront aussi être utilisées telles que les MFC prétraitées chimiquement avec l'agent TEMPO qui, grâce à leurs groupes carboxyles, peuvent créées des interactions plus fortes avec des molécules ou des polymères de cyclodextrines cationiques, favorisant ainsi une absorption ou un greffage de la cyclodextrine sans endommager la cellulose avec l'acide citrique.

Controlled release fiber-based system using cyclodextrin and microfibrillated cellulose

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Abstract

Further to previous studies proving the relevant interest in using MFC-coated papers as controlled release system, this study tried to develop a more performing delivery system using in addition a well-known one: the cyclodextrin.

In this study, an antibacterial molecule, the chlorhexidine digluconate (CHX), was added in the MFC suspension, the β -cyclodextrin solution or both before being coated onto a cellulosic substrate. The release study of the CHX was conducted following two strategies: its continuous diffusion and its intermittent diffusion, i.e. a diffusion interrupted by the renewal of the release medium, into an aqueous medium. SEM, FE-SEM and FTIR analyses were performed to give an interpretation of the release mechanism of the CHX.

As already proved in the literature, the cyclodextrin and the MFC showed their efficiency as controlled delivery system. Nevertheless, compared to the MFC, the β -cyclodextrins released the CHX over a longer period, mainly due to its ability to form an inclusion complex with the CHX.

When mixing both controlled release systems, no real synergistic action was deduced from the release study: the paper samples coated with the three compounds released over a same period of time than the paper coated with the MFC.

However, we showed in this study the high performance of the coated cyclodextrin as controlled delivery system. This system released, indeed, the CHX molecules much longer than the other samples, whereas the cyclodextrins were only coated onto the paper surface.

Thanks to the CHX and cellulose interactions and to the CHX:-cyclodextrin inclusion complex, this study paved the way for a future use of the cyclodextrin with cellulosic substrate: instead of grafting the molecules onto the substrate, its coating could be a new key solution, preventing the substrate from successive converting processes.

Inspired from Paper 7:

*Nathalie Lavoine, Nicolas Tabary, Isabelle Desloges, Bernard Martel and Julien Bras (2013)
Controlled release fiber-based system using cyclodextrin and microfibrillated cellulose.
Submitted to Material Science Engineering-Part C.*

I-1. Introduction

During the last two decades, a trip back to bio-based materials has been the main concern of lots of researchers. The next step is now the development of smarter bio-materials. For example, in different applications such as medical excipient or antibacterial food-packaging, the active agent introduced in the matrix must be released to be effective, but not too fast. To achieve a long-term activity, a controlled release is thus needed and several possibilities were already tested.

Among them, a first possibility consists in using cyclodextrins (CDs). CDs are cyclic oligosaccharides composed of D-glucose units that are joined by α -1,4-glycosidic linkages. The most commonly used CDs subtypes are α CD, β CD and γ CD, which consist of 6, 7 and 8 D-glucose units, respectively. The three-dimensional structure of these CDs makes them attractive: considered as truncated cone, the hydroxyl groups located at the outer surface of the molecule make it hydrophilic, whereas the inner cavity is hydrophobic. CDs have thus the capability to trap guest molecules and form host-guest inclusion complexes, mainly driving by hydrophobic or van der Waals interactions (van de Manakker, *et al.*, 2009). They are thus potential bio-based candidates to achieve a controlled release of active agent. Indeed, primarily used in pharmaceutical field as solubilizers for lipophilic drugs (Brewster and Loftsson, 2007, Jansook and Loftsson, 2009, Loftsson and Duchêne, 2007), they are more and more used as trap molecules for drug delivery systems especially for medical and pharmaceutical fields (Hoang Thi, *et al.*, 2010, Loftsson and Duchêne, 2007, Salústio, *et al.*, 2011, Vyas, *et al.*, 2008). From this perspective, CDs were associated with different kinds of materials. They were often combined with polymeric matrices under various shapes (hydrogels (Li, *et al.*, 2003), prostheses (Blanchemain, *et al.*, 2005), membranes (Tabary, *et al.*, 2007) or spheres (Krauland and Alonso, 2007)) and showed very attractive results in terms of controlled and prolonged release.

Other applications were also targeted such as the textile industry. For example, after grafting the inclusion complexes CD:-insecticides onto a fabric, this latter kept its efficacy for a longer time after treatment (Romj, *et al.*, 2005), and even after successive washing (Wang and Cai, 2008).

Further to these promising results, Cusola, *et al.*, 2013 grafted very recently CDs onto three different paper-based substrates using a patented process developed for different kinds of biomaterials (Martel, *et al.*, 2008). A polymerization reaction was achieved by the immersion of the cellulosic substrates into a solution composed of β CDs, citric acid and a catalyst, leading then to the grafting and the formation of a stable three dimensional CD-polymer network. The CD-polymer grafted papers were then immersed in an antibacterial solution of chlorhexidine digluconate (CHX). They presented thus a longer release (7 days more) compared to the non-

grafted paper-samples. Up to our knowledge, this study is the only one dealing with the grafting of CDs onto paper substrates in order to develop a new delivery system for antibacterial materials. It indeed proved that paper-substrates were able to be grafted with polymers of CDs and used as a carrier and controlled release system of an active molecule (Figure 4-I.1).

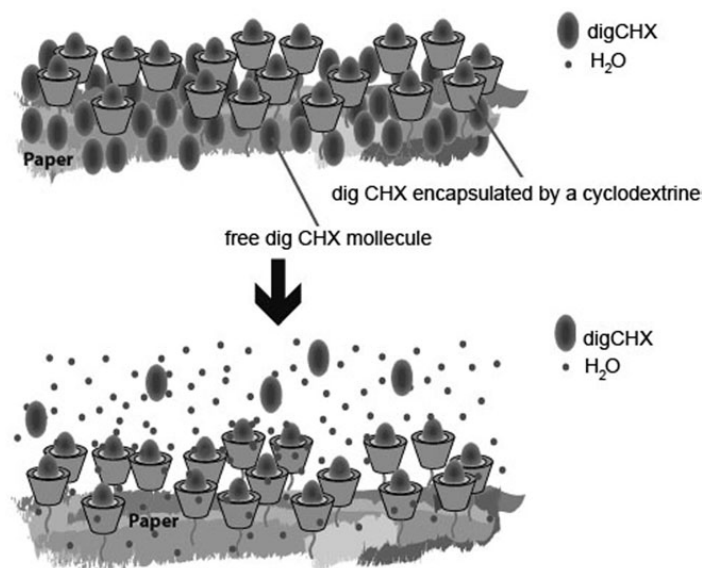


Figure 4-I.1. Schematic representation of the massive release of a paper grafted with cyclodextrins and loaded with a solution of chlorhexidine digluconate (dig CHX) (from Cusola, *et al.*, 2013)

The development of controlled release system based on paper substrates can also be achieved using microfibrillated cellulose (MFC).

MFC is made from cellulosic fibers thanks to their high shearing mechanical treatment. Its first manufacturing process was patented in 1985 by Turbak, *et al.*, 1985 and consisted in pumping the cellulose pulp at high pressure and through a spring-loaded valve assembly. Since then, other mechanical treatments were developed, and due to the high energy consumption induced, enzymatic and chemical pre-treatments are currently carried out before applying mechanical treatments. Whatever the manufacturing process the MFC obtained has in most of the case diameters between 10-50 nm and lengths exceeding 1 μm . The nanometric dimensions of MFC make it a very promising and astonishing material. In many studies, MFC clearly shows its ability to mechanically reinforce polymers and improve their barrier properties (Lavoine, *et al.*, 2012, Siró and Plackett, 2010). Besides, once dried, MFC is able to form films with competitive properties (Belbekhouche, *et al.*, 2011, Syverud and Stenius, 2009). Currently, the interest in MFC is drastically increasing as well as its use in various applications. Very recently, its application as carrier and drug delivery systems was investigated. MFC was used as tablet excipient (Kolakovic, *et al.*, 2011), as suspension (Valo, *et al.*, 2011), particles (Kolakovic, *et al.*, 2012a), films (Cozzolino, *et al.*, 2013, Kolakovic, *et al.*, 2012b) and aerogels (Valo, *et al.*, 2013).

Whatever its shape, it was able to release progressively the loaded drugs. In addition to these results, we demonstrated that MFC-coated papers were also able to release more slowly the loaded active molecules (Lavoine, *et al.*, 2013a). The ability of MFC to act as delivery and controlled release system was mainly due to its nanoporous network. Experiments were carried out with the similar antibacterial solution; chlorhexidine digluconate (CHX). The paper substrates coated with a mixture of MFC and CHX needed 24 h and around 23 washings in aqueous medium so that the entire amount of CHX was released (against 2 h and 18 washing steps for a substrate without MFC). These first results were very promising and paved the way for the use of MFC-coated paper substrates as new delivery systems of active molecules.

Based on these results, the present study aims, thus, to improve the release duration of active molecules loaded in MFC-coated paper substrates by using cyclodextrins. The novelty of this study lies thus in the simultaneous use of MFC and β CDs in order to develop a more efficient controlled release system. Compared to the previous strategies which grafted CD-polymers in the whole paper structure, β CDs are here only mixed with an antibacterial solution and/or MFC, so that they can be deposited onto the paper surface using a bar coating process. The antibacterial solution used in this study is the aqueous solution of CHX. As detailed before and reported in many studies (Cortés, *et al.*, 2001, Denadai, *et al.*, 2007, Tabary, *et al.*, 2007), CHX was often used in combination with CDs as the host-guest inclusion. Its release was then carried out in water by investigating two different protocols: (i) continuous and (ii) intermittent diffusions (with renew of release medium) so that a synergistic or complementary action of both β CDs and MFC can be highlighted and understood.

I-2. Materials and methods

I-2.1. Materials

The MFC suspension was produced and furnished by FCBA (France). The suspension was manufactured as following: Domsjö pulp was first enzymatically pre-treated with an endoglucanase for 1 h. Then, the pulp was mechanically treated using a homogenizer GEA Ariete Niro Soavi®. It was subjected to four passes under a pressure of 1,400 bars. The final concentration of the MFC suspension is 2%wt.

β -cyclodextrin (CAS n° 7585-39-9) was purchased from Sigma-Aldrich (France) and used as received.

Chlorhexidine digluconate solution (CAS n° 18472-51-0) was purchased from Sigma-Aldrich (France) at 20% in water. This solution was diluted at 2% in deionized water for its use in this study.

The cellulosic substrates were kindly delivered by Ahlstrom (France). With a basis weight of 42 g/m², this paper is one side coated. Its use is initially intended for food-packaging application.

I-2.2. Methods

Samples preparation

Four different kinds of paper samples were prepared as described by Figure 4-I.2: (1) CHX coated-paper samples, (2) CHX-MFC coated-paper samples, (3) CHX-βCD coated-paper samples and (4) CHX-MFC-βCD coated-paper samples.

Coating process

The samples were obtained by the coating of the base paper samples with a specific solution or suspension. The coating process applied was similar for each sample. It consisted in using a bar coating process with a 0.9 Mayer bar, at a speed of 5 cm/s.

Five successive layers of the solutions/suspensions (described below) were coated onto the untreated paper surface. Between each layer, the paper samples were dried for 3 min at 105°C using a contact drying system. After the last coating, a longer drying of 1 h at 105°C was carried out to determine the dried coat weight of each sample.

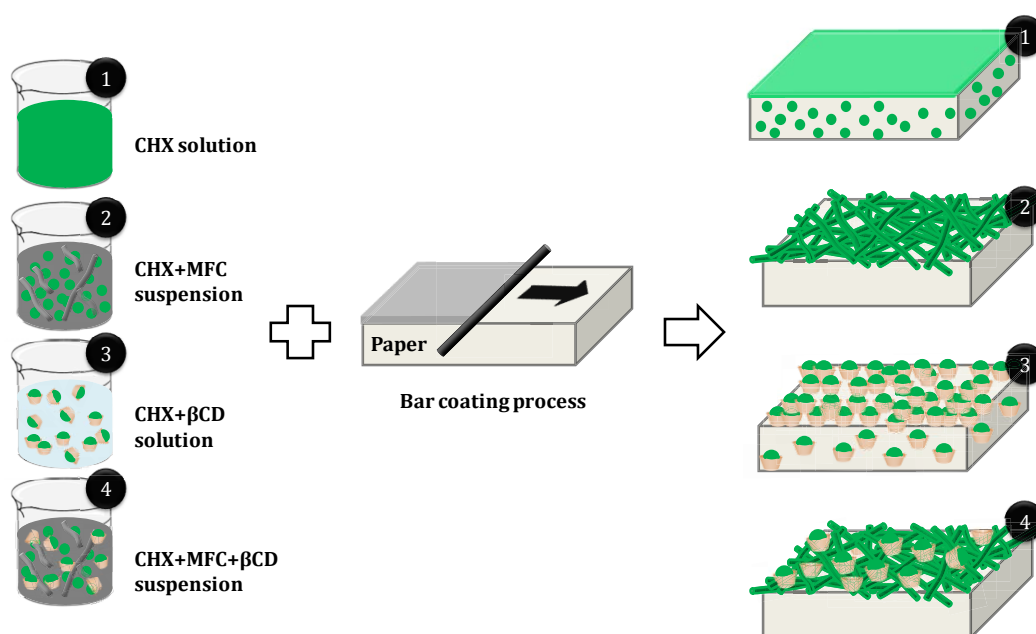


Figure 4-I.2. Strategies applied in this study to prepare the four different coated paper samples. Four solutions/suspensions were prepared: (1) CHX solution diluted at 2% in water, (2) CHX+MFC suspension, (3) CHX+βCD solution and (4) CHX+MFC+βCD suspension.

Five layers were successively coated onto the paper samples using a bar coating process.

Coating slurries

Four solutions and/or suspensions to be coated were prepared by mixing CHX with MFC or β CD or both. The exact amount of each component was expressed in dried mass (g) per mass of dried paper in the Table 4-I.1.

(1) The first coating solution consisted in the chlorhexidine digluconate solution diluted at 2%wt in deionized water. The solution was kept one night at 4°C before being coated five times onto paper samples.

(2) The second suspension was prepared by mixing CHX with MFC using an Ultra-Turax (France). CHX and MFC had both a concentration of 2%wt. A suspension of 75% of MFC and 25% of CHX was then prepared and kept one night at 4°C before being coated onto paper samples. From the measurement of the dried coat weight of the coated paper substrates, the quantity of both components was calculated and expressed in grams per grams of the dried mass of the paper substrate (Table 4-I.1).

(3) For the third solution, CHX and β CD were mixed in deionized water in proportion 67/33% respectively. The solution was also stirring using an Ultra-Turax and kept one night at 4°C before being coated onto paper samples. The table 4-I.1 gives the final quantities of both components present into the dried coat weight of the coated paper substrate.

(4) The last samples were coated with a suspension including CHX, MFC and β CD in proportion 22/67/11% respectively (see Table 4-I.1 for the quantity in grams). The β CD was first solubilized in the CHX solution (2%wt). The final solution was then added to the MFC suspension. The mixture was finally stirring using an Ultra-Turax and kept one night at 4°C before being coated onto paper surface.

Each coating color was prepared so that almost similar quantities of CHX, MFC and β CD were coated onto the paper substrates. For each coating color, at least six samples of 10×10 cm² were prepared and weighted for the further release study.

Coating color	Amount of (g/g of paper)		
	CHX	β CD	MFC
CHX	0.04±0.002	0	0
CHX/ β CD	0.01±0.007	0.010±0.006	0
CHX/MFC	0.02±0.01	0	0.078±0.008
CHX/ β CD/MFC	0.02±0.01	0.013±0.02	0.078±0.016

Table 4-I.1. Composition of each coating color

The quantity (in grams) of each component per gram of paper (dried) is expressed as a function of the coating color prepared.

Determination of the CHX quantity introduced in paper samples

The total amount of CHX introduced into the 10×10 cm² paper samples before the release study was determined by mass difference of the dried paper samples after and before coating. Before coating, each paper sample was indeed dried for 1 h at 105°C using an oven. The dried mass (g) was determined using a Lhormagy balance (±0.001 g). After coating, the similar process was applied to get the mass of the dried coated paper samples (g) and deduced the dried coat weight of each sample.

Knowing the exact quantity of CHX introduced in the four coating colors, the percentage of CHX remaining in the dried coat weight of each sample was then able to be calculated.

The determination of the CHX amount introduced into each paper sample is based on the hypothesis that each sample was homogeneously coated. Besides, to ensure the repeatability of this procedure, at least six specimens were done for each coating color.

Release study

Two protocols were carried out for analyzing the release of CHX into an aqueous medium (Lavoine, *et al.*, 2013c).

The coated-paper samples of 10×10 cm² was adhered to a light expanded polystyrene support using scotch tape in order to study only the release of CHX from the sample's surface. These experiments were repeated on three specimens of each sample for both release protocols, at room temperature and under sink conditions.

(i) Continuous diffusion experiments

The continuous diffusion was studied in 500 mL of deionized water that was continuously stirred with a magnetic stirring-bar at around 200 rpm. The samples were placed at the surface of the aqueous medium. At successive intervals, 3 mL of the solution were taken for UV analysis at a wavelength of 254 nm.

(ii) Intermittent diffusion experiments

This protocol was similar to the continuous diffusion experiments, except that between each hour sampling step, the aqueous medium was renewed.

The analysis of the absorbance of the solution sampled was carried out with a UV spectrophotometer SHIMADZU UV1800 at a wavelength of 254 nm. Using a calibration curve, the concentration of CHX released was then determined as a function of time.

$$A = 31.38 * C$$

Where *A*, is the absorbance of CHX in water at 254 nm and *C*, the concentration of CHX solution measured in g/L.

Infrared spectroscopy (FTIR)

The release mechanism of the CHX, β CD and of the inclusion complex CHX: β CD into water was studied using Infrared Spectroscopy (FTIR).

β CD was analyzed under its powder shape. The CHX solution (2%wt) was dried over night at 105°C in a ventilated oven, so that the dried CHX powder can be obtained. The release media of the paper samples coated with the slurry CHX/ β CD were dried over night at 105°C in a ventilated oven after 3 h and 24 h of release. The powders obtained were respectively used for the FTIR analyses.

KBr pellets containing 1 wt% of each different powder were consequently prepared and their IR spectra were recorded in absorbance mode using a PerkinElmer *Spectrum65* FTIR spectrometer in the 4,000-500 cm^{-1} wave number range, with a resolution of 4 cm^{-1} and 10 scans. At least three different pellets per sample were analyzed to confirm the reproducibility and a representative spectrum was selected for discussions.

Samples characterization

Before analyzing the coated-paper samples, the MFC suspension was observed using a Field-Emission Gun Scanning Electron Microscopy (FE-SEM, Zeiss® Ultra-55, France). The MFC suspension was spread onto a metal substrate using carbon tape, allowed to dry for two nights at room temperature, and coated with a thin layer of gold. The working distance used with the FE-SEM was 5.5 mm for an accelerating voltage of 2.00 kV at a magnitude of $\times 20.00\text{K}$ and $\times 50.00\text{K}$. The diameter of MFC was then determined by an image analysis using the ImageJ® software.

The base paper and coated-paper samples were analyzed by a Scanning Electron Microscopy (SEM) using a Quanta200® (USA). The Back-Scatter detector mode (BSE) was used in order to distinguish the cellulosic parts from the paper fillers. The respective surfaces and slices of the base and coated papers were precisely cut, and mounted onto a metal substrate using a carbon tape. The working distance employed was 9.6 mm with a voltage of 12.5 kV at a magnitude of $\times 150$ for surfaces and respectively 9.6 mm, 15.0 kV and $\times 1200$ for the cut views.

I-3. Results and discussion

I-3.1. Characterization of MFC and paper samples

Due to its high specific area, the MFC suspension (2%wt) looks like a white gel (Figure 4-I.3). According to literature (Iotti, *et al.*, 2010) and previous studies (Lavoine, *et al.*, 2013b), this gel has a shear-thinning behavior and is able to be coated onto different substrates. At higher magnification, an entanglement of nano-fibrils is observed thanks to FE-SEM and after an image analysis, diameters of about 33 ± 8 nm were measured. None conclusion can be drawn about the length of the nano-fibrils mainly because of their tight entanglement. Nevertheless, according to others studies (Pääkkö, *et al.*, 2007, Siró and Plackett, 2010), MFC has length exceeding 1 μm ; mainly when it has been enzymatically treated (like in our case).

The MFC suspension presented thus nano-scale dimensions and according to optical microscopy images (data not shown), it contained nano-fibrils of similar diameters with very low amount of aggregates or macro-scale fibers. The MFC used in this project were then of good quality and could be considered as nanofibrillated cellulose (NFC) or cellulose nanofibrils (CNF). Indeed, up to date, no standardization regarding its designation exists in spite of several actions (Marrapese, 2013). Because of that and the exponential increase of research in this area, different grade quality of MFC and similar denominations (MFC, NFC, CNF, etc.) can lead to confusion. In this study, the term MFC will be applied and used to describe the suspension of 2%wt used as paper coating.

