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Pierrick Paillot

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Pierrick Paillot. Modification chimique, greffage et dispersion d'agents fonctionnels pour des applications antimicrobiennes. Polymères. Université de Lyon, 2016. Français. NNT : 2016LYSES006 . tel-02020143v1

HAL Id: tel-02020143

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Submitted on 15 Feb 2019 (v1), last revised 18 Feb 2019 (v2)

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THESE

Présentée à

L'Université Jean Monnet, Saint-Etienne

Ecole doctorale – Science Ingénierie Santé (ED SIS 488)

Pour obtenir le diplôme de

DOCTEUR de l'université Jean Monnet

Spécialité « Chimie et Sciences des Matériaux Polymères »

Par

Pierrick PAILLOT

MODIFICATION CHIMIQUE, GREFFAGE ET DISPERSION D'AGENTS FONCTIONNELS POUR DES APPLICATIONS ANTIMICROBIENNES

Présentée et soutenue le 02/02/2016 Devant un jury composé de :

Rapporteurs	Madame Nadia Oulahal
Rapporteurs	Monsieur David Ruch
Examinatrice	Madame Françoise Fenouillot
Examinateur	Monsieur Christian Carrot
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Directeurs de thèse	Monsieur Mohamed Taha
Co-Directeurs de thèse	Monsieur Frédéric Becquart
Co-Directeurs de thèse	Madame Corinne Jegat

« L'envie de savoir est le plus puissant moteur humain »

« L'important n'est pas de convaincre, mais de donner à réfléchir »

« Dans la vie, il y a trois facteurs : le talent, la chance, le travail. Avec deux de ces facteurs, on peut réussir. Mais l'idéal est de disposer des trois. »

Bernard Werber (1961-…) Artiste, Ecrivain

Remerciements

Ces travaux de thèses ont été réalisés au laboratoire d'ingénierie des Matériaux polymères à l'université Jean Monnet de Saint-Etienne (IMP@UJM).
Je souhaiterais d'abord remercier Christian Carrot, directeur du laboratoire IMP pour l'accueil réservé au sein de son effectif. Je remercie mon directeur de thèse, Monsieur Mohamed Taha pour l'encadrement ainsi que le suivi qu'il a réalisé tout au long de ces trois années. Je remercie également Corinne Jegat, co-encadrante de ma thèse pour sa grande disponibilité, ses conseils et son grand soutien apporté. Je remercie Monsieur Frédéric Becquart, également co-encadrant de ces travaux pour les conseils qu'il a pu m'apporter.

Je remercie également les différents partenaires industriels du projet STABIPACK pour les accueils réservés dans leurs locaux ainsi que les nombreuses expertises apportées sur des domaines très variés.
Je tiens à remercier Mme Nadia Oulahal et M. David Ruch d'avoir accepté d'être les rapporteurs de cette thèse ainsi que Mme Françoise Fenouillot et M. Jean-Claude Jammet pour leur participation à ce jury et ainsi que du temps consacré pour la lecture et le jugement de ce travail.
Je remercie chacun des membres du laboratoire IMP de Saint-Etienne, les permanents et non permanents. Je souhaite remercier Caroline Pillon, Claude Raveyre, Jean-Charles Majesté, Frédéric Prochazka, Corinne Jegat, Frédéric Becquart pour les formations données ainsi que les nombreuses informations apportées dans les domaines de la conception et la caractérisation de polymères.
Je remercie finalement tous les doctorants, d'abord les filles, Alix, Fanny, Elodie, Marie, Sirine, puis les garçons, Olivier, Benjamin n°1 et n°2, Ked, Hang, Anouar.
Plus particulièrement, je remercie mon petit shab Anouar, pour m'avoir supporté dans « the office of love » et je remercie également Monsieur Benjamin CONVERS, avec qui j'ai eu de nombreuses discussions sportives et autres.

Je garde les meilleurs remerciements pour la fin. Je souhaiterais particulièrement dire un très GRAND MERCI à ma maman, Anne PAILLOT et mon papa, Gilbert PAILLOT qui m'ont toujours soutenu et cru en moi. C'est en grande partie grâce à eux si j'ai réussi à m'accrocher dans les moments difficiles et si j'ai pu réaliser tout ce travail.
Je remercie également tous les autres membres de ma famille qui ont également cru en moi, mon frangin Maxime, mes grands parents, tantes, oncles, cousins…

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INTRODUCTION GENERALE

Introduction Générale

L'être humain a depuis toujours eu le besoin de protéger ses proches ou ses biens. Pour ce qui est de ses objets, l'idée première est bien entendu la protection alimentaire et pour se faire l'utilisation d'emballages. Dans ce contexte, l'homme a commencé à développer ce type d'innovations pour permettre la conservation des produits et éviter ainsi les gaspillages. L'homme s'est servi de matériaux naturels pour la fabrication des premiers emballages. C'est par la suite qu'est venue la fabrication de matériaux synthétiques, comme les polymères afin d'optimiser au maximum la protection.

Les emballages concernent aujourd'hui divers secteurs, et entre autres, celui des cosmétiques, avec les shampoings ou autres crèmes de beauté. Ce secteur fait partie des marchés en plein essor et en constante progression. Ceci est lié à une demande accrue des consommateurs, de plus en plus soucieux de leur image. Les français sont de grands consommateurs de produits cosmétiques. En France, ce marché est aujourd'hui l'un des plus prolifiques avec un chiffre d'affaire de plus de 16 milliards d'euros en 2008 et en constante croissance. Ce dernier représente le quatrième secteur exportateur juste derrière l'aéronautique, l'automobile et les boissons.

Les produits cosmétiques sont des substances qui sont destinées à venir en contact direct avec des parties du corps humain. Ces produits nécessitent une très grande sureté sanitaire vis-à-vis des clients et c'est pour cela que depuis de nombreuses années, l'introduction d'agents dits « conservateurs » est fortement utilisée.
Cependant, depuis toujours, les mentalités évoluent, et l'homme cherche par tous les moyens d'améliorer leurs conditions de vie. Aujourd'hui, le consommateur souhaiterait un produit plus respectueux de son intégrité. Dans ce contexte, que l'idée serait de retirer les conservateurs pour obtenir un produit dit bio-naturel, sans conservateur ou contenant uniquement des conservateurs naturels.

Depuis peu de temps, des collaborations naissent entre les industriels de la formulation de produits cosmétiques et les industriels de packagings. Ils travaillent ensemble pour tenter d'atteindre le O% de conservateurs dans le produit cosmétique. Malgré l'existence de quelques travaux visant à développer des polymères antimicrobiens pour les packagings cosmétiques, ce secteur reste très ouvert aux nouveautés. De plus, l'innovation est aujourd'hui plus axée sur d'autres domaines, comme le médical ou le biomédical.
Le secteur le plus fortement développé en matière de recherche est celui des emballages alimentaires. Ces films ou matrices, généralement polymères ont des fonctionnalités antimicrobiennes par un ajout

d'actifs. L'utilisation de ces agents apporte plusieurs avantages, comme l'amélioration de la qualité ou du temps de conservation de l'aliment.

Les recherches menées sur les emballages cosmétiques sont peu avancées, peu d'études ont été publiées car la protection est centrée sur l'amélioration des formulations et non sur le packaging. [1-5] Une autre contrainte est que contrairement aux emballages alimentaires où la recherche est très avancée et où un grand nombre de travaux le démontre, le secteur du packaging cosmétique reste peu étudié. [6-12] Il est donc difficile actuellement de trouver des informations.