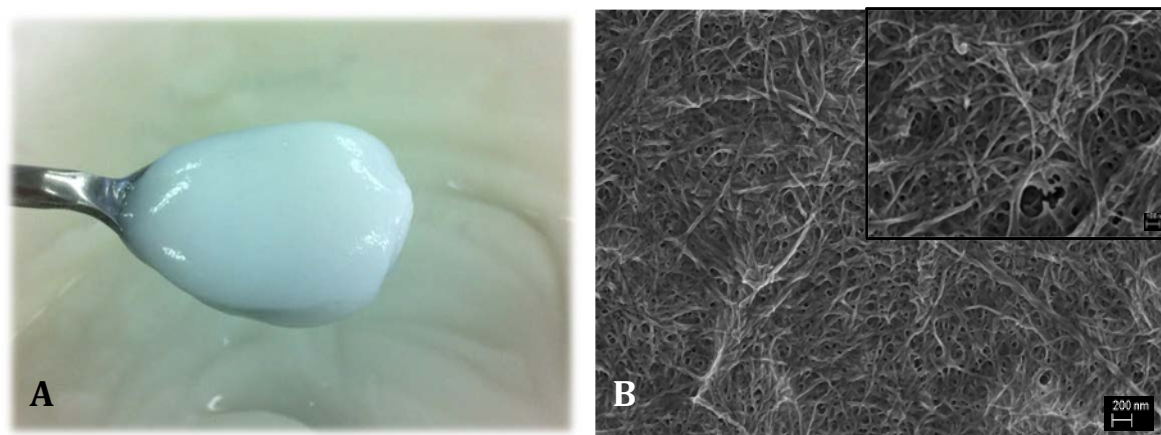


Figure 4-I.3. (A) Picture of the MFC suspension (2%wt) enzymatically pre-treated and mechanically treated with homogenizer and (B) FE-SEM images of this suspension at $\times 20.00\text{k}$ and $\times 50.00\text{k}$

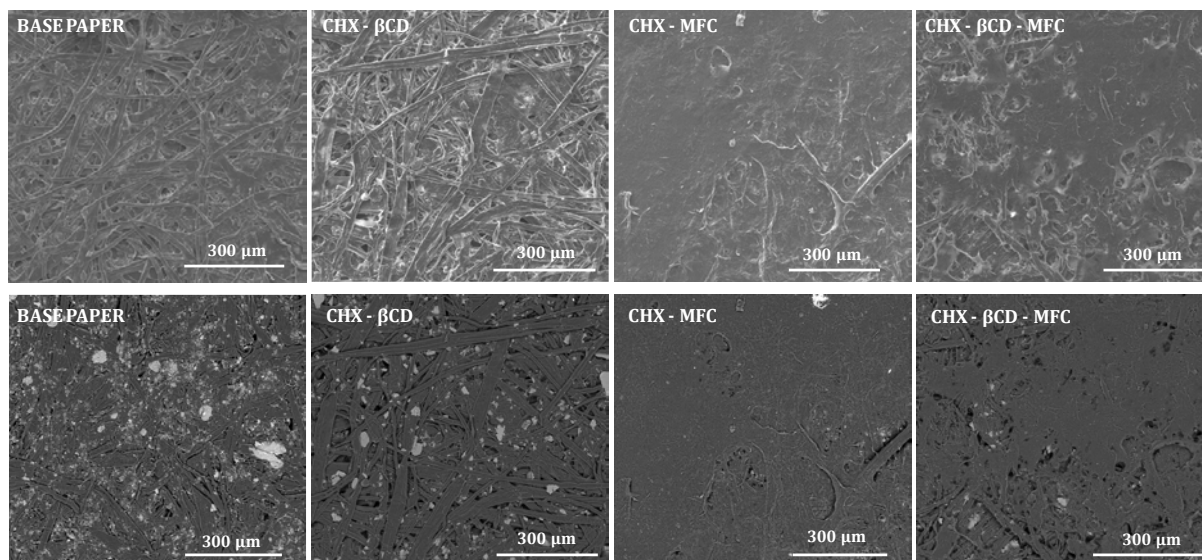


Figure 4-I.4. SEM images of paper sample surfaces ($\times 150$).

Four samples are represented: *(from left to right)* (i) base paper, (ii) paper samples coated with 5 layers of the solution (CHX + β CD), (iii) paper samples coated with 5 layers of the suspension (CHX + MFC) and (iv) paper samples coated with 5 layers of the suspension (CHX + β CD + MFC). On the top, the FE-SEM images are usual topography. The images below were taken using the BSE mode (*white spots: papers fillers; black spots: cellulosic parts*).

Paper samples were coated with four different coating colors comprising CHX, MFC and β CD in various proportions as described in Figure 4-I.2 and Table 4-I.1. The SEM image of each paper surface is presented in Figure 4-I.4. Their topography is represented with the usual detector on the top, and just below, the same images are shown with the Back-Scattered Electrons (BSE) mode, highlighting thus the fillers and cellulosic parts onto the samples surface. Compared to the base paper, the paper samples coated with the solution (CHX + β CD) present a similar topography. Fibers are obviously observed in each case. BSE mode highlights the high number of fillers (in white) initially introduced in base paper. With coating, the structure seems a little bit more opened with less filler than base paper. This is due to the coating of an aqueous solution, which opened the fiber network by the coating of a high quantity of water. The samples coated with the suspension (CHX + MFC) have a different topography than previous samples. Indeed, with the coating of MFC, the fibers network of paper is entirely covered. As shown in a previous study (Lavoine, *et al.*, 2013b), the paper surfaces were entirely covered after five successive MFC layers, i.e. with a coat weight of about 7 g/m^2 . The coating of the suspension (CHX + MFC) shows however slight defects. The sample surface has some holes and some filler are still visible. These defects are more consequent with the paper samples coated with the suspension (CHX + MFC + β CD). It is clear that the surface is not homogeneously covered. One explanation is the interactions between on the one hand β CD and MFC and on the other between CHX and MFC. β CD is composed of glucose units and is thus able to form hydrogen bonds with

MFC, which promotes their aggregation. The interactions between CHX and MFC play also a major role. According to literature (Blackburn, *et al.*, 2007, Giménez-Martín, *et al.*, 2009), chlorhexidine is able to be adsorbed onto the cellulosic fibers by hydrogen bonding between biguanide groups and *p*-chlorophenol of CHX molecule with the hydroxyl groups of cellulose polymer. MFC has a specific area ten times higher than cellulosic fibers (Siqueira, *et al.*, 2009). Its interactions with CHX are then multiplied, limiting thus the number of hydrogen interactions between MFC and promoting the aggregation.

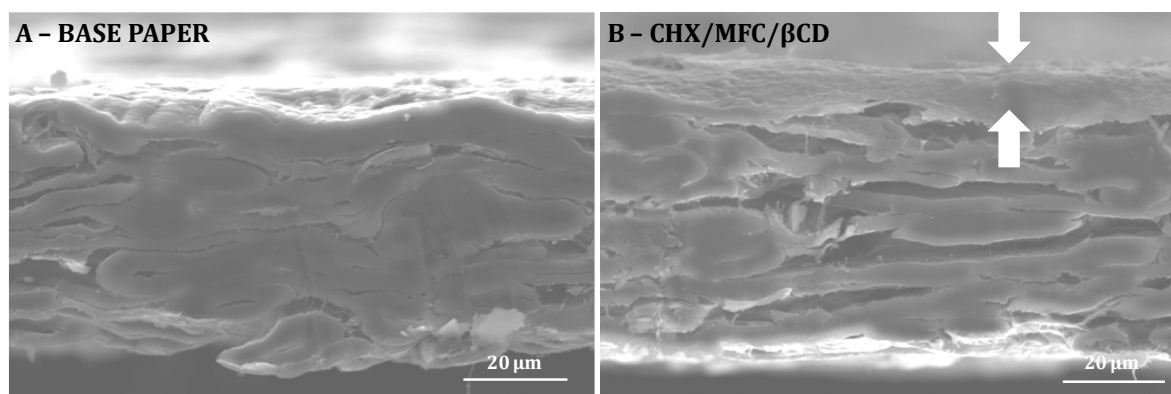


Figure 4-I.5. SEM images of paper samples slices: (A) base paper and (B) paper samples coated with (CHX + MFC + β CD) ($\times 1200$)

The impact of these interactions was also observed onto the SEM images of the paper sample slice. Figure 4-I.5 presents the cut view of the base paper compared to the paper samples coated with the suspension (CHX + MFC + β CD). As previously said, the fiber network of the coated-paper samples is more opened than that of base paper. Nevertheless, this is rather due to the effect of water during the coating process. Indeed, the coating of the aqueous MFC suspension was specifically located onto the paper surface.

1-3.2. Release study

The release study of CHX in an aqueous medium was studied following two protocols. The first one is a continuous diffusion as a function of time. The results are presented in Figure 4-I.6. The graph plots the released amount of CHX (%) for each kind of paper samples. During the diffusion, the released amount of CHX increased, until reaching a maximum value pointing out the end of the experiments. 100% of CHX was expected to be released for each sample. Nevertheless, maximum values between 50 and 70% of CHX only, were released. The explication resides in the interactions between CHX and cellulose. As explained before, CHX is able to be absorbed onto the cellulosic fibers. Besides, after coating, each paper sample was dried for 1 h at 105°C making the covalent links between the CHX molecules and cellulose possible by the

chlorination of hydroxyl groups. In a previous study (Lavoine, *et al.*, 2013c), we indeed proved experimentally these interactions: a paper samples impregnated in CHX solutions was submitted to an aqueous soxhlet for 10 h. Even after this washing, the Cl-atoms were still noticeable with an elemental map. A proportion of 0.46% of Cl was measured by the elemental analysis.

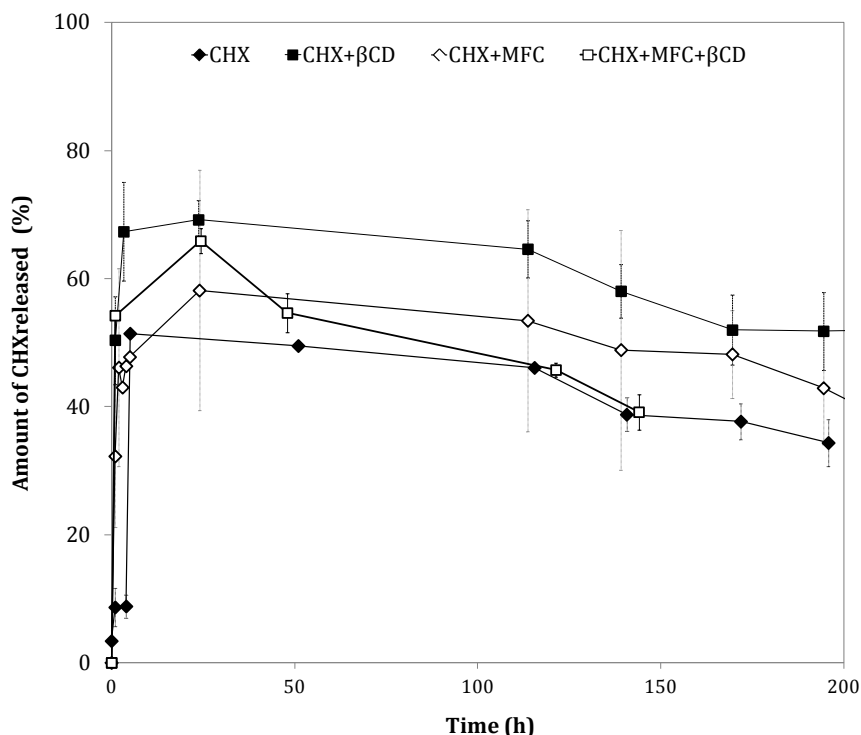


Figure 4-I.6. Release kinetic of paper samples in aqueous media

The released amount of CHX (%) is plotted as a function of time (h). Paper samples coated with (i) CHX (black diamond), (ii) CHX mixed with MFC (white diamond), (iii) CHX and βCD (black square), and (iv) CHX mixed with MFC and βCD (white square) are compared.

Considering the time needed to achieve the plateau value, i.e. the release of the total amount of CHX, a delay is observed for the paper samples coated with MFC, βCD and both: the total amount of CHX is released after one day (24 h), whereas the paper samples coated with CHX only released the whole CHX in 5 hours only.

Although a different release kinetic was not concluded (similar curves slopes), a delayed impact of both components was noticeable proving the efficiency of βCD and MFC to release more progressively the molecules. However, it is worth noting that no synergistic effect was observed between βCD and MFC. Indeed, the paper samples coated with (CHX + βCD + MFC) also released the total amount of CHX in 24 h.

Over time, a decrease of the released amount of CHX was observed. This decrease was due to the ability of the paper samples to re-absorb the CHX released. Even at sink conditions, the paper samples were able to release the CHX molecules and to absorb them again. Several

experiments proved this hypothesis. Firstly, the variations of the CHX concentration in a glass vessel were indeed checked by UV-Vis spectrophotometer, and measured constant over time. The same variations were also checked, but in the presence of an immersed paper samples into the CHX solution, and showed a decrease over time during one month. A similar experiment was also conducted with the presence of an immersed paper samples into the CHX solution. The U.V. analyses showed a decrease of the CHX concentration over time, proving the phenomenon of “re-absorption” of the paper samples.

This first protocol highlighted thus slightly the delayed effect of β CD and MFC onto the CHX release. A synergistic action of both was not concluded, and from this perspective a second protocol promoting the release of CHX was carried out.

The second release protocol was similar to the continuous diffusion, except that each hour the release medium was renewed promoting the release of CHX in the aqueous medium by moving the molecules balance. Figure 4-I.7 presents the final results of these experiments: the total number of washing steps required to release the whole CHX quantity is expressed for each sample. Owing to this renewal, the whole amount of CHX was released in each case, i.e. 100% of CHX initially introduced into the paper samples. However, accounting the standard deviations, the repeatability of the process remained sensitive as previously concluded with the continuous diffusion experiments. This seems mainly due to the heterogeneity of coating (shown in Figure 4-I.4) caused by the electrostatic interactions between CHX- β CD and MFC.

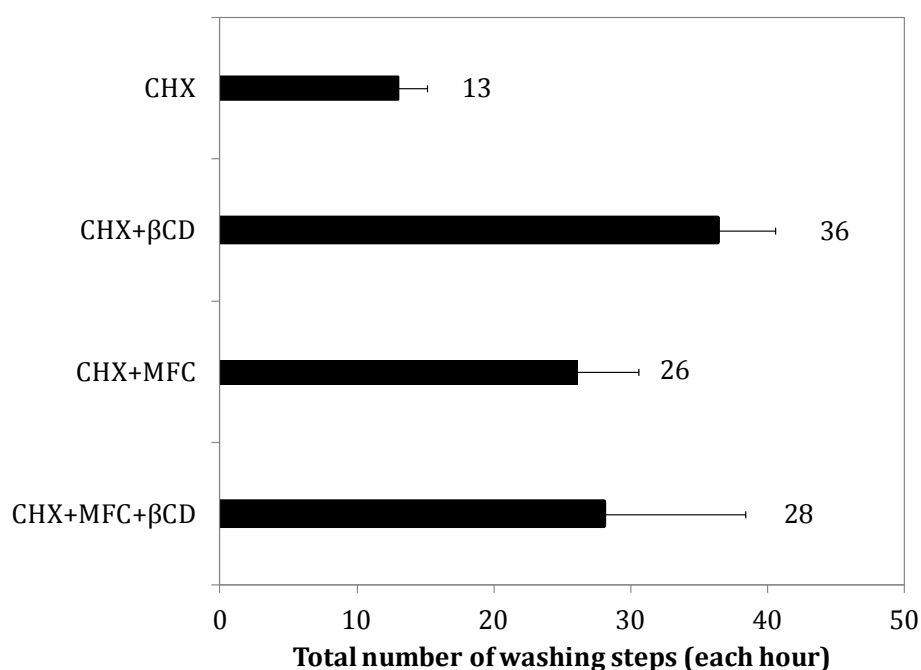


Figure 4-I.7. Results of the intermittent diffusion experiments
Total number of washing steps required to release the whole quantity of CHX per sample.

This second protocol highlighted also the positive effect of MFC and β CD on the CHX release. Indeed, the CHX-coated papers released the whole quantity of CHX within 13 washing steps; whereas, with the addition of MFC, the release went on for 26 washing steps. This result highlighted clearly the effect of MFC on a more progressive release of the CHX molecules into the aqueous medium. It confirmed the relevant interest in using MFC as delivery systems as proved in previous studies (Lavoine, *et al.*, 2013c).

In comparison with these first results, the paper samples coated with the solution (CHX + β CD) released over even a longer time, i.e. during 36 washing steps. The release was more progressive than with MFC, and, similar quantities were released during the first washing step proving the excess of CHX in both cases (burst effect). The host-guest inclusion with cyclodextrin was thus a more efficient method to obtain a more progressive release of a molecule, whereas the quantity of CHX was slightly lower in these samples than in the samples coated with (CHX + MFC)(Table 4-I.1).

This phenomenon is explained by the principle of each delivery system. MFC retains molecules by physical interactions: its nanoporous network prevents molecules from being released. As the MFC is highly hydrophilic, this network is moreover weakened in contact with water and tends to swell. On the contrary, β CD contains molecules thanks to chemical interactions. Even if the association constant between CHX and β CD is weak ($354 \pm 10 \text{M}^{-1}$) compared to other host-guest inclusion complexes (e.g. TBBA with an association constant of $16,600 \text{M}^{-1}$ (Janus, *et al.*, 1999)) the chemical inclusion remains stronger than physical interactions.

Indeed this second protocol compared the efficiency of the MFC and the cyclodextrin as controlled delivery system. The β CD clearly presented better results, whereas the cyclodextrins were only coated onto the paper surface and not grafted as usually done (detailed in the introduction).

It is also worth noting that the CHX/ β CD-coated paper released over a longer period than the CHX-coated paper alone, whereas no grafting prevented the complex CHX/ β CD from being released.

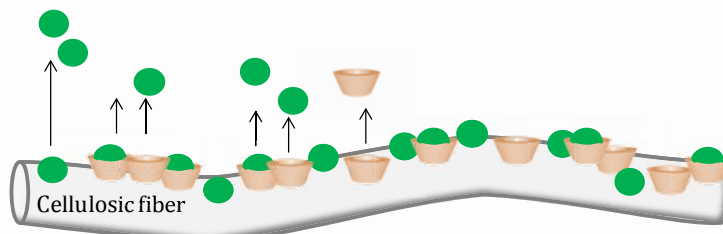
Three scenarii can, thus, be proposed.

- (1) The β CD:-CHX complex is linked to the cellulose thanks to the β CD, which could interact with cellulose; in this case, due to the interactions between the CHX and the β CD, the CHX could be released over a longer period.
- (2) The complex is linked to the cellulose by the CHX. In this case, the β CD could be firstly released, followed by the CHX molecules.

(3) The complex is linked to the cellulose by the CHX and the inclusion complex. The whole complex could be released over a longer period due to the chemical interactions between the CHX/βCD complex and the cellulose (Figure 4-I.8).

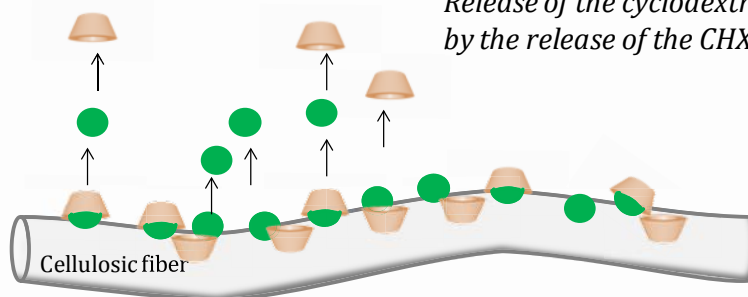
SCENARIO 1

Release of the CHX and of the excess of cyclodextrins



SCENARIO 2

Release of the cyclodextrin followed by the release of the CHX



SCENARIO 3

Release of the inclusion complex

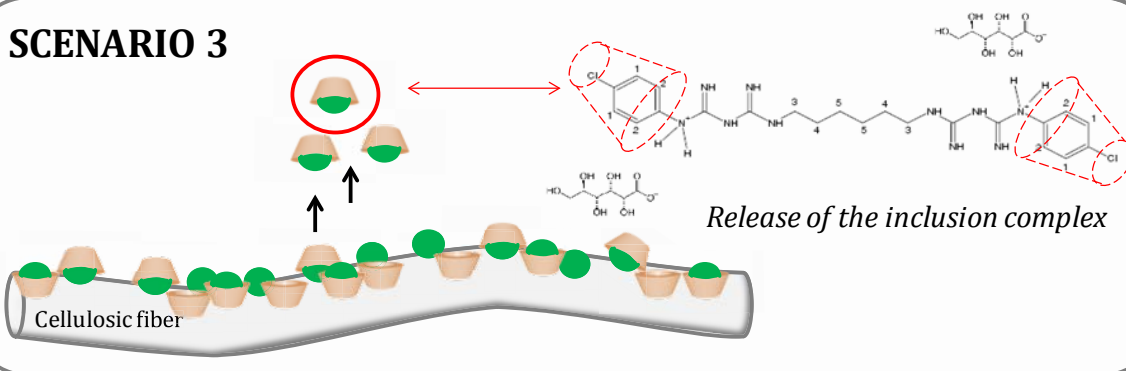


Figure 4-I.8. Three scenarii investigated to explain the release mechanism between the CHX, the cyclodextrins and the CHX-βCD inclusion complex.

To explain this phenomenon and approve one possible scenario, FTIR spectroscopy was conducted on the release media obtained after 3 h and 24 h of release of the CHX/βCD-coated paper (Figure 4-I.9). The spectra obtained were compared with the spectra of the neat CHX and βCD. When considering the spectra between 4,000 and 500 cm^{-1} , a specific peak of the βCD and the CHX is located at 3384 cm^{-1} and 3333 cm^{-1} , respectively. The spectra of the CHX/βCD media

showed nevertheless a nearby peak at 3303 cm^{-1} . This shift could possibly prove the release of the CHX: β CD inclusion complex.

This hypothesis is, indeed, confirmed with the spectra between 1,650 and 750 cm^{-1} . The β CD spectrum has only one peak at 1,650 cm^{-1} , and the CHX spectrum presents two peaks at 1,620 and 1,583 cm^{-1} , respectively. However, in the same wave number range, the CHX/ β CD samples have three main peaks instead of one or two:

- the first one at 1,650 cm^{-1} proved that the excess of β CD was released, mainly in 3 h, since the intensity of the peak is lower than that of the 24 h spectrum;
- the second one is shifted to 1,605 cm^{-1} instead of 1,620 cm^{-1} , which proved that the CHX: β CD inclusion complex was formed at the paper surface, and was released in higher quantity after 24 h;
- the last peak located at 1,583 cm^{-1} is specific to the CHX spectrum and assess that the excess of CHX was also released, mainly after 3 h.

Similar conclusions are drawn when considering the peaks between 900 cm^{-1} and 750 cm^{-1} .

These FTIR spectra confirmed, thus, the continuous diffusion experiments: (i) after 3 h, the excess of CHX was released (as proved by the kinetic of the CHX-coated paper samples); (ii) after 24 h, the β CD:-CHX complex was then released.

Furthermore, with another test consisting in releasing the β CD coated alone onto paper samples followed by FTIR analyses (data not shown), we also proved that the β CD had no strong interactions with the paper substrate and was released, after 3 h just like the CHX. In conclusion, the excess of β CD and the excess of CHX were released after 3 h in contact with the aqueous medium.

The CHX/ β CD-coated samples released over a long period, i.e. 36 washing steps, due to the interactions between the inclusion complex β CD:-CHX and the cellulose. The FTIR spectra proved, indeed, that the whole inclusion complex was released: in conclusion, the inclusion complex was much more linked to the cellulosic substrate compared to the CHX. This can be explained when considering the chemical configuration of the inclusion complex (Figure 4-I.8): the both hydrophobic aromatic cycles of the CHX are, indeed, included into the β CD. As a result, two hydrophobic moieties are replaced by two hydrophilic polysaccharides able to multiply the hydrogen bonds with the cellulose. The interactions between the CHX/ β CD complex and the cellulose are, thus, much more intensive than between the CHX and the cellulose. These conclusions support the scenario number 3, which considers the release of the whole inclusion complex, tightly linked to the cellulose thanks to the CHX/cellulose interactions.

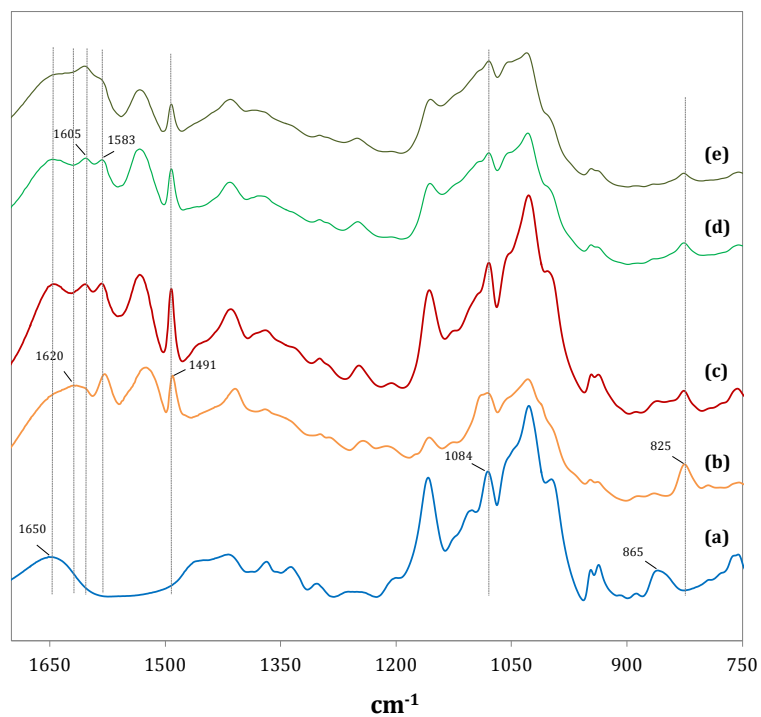
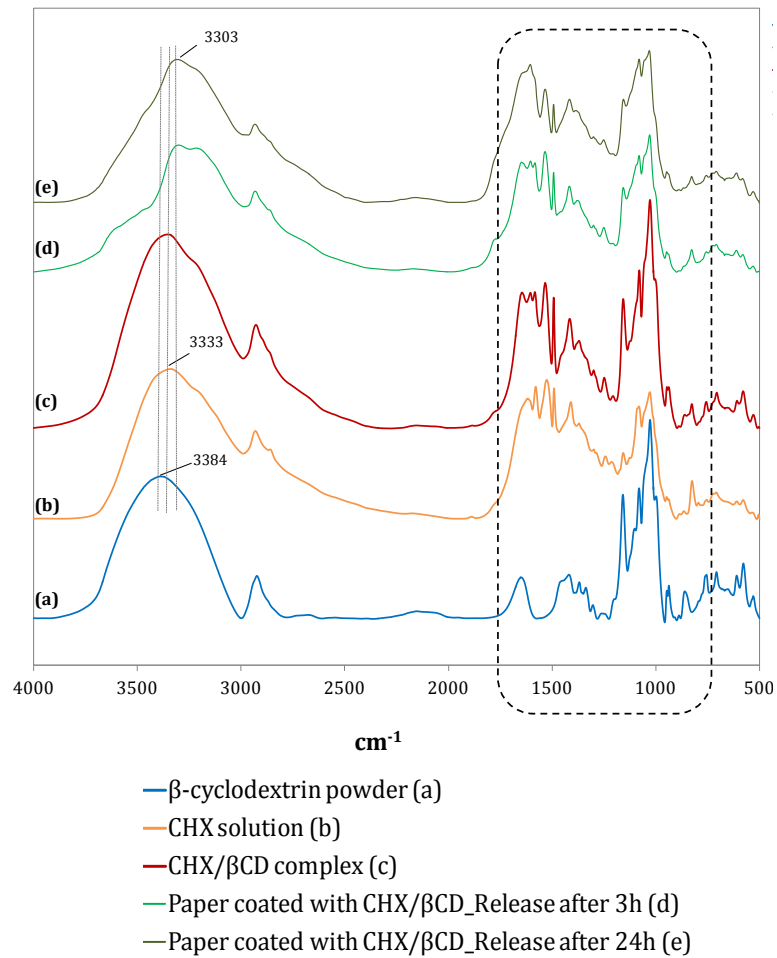


Figure 4-I.9. FTIR Spectra of the β CD (a), the CHX (b) and the CHX/ β CD mixture released by the coated paper after 3 h (c) and 24 h (d), respectively between 4,000-500 cm^{-1} (up) and 1,650-750 cm^{-1} (down)

The last samples analyzed in figure 4-I.6 were studied within the expectation of a synergy between the MFC and the β CD. The continuous diffusion did not prove this expectation, and for this reason the intermittent diffusion experiments were carried out.

The paper samples coated with the suspension (CHX + MFC + β CD) released the whole CHX after a mean value of 28 washing steps. Accounting the standard deviations, this value is quite similar to the result obtained with the paper samples coated with (CHX + MFC). None synergistic action could thus be concluded between β CD and MFC, mainly due to a lower penetration of the complexes CHX- β CD trapped into the nanoporous MFC network.

The authors considered indeed the following hypothesis to explain this result. Considering the experimental protocol followed to make the coating color (CHX + MFC + β CD), the complexes CHX:- β CD were formed before being mixed with the MFC. Nevertheless, as already shown in previous studies dealing with the MFC coating onto paper substrates (Lavoine, *et al.*, 2013), the MFC coating remains mainly at the paper surface. Thus, during the coating, the slurry (CHX + MFC + β CD), and consequently the complexes CHX:- β CD, were kept mostly at the paper surface.

On the contrary, during the coating of the (CHX + β CD) aqueous solution, the water as well as the CHX:- β CD complexes had the opportunities to penetrate into the paper structure. Consequently, we assisted to a longer release with the solution (CHX + β CD) because the complexes had to be released through the whole paper thickness.

Finally, the release study of the CHX molecules highlighted four main points:

- a) The release of CHX is more efficient with the β CD than with the MFC;
- b) The grafting of β CD is not necessary to obtain a controlled delivery system;
- c) The MFC is also able to release progressively the CHX in water, but acts mainly at the paper surface, which limits the duration of release;
- d) No synergistic action is concluded between the β CD and the MFC, since both are included into the coating color.

I-4. Conclusion

Usually used with polymeric matrices or textiles, cyclodextrin already proved its efficiency as drug delivery system. More recently, MFC also proved its interest as delivery system once coated onto paper substrates.

The idea of this study was to combine the β CD and the MFC to perform a more efficient controlled release system. The idea of this paper was, thus, to mix them both into the paper coating color in association with the active molecules targeted, the chlorhexidine digluconate (CHX).

After carrying out the release study of the CHX into an aqueous medium, various conclusions can be drawn.

Although a synergy between the MFC and the β CD was not observed, this study allowed a comparison of the efficiency of the MFC and the β CD as controlled delivery system, and paved the way for a future use of β CD with cellulosic substrates.

Indeed, compared to samples coated with the MFC, the samples containing the β CD released over a longer period the molecules. This was mainly due to the fact that the MFC concentrated the CHX at the paper surface, whereas the CHX/ β CD solution distributed the CHX into the whole paper structure. Besides, the CHX and β CD were strongly chemically linked and this complex has high interactions with cellulose, whereas the MFC only trapped the molecules thanks to its nanoporous network.

Finally, we also proved in this study the interest of coating the β CD onto the paper surface instead of grafting it. The grafting of β CD onto cellulosic matrices generally induced the use of citric acid and damaged considerably the properties of the paper substrate.

In this study, we showed that the coating of β CD was a very efficient controlled delivery system. This was mainly due to the specific use of CHX, which interacts with the cellulose. However, it is worth noting that the whole inclusion complex was released during the release study limiting successive uses of these samples.

As a result, different perspectives can thus be considered to develop a more performing controlled release system:

- (i) The chemical grafting of β CD followed by the coating of MFC could, nevertheless, be tested to avoid the release of the cyclodextrin. Besides, the MFC is able to reinforce the mechanical and barrier properties of cellulosic substrates and could possibly counterbalance the loss of properties induced by the grafting process of the cyclodextrin;

- (ii) The grafting of β CD directly onto the MFC could also be a solution to preserve the properties of the cellulosic substrate and allowed a longer release;
- (iii) Finally, the successive coating of β CD and MFC could be another solution to be investigated, so that a synergistic action between both components could be observed.

This chapter suggested a new kind of controlled delivery system based on the coating of cyclodextrins with MFC on a paper substrate.

The coating of cyclodextrins alone proved, in particular, its efficiency in that field. However, despite good results in terms of prolonged release system, the cyclodextrins were released at the same time as the active compound. As a result, the active paper substrates could not be reused.

To overcome this consequence, the grafting of cyclodextrins onto the paper substrate was investigated in the following study.

Elaboration of a new antibacterial bio-nano-material for food-packaging by synergistic action of cyclodextrin and microfibrillated cellulose

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Abstract

In response to the current society's requirements and challenges regarding the elaboration of new functional bio-materials for food-packaging application, this study proposes a new solution based on the synergistic action of beta-cyclodextrins (β CDs) and microfibrillated cellulose (MFC).

Carvacrol, an antibacterial molecule approved as safe food additive, was included by impregnation into β CDs previously grafted in a paper substrate. MFC was then coated onto the grafted paper containing carvacrol. Two release studies were conducted into deionized water and agar to conclude on the positive effect of β CDs and MFC on the release of carvacrol. Antibacterial tests were carried out in parallel. Besides, the mechanical and barrier properties of the final material were also tested, such as the Young's modulus, the zero-span breaking length, the air permeability and the water absorption. Into water, β CDs proved clearly its efficiency as progressive delivery system: the whole amount of carvacrol was released 21 h later with a release kinetic 24% slower. Into agar, β CDs and MFC acted simultaneously to preserve the antibacterial activity 12 h longer.

These results are very promising since they considered a new kind of delivery system with antibacterial properties based on the synergistic action of MFC and cyclodextrins.

Inspired from Paper 8:

Nathalie Lavoine, Clara Givord, Nicolas Tabary, Isabelle Desloges, Bernard Martel and Julien Bras (2013) Elaboration of a new antibacterial bio-nano-material for food-packaging by synergistic action of cyclodextrin and microfibrillated cellulose. Submitted to Journal of Controlled Release.

II-1. Introduction

The current society's requirements dealing with the elaboration of new food-packaging materials are inducing a strong development of active packaging, i.e. a material intended to extend the shelf-life, to maintain or improve the conditions of packaged food (Council, 2004). Since 2004, these new materials are included in the European regulations, but since the publication of the both regulations 1935/2004 and 450/2009 (EC), respectively, no more text of law was published.

Therefore, numerous kinds of active food-packaging materials are nowadays developed at research scale. Among them, two main categories can be distinguished: (i) the scavenger and (ii) the antibacterial packaging materials. Although some oxygen scavenger materials (e.g. sachets or labels) are already marketed in the European Union, none antibacterial materials are, up to our knowledge, nowadays commercialized.

It is true that the current antibacterial materials developed at research scale still have some main drawbacks preventing them from being produced at industrial scale. For example, microencapsulation, technique developed for two decades (Gibbs, *et al.*, 1999), of antibacterial agents (Abreu, *et al.*, 2012, Hosseini, *et al.*, 2013, Li, *et al.*, 2009), especially volatile compounds such as essential oils, is produced with still too expensive manufacturing processes, using mainly toxic chemicals. Besides, the release of the antibacterial agent is until now a non-controlled process with microcapsule, which limits their applications in the food-packaging or biomedical fields.

The elaboration of an efficient controlled delivery system is also a consequent challenge. Considering the problematic of drug delivery systems, they have to deliver the drug to the right area, at the right time and at the right concentration (Ward and Georgiou, 2011). These challenges are similar for antibacterial food-packaging. In addition, the antibacterial agent chosen must be safe for consumers and it must not alter the organoleptic properties of the packaged food.

All these requirements and challenges lead us to propose a new solution of antibacterial bio-material for food-packaging application. The purpose of this study is thus the elaboration of an antibacterial bio-nano-material able to achieve a controlled release of the antibacterial agent. Within this perspective, a fiber-based material uses the synergistic action of cyclodextrin and microfibrillated cellulose, both already used as efficient molecule carrier and delivery system, to release progressively carvacrol, a well-known and efficient antibacterial agent.

Carvacrol or 2-methyl-5-(1-methylethyl)-phenol is a monoterpenic phenol (Figure 4-II.1). It occurs in aromatic plants and in many essential oils of the Labiatae family (also known as mint

family) (Ortega-Nieblas, *et al.*, 2011). It is approved as a safe food additive in the USA (Nutrition, 2006) and Europe (Communities, 1999). Carvacrol is reported to have a wide variety of biological properties including anti-inflammatory (Hajhashemi, *et al.*, 2002), antioxidant (Prieto, *et al.*, 2007), insecticidal (Karpouhtsis, *et al.*, 1998) and antimicrobial (Ben Arfa, *et al.*, 2006) activities. Its hydrophobic nature and its volatility limit in many cases its applications. Indeed the effective antimicrobial concentration often exceeds the acceptable sensory levels, such as the odor or the taste (Nostro and Papalia, 2012). A solution consists in reducing its sensory impact and lowering the required dose by the use of synergistic association with a variety of treatments. In this regard, one idea aims to trap carvacrol in cyclodextrins by the formation of inclusion complexes. Cyclodextrins (CDs) are cyclic oligomers of α -D-glucopyranose that can be produced from starch by certain bacteria (Astray, *et al.*, 2009). The most widely used are the α -, β - and γ -CDs; with six, seven and eight (α -1,4)-linked α -D-glucopyranose units, respectively. In this study, the β -cyclodextrins (β CDs) are used to form inclusion complexes with carvacrol (Figure 4-II.1) owing to their 3-D structure, which is considered as a truncated cone with a hydrophobic cavity and a peripheral hydrophilic zone.

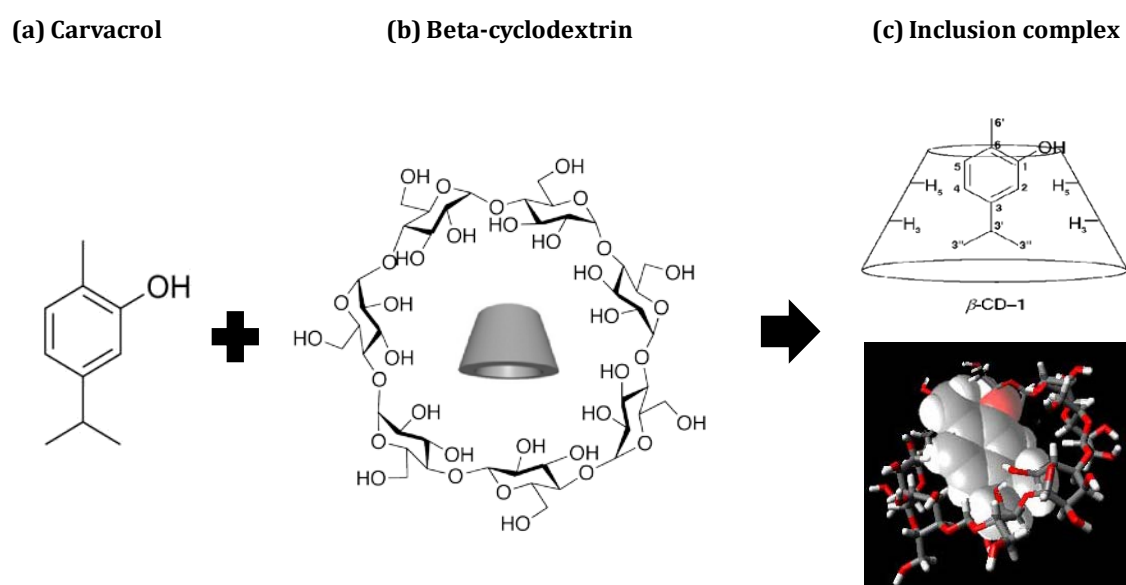


Figure 4-II.1. Molecules of carvacrol (a), β -cyclodextrin (b) and their respective inclusion complex (c)
The picture of the inclusion complex was designed with the Avogadro open source software.

They were primarily used in pharmaceutical applications as solubilizers for lipophilic drugs (van de Manakker, *et al.*, 2009) and so can be a perfect carrier of carvacrol. The inclusion complexes β CDs:-carvacrol were indeed already proved in different studies (Locci, *et al.*, 2004, Mulinacci, *et al.*, 1996) (Figure 4-II.1). Due to this inclusion, β CDs are able to release progressively the host molecules. For this reason, they are used in many applications: pharmaceutical (Brewster and Loftsson, 2007, Loftsson and Duchêne, 2007), textile (Szejtli, 2003), biomedical (Hoang Thi, *et*

al., 2010) and food-packaging (Koontz, *et al.*, 2010, López-de-Dicastillo, *et al.*, 2011). Its use with cellulosic materials remains nevertheless quite rare. A very recent publication (Cusola, *et al.*, 2013) succeeded in immobilizing β CDs into paper substrates based on a patented grafting process initially used for the textile industry (Weltrowski, *et al.*, 2000). Up to our knowledge, this is the unique study dealing with the grafting of β CDs into paper substrates. This study proved that β CDs grafted paper substrates were able to prolong the delivery of an active molecule.

This grafting process was applied in the present study so that the paper substrates were grafted with β CDs before carrying carvacrol. Indeed, the grafting of β CDs was essential to avoid the whole inclusion complex diffusion during the release study. In addition, we coated our grafted fiber-based materials with microfibrillated cellulose (MFC) to ensure a more efficient control of the release of carvacrol. Microfibrillated cellulose is a new kind of bio-nano-material, whose use is exponentially increasing (Dufresne, 2012, Lavoine, *et al.*, 2012, Siró and Plackett, 2010). MFC is a sub-division of the cellulosic fibers and is produced by applying a high shear mechanical treatment to the fibers (Turbak, *et al.*, 1985). Thus, the dimensions of MFC usually vary between 10 – 60 nm for the diameters and exceed 1 μ m for the length (Siró and Plackett, 2010). The entanglement of this nanometric microfibrillated cellulose allows the formation of strong and highly barrier films (Aulin, *et al.*, 2010, Syverud and Stenius, 2009) or very porous aerogels (Korhonen, *et al.*, 2011, Sehaqui, *et al.*, 2011), for example. This new material is nowadays used in various applications: composites (Shibata, *et al.*, 2011, Shields, *et al.*, 2008, Tingaut, *et al.*, 2009), printing (Ankerfors, *et al.*, 2009, Torvinen, *et al.*, 2012), electronic (Ummartyotin, *et al.*, 2011), paper industry (Ahola, *et al.*, 2007), food-packaging (Díez, *et al.*, 2011), medical (Cherian, *et al.*, 2011) etc. Very recently, its use as drug delivery system emerged. MFC was used as spray-dried powder (Kolakovic, *et al.*, 2012a, Kolakovic, *et al.*, 2011), as aerogels (Valo, *et al.*, 2013) or as films (Cozzolino, *et al.*, 2013, Kolakovic, *et al.*, 2012b) to release progressively medical drugs or more recently antibacterial agent. In parallel, we also very recently proved that the MFC coated onto fiber-based substrates was able to preserve its nanoporous network and to act as a delivery system of active molecules (Lavoine, *et al.*, 2013c). Compared to a paper without MFC, the substrates coated with MFC released indeed the active molecules 26 days more, achieving during this whole period an efficient antibacterial activity.

Based on these promising results, we tested in the present study the possible synergistic action between β CDs grafting and MFC coating to achieve a new antibacterial bio-nano-material. Different release studies were conducted in two different media: deionized water and agar. In parallel, the long-term antibacterial activity of the samples was analyzed. Each time, the proper action of β CDs was studied and compared or linked to the action of MFC on the release of carvacrol.

II-2. Experimental section

II-2.1. Materials

A 42 g/m² base paper was used as reference. It was kindly supplied by Ahlstrom society (France). Made of bleached hardwood virgin pulp, it was one side coated. Its main application is the food-packaging sector.

Microfibrillated cellulose (MFC) suspension (2%wt) was produced and supplied by the FCBA (France). It was made from sulfite pulp (Domsjö®), enzymatically pre-treated with an endoglucanase for 1 h and mechanically treated with a GEA Ariete® (GEA Niro Soavi, Italy) homogenizer. The parameters applied were 1 pass at 1,000 bars followed by 4 passes at 1,400 bars.

Beta-cyclodextrin (β CD) (CAS n°7585-39-9), citric acid (CA) (CAS n°77-92-9) and sodium hypophosphite (CAS n°7681-53-0) were purchased from Sigma-Aldrich (France) and used as received.

Carvacrol (CAS n°499-75-2) was purchased as a liquid from Sigma-Aldrich (France), and used as received (98%).

Methanol (CAS n°67-56-1, $\geq 99.9\%$) was also purchased from Sigma-Aldrich (France).

Bacillus subtilis spores suspension (10⁴ spores/ml), nutrient agar and Petri dishes (90 mm diameter) were purchased from Humeau Laboratories (France).

II-2.2. Methods

Characterization of the MFC suspension

Field Emission Gun Scanning Electron Microscopy (FE-SEM) images of the MFC suspension were taken using a Zeiss® Ultra-55 (France). The MFC suspension was spread onto a metal substrate using a carbon tape, allowed to dry for 48 h at room temperature, and was coated with a thin layer of gold. The working distance used was 5.5 mm for an accelerating voltage of 2.00 kV at a magnification of $\times 20.00$ k.

The diameter of the MFC was determined by image analyses using the ImageJ® software, based on at least 50 measurements.

Beta-cyclodextrin grafting

β CD-grafted papers were prepared following a previously published protocol (Cusola, *et al.*, 2013):

Base paper was firstly dried for 1 h at 105°C to determine its dried mass (M_i) with a Lhormargy balance (± 0.001 g). A 100 ml aqueous solution of CA/sodium hypophosphite/ β CD was prepared respectively in mass proportion 10/3/10 g. Base paper was impregnated in this

solution for 10 seconds. After squeezing the excess of solution using a “Labor Size Presse SP”, Mathis AG, Switzerland, (3 times, at 2 bars and 2 rpm), the samples were dried 5 min at 105°C in a ventilated oven. Then the grafting was done by curing the impregnated paper samples for 30 min at 160°C using a textile finisher range.