Le domaine des emballages cosmétiques pourrait ainsi aboutir à de nombreuses découvertes ou innovations.

Néanmoins, certains chercheurs tentent quand même de développer des emballages cosmétiques dans lesquels seront dispersés des agents et pourront agir sur la croissance de microorganismes. Ces produits antimicrobiens commencent à arriver sur le marché, alors qu'ils étaient plutôt réservés au secteur alimentaire. [13-14]

Un autre frein important à la création des emballages actifs concerne plutôt la partie fabrication. Actuellement, plusieurs voies de création de matériaux polymères existent. Ainsi, les conditions de mise en œuvre vont être variables. Certaines à haute température qui peut dégrader les agents antimicrobiens. D'autres technologies sont effectuées dans des conditions plus environnementales. Ces incorporations d'agents associées aux procédés de fabrication entrainent généralement des modifications de propriétés des emballages, comme les caractéristiques thermiques, mécaniques, ou encore physico-chimiques. Il est important dans le cadre d'un tel projet d'évaluer les modifications qui pourraient potentiellement empêcher toute utilisation des polymères modifiés dans la conception finale des emballages.

Dans le cadre du travail de thèse, les objectifs principaux consistent à développer des matériaux antimicrobiens destinés à protéger des zones spécifiques d'emballages cosmétiques.
Pour ce faire, différentes technologies et agents vont être utilisés pour la partie conception. Dans un premier temps, l'utilisation d'une technique à température ambiante devra permettre de s'affranchir des risques de dégradation des actifs par de trop fortes sollicitations thermiques et/ou mécaniques. Cette partie devra permettre de trouver les meilleurs antimicrobiens pour l'application industrielle finale, et ceci grâce à des tests bactériologiques.

Après sélection des actifs à partir du procédé à froid, la suite devra permettre de comparer les propriétés physiques, chimiques, thermiques… des matériaux préparés à température ambiante en fonction des concentrations et de la nature des actifs incorporés.

Finalement, un procédé de fabrication à haute température avec les agents sélectionnés sera mis en œuvre. Ces types de procédé sont généralement utilisés industriellement dans le secteur de l'emballage, comme l'extrusion ou l'injection. A haute température, la première étape de caractérisation devra apporter une vérification de l'activité antimicrobienne, c'est-à-dire constater si les agents actifs peuvent résister à de forte contraintes thermiques et de cisaillement. Une autre étude sera aussi menée sur les impacts de ces antimicrobiens sur les propriétés thermiques et mécaniques du matériau polymère.

Trois équipes de recherche transdisciplinaires se sont associées pour la réalisation de ce travail. Cette thèse rentre dans le cadre d'un projet collaboratif FUI (Fonds uniques interministériels) comprenant des partenaires industriels spécialisés dans la conception de packagings (ALBEA, PUMPART, PRP Création, Plastipolis), la formulation de crèmes (STRAND Cosmetic, Cosmetic Valley), et en bactériologie (ACM Pharma). Le travail présenté ici a été réalisé sur 3 sites différents :

- Laboratoire Ingénierie des Matériaux Polymères (UMR CNRS 5223) de l'Université Jean Monnet de Saint-Etienne (IMP@UJM) où j'ai réalisé une grande partie des travaux de fabrication de matériaux et la caractérisation de différentes propriétés thermiques, viscoélastiques, relargages…

- Le site INSA de Lyon du laboratoire Ingénierie des matériaux polymères (IMP@INSA) où j'ai réalisé les matériaux antimicrobiens par voie dite « froide » utilisant la polymérisation radicalaire sous UV.

- Le laboratoire ACM PHARMA situé à Bellegarde (LOIRET) a apporté son expertise sur l'aspect bactériologique. Ce laboratoire a aidé à concevoir des essais de caractérisation de l'activité antimicrobienne de matériaux polymères par deux méthodes différentes, soit en contact direct en milieu gélosé de culture, soit par immersion au sein de suspensions microbiennes.

Ce manuscrit est structuré en 6 chapitres.

Le premier chapitre sera consacré à une synthèse bibliographique visant à découvrir le secteur des polymères antimicrobiens. L'objectif sera de voir quels sont les secteurs industriels les plus représentés, évaluer quelles natures de matériaux et d'actifs ont pu être employés, et également quelles technologies de fabrication existent actuellement. Cela permettra d'apporter un éclaircissement sur ce qui pourrait être envisagé pour la suite des travaux expérimentaux. Dans ce chapitre, il sera possible d'effectuer une sélection de matériaux et d'agents antimicrobiens à tester pour tenter d'apporter des solutions de protection des emballages cosmétiques.

Le deuxième chapitre portera sur une étude comparative de l'action antimicrobienne de différents actifs. La première étape s'intéressera principalement à la mise en œuvre d'une technologie adaptée de polymérisation basse température. Nous ferons le choix d'une référence matricielle pour protéger les zones d'ouverture de l'emballage. Une fois cette référence sélectionnée, diverses familles d'antimicrobiens (choisis par l'étude bibliographique) seront incorporées au polymère dans des mêmes conditions de fabrication. Une étude de caractérisation thermique et d'activité antimicrobienne devra permettre d'établir un classement des actifs les plus efficaces pour les applications industrielles.

Le troisième chapitre présentera l'impact de la concentration des agents choisis sur les propriétés thermiques, thermomécaniques, viscoélastiques, morphologiques, ou encore sur la libération de la matrice de référence. Ceci toujours par une voie de fabrication à basse température.

Les quatrième et cinquième chapitres porteront sur le transfert de mise en œuvre à basse température vers la haute température. Pour cette partie, nous utiliserons une technologie d'extrusion de laboratoire sera effectuée. Le matériau employé sera un copolymère EVOH dans lequel seront introduits des agents antimicrobiens précédemment sélectionnés. Plus spécifiquement, le chapitre 4 va s'intéresser à l'incorporation d'acétate d'argent tandis que le chapitre 5 sera consacré à l étude d'un autre antimicrobien, de nature équivalente, l'acétate de cuivre (II).
La première étape devra permettre de vérifier la possibilité de mise en œuvre. La suite sera consacrée aux impacts des actifs sur les propriétés thermiques, viscoélastiques, de libération, ou encore sur la morphologie. Lors de ces caractérisations, un intérêt sera également porté sur la capacité de réaction des antimicrobiens sous de fortes sollicitations thermiques et/ou mécaniques par extrusion réactive et ceci au contact direct des EVOH.
Ces derniers chapitres présenteront précisément l'activité antimicrobienne des matrices fabriquées. Il devra permettre de vérifier si les antimicrobiens sélectionnés peuvent conserver une action de protection, malgré les fortes sollicitations imposées lors de leur extrusion.

Enfin, un chapitre annexe sera consacré à la dispersion dans le matériau via les deux procédés à chaud et à froid d'amines quaternaires commerciales ou synthétisées par nos soins. Pour ceci, des amines primaire, tertiaire et quaternaire seront introduites à l'état liquide ou solide dans le matériau méthacrylate fabriqué par polymérisation à basse température, mais aussi par extrusion dans les EVOH. Ce chapitre présentera une comparaison entre l'impact des différentes amines sur un même matériau au niveau des propriétés thermiques, thermomécaniques, et leur activité antimicrobienne. Dans cette annexe, un comparatif sera fait entre les deux voies de fabrication basse et haute température.