A water soxhlet extraction was then carried out for 3h30 to remove the non-grafted components. After that, the paper samples were once again dried for 2 h at 105°C. The mass of the dried β CD-grafted paper samples (M_f) was after that measured with a Lhormargy balance (± 0.001 g).

The grafting rate (%) was calculated as follows:

$$G(\%) = \frac{M_f - M_i}{M_i} * 100$$

Citric Acid-grafted (CA-grafted) paper samples were also prepared as references. The same protocol was applied with different CA/sodium hypophosphite/ β CD proportions: 10/3/0 g per 100 ml of solution.

Carvacrol introduction and quantification

Carvacrol was introduced by impregnation of samples ((i) base paper, (ii) β CD-grafted paper and (iii) CA-grafted paper) into a solution of carvacrol/ethanol in mass proportion 15/85%.

Samples were impregnated for 10 min in the impregnation bath and then dried at 30°C in a ventilated oven for 20 min.

The total quantity of carvacrol introduced into paper samples was measured by a complete release in a bath of methanol. The samples containing with carvacrol were entirely impregnated for one week at room temperature in a 500 ml bath of methanol placed into a hermetic plastic flask. The released amount of carvacrol was then analyzed using a UV-Vis spectrophotometer SHIMADZU UV 1800 at 275 nm, and calculated by the following calibration curve:

$$A = 19,526 * C$$

with $R^2 = 0.9991$

with A , the absorbance at 275 nm of carvacrol in methanol and C , the carvacrol concentration (g/L).

This protocol was repeated three times for each sort of paper samples to obtain a mean value of the total amount of carvacrol introduced.

Complexation study of carvacrol – β -cyclodextrin by NMR

The spectra were recorded using a spectrometer Bruker Avance™ 400 equipped with a Bruker Ultra-Shield 9.4 T (proton Larmor frequency of 400.33 MHz) and with a BBI probe (1H,

X). Two-dimensional NOESY experiments were acquired in the phase-sensitive mode using the Bruker ASX 400 (9.4 T) spectrometer. The probe temperature was regulated to 300 K. Spectra consisted of a matrix of 2 K (F2) by 2 K (F1) covering a sweep width of 4084 Hz. Spin-lock mixing periods of 500 ms was applied. Before Fourier transformation, the sine apodization functions were applied in both dimensions. 256 increments were collected with 80 transients.

Coating process

Samples were coated with the 2%wt MFC suspension using a bar coating process. A 0.9 mm Mayer bar was used at a speed of 5 cm/s. Each sample was coated with five MFC layers. Between each coating, coated samples without carvacrol were dried at 105°C using a contact drying system for about 3 min. The samples with carvacrol were dried at 50°C using the similar drying system.

The coat weight of each paper sample was then determined by weighting 10 × 10 cm² samples with a Lhormargy balance (± 0.001 g). Some samples were also coated five times with only water using the same coating process and parameters.

Following the protocols described above, different kinds of paper samples were prepared and compared. Figure 4-II.2 resumes the methods of preparation for each sample.

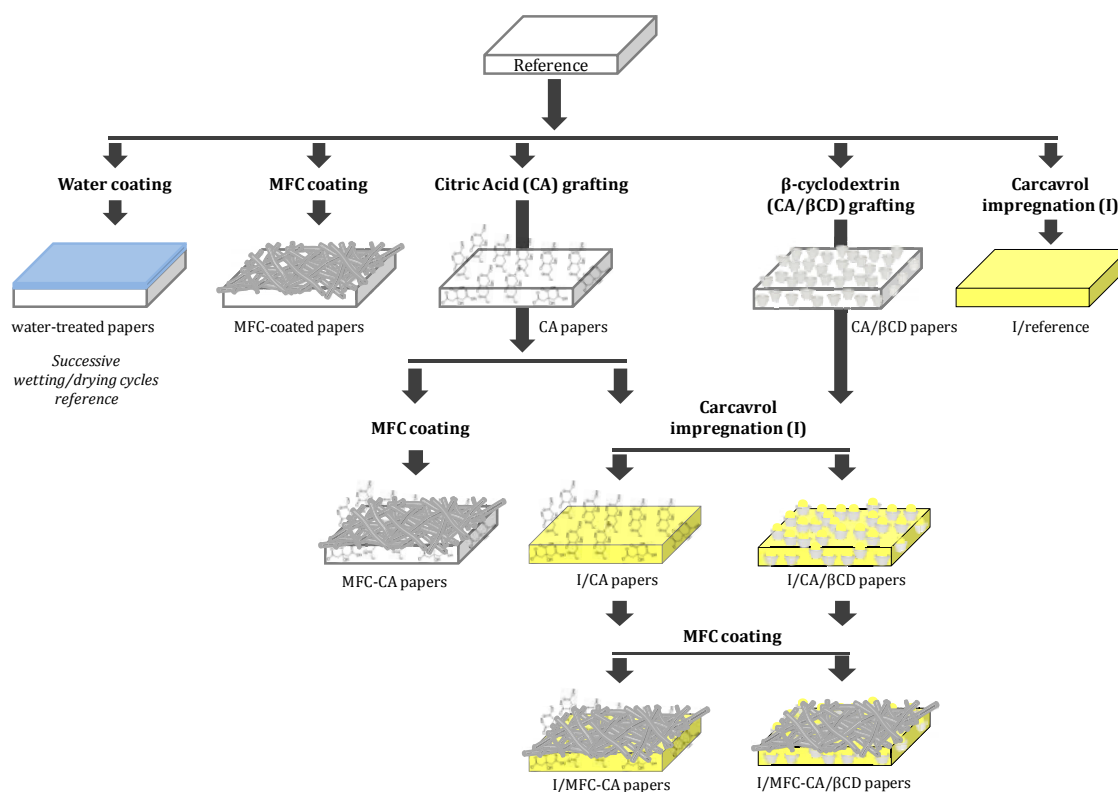


Figure 4-II.2. Strategies of preparation of the different samples

The impact of the successive wetting/drying cycles and of the grafting process was analyzed with the water-treated and the CA-grafted paper samples, respectively.

Samples characterization

The samples analyzed in this study are detailed in Table 4-II.1 as well as their designation and characterization.

Each sample was maintained at a temperature of 23°C, and a relative humidity (RH) of 50% for at least 24 h before each following characterization test.

Samples	Designation in the text	Structural properties			Barrier properties		Mechanical properties			Release study	Antibacterial tests
		Basis weight (g/m ²)	Thickness (μm)	SEM images	Water absorption (g/m ²)	Air permeability (cm ³ /Pa.s.m ²)	Young's modulus (GPa)	Elongation (%)	Zero-span breaking length (km)	in water	in agar
Base paper	Reference	X	X	X	X	X	X	X	X		
5× water-treated papers	water-treated papers	X	X		X	X	X	X	X		
5× MFC-coated papers	MFC-coated papers	X	X	X	X	X	X	X	X		
CA-grafted papers	CA papers	X	X			X	X	X	X		
βCD-grafted papers	CA/βCD papers	X	X			X	X	X	X		
5× MFC-coated CA-grafted papers	MFC-CA papers	X	X			X	X	X	X		
Carvacrol impregnated base papers	I/reference									X	X
Carvacrol impregnated CA-grafted papers	I/CA papers									X	X
Carvacrol impregnated βCD-grafted papers	I/CA/βCD papers									X	X
Carvacrol impregnated CA-grafted papers coated with 5× MFC layers	I/MFC-CA papers									X	X
Carvacrol impregnated βCD-grafted papers coated with 5× MFC layers	I/MFC-CA/βCD papers									X	X

Table 4-II.1. Recap chart of different samples prepared and the respective analysis carried out with each

Structural properties

The basis weight and MFC coat weights of samples were determined by weighting at least ten samples of 10 × 10 cm² with a scale of ±0.001 g of precision. The data given is an average value of ten measurements.

The thickness was measured with a Lhomargy micrometer (±0.01 mm) and was expressed as an average of at least five measurements for each 10 × 10 cm² sample according to the standard ISO 534:2011.

Scanning Electron Microscopy (SEM) images of the cross-section of non-coated and coated papers were performed using a Quanta200® (The Netherlands). Cross-section and surface of the base and coated papers were mounted onto a metal substrate recovered with a carbon tape to be analyzed with SEM. The Back-Scattered Electron Detector (BSE) was also used to analyze the coated paper surfaces. The working distance employed was 25.8 mm with a voltage of 12.5 kV at a magnification of ×150 for surfaces and 10.1 mm, 15 kV at ×600 for cross-sections, respectively.

Mass transport properties

The water absorption was measured following the Cobb 60 method (ISO 535). For each sample, at least five measurements were done to calculate an average value of the Cobb Index. The air permeability tests were carried out with the system of Mariotte vases (ISO 5636) using a sample area of 2 cm² and a vacuum of 1.0 or 2.5 kPa depending on the sample permeability. The results were expressed as an average of at least five measurements.

Mechanical properties

Young's modulus (GPa) and Elongation at break (%) were determined using a Lorentzen & Wettre Tensile Tester and following the standard ISO 1924-2/3 (at 10 mm/min and span of 180 mm) for references, water-treated and MFC-coated samples. An adapted method from this standard was applied for references, CA, CA/ β CD and MFC-CA paper samples (modified speed of 100 mm/min and span of 100 mm). Ten measurements were carried out to obtain an average value.

Zero-span breaking length test was carried out using a Trouble-Shooter TS-100 (Pulmac Instruments International Inc., France) and following the TAPPI-231 standard. Ten measurements were done per sample and averaged.

Release study in aqueous medium

A release study was conducted in an aqueous medium with the samples containing carvacrol. The test was carried out at least three times for each sample and under sink conditions so that the release medium was never saturated with carvacrol.

Samples of $10 \times 10 \text{ cm}^2$ were stuck to an expanded polystyrene (PS) support using scotch tape in order to study only the release of carvacrol from the sample's surface. The perimeter of each sample was then taped to prevent carvacrol from being released through the sample thickness.

The release was performed in an aqueous bath of 500 ml of deionized water that was continuously stirred with a magnetic stirring-bar at 200 rpm, at room temperature. The samples were placed at the surface of the aqueous medium. At successive intervals (10, 20, 40, 60 min; 1, 2, 4, 6 h, and then, once per day), 3 ml of the solution were taken. Their absorbance was then analyzed using a UV-Vis spectrophotometer SHIMADZU UV 1800 at a wavelength of 273 nm. Using the following calibration curve, the concentration of carvacrol released was then determined over time:

$$A = 0.0103 * C$$

(with $R^2 = 0.9979$)

with A , the absorbance at 273 nm of carvacrol in water and C , the carvacrol concentration (mg/L).

Antibacterial tests

The antibacterial tests were conducted following the first steps of the standard EN 1104 procedure (AFNOR, 2005). This procedure consisted in placing the samples to be tested (previously cut into circles of 16 mm in diameter) onto a nutrient agar inoculated with the bacteria. The nutrient agar was previously inoculated with *Bacillus subtilis* to reach a final concentration of 10^4 spores/mL. This solution was poured into Petri dishes and the samples to

be tested were placed on the surface before cooling. Each Petri dish was maintained in the fridge for 2 h and was subsequently stored at 30°C for an incubation period of 3 days.

This procedure was slightly modified in order to study the antibacterial activity of the samples over time. Before storing the Petri dishes at 30°C for 3 days, the paper samples were removed from each Petri dish right after the 2 h spent in the fridge. The Petri dishes without the paper samples were then stored at 30°C for 3 days, and the paper samples were placed onto new Petri dishes with freshly inoculated agar for a new antibacterial test during the 2 h cooling.

In the event of any antibacterial activity, the Petri dish will present inhibition zones around each sample; the larger the diameter of the inhibition zone, the more efficient the antibacterial activity. For each test, the diameter of the inhibition zone was, thus, measured with a ruler (± 0.5 mm). At least five diameter measurements were performed for each three specimens (three circles of 16 mm in diameter) per Petri dish, and three Petri dishes were done for each sort of sample. From these results, the inhibited area was calculated by considering only the surface of diffusion and not the sample surface. These steps were repeated until the inhibition zones of the paper samples tested could no longer be measured.

Three types of control Petri dishes were also prepared to evaluate and confirm the test results, namely using: (i) non-inoculated agar, (ii) inoculated agar, and (iii) inoculated agar with Penicillin discs, which is a well-known *Bacillus subtilis*-specific antibiotic.

II-3. Results and discussion

II-3.1. Characterization of the MFC suspension and paper samples

The nanoporous MFC network

The 2%wt MFC suspension is a white gel composed of an entanglement of microfibrils in water. FE-SEM analyses were conducted so that this entanglement could be conserved once dried. Figure 4-II.3 presents the image obtained. A tight network of nanometric fibers is clearly represented, and with an image analysis using the ImageJ® software, a mean diameter of 33 ± 8 nm was determined. This value corroborates those described in previous studies (Siró and Plackett, 2010, Zhang *et al.*, 2012). However, due to the tight entanglement, the estimation of the MFC length was impossible to measure accurately at a low magnification. Nevertheless, according to literature (Siqueira, *et al.*, 2010) the estimated value exceeds usually 1 μm .

Similar kinds of MFC suspension were already coated onto paper substrates but often diluted to lower concentrations (Aulin, *et al.*, 2010, Nygård, *et al.*, 2011). In this study, the suspension was used as received after the homogenization step, i.e. at 2%wt. The white viscous gel suspension

pictures shown in Figure 4-II.3 confirmed that a good quality and homogeneous MFC suspension was produced. Before checking synergistic effect of MFC coating and β CDs grafting on the antimicrobial properties, it is key to check influence of both processes, i.e. coating and grafting, onto the ensued fiber based materials.

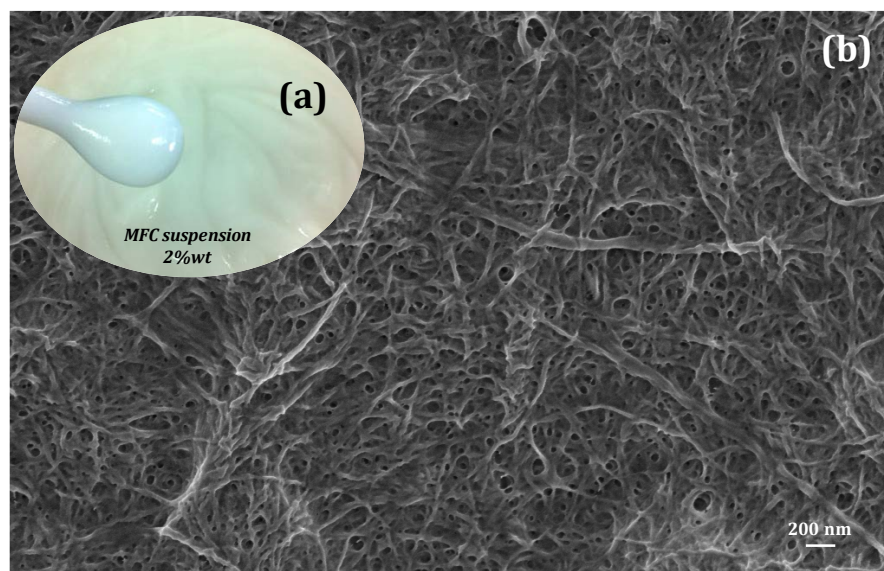


Figure 4-II.3. (a) Picture of the gel-like MFC suspension at 2%wt and (b) FE-SEM image of the dried 2%wt MFC suspension (WD: 5.5 mm, Mag. $\times 20.00$ k)

Influence of the grafting process on the final material properties

The grafting protocol conducted in this study was based on the impregnation of the reference into a solution of β -cyclodextrin (β CD), citric acid (CA) and a catalyst (sodium hypophosphite). The grafting of β CD was successful since a grafting rate of about 9% was determined. However, β CD was not the only one to be grafted into the reference paper. CA-grafted papers were indeed prepared following the same protocol of grafting, without adding β CD into the solution of impregnation. A grafting rate of about 4% was measured proving also the grafting of citric acid into the reference paper. It is well-known that citric acid can strongly modified cellulose based material properties (Olsson, *et al.*, 2013).

That is why, the impact of the grafting process on the mechanical and barrier properties of the samples was thus analyzed onto CA and CA/ β CD grafted samples.

In addition to the structure property (e.g. bulk) which was not impacted by the grafting process, three main mechanical properties were also studied: the Young's modulus, the zero-span breaking length and the elongation (Table 4-II.2). These properties were all three drastically damaged: in comparison to the reference paper, an overall decrease of about 40 to 50% was indeed observed in machine and cross direction due to the grafting process.

This loss of mechanical properties was mainly due to the presence of citric acid in the solution of impregnation. During the grafting process, citric acid was grafted with cellulose after drying at successive high temperatures. In addition to the pH effect, citric acid can covalently bond the fiber surface and consequently interact or limit hydrogen bonds between cellulosic fibers. Furthermore, citric acid can also crosslink cellulose macromolecules inside the fiber, which renders it more fragile.

The air permeability, however, was improved by the presence of grafted CA and β CD into papers (Table 4-II.2). The enhancement was significant since the air resistance was increased by 50%. Despite the weakening of the mechanical properties induced by the hydrogen bonds between CA and cellulose, CA created bridges between cellulosic fibers tightening thus the fiber network by the well-known crosslinking.

These first analyses showed a very significant effect of the grafting process on the paper properties: drastic damages of the mechanical properties but consequent improvement of the air permeability. Nevertheless, the final paper material proposed in this study as a new solution of bio-material for food-packaging application comprises besides MFC layers. Consequently, the effect of MFC on the final paper properties was also studied.

Paper samples	Basis weight (g/m ²)	Thickness (μm)	Bulk	Young's modulus (GPa)		Zero-span breaking length (km)		Elongation at break (%)		Air permeability (cm ³ /Pa.s.m ²)
				MD	CD	MD	CD	MD	CD	
Reference	42±0	57±1	1.35	7.3±0.2	3.4±0.1	13.6±1.2	12.3±0.8	1.0±0.0	4.0±0.0	5.7±0.2
H ₂ O-treated	43±0	60±1	1.39	4.4±0.9	2.7±0.1			2.0±0.4	3.9±0.2	
MFC-coated	47±1	64±2	1.37	5.9±0	3.9±0.1			0.5±0.2	1.9±0.4	
CA	45±0	61±1	1.35	4.6±0.2	1.9±0.1	8.4±0.6	7.6±0.7	0.6±0.1	0.8±0.2	2.7±0.3
CA/βCD	48±0	67±1	1.39	4.3±0.2	-	6.7±0.4	6.4±0.2	0.7±0.1	-	2.5±0.2
MFC-CA	53±0	67±2	1.26	3.9±0.2	2.3±0.1	8.7±0.4	7.0±0.2	0.9±0.2	1.8±0.4	1.8±0.1

Table 4-II.2. Influence of the bar coating and grafting processes on the paper properties in machine and cross-directions (MD and CD, respectively) and impact of the MFC suspension on each treated sample.

The samples were maintained at least for 24h at 23°C and 50% of relative humidity (RH) before characterization. Each test was conducted at 23°C and 50%RH on a least five to ten specimens per samples in machine and cross-directions (MD and CD respectively).

Influence of the coating process and MFC on the final material properties

According to previous studies (Aulin, *et al.*, 2010, Lavoine, *et al.*, 2013b, Nygård, *et al.*, 2011), the coating of MFC onto paper surfaces enhances the properties of the final papers whatever the coating process used. Nevertheless, the achievement of a homogeneous coverage of the surface of the paper was only possible with the laboratory coating of five successive layers

of MFC. This protocol was already noticed by a study recently submitted (Lavoine, *et al.*, 2013b), which detailed the impact of such a MFC coating on the final paper properties. In this previous study, we proved also that this coating process used with water had also a main influence on the paper properties. Since the MFC suspension had a high water content (98%wt), its coating induced simultaneously a major addition of water into paper substrates and so decreased properties. Over a first phase in this study, the influence of the coating process and of the MFC suspension was studied on the paper properties to corroborate with literature. In a second phase, the effect of MFC coated onto grafted papers was investigated so that an eventual improvement of the paper properties could be observed.

Table 4-II.2 presents the mechanical and barrier properties of the reference, water-treated and MFC-coated papers (samples presented in Figure 4-II.2). A similar coating process was conducted to produce the water-treated and MFC-coated samples.

With five successive layers, a MFC coat weight of about 5 g/m² was deposited. Simultaneously, the thickness was also increased by about 7 µm. However despite this addition of microfibrils, the MFC-coated papers presented a similar bulk value as the reference. Moreover, the addition of MFC counterbalanced also slightly the opening of fiber structure induced by the coating process: water-treated papers had, indeed, a higher bulk value than reference.

The successive wetting/drying steps weakened the Young's modulus as the fiber network was more and more opened, whereas the elongation at break was enhanced owing to the successive drying under tension, which promoted a slight shrinkage of paper samples.

The addition of MFC counterbalanced these damages, especially by an increase by 35-40% of the Young's modulus. These results corroborated those in literature and especially those of our previous study (Lavoine, *et al.*, 2013b), although the MFC source was different (eucalyptus vs. Domsjö®). A different paper substrate was also used in this case, but the same improvements of mechanical properties were observed for both paper substrates.

Further to mechanical tests, air permeability and water absorption tests were also conducted on water-treated and MFC-coated samples. Figure 4-II.4 presents the results of both tests. The air permeability is plotted as a function of the water absorption. Usually, due to the refining of cellulosic pulp and consequently to the fiber fibrillation and hydration, a decrease of air permeability and water absorption is observed owing to a closest fiber network. Conversely, with the addition of MFC, which could also be considered as very well refined pulp fibers, the air permeability was well decreased but the water absorption was drastically increased.

Exceptionally, we also plotted the results obtained for ten times water-treated and MFC-coated samples. These samples were not considered in the rest of this study, as the coating of 10

successive MFC layers is obviously inconceivable within a future industrial application. Nevertheless, these results were quite significant to be highlighted, as a MFC coat weight of only 9 g/m^2 decreased again more drastically the air permeability.