Cela afin de vérifier la thermo stabilité de ces amines et voir si celles-ci peuvent représenter une perspective d'obtention de matériaux antimicrobiens à haute température.

CHAPITRE 1 :

ETAT DE L’ART

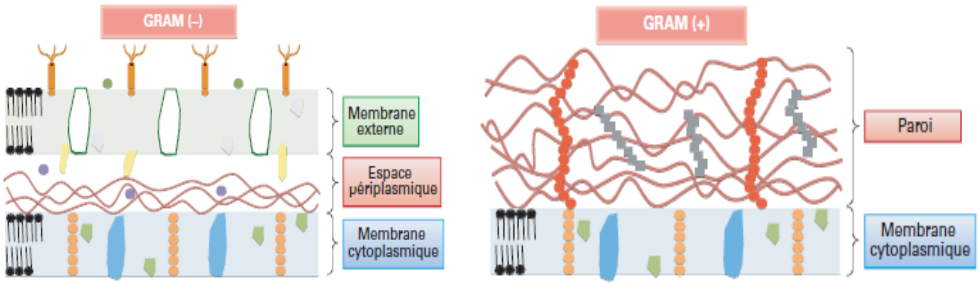
A- Généralités sur la microbiologie

Cette première étape de recherche a consisté à découvrir le monde antimicrobien. En effet, pour lutter contre une prolifération bactérienne, il est essentiel de connaitre d'une part, les différents agents antimicrobiens, mais également les cibles visées que sont les microorganismes. Cela nous permettra d'appréhender quelques généralités sur la vie de ces agents pathogènes.

A-1- La microbiologie

Dans le secteur de la cosmétique, il existe trois grands types de microorganismes contre lesquels les agents actifs présents dans les formulations doivent lutter. Ce sont les bactéries, les levures et les moisissures ou champignons. Il est important de faire une distinction entre les bactéries et les levures. Une bactérie est un organisme unicellulaire qui va entraîner l'apparition d'une maladie. En réalité, le terme bactérie est employé pour définir un microorganisme bien précis. On emploiera le terme de microbes pour définir un ensemble d'espèces pathogènes pouvant provoquer des infections. Le terme « microbe » désigne l'ensemble des agents pathogènes : bactéries, levures mais aussi les virus.

Ces microorganismes présentent comme chaque cellule vivante une membrane cytoplasmique constituée de deux couches lipidiques à base de protéines. La composition de cette membrane va varier selon le microorganisme. Chez les levures, ou champignons, celle-ci est recouverte d'une paroi externe à base de chitine et de glucane. [15-17] Pour les bactéries, il s'agit d'organismes unicellulaires sans noyau (procaryote). Elles sont de petites tailles pouvant aller jusqu'à des valeurs de quelques dizaines de micromètres. Elles présentent la particularité de posséder une membrane plasmique. Elles sont capables via l'utilisation de certaines enzymes de fabriquer une paroi autour de cette membrane. C'est cette paroi qui permet de faire la distinction entre deux grandes familles, les bactéries à Gram-positif et Gram-négatif. La figure 1 montre un schéma des deux types de membranes cellulaires que l'on retrouve sur les bactéries.



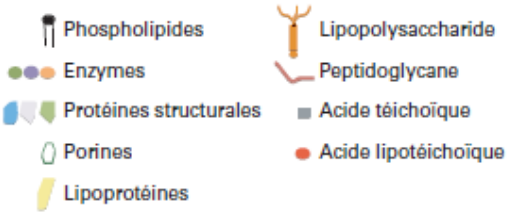


Figure 1 : Représentation schématique de membranes cellulaires de deux types de bactéries : bactérie Gram(+) (à droite) ; bactérie Gram (-) (à gauche) [16]

La paroi des bactéries à Gram-négatif comme le Pseudomonas Aeruginosa possède plusieurs couches dont l'une est constituée de peptidoglycane généralement plus petite que pour les bactéries à Gram-positif. Cette paroi est composée d'une couche de lipoprotéines et d'une couche superficielle de lipopolysaccharides. On remarquera deux choses concernant cette famille de bactéries. La première est que la membrane extérieure présente un nombre important de pores. La seconde est que l'ensemble des couches qui composent la membrane externe de la bactérie est séparée de la membrane cytoplasmique par un espace appelé « périplasme ». [18] Ce périplasme désigne un espace qui contient une fine paroi de peptidoglycane. C'est là que se produisent de nombreux phénomènes biochimiques comme la respiration bactérienne. De plus, le périplasme peut jouer un rôle dans la résistance des bactéries aux antimicrobiens. Dans le cas des bactéries Gram négatifs, les antibactériens doivent pénétrer dans le périplasme pour atteindre le cytoplasme bactérien. Cette zone périplasmique peut contenir certaines enzymes qui vont fournir une résistance face aux antibiotiques. L'autre grande famille sont les bactéries à Gram-positif comme le Staphylococcus Aureus. Ces microorganismes sont constitués d'une paroi de peptidoglycane en présence d'acide teichoique. Cette paroi est en contact direct avec la membrane cytoplasmique. [16]

Le fait est que, les actifs doivent agir par réactions ou interactions sur la paroi des bactéries ou encore sur la membrane cytoplasmique. C'est pour cette raison que la nature des antimicrobiens va jouer un rôle important sur la dégradation des bactéries. Certains agents seront ainsi plus actifs sur des bactéries à Gram(+) et d'autres sur les Gram(-). De manière générale, les recherches montrent que ce sont ces interactions ou réactions sur les parois membranaires des cellules microbiennes qui provoquent leur destruction. [19-29] Par exemple, le contact entre germes et antimicrobiens peut dégrader la perméabilité de la membrane qui est finalement modifiée. Certains antimicrobiens vont alors générer des ions dont l'absorption sera possible par les germes. Une fois absorbés, ces ions sont assimilés par les germes et vont empêcher la croissance des enzymes requises pour la respiration cellulaire entrainant leur destruction. [23, 24, 27-29]

Une autre possibilité d'action évoquée concerne les modifications du pHi intracellulaire, un paramètre important dans la prolifération des microbes [20]. Certains pHis optimaux sont nécessaires pour la prolifération des microbes.

A-2- Les bactéries, moisissures et levures dans la cosmétique

Dans le domaine de la cosmétique, les chercheurs qui travaillent sur l'élaboration de formulations. Pour les analyses bactériologiques, ils utilisent 5 références de microbe : 3 bactéries, 1 moisissure et 1 levure.

Les 3 bactéries sont Escherichia Coli [30] ; Staphylococcus Aureus [31-32] et Pseudomonas Aeruginosa. La levure est le Candida Albicans [33] et la moisissure un Aspergillus Niger. Ceux sont des microorganismes pathogènes pour l'homme.

A-3- Les tests pour l'évaluation de l'activité antimicrobienne dans des formulations cosmétiques

Le plus connu des tests permettant de mettre en évidence l'activité microbienne au sein des produits cosmétiques est le « challenge test ». Lors de la fabrication des produits cosmétiques, les industriels suivent une norme NF EN ISO 29621 de juin 2011 afin de garantir aux consommateurs une certaine assurance de sécurité et de qualité. Il s'agit pour les fabricants de suivre tout au long de la chaine de production la qualité microbiologique des cosmétiques. Aujourd'hui, une attention particulière est portée sur la qualité des installations (température, pression, humidité, stérilité …) afin d'éviter une prolifération de bactéries au moment de la conception des produits.

Le challenge test permet d'évaluer le comportement d'une formulation par rapport à une contamination bactérienne provoquée in vitro. Une fois le produit conçu, des microorganismes y sont introduits volontairement avec une quantité définie. Une évaluation du nombre de la population est ensuite réalisée à intervalles de temps réguliers pouvant aller de 2 à 28 jours. [34]

Ces diverses techniques d'analyses peuvent apporter deux types de résultats, qualitatif, ou quantitatif. Il est alors possible de visualiser l'évolution microbienne sur un produit. Des résultats chiffrés peuvent également être obtenus, comme la détermination de la concentration minimale à incorporer aux cosmétiques pour inhiber l'action des microbes (CMI : concentration minimale inhibitrice). Ces tests peuvent être mis en œuvre en milieu solide, liquide ou gazeux. Néanmoins, plus couramment, le challenge test se pratique sur milieu solide.

B- Les agents antimicrobiens

Un antimicrobien est une substance qui va avoir une action néfaste pour les microorganismes. Ces actifs peuvent agir de deux manières différentes, soit ils vont détruire les microorganismes, c'est l'effet bactéricide, soit ils vont empêcher leur croissance, c'est l'effet bactériostatique. Les antibiotiques sont des molécules antimicrobiennes qui sont utilisées pour lutter contre certaines infections dans le secteur médical. De manière générale, il existe deux grandes classes d'agents antimicrobiens, les agents organiques et inorganiques. Tous ces agents sont regroupés en différentes familles comme par exemples, les acides, les alcools, les terpènes, les antibiotiques, …

B-1- Agents antimicrobiens organiques

Beaucoup d'applications comme la production ou encore le stockage des aliments peuvent bénéficier de l'incorporation de biocides dans les polymères.[6, 35] Cette partie présente un tableau récapitulatif d'exemples d'études ayant été réalisées pour la fabrication de matériaux polymères antimicrobiens avec incorporation d'actifs organiques. Les techniques de fabrication utilisées y sont mentionnées, ainsi que les propriétés étudiées et le type de microorganismes ciblés.

Tableau 1 : Exemples de matériaux polymères antimicrobiens fabriqués avec introduction d'agents organiques

Agent antimicrobien	**Matériaux polymères**	**Techniques de fabrication**	**Propriétés étudiées**	**Microorganismes ciblés**
CHITOSANE [36]	Chitosane	1) Matrice réalisée en solution (activité antimicrobienne naturelle) 2) Dispersion d'agents par simple mélange physique (procédé de suspension) formation de films minces [37-40]	1) Propriétés mécaniques, thermiques, durabilité… [35] 2) Etude de libération des antimicrobiens	*Salmonella enteric; serovar Typhimurium, Escherichia coli et Staphylococcus aureus* [35, 39, 41]*; Lactobacillus acidophilus et Lactobacillus casei* [38] *Proteus vulgaris; Absidia coerulea fungi; Saccharomyces ; cerevisiae and Rhodotorula glutensis ; Aspergillus niger ; Aspergillus parasiticus …* [19]

			3) Etude de l'activité antimicrobienne [36, 41, 42]	
NISINE [36, 44-48]	Poly(éthylène-co-acétate de vinyle) [43] Acide polylactique Films de protéines	1) Dispersion dans des solutions liantes de polymère puis dépôt sur un support avant polymérisation [43-44, 49]	1) Etude de libération des antimicrobiens [43, 50] 2) Etude de dispersion des agents [51]	*Micrococcus flavus* [44] ; *Micrococcus flavus et Listeria monocytogenes* [45, 51] ; *Lactobacillus helveticus et Brochothrix thermosphacta* [47]; *Listeria innocua et Escherichia Coli* [48, 51]; *Salmonella Enteritidis* [51]
ACIDE SORBIQUE	Cellulose [52-54]	1) Technique d'enrobage par des particules de gélose de nanoparticules d'acide sorbique 2) Fabrication de films de cellulose par simple mélanges physiques	1) Etude de libération des antimicrobiens [54] 2) Etude antimicrobienne [54]	*Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus delbrueckii, Lactobacillus acidophilus et Lactobacillus casei* [52] ; *Aspergillus niger ; Escherichia coli* [54]
SORBATE DE POTASSIUM [45-49]		1) Fabrication de films par simple mélange physique	1) Etude de libération des antimicrobiens [55-56]	*Zygosaccharomyces bailii* [55]
LYSOZYME	PVOH [60] Cellulose [61, 62, 63, 64] Acétate de cellulose [62] Carboxyle méthyle cellulose [63]	1) Fabrication de films par simple dispersion physique [60, 62, 63] 2) Immobilisation du lysozyme sur certains polymères [61, 64] 3) Fabrication de films minces	1) Etude de libération des antimicrobiens [62, 65-67, 62, 64] 2) Modification de paramètres procédés [62] (modifications de la morphologie qui joue	*Escherichia coli et B. amyloliquefaciens* [62, 63]; *L. innocua* [63] ; *Micrococcus lysodeikticus* [64]

		par mélange en solutions puis déposition sur supports avant séchage (couche protectrice) [62]	sur la libération) 3) Etude antimicrobienne [62]	
ACIDE PROPANOIQUE [16, 68, 20]	Matrice comestible à base de protéines [69]; Chitosane [70]	1) Fabrication à partir d'une solution aqueuse déposée sur un support (film mince protecteur) [71]	1) Etude de libération des antimicrobiens [71] 2) Etude de l'activité antimicrobienne [69, 72]	*bacteria d'acide lactique, Enterobacteriaceae, Lactobacillus sakei ou Serratia liquefaciens, S. liquefaciens* [71]
ENZYME GLUCOSE OXYDASE	Emballages « Bioka » [73]) ou Tetra Pak [74] Poly-lactiques acide (PLA) [75] Polypropylène [76] Polyéthylène [77, 78]	1) Introduction d'actif dans des couches laminées [79] 2) Fabrication par électro-filage (nano-fibres ou micro-fibres antimicrobiennes) [80, 81] 3) Immobilisation d'enzyme [75, 76] (Création de sites actifs sur le polymère) 4) Fabrication par polymérisation UV [77, 78]	1) Etude morphologique [75] 2) Etude de libération des antimicrobiens [77, 78]	*propionibacteria, Propionibacterium acidipropionici, P. jensenii, P. thoenii, P. freudenreichii subsp. freudenreichii and P. freudenreichii subsp. Shermanii* [64] *; Streptococcus lactis Streptococcus mutans Candida albicans …* [21, 22]
CARVACROL ET THYMOL (huiles essentielles) [82-93]			1) Etude de libération des antimicrobiens 2) Activité antimicrobienne [88-94]	*Acinetobacter baumanii, Aeromonas veronii biogroup sobria, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,*

				Salmonella enterica. enterica serotype typhimurium, Serratia marcescens et Staphylococcus aureus [23, 24, 82,84-88, 91, 94] ; *Neisseria gonorrhoeae* [85] ; *Corynebacterium, Streptococcus, Lactobacillus, Micrococcus, E. faecalis S. cerevisiae…* [86] ; *Proteus mirabilis, Citrobacter koseri, Enterobacter aerogenes Streptococcus pneumoniae, Micrococcus luteus, Staphylococcus epidermidis* [87] ; *Listeria innocua* [88, 91]; *Saccharomyces cerevisiae et Aspergillus niger* [91]; *Bacillus subtilis et Saccharomyces cerevisiae* [23] ; *Listeria monocytogenes, Streptococcus pyogenes, Proteus vulgaris* [24]

Ces études ont montré que des polymères pouvaient être améliorés en ajoutant des actifs organiques antimicrobiens et aboutir à la fabrication de matériaux protecteurs.