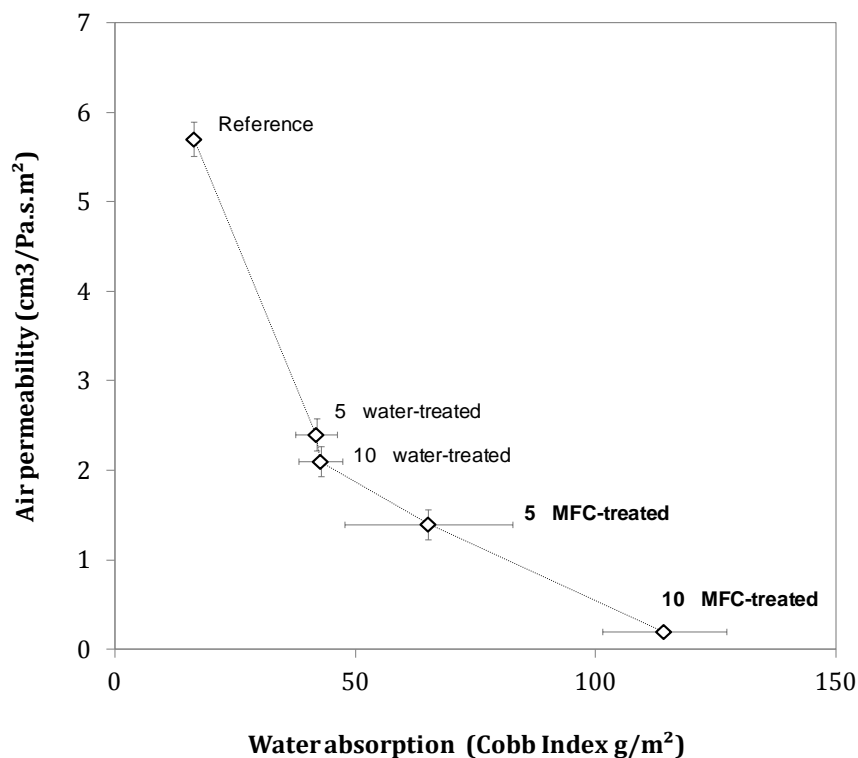


Figure 4-II.4. Influence of the coating process and the MFC suspension on barrier paper properties. The air permeability is plotted as a function of the water absorption. The tests were conducted on at least five specimens. Samples coated with ten MFC layers and treated ten times with water were also added to underline the drastic effect of MFC onto these both properties.

$10\times$ MFC-coated papers had a basis weight of 51 g/m^2 (i.e. a MFC coat weight of 9 g/m^2) and a thickness of $65 \mu\text{m}$. $10\times$ water-treated papers had a basis weight of 43 g/m^2 and a thickness of $60 \mu\text{m}$. Similar bulk values of 1.36 ± 0.01 were calculated for both samples.

Indeed, when considering the $5\times$ water-treated papers compared to the reference, the air permeability was decreased by 60%. This decrease was preserved with five water treatments more. The successive wetting/drying steps tightened, indeed, the fiber network until a certain limit. On a similar way, the water absorption was also increased by 50% for both samples. The coating process induced thus in primary step the improvement of the air resistance. This improvement was again drastically reinforced with the MFC coating. Compared to the water-treated samples, the air permeability was indeed decreased by 42% and 90% for the $5\times$ MFC-coated and $10\times$ MFC-coated samples, respectively. These results also corroborated those in literature. Other kinds of MFC were usually used and coated onto different paper substrates, but

the decrease of air permeability was always drastic even with a low MFC coat weight (Aulin, *et al.*, 2010, Hult, *et al.*, 2010, Syverud and Stenius, 2009).

Conversely, the water absorption was significantly increased with the increase of the MFC coat weight. The values were already doubled with the successive wetting/drying steps, and with the addition of MFC, these latter increased again and even doubled in the case of the 10 times coated samples. This was due to the high specific area and the hydrophilic nature of MFC.

Further to these results, the impact of the MFC coating was analyzed on the grafted samples as described in Table 4-II.2.

A MFC coat weight of 8 g/m² was also deposited onto CA grafted papers. The bulk of the MFC-CA grafted samples was slightly decreased compared to CA grafted papers. The trend was reversed when making the comparison between the reference and the MFC-coated samples.

The effect of MFC was not as significant as previously described: the Young's modulus and elongation at break were slightly increased, but in comparison with the reference, the effect of the grafting process was not enough counterbalanced by the addition of MFC.

Regarding the zero-span breaking length, similar values were obtained for coated and non-coated grafted papers. This result was expected as the zero-span breaking length gives an estimation of the fiber strength.

Contrary to our expectation, the damages induced by the grafting process were not enough counterbalanced by the MFC coating. Indeed, not only the fiber network but also each individual fiber was impacted by citric acid, and as described before, the MFC mainly had an effect on the surface properties of the paper samples. Nevertheless, the MFC coating enhanced again the air resistance: the same value was reached for MFC-CA grafted papers and MFC-coated papers. Better barrier properties to oxygen gas and higher water absorption, could be expected also with these samples based on literature.

Study of the β CD/carvacrol inclusion complex

A proton NMR study was developed to evidence the complexation between the carvacrol and the β CD. The spectrum in Figure 4-II.5a displayed the NMR spectrum of carvacrol where aromatic (doublets 7.10–7.08, doublet 6.80–6.77 and singlet at 6.75 ppm) and protons belonging to the methyl group (singlet 2.10 ppm) and isopropyl group (2.78 and 1.12 ppm) are observed.

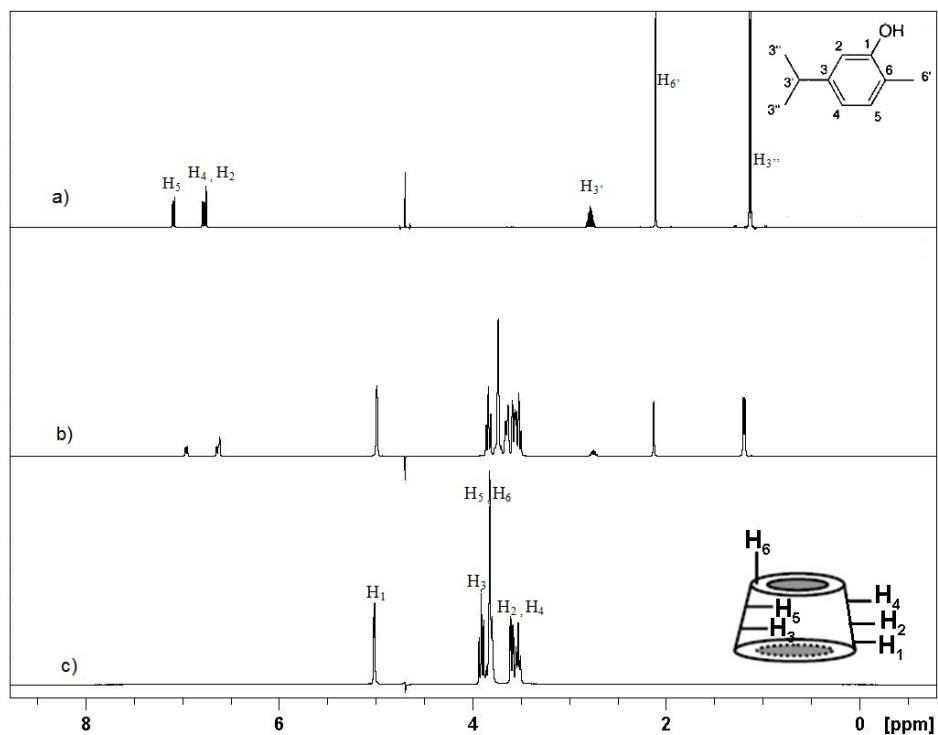


Figure 4-II.5. ¹H NMR spectra in D₂O (400 MHz) of carvacrol (a), carvacrol-βCD complex (b) and βCD (c)

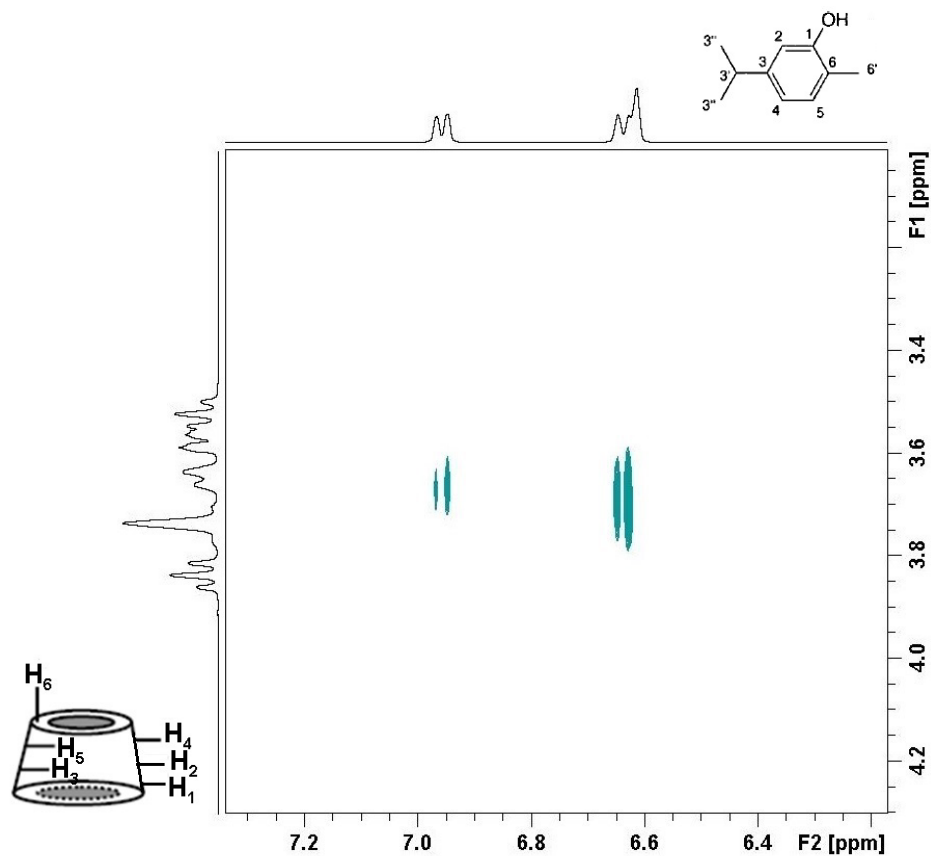


Figure 4-II.6. NOESY NMR spectrum focused on the cyclodextrin protons of βCD (*vertical*) and the aromatic protons signal of carvacrol (*horizontal*) in D₂O.

Besides, the spectrum in Figure 4-II.5c relative to the β CD displayed the signal of the glucopyranosic units of cyclodextrins, H1 at 5.01 ppm, H3, H5, H6, H4 and H2 situated between 3.50 and 3.93 ppm.

The spectrum in Figure 4-II.5b is relative to the carvacrol/ β CD mixture, where the aromatic protons of carvacrol shifted to 6.97–6.95, 6.65–6.63 and 6.61 ppm, and the isopropyl signal shifted upfield and downfield to 2.74 and 1.19 ppm. As observed in Figure 4-II.6, the 2D NOESY NMR spectrum obtained from the NOESY sequence displayed a correlation due to the dipolar interaction between the carvacrol aromatic group and the H5 of β CD situated inside the cavity.

This study clearly confirmed the inclusion complexation of carvacrol in the β CD, as already proved in the literature (Mulinacci, *et al.*, 1996; Locci, *et al.*, 2004).

The association constant (K_a) between free β CD in solution and carvacrol for a 1:1 complex values was determined by applying the Benesi–Hildebrand's equation adapted to NMR by Scott (Bakkour, *et al.*, 2006). A constant of $2141 \pm 279 \text{ M}^{-1}$ was calculated.

Carvacrol quantification

After the grafting of the paper samples, each one was impregnated for 10 min in the solution of carvacrol/ethanol. The samples were then dried for 20 min at 30°C. Some of these samples were then coated with five successive layers of MFC. Compared to the samples without carvacrol, these latter were dried at 50°C avoiding the complete evaporation of carvacrol. According to the study of Chalier, *et al.*, 2007 dealing with the effect of drying on the quantity of carvacrol remaining into paper coating, a drying at 50°C for 210s induced only losses of about 25% of the carvacrol initially introduced. These parameters were applied for soy protein coated papers containing carvacrol, and were a good analogy to our case.

Besides, it was necessary to determine the exact quantity of carvacrol introduced by impregnation into the paper samples. In that perspective, a complete release of samples containing carvacrol was conducted into a methanol bath. Methanol is, indeed, well-known to separate the cyclodextrins and their guest without releasing the cyclodextrin grafted into paper samples.

The absorbance of the solution containing methanol and the carvacrol released was measured at 275 nm each day until no more carvacrol was released. The total amounts of carvacrol released per sample are reported in the Table 4-II.3.

I/References and I/CA/ β CD papers contained a similar amount of carvacrol. On the contrary and, as expected, the samples coated with MFC after impregnation of carvacrol had a lower amount of carvacrol due to the successive wetting/drying steps. Nevertheless, the 35 mg of

carvacrol still present are sufficient to make inclusion complexes with the whole β CD grafted. Indeed, considering a grafting rate of about 9% of β CD and CA (in proportions 50/50) for a 600 mg paper sample (mean mass of the samples used) and a 1:1 complex between β CD and carvacrol, the maximal theoretical amount of carvacrol, which is able to complex with β CD, is about 4 mg.

Samples	Grafting rate of β -cyclodextrin and/or citric acid into papers (%)	Total amount of carvacrol released (mg)
I/Reference	0	112 \pm 42
I/CA/ β CD papers	8.94 \pm 0.01	116 \pm 7
I/CA papers	3.61 \pm 0.01	176 \pm 21
I/MFC- CA/ β CD papers	8.94 \pm 0.01	35 \pm 2

Table 4-II.3. Quantification of the total amount of carvacrol introduced into paper samples by impregnation method and respective mean grafting rates of β - cyclodextrin and acid citric for each sample.

As the three samples were prepared with an excess amount of carvacrol, a phenomenon of burst effect is consequently expected at the beginning of the release. In conclusion, the kinetic pattern obtained directly after the burst effect would be the most significant part of the release mechanism, and will be, as a result, studied in details hereinafter.

II-3.2. Release study of carvacrol into aqueous medium

The release of carvacrol was studied into an aqueous medium. The solubility of carvacrol is very low, but nevertheless its release was able to be carried out into deionized water. The results are shown in the Figure 4-II.7.

Due to the high volatility of carvacrol in aqueous solution, the release was not easily repeatable. This explains mainly the high standard deviations obtained for each different sample. Besides, the release was also very rapid: the reference and I/CA-grafted papers reached the approximate plateau value after 3 h, and the I/CA/ β CD-grafted papers released its whole quantity of carvacrol after 24 h (accounting for the standard deviations). The impact of β CD was thus clearly noticeable: the release of carvacrol was slowed down since these samples released the whole amount of carvacrol 21 h later than the reference and the I/CA-grafted papers (Figure 4-II.7a). It is worth noting that the initial carvacrol amount was quite similar for these three samples (I/Reference, I/CA and I/CA/ β CD Table 4-II.1.).

When considering the beginning of the curves (Figure 4-II.7b), the influence of the grafted paper on the carvacrol release was however slight compared to the reference: the proportion of

carvacrol released as a function of the time were indeed a little bit lower for the grafted papers than for the reference (in 1 h: 58% and 62% were released for the CA and CA/ β CD respectively against 74% for the reference). This observation was also confirmed when plotting the carvacrol quantity released in mg as a function of the time. The kinetic of the samples was quite similar: about 123 mg/h for the I/Reference compared to 148 and 105 mg/h for the I/CA and I/CA/ β CD grafted papers, respectively.

This might be due to the excess of carvacrol released during the beginning of the experiment. Owing to the grafted citric acid and the hydrogen bonds formed between the carvacrol and citric acid, the I/CA papers released the carvacrol as slowly as the I/CA/ β CD papers (almost similar slopes). Nevertheless, from 40 min, i.e. about 0.67 h, the I/CA/ β CD papers continued its slow and progressive release of carvacrol for 23h20, whereas the I/CA papers released more rapidly the carvacrol until reaching the plateau value at 3 h. Thus, the use of β CD induced clearly a slower and more progressive release of carvacrol, although the grafted citric acid also had a significant influence. The effect of β CD grafted into papers on the release of molecules was already proved in literature²⁶. Nevertheless, the CA grafted papers were not studied, and we just showed the importance to also consider this reference.

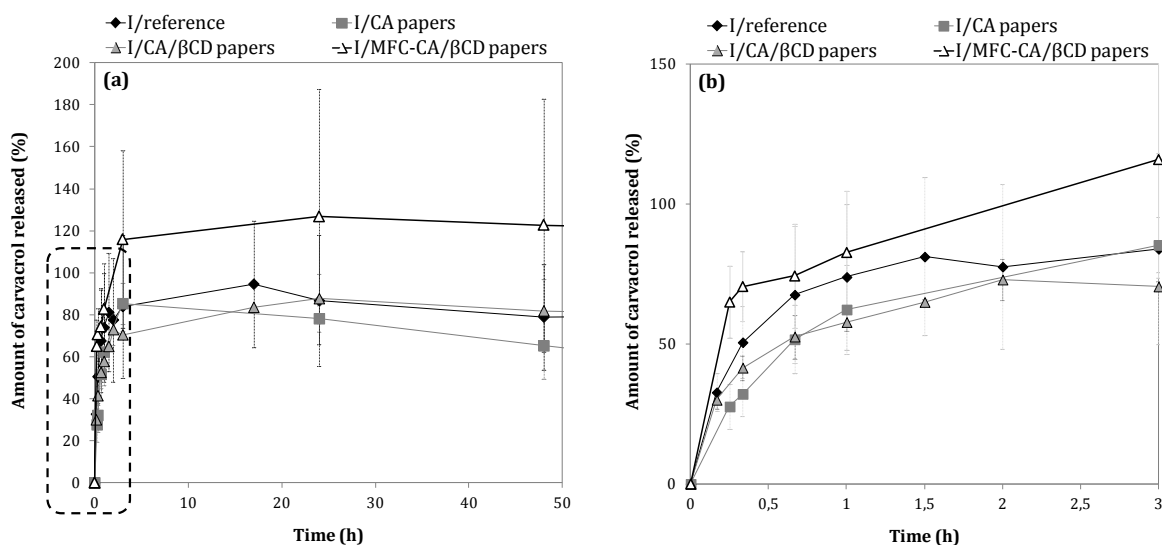


Figure 4-II.7. Release study of carvacrol into aqueous medium.

Impact of β -cyclodextrin and MFC on the release kinetic of carvacrol in comparison with the reference and CA grafted papers. Tests were conducted at 23°C and 50%RH for three specimens of each sort of papers. The graph (a) plots the amount of carvacrol released as a function of time for 50 h; the graph (b) is a zoom of the graph (a) on the first 3 h.

The novelty of this study consists also in using simultaneously β CD and MFC to have a better control of the carvacrol release. Figure 4-II.7 also presents the release study conducted with the I/MFC-CA/ β CD papers. Although the plateau value was also reached after 24 h like the

I/CA/ β CD papers, the slope of the curve was much higher than the others, i.e. the proportion of carvacrol released was higher (at a similar time) than the other samples when considering the initial amount of carvacrol introduced.

This result was unexpected since the samples were grafted with β CD and then coated with MFC. Nevertheless, the standard deviations were quite significant compared to the other samples, and when considering the plateau value, more than 100% of the carvacrol introduced were released, i.e. more than 35 mg. Obviously, although each sample was loaded on a similar way with carvacrol, the MFC coating induced some heterogeneities, which explained these high standard deviations.

The low impact of MFC on the carvacrol release might also be due to the experimental process applied to coat the MFC. Indeed, the coated MFC was dried at 50°C only, instead of 105°C because of the presence of carvacrol. Thus, the nanoporous MFC network was not as tight as it had to be when dried at 105°C in our previous study⁴⁹. During the release study, the MFC layers in contact with water began effectively to crumble, releasing then more rapidly the carvacrol.

This release study did not allow to clearly conclude on the effect of the MFC on the release of carvacrol. However, even with a lower quantity of carvacrol, i.e. 4 to 5 times less than the quantity introduced into the other samples, the MFC released the whole amount after 24h, and the small quantities released were also sufficient to bring an antibacterial property as it will be detailed below. Another release study was consequently performed with a solid aqueous medium to also avoid the crumbling of the MFC layers. The influence of the MFC, and thus the synergistic action between β CD and MFC was highlighted by the release study into agar, conducted in parallel with antibacterial tests.

II-3.3. Release study of carvacrol into agar: Antibacterial tests

The antibacterial action of carvacrol was already analyzed in many studies (Ben Arfa, *et al.*, 2006, Ben Arfa, *et al.*, 2007, Lambert, *et al.*, 2001, Nostro, *et al.*, 2012). Its hydrophobic nature as well as its free hydroxyl function are the two main factors responsible of its antibacterial nature. Owing to its hydrophobic nature, it interacts with the lipid bilayer of the cytoplasmic membrane and itself aligns between the fatty acid chains causing the expansion and the destabilization of the membrane structure and increasing its fluidity and its permeability (Nostro and Papalia, 2012). The presence of the free hydroxyl groups and of a system of delocalized electrons is also important. Acting as proton exchanger, the gradient across the cytoplasmic membrane is reduced, which can eventually lead to the cell death due to the decrease of the ATP (Adenosine Triphosphate, which transports the chemical energy within cells for metabolism) (Ben Arfa, *et al.*, 2006).

Carvacrol is thus a strong antibacterial agent against a large range of bacteria. It is a volatile molecule, in the presence of humidity or water, able to diffuse both in the air (head space) and in the agar. Before carrying out diffusion tests into agar, the diffusion of carvacrol was then tested in the air. The samples containing carvacrol were stuck on the lid of the Petri dish, and after 2 h in the fridge and 3 days at 30°C, an inhibition zone was noticeable on the inoculated agar (results not shown). The carvacrol was thus able to diffuse in the air and be antibacterial. Nevertheless, this step was repeatable only once, since no more inhibition zone appeared with successive antibacterial tests.

Further to this result, the diffusion of carvacrol was carried out into inoculated agar, and results proved that carvacrol was highly efficient against *Bacillus subtilis* when diffused into agar. Its long-term activity was still expected and was consequently tested with successive antibacterial tests.

As detailed in the experimental section, the antibacterial tests were successively repeated. Once the active samples were put in contact with the inoculated agar, they were let in the fridge for 2 h so that carvacrol could easily diffuse into the agar instead of diffusing in the air. After that, the samples were removed from these Petri dishes (1) and put in contact with new Petri dishes (2) containing a new inoculated agar. The dishes (1) were then put into the oven at 30°C for 3 days so that the bacteria can grow. The dishes (2) were submitted to the same steps than the dishes (1).

These tests were repeated until no more inhibition area was distinguished. The results are presented in the Figure 4-II.8. Compared to the release into water, the release of carvacrol into agar was overall slower. It seems obvious that the molecule release into a liquid medium happens faster than into a solid medium owing to the molecule motion.

Consequently, these antibacterial tests emphasized more the impact of the grafted β CD on the release of carvacrol. The reference and the CA papers impregnated with carvacrol lasted only twice 2 h-periods. Indeed, no more inhibition zone was still measureable after the second antibacterial tests. The I/CA/ β CD papers released carvacrol for seven successive 2 h-periods, which means successively for 14 h. The enhancement of the duration of the antibacterial activity was quite significant compared to the reference and CA papers. β CD allowed thus a more progressive release of carvacrol prolonging the antibacterial activity for 10 h. This result was in accordance to the previous conclusions drawn from the release study into water. Indeed, compared to the I/reference and I/CA papers, the I/CA/ β CD papers released the whole amount of carvacrol also later; within 24 h instead of 3 h.

As expected, these antibacterial tests also allowed underlining the effect of MFC on the release of carvacrol. Considering the I/MFC-CA/ β CD papers, they released carvacrol for eight (8)

successive 2 h-periods, i.e. one period more than the I/CA/ β CD papers (repeatable with each three Petri dish prepared). This increase of the duration of the antibacterial activity was thus mainly due to the presence of the MFC layers. Although this enhancement was not so consequent, it is worth noting that the MFC network was not as tight as it used to be due to the low temperature applied for the coating process. Indeed, in our previous study (Lavoine, *et al.*, 2013c), we clearly proved that the MFC coating was able to improve of 12 days the antibacterial activity of papers impregnated with the chlorhexidine digluconate solution; and this, with 7 g/m² of MFC deposited with a bar coating process and dried at 105°C. We have also to keep in mind that the carvacrol quantity was much lower into I/MFC-CA/ β CD papers than the quantity into the I/CA/ β CD samples. However, even with a lower quantity, the antibacterial activity lasted slightly longer owing to the presence of MFC. This result is thus very positive regarding the impact of the antibacterial agent on the organoleptic properties of food. Indeed, a packaging material including a much lower quantity of carvacrol is expected to limit even to avoid any food damages.

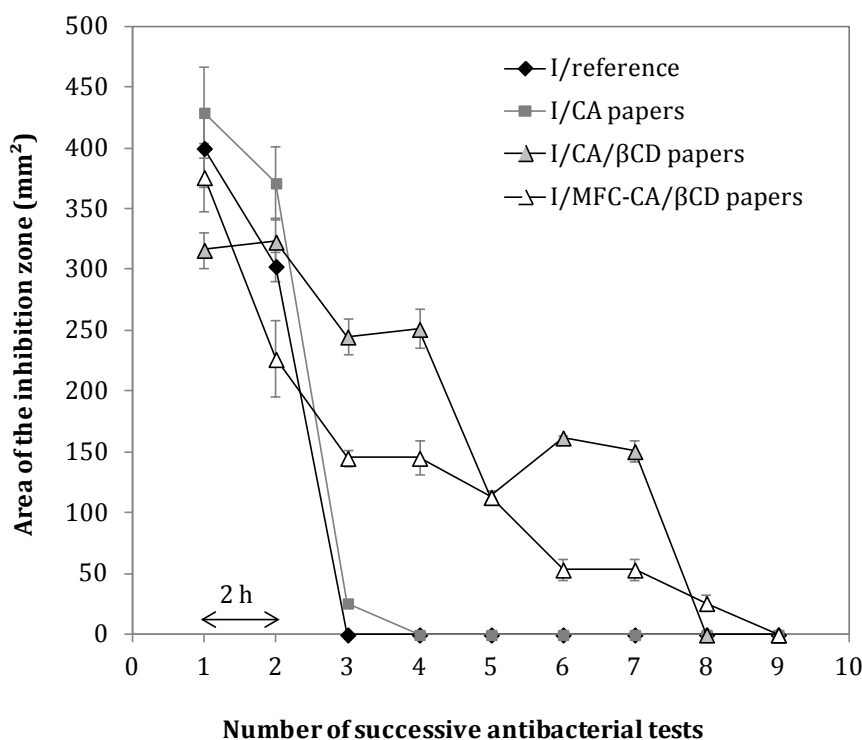


Figure 4-II.8. Area of the inhibition zone as a function of the number of successive antibacterial tests
The area of the inhibition zone was calculated from the measurement of the diameter of the zone. The final area represented here is the difference between the effective inhibited area and the area of the samples of 16 mm diameter. The number of successive antibacterial tests corresponded to the number of 2 h-period spent in the fridge, which corresponded to the time during which the active sample was in contact with the inoculated medium.

As a result, we can conclude on a synergistic action between the β CD grafting and the MFC coating allowing the more progressive release of a lower quantity of carvacrol and the preservation of a long-term antibacterial activity.

This result is thus very promising for the development of a new delivery system able to release progressively an active molecule.

II-4. Conclusion

This study presented the development of a new antibacterial bio-material for food-packaging application based on the synergistic action of the beta-cyclodextrin and microfibrillated cellulose. The β -cyclodextrin as well as the MFC already proved their efficiency as delivery system of active molecules. Nevertheless, the combination of both was never achieved, and this study proved its efficiency.

Indeed, paper substrates grafted with β -cyclodextrin and then coated with MFC were able to release longer and more progressively carvacrol. Furthermore, these MFC-coated substrates preserved the antibacterial activity of carvacrol in spite of a lower quantity. This quantity can, thus, be targeted to limit the modification of the organoleptic properties of food. Moreover, despite this very low amount of carvacrol, the antibacterial activity was maintained longer with the MFC-coated substrates than with other samples without MFC or only with β -cyclodextrin.

These results are thus very promising for the elaboration of new delivery systems for food-packaging application. Due to its hydrophobic and volatile nature, carvacrol is indeed difficult to use in usual food-packaging materials. With this study, a new solution was achieved allowing the development of a bio-material with a long-term antibacterial activity.

Chapter 4-III Conclusions

The Chapter 4 paved the way for an innovative controlled release system based on the synergistic action of MFC and cyclodextrin. Owing to the trapped mechanism of the cyclodextrin and the nanoporous MFC network, the active molecules introduced in this new matrix were released and kept active over a longer period.

This Chapter presented two different strategies to combine the MFC and the cyclodextrin. Either the cyclodextrins were coated with the MFC suspension onto the paper surface, or they were firstly grafted onto the paper substrate, which was in a second time coated with the MFC suspension.

Two active molecules were also tested. The chlorhexidine digluconate (CHX) was used with the first strategy since its interactions with the cellulose promoted its more progressive release into the aqueous medium. Indeed, in this case, no specific synergistic action was observed between the MFC and the cyclodextrins, but the coating of the cyclodextrin mixed with the CHX alone led to very promising results. This result is clearly related to the cyclodextrin:-CHX complex, which has much more interactions with cellulose than the CHX alone. However, with this process, the cyclodextrin was also released, which limits the re-use of the material.

With the second strategy, the cyclodextrin were consequently grafted to avoid this phenomenon, and the molecule tested was the carvacrol. This molecule was firstly used owing to its volatile nature in humid environment. This ability was, indeed, targeted so that this molecule can diffuse directly into the cyclodextrin-grafted paper samples, avoiding thus to load the samples with an excess of active molecules. Nevertheless, the quantity released into the paper substrate was not efficient against bacteria, and the impregnation method was rather selected to introduce the carvacrol.

In spite of an excess of carvacrol and slightly “oily” paper samples, a slight synergy between the MFC and cyclodextrin was observed with the release study into the inoculated agar. The cyclodextrin-grafted paper samples released, indeed, the carvacrol over 14 h, whereas with the presence of the MFC, this release was extended by 2 h. The enhancement was, nevertheless, not so significant. This can be explained by the formation of a “weak” nanoporous MFC network, induced by the low drying temperature of the MFC layers, required by the presence of carvacrol. The nanoporous MFC network was, indeed, not dried as usual and, as a result, was less tight. This was also confirmed with the release study conducted into water, where the MFC layer began to lose cohesion.

The Chapter 4 set, thus, the basics for a future development of paper-based controlled release systems using the synergistic action of the MFC and cyclodextrin.

A main advantage of this development was the achievement of a reusable controlled delivery system obtained by a new impregnation of the material into an active solution.

Following these promising results, various perspectives have been suggested to overcome the different drawbacks underlined in the two sub-chapters and to improve the primary concept:

- (1) Based on the “Layer-by-Layer” strategy, the successive coating of cyclodextrin and MFC could be firstly considered;
- (2) Then, before the introduction of the active molecules and the coating of the suspension onto the paper surface, the MFC could in first step be grafted with the cyclodextrin;
- (3) The use of a polymer of cyclodextrins, produced by the UMET laboratory, could also be investigated, so that the grafting process using citric acid could be avoided, as well as the release of the cyclodextrin in parallel to the release of the active molecules;
- (4) Finally, other kinds of MFC suspensions could also be used as paper coating. For example the use of TEMPO-oxidized MFC could be a key solution, since its carboxyl groups would be able to interact more strongly with cationic molecules or with cationic polymers of cyclodextrins, currently developed by the UMET laboratory. They can also allow the grafting of cyclodextrins without using citric acid.

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GENERAL CONCLUSION AND PERSPECTIVES

General Conclusion and Perspectives

In a context of a sustainable development and a food security policy, the objective of this PhD was to develop an innovative functional bio-nano-material for food-packaging.

This project, indeed, was set up to meet the requirements of the current society, looking for a more biodegradable, recyclable and convenient packaging material able to protect more efficiently the food.

From this perspective, cellulosic material was chosen as based packaging material. Its commercial image has, indeed, always been linked to recyclability and biodegradability, even if, in comparison to plastic packaging material, it is restrained to some applications due to its barrier properties.

To overcome these drawbacks and to response to the society's demands, we selected an innovative bio-nano-material called microfibrillated cellulose (MFC) within the framework of this project.

At the beginning of the project, in 2010, the MFC was mainly used in nanocomposites, as films or aerogels. Its application with cellulosic substrates was limited to one patent, four scientific studies published in 2009 and 2010, and one European project launched in 2009 (SUNPAP). Although these first results were very promising, we had to wait 2013 for exceeding twenty scientific works on this topic.

The innovative side of this PhD project is not only the use of MFC with cellulosic substrates, but, also and, above all, the use of MFC as controlled delivery system for packaging applications. In 2010, none scientific papers dealt with this topic. The first paper suggested the use of MFC as drug carrier matrix appeared in 2011, and we have to wait until 2013, i.e. the last six months of this PhD, to assist to the publication of six scientific papers dealing with the use of MFC as films, aerogel, tablet or spray-dried suspension drug delivery system.

The publication of the first works dealing with a similar topic strengthened our primary idea: the necessity to develop a functional bio-nano-material for packaging applications using MFC as controlled delivery system.

As presented in this PhD report, this project was divided in four main parts: design, processing, characterization and developments.

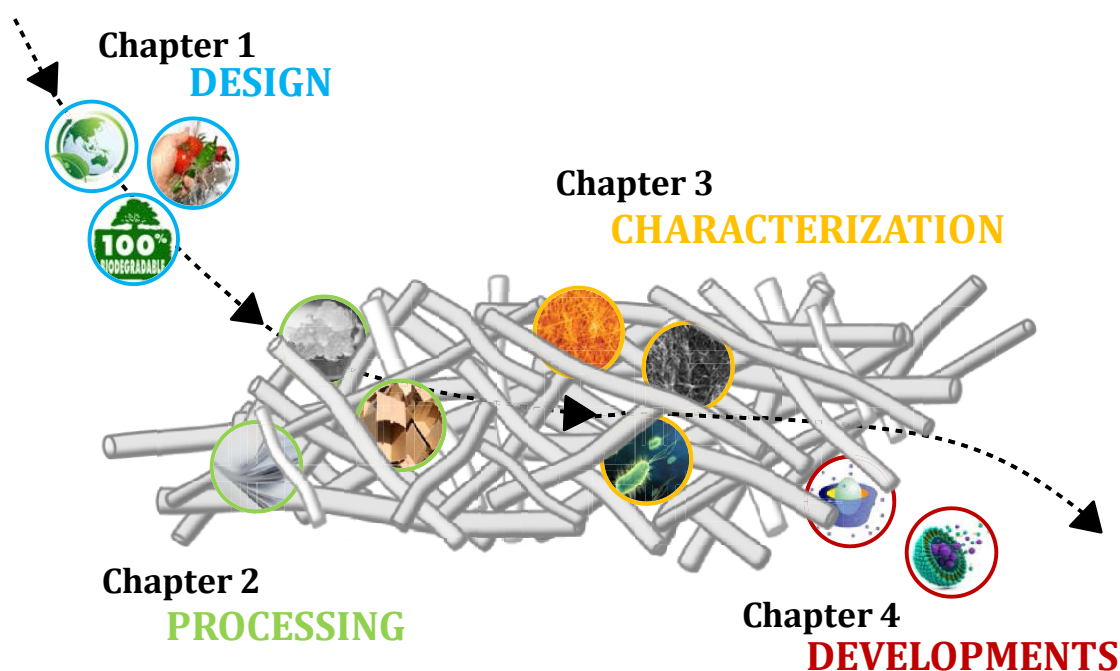


Figure 1. Manuscript organization

These four parts were tightly connected such as the nanoporous MFC network.

In the first part, the context of this project has been detailed. The society's requirements on the food-packaging materials, the current concerns on the food spoilage and the food poisoning lead to find new solutions of food-packaging materials. The **design** of an innovative bio-material has, therefore, been assessed by focusing on the use of MFC, considered as a key solution to the listed requirements.

Consequently, our strategy consisted firstly in coating the MFC suspension on the surface of our selected cellulosic materials. The Chapter 2 detailed the **processing** and the impact of the MFC coating on the mechanical and barrier properties of a paper and a cardboard substrate. Although the improvement of these properties were not as drastic as expected, we highlighted an essential point, which was, until now, never taking into account in any scientific works: the negative impact of the successive wetting/drying cycles.

Indeed the MFC suspension is mostly composed of water (98%). As a result, it was essential to consider the base cellulosic materials coated with water only, since its influence on the properties of cellulosic materials is a well-known phenomenon. We proved that the

improvement or damage of some properties was mainly due to the wetting/drying cycles, which modified considerably the structure of the fiber network.

When considering the water-treated references, the MFC slightly improved the mechanical properties of paper and cardboard substrates but drastically enhanced the air resistance and the water absorption.

Within the framework of this Chapter 2, further works could be investigated. For example, it could be interesting to increase the dried MFC content of the coating suspensions. Otherwise, other kinds of MFC such as the TEMPO oxidized MFC could be tested since their nanoporous network is more tightened and would possibly enhance better the final properties. A mix of the MFC suspension with other solid (nano)particles (e.g. Montmorillonites) might also be a solution to increase the solid content of the coating slurry and to improve the end-use properties. Finally, another way would consist in using the MFC suspension in mass, especially with a cardboard substrate. Some previous works, indeed, showed very promising results from a mechanical and barrier properties point of view.

In this second part, we were also able to observe our MFC coating with microscopy techniques. Usually, SEM is used to conclude on a homogeneous coating or to measure the thickness of the MFC layer deposited. But no nanoscale dimensions can be distinguished at the surface of a material with such equipment. In our case, we tried to observe the nanoporous MFC network with the Atomic Force Microscopy technique. The pictures obtained clearly highlighted the formation of a network as similar as the one of the MFC suspension. The conclusion was that even after the coating of the suspension and its drying, the nanoporous MFC network was preserved.

This finding leads us to develop a functional bio-nano-material, which consisted in the introduction of an active compound into the coated nanoporous MFC network. **The Chapter 3** is the key part of our study. It presented the proof of concept: the MFC network is able to release more progressively the active molecule into an aqueous medium. It was the first study using the MFC coating as controlled release system.

An antibacterial molecule (CHX) was, consequently, introduced in this network. It was not only more progressively released owing to the MFC, but it also kept its antibacterial activity over a longer period thanks to the tightened nanoporous MFC network. Whatever the substrate used, paper or cardboard, the MFC coating showed very promising results as controlled delivery system. The impact of the interactions between the active molecule and the cellulosic substrate was also highlighted. Contrary to the caffeine, the CHX was able to create hydrogen bonds and ionic interactions with the substrates, which promoted a different release pattern.

Further works could then be investigated in this thematic. The use of other active compounds with or without interactions or such as proteins (bigger one) could be interesting to study. The use of other types of MFC suspensions (TEMPO pre-treated and their charge interactions) is also an idea for next steps. The modeling of the mass transport phenomena might also be studied in detail. It is currently one of the perspectives that we have already launched (see more details later on). Besides, within the context of food-packaging materials, it would be interesting to deal with antibacterial and food tests in depth, so that the migration of the active compound could be evaluated against more resistant bacteria and under more complex conditions.

In the **last chapter**, we broadened up our strategy to new applications such as biomedicine. Within the context of a collaboration with the research laboratory “UMET” in Lille (France), we developed a new kind of controlled release system based on the use of MFC and cyclodextrins. Cyclodextrins are, indeed, well-known in the pharmaceutical/medicine field as drug delivery systems.

The challenge, here, was first to compare the action of MFC and cyclodextrins as controlled delivery system, and then to use them together in order to create a synergistic effect.

Two strategies were followed to introduce cyclodextrins in our cellulosic substrate. Firstly, they were directly coated onto the surface of our substrate, either alone with the antibacterial molecules or mixed with the MFC suspension. None synergistic effect was observed, but the only use of cyclodextrin as coating proved to be very efficient as controlled delivery system.

The second strategy consisted in grafting the cyclodextrins into the paper substrate following a protocol patented by the UMET laboratory. The main advantage of this strategy was that the cyclodextrins were well trapped into the paper structure, and thus, were not able to be released as we proved in the first strategy. However, a clear drawback of this method was the use of citric acid, which damaged consequently the paper properties. The coating of MFC only slightly counterbalanced this loss of properties. Nevertheless, the grafting of the cyclodextrins followed by the MFC coating proved to be an efficient controlled release system: in this case, a synergistic action was observed between both MFC and cyclodextrins.

As perspectives, the grafting of cyclodextrin with MFC could be investigated before coating the final suspension onto the cellulosic substrate. As the coating of cyclodextrins gave promising results, further studies could also be considered in that way by using other kinds of active compounds so that the exact mechanism of release could be well understand.

Finally, this PhD project paved the way for a new kind of application of the MFC combined with cellulosic substrate: the use of MFC-coated cellulosic substrates as controlled release system of active molecules.

It contributes thus:

- 1) to highlight the essential process parameters to consider when the MFC coating is investigated with a cellulosic substrate: the MFC concentration, the water-treated reference, the properties of the base material;
- 2) to prove that the nanoporous MFC network is preserved once coated onto a cellulosic substrate,
- 3) to show that MFC can be used as controlled release system of antibacterial molecules with a strong influence of this latter;
- 4) to pave the way for future high-added value applications in the fields of food-packaging and biomedicine.

Since the beginning of this project, the number of publications dealing with the use of MFC continues to increase exponentially. Nevertheless, in the domain of controlled release system, the research has just begun as proved by the Table 1 presenting the evolutions of the field for the past three years.

THEMES	BEFORE 2010	AFTER 2013
	5 scientific works (4 publications and 1 patents)	Around 50 scientific works (8) (≈ 35 publications and 12 patents)
MFC and cellulosic substrates	MFC coating	MFC coating
	<ul style="list-style-type: none"> - onto handsheet, kraft and grease proof papers, and cardboard - using bar coating process - alone or mixed with fillers or resins/polymers - to improve the barrier and printing properties 	<ul style="list-style-type: none"> - onto different kinds of <i>papers and cardboard with detailed analysis of mechanical and barrier properties</i> - as blend, <i>coating (at 2%wt)</i>, or multilayers - using blade, curtain, <i>bar coating and size press</i> - to improve mechanical, barrier and printing properties (<i>+1 review</i>)
MFC as controlled delivery system	0 scientific works with MFC produced from cellulosic sources	13 scientific works (5)
		MFC release systems as <ul style="list-style-type: none"> - films (2), aerogels (1), spray-dried suspension (4), suspension (1), <i>coating (5)</i> - <i>Antibacterial and food tests</i>

Table 1. Main evolutions in the field of MFC used with cellulosic substrates and as controlled delivery systems from 2010 to 2013. Our contribution is underlined in bold italic font.

This project set, thus, the basics for further developments in that field. It would not have been achievable without the numerous collaborations we established over these three years.

Thanks to the FCBA (Grenoble), very good quality of MFC suspensions were provided during the entire PhD project. We also had the opportunity to develop a project on the use of MFC films as controlled delivery system. This 1-year project led to the presentation of an awarded poster at the TAPPI International Conference on Nanotechnology for Renewable Materials in Stockholm, in 2013. This poster is presented in the Appendix of this manuscript.

Without this second collaboration, the Chapter 4 of this manuscript would not have been achievable. The UMET laboratory shared its knowledge about cyclodextrins and drug release so that a new kind of controlled release system could be developed.

A last collaboration was also established these last final months of this PhD project with the laboratory of the University of Montpellier II.

Indeed, over this whole manuscript, we only slightly discussed on the mechanism of diffusion of the various antibacterial molecules tested. Within the context of controlled release systems, it is necessary to consider the fundamental part behind these experimental results. We suggested, therefore, over this manuscript, some mathematical models or release mechanism, which were able to describe the behavior of the molecules released through the paper matrix with or without MFC.

The final discussion on this topic is, however, still under progress, since the release mechanisms happening through a swelling-polymeric matrix are complex.

Nevertheless, we would like to give an overview of this work. The context of this collaboration as well as the first results obtained with the University of Montpellier II are described in the Appendix of this manuscript.

The final results will be presented in the PhD defense.

We hope that this manuscript will contribute to further developments of controlled delivery systems using MFC for food-packaging and biomedicine applications, and will arouse the curiosity of the scientific community on this innovative and astonishing bio-nano-material, which is the microfibrillated cellulose.