Des études de dispersion et de morphologie des polymères, en microscopie sont souvent menées lors de la conception des matériaux [51, 75]. L'objectif est d'obtenir par exemple une dispersion la plus homogène possible afin d'optimiser les diffusions et les relargages d'actifs dans le milieu environnant. C'est ce paramètre de libération qui conditionne généralement l'activité antimicrobienne finale. Plus la quantité libérée sera importante et plus l'activité finale contre la prolifération de germes sera améliorée.

Le paramètre de libération peut également dépendre de plusieurs autres critères, comme la morphologie finale du polymère. Un matériau poreux va par exemple libérer une quantité d'agent plus importante qu'un matériau dense, augmentant l'activité antimicrobienne finale. [62]

La libération des actifs peut aussi dépendre des propriétés physico-chimiques des polymères utilisés et donc des affinités entre agents antimicrobiens et matériaux. Ces caractéristiques peuvent générer par exemple des diffusions Fickiennes [50, 54-56].

Cet état de l'art attire l'attention sur quelques paramètres importants à prendre en considération lors de la conception de matériaux antimicrobiens.

Il existe également des matériaux fabriqués à partir d'une autre catégorie d'agents, des antimicrobiens inorganiques comme les métaux ou les oxydes.

B-2- Agents antimicrobiens inorganiques

Certains métaux ioniques, tels que le Cuivre, l'Argent, le Zinc, le Palladium ou encore le Titane sont utilisés dans le commerce à cause de leur intérêt sanitaire. Ces métaux présentent comme avantage l'action antimicrobienne ou encore une excellente protection contre certaines sollicitations telles que les UV [95]. Le tableau 2 présente toute une gamme de matériaux à base de métaux employés dans la lutte antimicrobienne

Tableau 2 : Liste de particules antimicrobiennes à base métallique et de nano-composites

Agent antimicrobien	**Matériaux employés**	**Microorganismes cibles**
1) Ag Zéolites	1) Acier inox	*1) Bacillus spp*
2) Ag Zéolites	2) Acier inox	*2) E. Coli S. Aureus*
		P. aeruginosa
3) AgNPs	3) PE	*3) A. acidoterrestris*
4) Ag-argile ; Ag-Zéolite	4) Chitosane	*4) E. Coli, Listeria*
		S. aureus
5) AgNPs	5) PVP	*5) Psychrotrophe*
6) Ag-MMT		*6) Pseudomonas spp*
1) CuNPs	1) Cellulose	*1) E. Coli*
2) CuNPs	2) Chitosane	*2) S. Aureus, Salmonella*
1) ZnO	1) PVC	*1) E. Coli, S. Aureus, Psychrotrophe*
1) TiO_2	1) Acier inox	*1) E. Coli*
2) TiO_2	2) EVOH	*2) Bacillus spp, E. Coli, …*

3) TiO_2	3) Acier inox	*3) Biofilms de Listeria* *E. Coli, Listeria, S. aureus, Salmonella*
4) TiO_2	4) Verre	*4) E. Coli*
5) TiO_2	5) PP	

Construction de ce tableau à l'aide de quelques revues évoquant la dispersion d'agents inorganiques dans des polymères : [68, 95, 96]

A. Llorens, E. Lloret et al (2011) ont étudié l'efficacité antimicrobienne, la résistance UV, la résistance à l'humidité, la capacité de diffusion, … de composés métalliques que l'on retrouve aujourd'hui dans le commerce.

Cependant, le nombre de composés métalliques utilisés étant très important. Le tableau 3 cible les deux antimicrobiens les plus employés, à savoir les oxydes de Titane ainsi que les nanoparticules d'argent. [95]

Tableau 3 : Exemples de matériaux polymères antimicrobiens fabriqués avec introduction d'agents inorganiques

Agents antimicrobiens	**Matériaux employés**	**Techniques de fabrication**	**propriétés étudiées**	**Microorganismes ciblés**
OXYDE DE TITANE (TiO_2)	Acier inoxydable, polypropylène, EVOH [25, 104-112] Polyméthyle méthacrylate (PMMA) [107], PVC [106], Polystyrène [109]	1) Dispersion de TiO_2 via un mélangeur interne à haute T°C (160°C) (0.5, 1, 2 et 5%) [104] 2) Fabrication de matrice polymère par réaction sol/gel (principe d'hydrolyse et de condensation des métaux alcoolates dans la matrice) [108]	Etude de l'activité antimicrobienne [104, 108]	*Escherichia coli, Salmonella enterica serovar Enteritidis et Pseudomonas aeruginosa* [25, 97-100, 102, 103, 107, 109, 111] ; *Pseudomonas aeruginosa* [101, 25, 111]; *Staphylococcus aureus* [25, 107, 109, 111] ; E. *faecalis* [104, 108] ; *Micrococcus lutes* [106] *Pichia jadinii* [111]
NANOPARTICULES D'ARGENT [113, 114]	Alcool polyvinylique (PVA),	1) Réduction d'un sel d'argent en présence de particules core-shell	Etude de dispersion [115, 123-124, 126]	*Escherichia coli, Staphylococcus aureus* [113, 115, 117-119, 125]; *Pseudomonas fluorescens,*

	Polyamide, polystyrène, polyéthylène téréphtalate, polyaniline [115-119]	(polystyrène (core) et polyamide acrylique (shell) ou polyéthylène glycol (shell) [120-121]) 2) Implantation directe d'ions argent dans les polymères (PVC, caoutchouc, PU, PE) qui sont ensuite réduits pour créer les nanoparticules en surface [122] ou en masse [117, 123, 124] 3) co-sputtering du di(éthylène) glycol méthacrylate avec l'argent [125] 4) Co-extrusion bi-vis co-rotative à 180°C d'un polymère avec nanoparticules	Etude de libération [115] Etude de l'activité antimicrobienne [117]	*Bacillus circulens* [117, 118]; *Pseudomonas aeruginosa, et B. cereus* [118] ; *Staphylococcus epidermis* [122, 125]

On constate comme pour les agents organiques, que les mêmes propriétés morphologiques, la libération et les activités antimicrobiennes sont étudiées dans le cas des oxydes de titane ou des nanoparticules d'argent. C'est une dispersion homogène [115, 123-124, 126] qui va par exemple favoriser une bonne libération des actifs (TiO_2 [104, 108] ; ions argent [115]) et permettre une importante activité antimicrobienne.

De cette partie sur les composés organiques et inorganiques, il ressort que chaque agent possède des caractéristiques qui lui sont propres. Certains actifs possèdent des avantages lors de la fabrication de matériaux antimicrobiens. Le chitosane possède par exemple une activité antimicrobienne naturelle lui permettant d'être utilisé directement en tant que matériaux. D'autres agents ont des capacités de conservation de leur activité antimicrobienne après un contact avec des formulations cosmétiques. C'est le cas par exemple de l'acide propanoïque, un conservateur actuellement utilisé dans des formules cosmétiques. [16, 20, 68]

Malgré les avantages apportés, d'autres actifs sont plus contraignants dans leur utilisation. Les oxydes de Titane ou encore les nanoparticules d'argent, qui rentrent dans la catégorie des antimicrobiens inorganiques, sont aujourd'hui soumis à controverses. La question du caractère toxique de ces composés chez l'être humain sous leurs formes micrométriques ou nanométriques est souvent posée. Ces agents pourraient potentiellement engendrer des risques sanitaires pour l'homme, par exemples, des irritations oculaires ou des voies respiratoires mais aussi certains risques allergiques ou cancérigènes. [83]

En conclusion, actuellement, des possibilités existent avec un nombre de polymères et d'antimicrobiens extrêmement important et des mises en œuvre via une grande variété de technologies. Ces études évoquent, entre autres, l'importance de la morphologie des polymères qui va conditionner la libération des agents actifs dans un environnement extérieur et au final l'action antimicrobienne.

Après avoir découvert les nombreuses possibilités concernant les agents antimicrobiens, il semblait évident de s'intéresser aux réglementations existantes dans le secteur de la cosmétique.

B-3- Les agents antimicrobiens autorisés dans les produits cosmétiques

Les réglementations dépendent des pays ou régions du monde. En Europe, l'annexe V de la directive cosmétique européenne de l'arrêté du 6 février 2001 pour la législation française a permis de lister les antimicrobiens pouvant être introduits au sein des formulations cosmétiques. [127] Cette norme permet de définir des limites et contraintes d'utilisation (concentration maximale, nature et conditions d'utilisation). L'exemple du formaldéhyde peut être cité car tous les produits qui en contiennent sont susceptibles de diffuser dans les formulations et doivent présenter la mention « contient du formaldéhyde » (si cette concentration est supérieure à 0,05%).
Il existe d'autres produits qui ne sont pas dans la réglementation mais qui présentent des propriétés antimicrobiennes. C'est le cas de certains alcools ou autres huiles essentielles dont des études ont

démontré leur efficacité. Cependant, ces derniers n'étant pas dans les normes cosmétiques, ils ne peuvent pas être listés en tant que conservateurs.

Les conservateurs sont classés en deux grandes catégories, les agents antimicrobiens synthétiques et naturels. Il existe une dizaine de conservateurs synthétiques autorisés comme le montre le tableau 4. Parmi eux, le plus connu reste l'ester de l'acide 4-hydroxybenzoïque, plus communément appelé « parabène ». Il s'agit du conservateur le plus utilisé dans les produits cosmétiques. En 2010, des enquêtes ont montré que près de 80% des produits cosmétiques en contenaient. Cela est dû notamment au fait qu'il est bon marché et a une bonne activité antimicrobienne. Cependant, l utilisation des parabènes fait polémique. Il semble d'après des études [127] qu'ils seraient potentiellement cancérigènes. C'est pour cette raison que les fabricants cherchent aujourd'hui à les retirer de leurs produits.

Tableau 4 : Quelques conservateurs autorisés en cosmétique

Conservateur	**Concentration massique maximale autorisée**
Acide Benzoïque et ses sels ainsi que ses esters	0,5% en acide
Acide Propionique ou propanoïque et ses sels	2% en acide
Acide salicylique et ses sels	0,5% en acide
Acide sorbique et ses sels	0,6% en acide
Formaldéhyde et paraformaldhéhyde	< 0,2% sauf pour l'hygiène buccale < 0,1% pour hygiène buccale : concentration exprimée en formaldéhyde
Sels du zinc du pyridine-1-oxy-2-thiol (pyrithione de Zinc)	0,5%

Pour finir sur les conservateurs de synthèse, on peut en citer d'autres tels que les acides sorbique, benzoïque, salicylique, les formols, le triclosan ou encore les alcools. Hormis ces agents synthétiques, il existe une autre grande famille de conservateur, ce sont les agents naturels. On y retrouve notamment les huiles végétales ou huiles essentielles. Ces agents ne sont pas listés dans les 56 autorisés par les normes. Cependant, le consommateur d'aujourd'hui ressent un fort rejet du conservateur synthétique notamment à cause des parabènes. C'est pour cette raison que de plus en plus, les fabricants se tournent vers des agents naturels. Ils tentent également de réduire les antimicrobiens des formulations pour les ajouter aux emballagesCe paramètre est important pour le fabricant qui en dessous d'une valeur limite peut ne pas déclarer la présence des antimicrobiens au moment de la vente. Ce point a un intérêt marketing majeur pour les concepteurs d'emballages intelligents.

Ainsi, toutes ces réglementations du secteur cosmétique doivent être rigoureusement étudiées par les industries lors la fabrication de formules, mais aussi d'emballage.

Cette introduction sur les agents antimicrobiens a permis de prendre connaissance de la grande variété de solutions qu'il existe aujourd'hui dans le secteur de la recherche. Cette première étape bibliographique va permettre de tendre vers le secteur des emballages antimicrobiens.

C- Les emballages antimicrobiens

Actuellement, peu de recherches académiques s'intéressent à l'emballage cosmétique. La protection antimicrobienne se fait majoritairement par des ajouts d'actifs directement dans les formulations. Cela explique pourquoi le nombre de travaux sur le packaging est minime [1-5].
Ces modifications de polymères notamment pour fabriquer des emballages antimicrobiens concernent principalement le secteur des emballages alimentaires. En ce sens, il paraissait possible dans le cadre de ce projet de s'inspirer de ce secteur afin de le transposer à une utilisation pour le domaine de la cosmétique.

Il est apparu au début de ce projet certaines similitudes entre ces deux secteurs, dans l'utilisation des polymères, de nature similaire pour la fabrication des emballages, mais également de certains antimicrobiens utilisés à la fois dans l'alimentaire (emballage) et dans la cosmétique via l'introduction de conservateurs dans les formulations cosmétiques.

L'examen de l'état de l'art doit permettre un apport d'informations sur les critères importants pour la conception d'emballages alimentaires. Ce sont ces critères qui doivent inspirer les choix d'actions afin de répondre aux objectifs du projet et trouver des solutions innovantes pour la fabrication finale d'emballages cosmétiques.

Il est très intéressant de constater qu'il existe aujourd'hui de nombreux exemples d'emballages actifs. [128] Des paramètres comme la nature physico-chimique des matrices, le procédé de fabrication, l'activité antimicrobienne ou encore les conditions de stockage des aliments doivent être considérés dans la conception des emballages ou des films antimicrobiens. [129]

Concernant la nature des antimicrobiens, le choix se limite généralement à la thermo-stabilité nécessaire pour garder les propriétés bactéricides ou bactériostatiques. En effet, des travaux ont montré que certains actifs comme les huiles essentielles « allium sativum » peuvent perdre leur activité antimicrobienne sous l'action d'une trop forte chaleur. [130, 131, 132] Ce paramètre est donc très important à prendre en compte lors de la dispersion de l'antimicrobien au sein du polymère.

Pour ce qui concerne les matériaux qui rentrent dans la conception des emballages, les propriétés physiques ou mécaniques sont importantes car l'ajout d'actifs peut entraîner des modifications non négligeables. Cela peut ainsi rendre le matériau inutilisable. On peut citer, en exemple, certaines propriétés de résistance aux chocs, viscosité, contrainte en traction, ou encore souplesse. Han et al en 1997 [130] ont observé une diminution de la transparence des films polymères par un ajout d'actifs.