APPENDICES

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APPENDIX 1

Modeling of molecules diffusion through a cellulosic substrate coated with microfibrillated cellulose

Context of the study

Following the promising results developed throughout this 3-year PhD project, we wanted to extend and deepen the understanding of the diffusion phenomena, which take place during the release of an antibacterial compound with microfibrillated cellulose (MFC). In this particular instance, the mechanism of release is quite complex to describe, since we are in the presence of porous and swellable systems, which interact with the other by hydrogen bonds, and eventually chemically interact with the molecules to be released.

Based on our previous experimental results, the idea of this study is, thus, to develop a mathematical model, which aims to explain and describe the molecules diffusion through 1) a paper substrate, a 2) paper substrate coated with MFC and a 3) MFC coating.

Within this perspective, we have established collaboration with the Professor Nathalie Gontard and the Associate Professor Valérie Guillard of the University of Montpellier II (Montpellier, France)..

The first step of this collaboration consisted in conducting an extended literature review, in order to screen the current mathematical models, which could be applied in our study. This led us to make some hypotheses regarding the release systems studied. Indeed, depending on the geometry and the structure of the release matrix, the mathematical model needs to be adapted.

When considering our controlled release systems, two main hypotheses can be investigated as regard the structure of the release matrix. The paper substrate as well as the MFC coating can be considered either as **(i) dense or as (ii) porous materials**.

The **hypothesis (i)** has been investigated by the two only one studies dealing with the release of molecules introduced into a neat MFC film (Kolakovic, *et al.*, 2013ⁱ; Cozzolino, *et al.*, 2013ⁱⁱ). Both considered the MFC film as a dense material and applied a mathematical model based on the Fickian diffusion.

Kolakovic, et al., 2013 used the following Higuchi model to describe the release of hydrophobic drugs (Equation 1):

$$Q = A \sqrt{\frac{D\varepsilon}{\tau} (2\rho - \varepsilon C_s) C_s t} \quad (1)$$

where Q is the amount of drug released, A is the surface area of the film, D is the diffusion coefficient of the drug inside the film, ε is the porosity of the film, τ is the tortuosity of the film, ρ is the density of the drug material in the film, C_s is the saturated solubility of the drug inside the film, and t is time.

The Higuchi equation quantifies the drug release from “monolithic dispersions” with slab geometry. It considers that the drug is homogeneously distributed within the matrix at an initial concentration that exceeds drug solubility (in the wetted system) (Siepmann and Siepmann, 2011ⁱⁱⁱ).

This model was especially well adapted to their experiments. They assumed, indeed, that the MFC films are flat and have a large aspect ratio of length to thickness, and used hydrophobic drugs.

With this model, they obtained good fitting parameters ($R^2 > 0.995$), and concluded that the diffusion, only, was the limiting factor to the release of the hydrophobic drug studied (e.g. indomethacin). With the study of another drug, e.g. itraconazole, they observed a more progressive release pattern, and suggested a zero order kinetic ($R^2 > 0.982$). This difference was mainly explained by the particle sizes of the incorporated drugs: with larger particles (in the case of the indomethacin), the lamellar structure of the MFC films is more disrupted, which induce a faster release. Other reasons explaining the release difference were also considered, such as the possible binding of the drug to the fibers (which is one main parameter to be investigated in our study). Nevertheless, the authors did not precise the influence and the extent of these factors.

In the second study on this thematic (Cozzolino, et al., 2013), a similar release study was conducted on MFC film loaded with an antibacterial protein, the lysozyme (a large macromolecule). Compared to the previous scientific work, the food-packaging sector was targeted as end-use application.

The experimental release data were modeled using the solution of Fick’s second law for a planar sheet with constant boundary conditions and assuming the following hypotheses:

- Both water diffusion and relaxation of molecular chains occur faster than the diffusion of the high molecular-weight antimicrobial compound (i.e. lysozyme) through the swollen network
- Lysozyme diffusion takes place in an homogeneous, symmetric medium

- The volume of the liquid medium is infinite (sink condition)
- The external mass transfer coefficient at the solid-liquid interface is negligible.

Their hypotheses as well as their study did not consider the MFC network as a dense material, although they applied the following equation based on Fickian diffusion (Equation 3):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{(2n+1)^2}{L^2} \pi^2 D t\right) \quad (3)$$

where M_t represents the amount (mg/kg) of the diffusing compound released at time t (s); M_∞ is the corresponding amount at infinite time (e.g. at equilibrium), taken as the initial quantity loaded on the film; D is the lysozyme apparent diffusion coefficient ($\text{cm}^2 \cdot \text{s}^{-1}$) through the MFC polymer matrix; and L is the thickness (cm) of dry films.

Compared to the other study, they associated their release system to a “monolithic solution”. In this case, the drug is not “completely” physically separated from the release rate controlling barrier, but more or less homogeneously distributed within the latter (although lysozyme is highly soluble in water).

From this mathematical model, the author obtained the value of the apparent diffusion coefficient so that the influence of different parameters such as the stimulant or the pH can be evaluated on the release of lysozyme. They also underlined the main factor influencing and explaining the progressive release observed. Just like the previous study, the MFC/lysozyme interactions was a major factor in determining the final release performance of the MFC matrix.

Both studies proposed two different mathematical models to explain the release of molecules loaded into neat MFC films. Interestingly, they suggested different hypotheses depending on their experimental conditions, but did not conclude on the suitability of their model. It is worth noting that these studies (published in 2013) are the first trying to explain the transfer phenomena through the complex nanoporous MFC network.

Following these two first approaches, we have also started our project with the assumption that the paper substrate and the MFC network are **(i) dense and unique materials** (i.e. one apparent diffusion coefficient for each system). We investigated different hypotheses, keeping in mind the influence of the interactions molecules/MFC or molecules/paper (which is the case when using CHX) as well as the swelling of the cellulose in contact with water.

A first model based on Fickian diffusion has been applied on the experimental part dealing with the release of **caffeine** introduced in our three different matrices: 1) paper substrate, 2) paper substrate coated with MFC and 3) MFC coating. The purpose, here, is to find a model explaining the release mechanism through these three matrices without including any other influencing parameters such as chemical interactions.

If this model is approved with further experimental data, the objective will be to apply this similar model to the release of CHX. In this case, the interactions between the CHX and the cellulose would be highlighted and a comparison between both molecules could be investigated. If the model is not approved, we will investigate other mathematical models. In a first step, the idea will be to test and fit the models described in the literature to our experimental data. The comparison could be useful in order to confirm or not that the MFC coating performs as a neat MFC film. A last step of validation will also close this part.

This whole modeling study is in progress and first models have been plotted with our experimental data. This will conclude our PhD project with fundamental perspectives and will be the subject of a last scientific publication in collaboration with the University of Montpellier II. The outline of this scientific paper is already in discussion and presented on the next page. The final results will be introduced during the PhD defense.

This scientific paper will aim to bring first answers explaining the diffusion mechanisms, which take place during our release experiments. Nevertheless, as introduced, this analysis is based on the assumption that the system is a **(i) dense material**.

It is clear that the paper substrate as well as the MFC network cannot be fundamentally considered as dense material, since we used their porosity to release more progressively the active compounds. Following this first publication, the further work will consist in the development of mathematical models for **(ii) swellable-polymeric systems including porous materials**. The literature already suggests some models, usually applied to study the water vapor diffusion through paper substrate (e.g. Knudsen (Spence, *et al.*, 2011^{iv})). In particular, the mathematical model describing the diffusion-controlled and swelling-controlled systems is (Huang, *et al.*, 2001^v):

$$\frac{M_t}{M_\infty} = kt^n + \alpha \quad (4)$$

where $\frac{M_t}{M_\infty}$ is the fractional drug release, n the diffusional exponent, and k the pre-exponential factor. In this model, the constant α is added to fit experimental data accounting for a rapid jump in concentration at $t=0$. This constant represents the notion of “burst effect”, we observed and described throughout this PhD.

These models will conclude our research project and pave the way for future fundamental discussions on the release mechanism taking place with MFC.

Modeling of molecules diffusion through cellulosic substrates coated with microfibrillated cellulose

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1. INTRODUCTION

Literature review on the current mathematical models developed to describe diffusion controlled drug delivery. This is based on the recent review of Siepmann and Siepmann (2011) published in Journal of Controlled Release. This article gives an overview on the current state of the art of modeling drug release from delivery systems, which are predominantly controlled by diffusional mass transport.

Some examples will illustrate the theoretical models described in this first introduction part leading, then, to the models applied to MFC-based controlled delivery systems (Kolakovic, et al., 2013; Cozzolino, et al., 2013).

The context of our experimental study will be here developed to introduce the present paper.

2. MATERIALS AND METHODS

2.1. Materials

The MFC suspension was supplied by the FCBA (France). It was produced from sulfite pulp (Domsjö®), enzymatically pre-treated with an endoglucanase for 1 h, and passed through a GEA Ariete® homogenizer (4 passes, at 1,400 bars). The concentration was 2% (w/w).

The base paper material was calendered paper with a basis weight of 41 g/m² made with non-bleached pulp.

Caffeine (99%), C₈H₁₀N₄O₂, was purchased from Sigma-Aldrich (France) and used as received.

2.2. Sample preparation

This part will describe the three strategies followed (Lavoine, et al., 2013^{vi}) to prepare the samples studied.

The first strategy consisted in the impregnation of the base paper in the caffeine solution. The second one involved the impregnation of the base paper followed by coating with five MFC layers. The last one involved coating of the paper with the MFC suspension, in which the caffeine was previously introduced.

2.3. Release study

The release protocol of continuous diffusion will be here reminded. It was already presented in detail in Lavoine, et al., 2013.

Each sample was adhered to a light expanded polystyrene support using scotch tape in order to study only the release of caffeine from the sample's surface. These experiments were repeated on three specimens of each sample, at room temperature and under sink conditions.

The continuous diffusion was studied in 500 mL of deionized water that was continuously stirred with a magnetic stirring-bar. The samples were placed at the surface of the aqueous media. At successive intervals, 3 mL of the solution was taken for UV analysis at a wavelength of 272 nm. Using a calibration curve, the concentration of the caffeine released was then determined as a function of time.

2.4. Model development

The first model investigated is based on Fickian diffusion and considers the following hypotheses:

- The volume of the liquid medium is finite
- The diffusion depends only on time
- The total amount of diffusing substance in the sheet and the liquid remains constant when diffusion proceeds
- At $t=0$, the swelling of the paper and the MFC network is achieved
- The external mass transfer coefficient at the solid-liquid interface is negligible.

The boundary condition is:

$$t > 0, \quad x = \pm L, \quad -D \frac{\partial C}{\partial x} = \frac{V^L}{A^P} \frac{\partial C}{\partial t}$$

where A^P is the film area, V^L is the volume of solution

With a constant diffusivity and the condition of the uniform concentration of the diffusing substance in the samples, the kinetics of transport of the substance out of the samples of thickness $2L$ is expressed by the following relationship (Mascheroni, *et al.*, 2010^{vii}):

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1 + \alpha)}{1 + \alpha + \alpha^2 q_n^2} \exp\left(-\frac{q_n^2 D t}{L^2}\right)$$

With $\alpha = \frac{V^L}{kV^p}$ where V^p represents the volume of sample (paper substrate, or paper and the MFC layers) and K the partition coefficient of the migrant in the system between the paper sample and the solution, which can be assumed as constant for low concentrations:

$$K = \frac{C_{sample,\infty}}{C_{solution,\infty}}$$

q_n are the non-zero positive roots of $\tan q_n = -\alpha q_n$ (Crank, 1975^{viii}).

2.5. Calculation procedure

Simulations of caffeine release were performed using the models developed and elaborated with MATLAB® software (The Mathworks Inc., Natick, MA, USA).

Theoretical kinetic release and concentration profiles were calculated with a half thickness L (μm).

The initial amount of caffeine was deduced from the sample preparation procedure and the initial quantity of caffeine in each sample was expressed in g of caffeine/g of samples as reported in *Table 1*. The partition coefficient of caffeine in the system sample/solution corresponded to the ratio of concentrations of caffeine in the sample and in the release medium at equilibrium was obtained at the end of the release study by calculations.

Positive roots q_n and β_n are calculated by using a routine programmed in MATLAB software.

2.6. Statistical analysis

The root mean square error (*RMSE*) was used to estimate the quality of model fitting and was calculated as follows:

$$RMSE = \sqrt{\frac{(\hat{y} - y)^2}{(N - p)}}$$

where y and \hat{y} are respectively the experimental and predicted residual value, N is the number of experimental measurements and p the number of identified parameters. If *RMSE* tends toward 0 or is very close to the experimental error, it means that model is able to represent the experimental data.

3. RESULTS AND DISCUSSION

- 3.1. *Release study: Experimental data (from Lavoine, et al., 2013)*
- 3.2. *Partition coefficient between the samples and water*
- 3.3. *Diffusivity values of caffeine in the three different matrices*
- 3.4. *Application of the model to another antibacterial compound, the chlorhexidine digluconate*

4. CONCLUSIONS AND PERSPECTIVES

Investigation of further mathematical models based on the diffusion through swellable polymeric matrices.

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APPENDIX 2

Posters

Impact of different coating processes of MFC on barrier and mechanical properties

Nathalie Lavoine, Isabelle Desloges and Julien Bras*

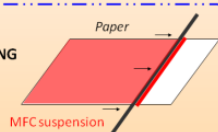
Grenoble INP Pagora - 461, rue de la Papeterie - BP 65 - 38402 Saint Martin d'Hères Cedex - FRANCE

Key words : MFC, paper coating processes, barrier properties, mechanical properties

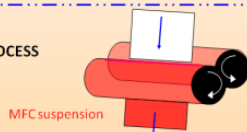
Abstract

The objective of this work is to **compare** and **understand** the impact of **three different coating processes** of MFC suspension on **barrier** and **mechanical properties** of the coated papers. For this, a 2% MFC suspension, obtained by mechanical treatment preceded by an enzymatic processing, was used. An already calendered paper of 41 g/m² was chosen as raw material. Three different coating processes were thus applied to coat MFC suspension on paper. Very promising results have been obtained, e.g. the **air permeability of a wet multilayer coated paper with 10 g/m² of the MFC suspension was reduced up to 5 times** when a **bar coated paper became 18 times less permeable** with only 6 g/m² of MFC suspension.

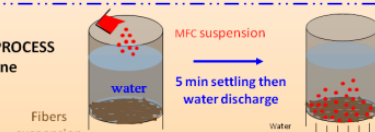
1 - BAR COATING PROCESS



2 - SIZE PRESS PROCESS



3 - WET MULTILAYER PROCESS Handsheet machine



Current work

Introduction

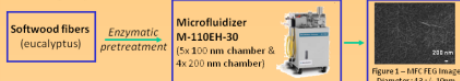
Among the different kinds of nanocelluloses, microfibrillated cellulose (MFC) has particularly unveiled outstanding properties [1]. Many applications are nowadays targeted for MFC like bio-nanocomposites [2-4], dispersion stabilizers [5] or barrier films [6, 7] for food packaging. This last application is more and more studied [8] but the combination of MFC with cellulosic materials remains still rare.



Materials & Methods

Materials

A 2% MFC suspension was furnished by FCBA (FR) and produced as the following way:



A calendered paper (G = 41 g/m², e = 54 μm) was used as base paper. **Fiber suspension.** Softwood pulp was beaten with a Valley refiner to obtain 25°SR and diluted to 20g/L.

Coated papers preparation in labor

Bar coating : 3 mL of the MFC suspension was coated on paper with a 0.6 mm Meyer bar, at 5 cm/s. After each coat, samples were dried at 100°C for 3 min by contact drying system.

Size press : A "Labor Size Presse SP" (Mathis AG) was used and filled with MFC suspension. Papers pass through two cylinders, at 8 bars and 50 m/min. Paper samples were dried at 100°C with a contact drying system.



Wet multilayer : Handsheet machine with a wire of 140 mesh was used. It was filled with about 9L of water. 2g/L of fibers suspension was added to water and allowed to settle on the wire for 5 minutes. 6g/m² to 10g/m² of MFC suspensions were added delicately after that and allowed to settle too. Water was finally drained off. Handsheet was dried at 100°C with a contact drying system.

Characterizations

Scanning Electron Microscopy (SEM) and Field Emission Gun (FEGSEM, Zeiss Ultra-55) were used to observe MFC coated papers. Burst index, Young's modulus and bending stiffness (resonance method) were carried out according standards ISO 2758/2759, ISO 1924-2/3 and ISO 5629. Air permeability tests were carried out with Mariotte vases (ISO 5636). Water absorption was measured according ISO 535 (Cobb method).

Results & Discussions

SEM and FEG images of MFC coated samples with bar coating

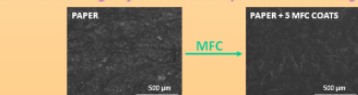


Figure 3 - SEM images of MFC coated samples with bar coating

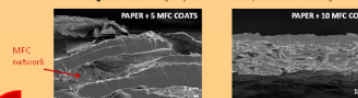


Figure 4 - FEG edge view images of MFC coated samples

After 5 MFC coats, coating is **homogeneous** and the surface is totally recovered. Most of MFC remain at the **surface of paper**.

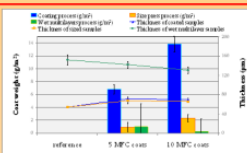


Figure 5 - Coat weights and thicknesses as a function of the number of MFC coats

The **maximum MFC weight coat** is obtained with the coating process : **14 g/m² for 10 MFC coats** whereas only 3 g/m² is coated with size press. No strong variations are noticed for the thickness.

Bulk properties

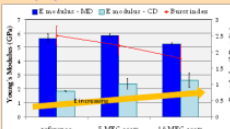


Figure 6 - Young's Modulus & Burst Index of bar coated samples

E modulus increases with the increasing MFC weight coat. Burst index remains stable whatever the samples.

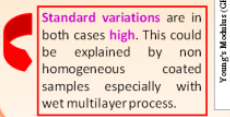


Figure 7 - Young's Modulus & Burst Index of wet multilayer coated samples

Standard variations are in both cases high. This could be explained by non homogeneous coated samples especially with wet multilayer process.

Results & Discussions

Barrier and surface properties

	Cobb Index (g/m ²)		
	COATING PROCESS	SIZE PRESS PROCESS	WET MULTILAYER PROCESS
Reference	60.80 ± 7.0	60.80 ± 7.7	145.86 ± 35.4
5 MFC coats	65.69 ± 4.2	60.49 ± 7.7	160.95 ± 22.7
10 MFC coats	67.36 ± 9.9	62.36 ± 1.8	128.47 ± 21.4

Figure 8 - Water absorption of each samples

Whatever the quantity of MFC, **no impact** is noticed on the **water absorption** for coated and sized press samples. Due to their unpredictable fibers orientation, multilayers present higher water absorption values which decrease with the increasing MFC weight. The standard deviations obtained moderate however the interpretations of the results.

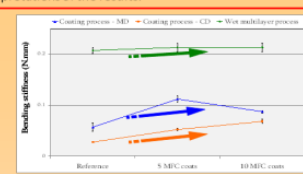


Figure 9 - Bending stiffness of wet multilayer and bar coated samples

For the three processes, bending stiffness increases with the increasing MFC weight. MFC has thus more impact on the paper surface than on its structure.

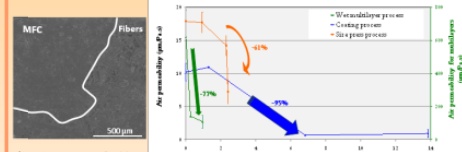


Figure 10.1 - SEM image of multilayer surface with and without MFC

Figure 10.2 - Air permeability as a function of MFC weight coat

Air permeability decreases drastically from 6 g/m² of MFC (5 coats) for the coating process (-95%) and from 5 g/m² (12 coats) for the size press process (-61%). Wet multilayer samples show higher permeability values : the impact of MFC on the air resistance is thus clearly noticed (decrease of 77% with only 1 g/m²).

Conclusions & Perspectives

- > Among the three processes, **bar coating** coats a **bigger MFC weight** per layer (= 1 g/m²).
- > The impact of MFC is clearly noticed on the **air permeability** : 6 g/m² of MFC **reduce the permeability of 95%** (coating process). The transmission rate pass from 3 mL/s to 0,1 mL/s.
- > MFC brings too a slight increase of the **mechanical properties**, especially for **Young's modulus and bending stiffness**.
- > Concerning the perspectives, experiments with the **bar coating process** need to be carried on and improved. Even if this process offers interesting results and few repeatability problems, drying process should be improved.
- > **Other characterization tests** could be carry out to study more precisely the barrier and mechanical properties brought by MFC (oxygen and water vapor permeabilities, tear strength, ...).

Acknowledgements

We would like to thank Bettina Ehrlich (Grenoble INP-Pagora) for her expertise in SEM - FEG imaging and Sandra Tiquin-Languin (FCBA) for her supply of MFC.

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BARRIER PROPERTIES OF SPECIALTY PAPERS COATED WITH MICROFIBRILLATED CELLULOSE:

INFLUENCE OF BASE PAPER AND COAT WEIGHT

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KEY WORDS: *MFC, SPECIALTY PAPERS, BARRIER PROPERTIES*

Introduction & Background

New packaging materials from sustainable resources, biodegradable and designed for food contact are expected. Since the 2000s, one innovative solution has been more and more studied and consists in using **microfibrillated cellulose (MFC)** as **surface layer onto a substrate**.

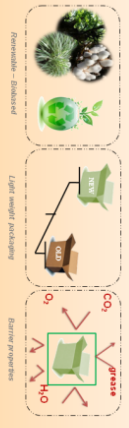


Fig 1. Main objectives for new packaging materials

The MFC presents a high surface area and has the ability to form a **nanoporous network**. It was firstly used with nanocomposites^{1,2} and as dispersion stabilizer³. More recently, MFC has shown its **capacity to improve the barrier properties of composites of fibers**^{4,5}. Its combination with cellulosic materials however remains rare^{6,7}. The present work investigates thus this still unknown domain and proposes a **study on the barrier properties of various MFC coated specialty papers**.

Strategies & Objectives

Strategies

- Impact of MFC surface layer?
- Impact of substrate?

Objectives

- Lightweight packaging with:
- Fit for food contact
- 100% Biobased and renewable

Fig 2. Strategies and objectives of the study

Materials & Methods

1. Lightweight specialty papers
 The substrates were selected among various kinds of **industrial specialty papers** in order to study the influence of the substrate (table 1).