Un autre point important à prendre en compte lors de la conception est l'interaction entre les agents actifs et les produits. En effet, les aliments affectent de manière plus ou moins importante les effets antimicrobiens ou la libération. [129] Les propriétés physico-chimiques de certains aliments vont agir sur des paramètres essentiels à l'action bactéricide ou bactériostatique. Citons par exemple le pH, qui dans certains cas est préférentiel dans la croissance ou la prolifération de microorganismes. Chaque aliment possède ainsi ses propres caractéristiques microbiologiques qui favorisent la prolifération des microorganismes. [129, 133] Il sera donc important dans le cadre des emballages cosmétiques de prendre en considération les interactions entre formules et antimicrobiens qui pourraient réduire l'activité de ces derniers.

Enfin, un paramètre important et également à prendre en compte concerne les coefficients de transfert ou de diffusion qui conditionnent la libération des actifs. [128]Ce dernier point sera en lien avec le design final de l'emballage. En effet, les packagings antimicrobiens peuvent être classés en cinq catégories qui sont fonction du mode de fixation de l'agent actif ou encore de la nature de ce dernier.

- Incorporation de l'agent actif directement au sein de la matrice polymère
- Immobilisation d'un antimicrobien sur une matrice polymère via des liaisons spécifiques
- Revêtement ajouté post-fabrication de l'emballage (traitement de zones par une couche protectrice)
- Utilisation de microcapsules dans lesquelles se trouve l'agent antimicrobien et qui seront ajoutées ultérieurement à la matrice polymère

- ⇨ Utilisation d'une matrice polymère possédant naturellement des propriétés antimicrobiennes

C-1- Différents designs d'emballages

C-1-a- Incorporation de l'agent actif au sein de la matrice polymère

Ce type d'emballage antimicrobien est très utilisé dans le domaine médical et commence également à se développer dans le secteur de l'alimentaire. De nombreux chercheurs travaillent sur ces techniques dans l'optique de l'appliquer aux secteurs de l'aliment comme la salade ou encore la viande. Différents actifs existent pour ces applications, mais certains sont utilisés plus souvent comme les oxydes de titane ($Ti0_2$) ou encore les nanoparticules d'argent.
Ces agents peuvent être incorporés dans des matrices polymères telles que, le polyéthylène, le polyamide, le polystyrène, le polypropylène, le poly(éthylène-co-vinyle alcool) (EVOH) ou encore le polychlorure de vinyle (PVC) (figure 2).

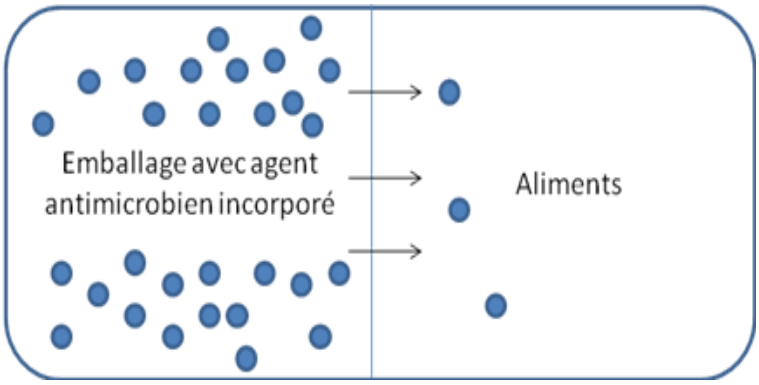


Figure 2 : Représentation schématique d'un emballage avec incorporation directe de l'agent antimicrobien dans le matériau constitutif

Pour évoquer l'EVOH, différents travaux de recherches traitent de la simple dispersion de composés antimicrobiens dans ce copolymère souvent utilisé dans la conception d'emballage.
P. Fernandez-Saiz, M. J. Ocio et J. M. Lagaron (2009) [134-135] ont travaillé sur une nouvelle formulation de revêtement. Ces derniers avaient pour objectif de contrôler le relargage du glucosamine au sein des produits alimentaires. Une large gamme de Chitosane a été introduit dans une matrice de poly(éthylène-co-vinyle alcool) (EVOH) afin de vérifier l'action antimicrobienne de l'emballage final.

Un regard a été porté sur la corrélation entre les capacités antimicrobiennes apportées par ce matériau et les caractéristiques de diffusion (migration) du glucosamine.
Concernant la synthèse de ces films minces (épaisseurs : environ 50µm), ceux-ci ont été fabriqués par un simple mélange entre deux solutions chimiques avec un passage à l'autoclave en faisant varier les paramètres de procédé. Ces chercheurs ont ainsi générer une réaction au sein de solutions constituées d'EVOH et d'acétate de chitosium, en présence d'un catalyseur acide acétique.

D'autre chercheurs, Maria L. Cerrada, Cristina Serrano et al (2008) [136] ont eux aussi travaillé sur ce même copolymère EVOH. Elles ont mis au point un nano-composite par un procédé à l'état fondu en présence d'oxyde de Titane (2 à 5% en masse de TiO_2) et ce pour des applications d'emballages alimentaires.

D'autres agents actifs ont été dispersés dans une matrice, citons l'Hexaméthylène tétramine (HMT) qui a connu l'une de ses premières utilisations dans le secteur médical. Ce composé fonctionne sur le principe suivant, à savoir la libération de formaldéhyde qui va réagir de façon spécifique avec certaines enzymes ou autres protéines présentes dans les microorganismes.

M. Ramos et al (2011) ont eux travaillé sur le Carvacrol et le Thymol, agents actifs que l'on retrouve dans le thym ou autres huiles essentielles et possédant de bonnes propriétés antibactériennes [94]. Ces derniers ont étudié le comportement de ces agents destinés à des applications d'emballages actifs à partir d'une matrice polymère de type polypropylène. Ces emballages ont la capacité de lutter contre des bactéries comme E. Coli ou le S. Aureus et ceci grâce à une libération d'additifs dans le milieu environnant. Pour la fabrication de ces échantillons, un simple mélange à 190°C, pendant 6 minutes et à 50tr/min a été réalisé dans un mélangeur interne entre le polypropylène et les antimicrobiens. Différents mélanges, avec des quantités en actifs variant de 5 à 10% massique ont été finalement mise sous forme de films de 200µm complètement transparent et homogène.

On peut également citer en exemple, des films composites à base d'acétate de cellulose fabriqués par voie solvant dans de l'acétone et en présence de propionate de sodium (4% massique). Ces matériaux permettent finalement de lutter efficacement contre la prolifération de certains microorganismes. [137].

D'autres techniques permettant l'obtention de ce design existent et utilisent comme matériaux initiaux, des monomères liquides dans lesquels sont dispersés des agents antimicrobiens. Cette technologie présente comme principal intérêt une grande facilité de mise en œuvre, car les mélanges vont se faire avant les étapes de polymérisation. [138-139]

Tai-Li Tsou et al (2005) [138] ont travaillé sur la réalisation de matériau polymère en partant d'une solution aqueuse concentrée avec deux monomères, l'HEMA et l'EGDMA. A cette solution, un amorceur de polymérisation ainsi qu'une quantité de 0.5% à 2% en agent antimicrobien, la ciprofloxacine ont été ajoutés avant une dernière étape de photo-polymérisation. C'est l'irradiation UV qui génère la polymérisation radicalaire du réseau dans lequel est finalement piégé l'antimicrobien qui pourra alors être libéré dans un milieu environnant à protéger. Bojana, D. Krezovic et al (2012) ont également travaillé à partir d'une technologie similaire de polymérisation radicalaire d'un HEMA en présence d'un amorceur. Ces derniers ont utilisé une voie en solution aqueuse, en présence d'HEMA, de polyvinylpyrrolidone (2 à 10% molaire) et d'acide itaconique (5% molaire) pour fabriquer des matériaux antimicrobiens.