Ref	Application	Basic weight (gsm)	Thickness (mm)	Porosity treatment	Area
A	Specialty	11	15	●●●	50x50 cm
B	Specialty	21	32	●●●	50x50 cm
C	Thinning	28	35	●●●	50x50 cm
D	Thinning	28	28	●●●	50x50 cm
E	Specialty	42	57	●●●	50x50 cm

Table 1. Basic features of selected base substrates

2. Microfibrillated Cellulose (MFC)
 The MFC suspension has been produced following process⁸ detailed in table 2 and characterized in table 3.

MFC production

Parameter	Selected condition (after 100% chemical recovery)
Enzymatic treatment	Celvolux (Novozym 4789), 25 mg, 5 h, 55 °C
Mechanical treatment	20 minutes at 6000 rpm

MFC characterization

Parameter	Value
Concentration	2.1 g/l in water
Viscosity	2480 mPa.s (Brookfield Viscosity 60 rpm, 23°C)
Dry matter	27 %



Fig 3. The 27 % gelatin equivalent of MFC

3. Surface treatment operation & investigated properties

- Before the coating and characterization steps, the paper samples were kept during 24h at 23°C, 50%RH.
- Due to the high water content of the MFC suspension, MFC coated papers were systematically compared to water surface sized papers with a view to taking into account the impact of the homionization (water uptake and drying).
- The MFC suspension is previously homogenized during 2 min, at 10,000 rpm (IKA Ultra-Turrax T18).
- A specific test has been proposed to check the grease barrier. A sample is coated with colored commercial oil during 30 min onto an absorbent measuring (Imapad®) the surface of the grease reported onto the absorbent paper.



Fig 4. MFC range of investigation

Part 1: Structure & Morphology

- FE-SEM, Environmental Scanning Electron Microscopy
- AFM, Atomic Force Microscopy
- FTIR, Fourier Transform Infrared Spectroscopy
- FT-Raman, FT-Raman Spectroscopy
- FTIR-ATR, FTIR-ATR Spectroscopy

Part 2: Barrier properties

- Gas Permeability (cm³/m².Pa.s)
- Water Vapor Transmission Coefficient (WVTR)
- CO₂ Diffusivity (cm²/s)
- Water absorption gain (%)
- Grease Resistance

Fig 5. Investigation

Results & Discussions

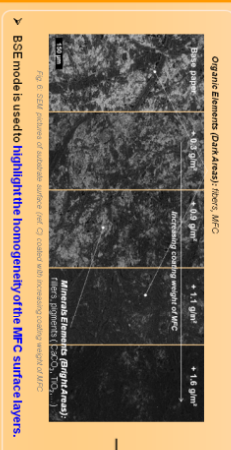


Fig 6. SEM images of MFC-coated surface (ref. C) at various magnification (ref. C) (base paper is 11 gsm (ref. A)).

Part 1: Structure & Morphology of MFC layers

> **BSE mode is used to highlight the homogeneity of the MFC surface layers.**

> FE-SEM & AFM are used to highlight the surface nanoporous network.

Part 2: Barrier properties

Three observation techniques ranging from **micrometric to nanometric** scale were used to characterize the MFC coated layer. It confirms that a MFC suspension deposited onto a porous substrate leads to the **formation of homogeneous layer for a coat weight higher than 1 g/m²** (Fig 6)

> a nanoporous network of entangled MFCs with an average pores dimension at the surface of 50 nm 4-14 nm (Fig 7).

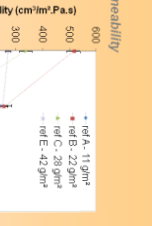
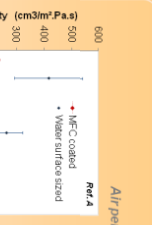


Fig 7. Air permeability as a function of the number of passes for MFC-coated and water surface sized papers (ref. B).

Fig 8. Air permeability (ref. A) as a function of the MFC layer weight (ref. B).

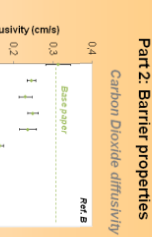


Fig 9. CO₂ Diffusivity as a function of the MFC coat weight (ref. B).

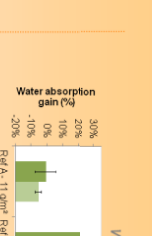


Fig 10. Water absorption gain of MFC-coated samples compared to water surface sized papers (ref. A).

Fig 11. Relative water absorption gain of MFC-coated samples compared to water surface sized papers (ref. A).

Ref	Base paper	Water surface sized	MFC coat weight	Water absorption gain (%)
A11 g/m ²	28 ± 2	24 ± 3	7.4	4.1 ± 1
C-28 g/m ²	69 ± 2	66 ± 1	60.1	41 ± 3
E-28 g/m ²	43 ± 2	25 ± 1	22 ± 4	10 ± 2
E-42 g/m ²	69 ± 2	71 ± 1	53 ± 1	16 ± 2

- > Compared to the base paper, the air permeability is about **3 times lower with only 1.2 g/m² of MFC** (Fig 8).
- > An improvement of the standard variations is also revealed (Fig 8).
- > Starting from a MFC ratio of 6-8%, the air permeability is reduced to 6-12 times depending on the base weight (Fig 9).

- > The MFC layers have a **strong impact** on CO₂ diffusivity with a drop of **300** compared to the base and water surface sized papers.

> Although neat MFC has no chemical gasproof property, nanoporous network of the MFC surface layer led to a **substantial reduction of oil migration** (Fig 11).

> Whatever the base paper, this represents a significant improvement of grease resistance ranging from **54 to 80%** (table 4).

Conclusions & Perspectives

- > **Athin MFC layers has an impact on the gas and liquid barrier properties.**
- > In order to have a homogeneous surface layer, a **minimal ratio of 6-8% of MFC is required whatever the basis weight.**
- > Barrier properties are certainly **due to the homogeneous layer and the nanoporous network.**
- > Some limits are however **highlighted: final barrier properties are closely linked to the interactions between water and the base paper.**
- > **Additional barriers & mechanical tests** have to be carried out to emphasize further the influence of a thin MFC layer.
- > This new **kind of lightweight coated papers** may represent an interest for:
 - > **Filtration application markets**

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Caffeine release study in a microfibrillated cellulose nanoporous structure

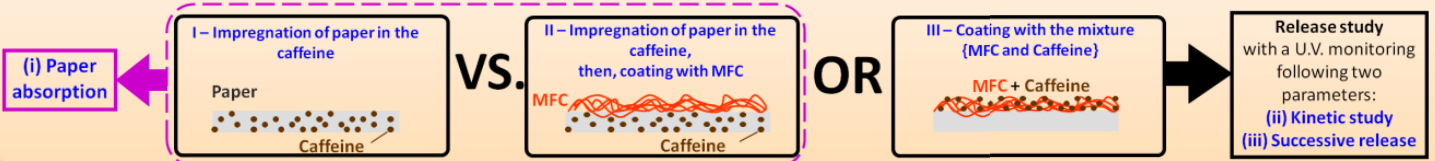
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Key words: Microfibrillated cellulose (MFC), Nanoporous network, Molecule release

Objectives

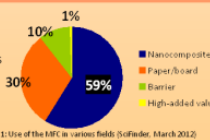
This study aims to take benefit of the nanoporous network formed by the microfibrillated cellulose (MFC) for obtaining a controlled release of a model molecule, the caffeine.



Current work

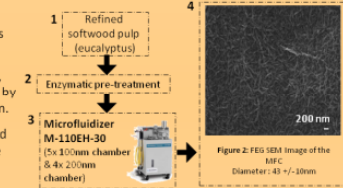
Introduction

The microfibrillated cellulose (MFC) was essentially used in nanocomposites for its reinforcement property¹. Thanks to its nano-scale dimensions and its ability to form a strong entangled nanoporous network, new high-value applications are emerging^{2,3}. Its application with paper remains, however, rare, especially its use as a matrix of controlled release⁴.



Materials

- o A **calendered paper** (basic weight = 41 g/m², thickness = 54 μm) is used as base paper.
- o The model molecule is the **caffeine**, C₈H₁₀N₄O₂, CAS n°58-08-2, purchased by Sigma-Aldrich. It is visible at λ=272 nm.
- o A **2% MFC suspension** was furnished by the FCBA (FR) and produced as the following way:



Samples preparation

I- Impregnation of paper in the caffeine solution
Paper samples of 10x10 cm² are dried 1h at 105°C. They are immersed in a caffeine solution of 21,4 g/L, during 10 minutes (according to the study of the paper absorption) and dried again 1h at 105°C. For each sample, the quantity of caffeine absorbed is weighted.

II- Impregnation of paper in the caffeine solution, then coating with MFC
The samples are prepared as I-. They are then coated with 5 layers of MFC suspension according to the previous study⁵ with a bar coating process.

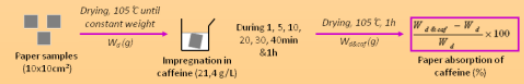
III- Coating with the mixture (MFC + caffeine)
The samples are coated with the MFC suspension mixed previously with the caffeine (21,4g/L of suspension).

Methods

Release study

(i) Paper absorption

Between each step, the weight of the samples is determined.

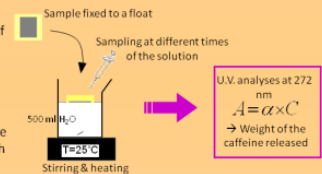


(ii) Kinetic study

This study was applied for each kind of samples (I, II and III). The release is only studied on surface as each sample is one-face coated.

(iii) Successive release

The principle is exactly the same as the protocol (ii), except that between each sampling, the water is renewed.



Results & Discussion

(i) Paper absorption

The samples absorb the maximum weight of caffeine in the space of 10 minutes as shown with the figure 3. The absorption obtained for 5 minutes present a too high standard variation, that is why this result was not considered.

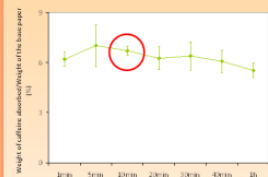


Figure 3: Weight of the caffeine absorbed by the base paper/Weight of the base paper (%) as a function of the time of impregnation.

This time will be used as reference for each impregnation of the samples in the caffeine solution.

(ii) Kinetic study

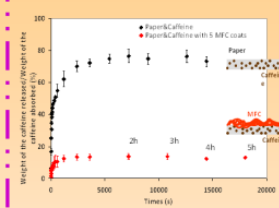


Figure 4: Weight of the caffeine released/Weight of the caffeine absorbed (%) as a function of the time of sampling.

The MFC layers slow down the release of the caffeine (Figure 4). With MFC, the release lasts more than 5 hours and there is still caffeine in the samples (not shown). The release in presence of MFC is much more progressive: only 15% of the caffeine is first released compared to 80%, without MFC.

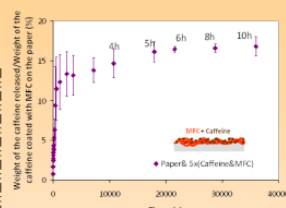


Figure 5: Weight of the caffeine released/Weight of the caffeine coated with MFC on the paper (%) as a function of the time of sampling.

The whole caffeine is not released after 10 hours: only about 16% is released (Figure 5). Two hypothesis: (i) the MFC prevents the caffeine from releasing or, (ii) the MFC slows down the release of the caffeine and the experiment has to be performed longer.

To confirm this point, the quantity of caffeine which remains in the paper, has to be measured and quantified.

(iii) Successive release

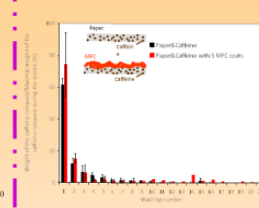


Figure 6: Weight of the caffeine released/Maximal weight of the caffeine released during the kinetic (%) as a function of the number of washing.

After 16 washings, no caffeine remains in the samples anymore compared to the samples coated with MFC: after 21 washings, the samples still contain the molecule (Figure 6).

This confirms the slower release obtained when the MFC is used.

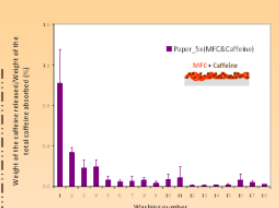


Figure 7: Weight of the caffeine released/Weight of the caffeine coated with MFC on the paper (%) as a function of the number of washing.

The caffeine remains in the samples after 18 washings. This experiment could last longer until no more caffeine remains. However, due to the hydrophilic and fragile nature of the paper, the samples begin to disintegrate after a long time in water.

Conclusions & Perspectives

- o To the best our knowledge, it is the first time that MFC is used as a **nanoporous network for a controlled release**.
 - o The first results are **very promising**: the MFC seems indeed to **strongly slow down the release** of the caffeine and to release it more progressively: in the space of **two hours**, a paper without MFC releases about **80%** of the caffeine previously absorbed whereas a paper with MFC only releases about **15%**.
 - o Whatever the strategy: (ii) the impregnation of the paper then coating with MFC or (iii) coating of the paper with the mixture MFC and caffeine, the release studies seem similar. For the third strategy, however, it might be useful to **study the release of the sample immersed** in water. Moreover, the method applied for the analyse of the surface of the sample should be renewed: the tape may also prevent the caffeine from releasing.
 - o One question remains: **Does the MFC slow down the release of the caffeine ? Or, does the MFC prevent the 80% left from releasing ?**
- The next step of this study will be to answer to this question and thus to **measure and quantify the caffeine remaining** in the paper sample. Eventually, this study will be done with an active molecule in order to check if the quantity is sufficient to achieve an antimicrobial activity.

Acknowledgements

We would like to thank Sandra Tapin-Lingua (FCBA) for her supply of MFC as well as the CTP and the CERBAIV for the loan of their U.V. spectrophotometer.

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USE OF DIFFERENT QUALITY OF MFC FOR PRODUCING CONTROLLED RELEASE FILMS

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ABSTRACT

OBJECTIVES: DEVELOPMENT OF NEW DELIVERY SYSTEMS OF ANTIBACTERIAL MOLECULE FOR FOOD-PACKAGING APPLICATIONS

Microfibrillated cellulose (MFC) is here used as films and produced using a handsheet machine. Thanks to its **nanoporous network**, MFC films are perfect carrier of antibacterial molecule, especially of **chlorhexidine digluconate (CHX)**. Well-known as strong oral hygienic agent, CHX is efficient against a wide range of bacteria and represents here an **antibacterial model** for future food-packaging applications.

Production of MFC films including active molecule CHX → **Release study into model food** → **Antibacterial tests** → **Food-packaging application**

INTRODUCTION

The numerous assets of MFC^{1,2} make it an ideal material demanded by various end user markets³ such as composites, pulp&paper, aerogel, films&coatings, rheological modifiers etc. Its use as drug delivery system is an emerging area. Indeed, few papers are nowadays focused on it^{4,5}: the current number of journal/review represent less than 1% of the about 4000 scientific studies about MFC (Fig.1).

Key words selected:

- MFC/Microfibrillated cellulose/Celulose microfibrils
- NFC/Nanofibrillated cellulose
- Celulose nanofibrils/CNF/Celulose nanofibers

Fig. 1: Number of journal/review published about MFC/NFC and % of these article works dealing with drug release/delivery systems. Statistics obtained with SciFinder (June 2013)

MATERIALS

• **MFC suspensions**
Two different kinds of MFC suspensions have been furnished and produced by FCBA (France). Their manufacturing process described Fig. 1.

Antibacterial agent
Chlorhexidine digluconate (CHX) aqueous solution (@20%wt) was purchased from Sigma Aldrich (Fig. 3)

Fig. 3: Molecule of CHX aqueous solution

METHODS

MFC films manufacturing process
The protocol has been adapted from Sehaqui et al.⁶(modifications in blue writing).

Fig. 4: Manufacturing process of MFC films

CHX solution was added in different proportions (0.1, 0.5 and 1%) in MFC suspension before films processing. Final suspension was stirred with an Ultra-Turax for 10 min, and then, mechanically for 30 min.

MFC films properties

MFC films were kept at 23°C, 50% of relative humidity.

- **Basis weight** – Balance (±0.001 g)
- **Thickness** – Micrometer (±0.001 mm)
- **Scanning Electron Microscopy (SEM)**
- **Atomic Force Microscopy (AFM)**
- **Air permeability** Mariotte Vase (ISO 5636)
- **Water absorption** Cobb (ISO 535)
- **E-Modulus, Tensile strength, Length at break** L&W Tensile tester (ISO 1924-2/3)

Structure properties
Barrier properties
Mechanical properties

Release study

Release study of MFC films + CHX was carried out into agar. Agar is considered as solid model food.

Fig. 5: Release study protocol

Antibacterial activity and food tests

Antibacterial activity was carried out against *B. Subtilis* (EN 1104).

Antibacterial activity?
Long-term antibacterial activity?

Activity of MFC films was also tested onto bread inoculated with *B. Subtilis*. Films were put onto bread keeping humid by contact with pure agar.

RESULTS AND DISCUSSION

MFC films - Structure properties

- **Nanoporous networks and high density**

Fig. 5: AFM images of each MFC films (1x1 µm)
Fig. 6: SEM images of MFC films slices (x1200)

- Different structural organisation for the two MFC types in films
- Layered structure in the case of Tempo-MFC

Characteristics of MFC films

	Basis weight (g/m ²)	Thickness (µm)	Density (g/cm ³)
E-MFC-GR	63 ± 1	42 ± 1	1.5
E-MFC-GR + CHX	80 ± 1	67 ± 1	1.2
T-MFC-H	70 ± 2	59 ± 1	1.3
T-MFC-H + CHX	76 ± 2	61 ± 1	1.2

- Increase of films thickness by addition of CHX
- Slight decrease of density

Retained amount of CHX in MFC films

	CHX mass introduced per film* (µg)	CHX mass retained in film	% CHX in film
E-MFC-GR + CHX	0.4	0.021	0.8%
	0.8	0.070	2.8%
	4	0.095	3.3%
T-MFC-H + CHX	0.8	0.8	42%

*diameter of MFC = 2g

- **Very high retention capacity for TEMPO-MFC film** due to the presence of carboxylate groups (100%)
- Strong ionic interactions with CHX molecule and TEMPO-MFC

MFC films - Barrier properties

Fig. 7: Water absorption and air permeability

- **Variation of Water absorption linked to chemical structure of the network** (High Cobb index for TEMPO-MFC due to the hydrophilic carboxylate groups, high decrease by introduction of CHX molecule)
- **Variations of Air permeability linked to the physical structure of the network** (more opened for the E-MFC film)

MFC films - Mechanical properties

Fig. 8: Mechanical properties

- Comparable mechanical properties for all films
- Low impact of CHX introduction on film properties

Release study: Kinetic

Released amount of CHX (%) from both MFC type films

Fig. 9: Released amount of CHX (%) as a function of time

- Different release profile for both MFC types
- **Very slow release of the active molecule for the T-MFC (13% in 7 days)** due to the tight network and strong interactions with CHX molecule
- **Faster release and plateau value reached for E-MFC-GR**

Food tests

Fig. 10: Food tests

- Antibacterial effect on food clearly showed
- **Antibacterial action effective by contact and diffusion in solid medium**

Antibacterial tests

Successive 3-days periods - 30°C

Fig. 11: Antibacterial tests

- Similar activities for both MFC types despite different CHX contents
- **Good antibacterial activity for T-MFC film despite the very low amounts of CHX released in time**

3-days periods

- Antibacterial activity of E-MFC films for 4 to 5 consecutive 3-days periods (= 15 days with medium renewal every 3 days)

Model conditions for food storage (4°C, 2h-periods)

Fig. 12: Model conditions for food storage

- Higher antibacterial activity for T-MFC-H from the 4th period
- Antibacterial activity tends to stop for E-MFC films
- **Longer activity for T-MFC films due to slower release**

CONCLUSIONS & PERSPECTIVES

- **Very high retention of the active molecule for the TEMPO-MFC film**
- **Modification of water absorption due to strong interactions with CHX molecule**
- **Very low and controlled release kinetic**
- **Tight network & covalent bonds between COO⁻ and CHX molecule**
- **Significant antibacterial activity despite the low released amount of CHX in time for TEMPO-MFC films**
- **TEMPO-MFC films seems to have a longer antibacterial activity**
- **Perspectives: Define the antibacterial activity duration, amount of CHX released in food**
- **Promising results for food packaging application**

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ABSTRACT/RESUME

The present work investigates the potential of microfibrillated cellulose (MFC) coated onto cellulosic substrates as controlled delivery system (CDS) of antibacterial molecules for food-packaging.

Two coating processes and three substrates were compared. Using a bar coating process, MFC was coated onto paper and cardboard substrates, enhancing their air resistance and bending stiffness with a minimum coat weight of 8 g/m². Microscopic analyses at nanoscale underlined the nanoporous MFC network preserved onto the substrate surface after coating. For the first time, this network was used as CDS of various molecules and proved its efficiency by releasing molecules more progressively and over a longer period. The antibacterial activity was effective against non-pathogenic bacteria, leading to the improvement of the food shelf-life. The application of this new material was broadened up by using simultaneously cyclodextrins and MFC, which also led to very promising results.

This PhD paves the way for new high-added value applications in the field of controlled delivery systems by using MFC-based materials.

Key words: *Microfibrillated cellulose, Cellulosic substrates, Barrier, Coating, Antibacterial property, Release study, Controlled delivery system, Cyclodextrins, Food-packaging*

Ce travail propose un nouveau type de matériau papier/carton pour l'emballage alimentaire. Ces substrats, enduits de microfibrilles de cellulose (MFC), ont montré un réel potentiel en tant que systèmes de libération contrôlée de molécules antibactériennes.

Deux procédés de couchage et trois substrats cellulosiques ont été comparés. Un poids de couche de 8 g/m² de MFC a été atteint avec le procédé de couchage à barre, améliorant la rigidité à la flexion et la résistance à l'air des matériaux.

Des analyses microscopiques ont mis en évidence la présence, après couchage, du réseau nano-poreux des MFC en surface des matériaux enduits. Pour la première fois, ce réseau a été utilisé afin de libérer plus progressivement et sur une période prolongée des molécules actives. Leur action antibactérienne a inhibé sur le long terme la croissance de bactéries non-pathogènes et a prolongé la durée de conservation d'aliment. L'utilisation simultanée de cyclodextrines et de MFC a élargi le domaine d'application de ce nouveau matériau, donnant lieu à des résultats très prometteurs.

Ce projet de thèse a ainsi favorisé le développement de nouvelles applications à haute valeur ajoutée à base de MFC, pour des systèmes de libération contrôlée de molécules actives.

Mots clés : *microfibrilles de cellulose (MFC), papiers/cartons, propriétés barrières, couchage, action antibactérienne, étude de libération de molécules actives, système de libération contrôlée, cyclodextrines, emballages alimentaires*