Ces deux exemples montrent clairement qu'il est possible de facilement introduire des composés antimicrobiens dans des monomères en solution avec une étape de polymérisation pour former un réseau. Cependant, cette voie n'est pas unique, puisqu'il est également possible grâce à la capacité de gonflement de certains réseaux de les doper en antimicrobiens après polymérisation. Pour ce faire, une seconde étape d'immersion dans une solution aqueuse concentrée en antimicrobiens et après création du polymère permet d'obtenir un matériau utilisable pour venir protéger des zones contre toutes proliférations de germes. [140-142]

Yungfen Shi et al (2013) ont démarré leurs travaux par la fabrication de copolymères radicalaires en partant de différents mélanges de monomères liquides (HEMA, MAA, GMA, DMAEMA…), auxquels ils ont incorporé un amorceur thermique de polymérisation AIBN. Une fois ces films fabriqués, ils ont utilisé la capacité de gonflement des réseaux en milieu humide pour les charger en antimicrobiens. Tous les échantillons copolymères ont finalement été immergés dans une solution concentrée de Gatifloxacine (500mg/L) pour permettre l'obtention de films antimicrobiens. [140] Bako et al (2007) ont également travaillé sur une étude similaire. Ils ont synthétisé des capsules de polymères par photo-polymérisation à partir de monomères HEMA et PEGDMA qu'ils ont ensuite immergé dans des solutions concentrées en chlorhexidine pendant 48h. [142] Une remarque intéressante concernant ces travaux est qu'il serait possible d'utiliser ces capsules pour les introduire dans un autre matériau afin de ralentir une diffusion des actifs dans le milieu environnant. Cela permettrait de se rapprocher du design d'emballages décrit dans une partie suivante (C) 1) d)).

Cette première partie a décrit le grand nombre de technologies existantes pour la réalisation de ce design d'emballage. Cette large variété de solution est justifiée par la facilité de mise en œuvre qui offre généralement de nombreuses possibilités d'adaptation au monde de l'industrie.

C-1-b- Immobilisation d'un agent actif sur une matrice polymère via des liaisons spécifiques (liaisons covalentes, liaisons ioniques, …)

Ces matériaux correspondent à une gamme de polymère où une fixation d'un agent sur une matrice via une réaction de fonctionnalisation permet de créer des liaisons spécifiques (liaisons covalentes, liaisons ioniques, …) (figure 3). Jeffrey A. Barish et Julie M. Goddard (2011) ont proposé un matériau polyéthylène sur lequel un greffage de polyéthylène glycol (PEG) est réalisé. La technique repose sur une fonctionnalisation du polyéthylène basse densité (LDPE) (traitement sous ozone après le greffage du PEG) et sur lequel des molécules bioactives vont être immobilisées. [143] Le traitement ozone est généré par une lampe mercure. Le polyéthylène LDPE est traité pendant 90 minutes pour permettre la création de peroxydes en surface. Cette présence de peroxydes sous une certaine chaleur va alors former des radicaux oxygène, ce qui va initier le greffage de polymérisation radicalaire du polyéthylène glycol diacrylate sur le polyéthylène.Cette technologie repose sur la création de liaisons covalentes qui permet de former des sites actifs en surface. Barish et al (2011) évoque également la possibilité de venir créer des sites actifs avec des enzymes, des peptides, des bactériocines … . Les composés antimicrobiens ne peuvent pas diffuser ou migrer dans un milieu environnant ce qui peut constituer un avantage pour certaines applications. [143]

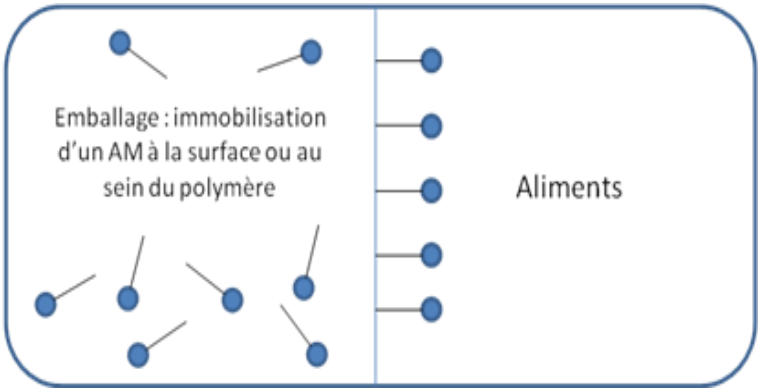


Figure 3 : Représentation schématique d'un emballage avec immobilisation de l'agent antimicrobien sur et dans le matériau constitutif

Appendini et Hotchkiss en 1996 ont démontré l'efficacité antibactérienne de molécules telles que le lysozyme ou encore des enzymes naturelles contre certains germes à Gram positif (L. Monocytogenes) [128]. Ils ont également travaillé sur l'immobilisation d'enzymes sur un polymère de type tri-acétate de cellulose qui permet d'éliminer près de 90% de M. Lysodeiktius.

Anthierens et al (2010) ont eux travaillé sur une matrice poly(butylène adipate) sur laquelle un greffage covalent d'ions phosphonium quaternaire permet de réduire la prolifération de

microorganisme E. Coli. [144] Cette réaction est réalisée par un mélange des composés (polymère fonctionnalisé et antimicrobien) en voie solvant au sein d'une solution de THF. Ces chercheurs ont mis en évidence la fonctionnalisation de cette matrice par une réaction de type « click » (Utilisation d'un catalyseur cuivre) et dont les groupes ioniques vont pouvoir réagir avec les fonctions réactives des microorganismes pour une utilisation finale dans les emballages alimentaires ou encore dans le domaine médical. Finalement, le polymère final, de nature polyester est fonctionnalisé par des groupements alkynes via la génération de liaisons covalentes.

Une autre possibilité existe actuellement et consiste à générer un hydrogel avec des sites actifs par greffage au moment de la polymérisation. Dans ce cas également, aucune libération n'est possible, et l'action va être réalisée par un contact entre les germes pathogènes et des sites antimicrobiens [145-148]. Cette possibilité peut s'avérer intéressante dans l'hypothèse où la fixation des agents peut être nécessaire, évitant ainsi toutes libérations d'agents actifs dans un milieu environnant. [149].

Pour terminer cette partie sur le design d'emballage avec immobilisation, il est une fois encore, possible d'évoquer l'utilisation des EVOH. Ces matériaux sont constitués de groupes fonctionnels OH, susceptibles de réagir pour former des liaisons hydrogènes. Du fait de cette caractéristique, V. Muriel-Galet et al (2012) [151] ont développé des films antimicrobiens pour contrôler la microbiologie sur les emballages de salade. Ces matrices ont été fabriquées avec comme matériaux de base un composite polypropylène associé à un poly(éthylène-co-vinyle alcool) dans lesquelles des huiles essentielles organiques ont été introduites. Les agents actifs employés sont notamment connus pour leur activité antimicrobienne [150, 151] Dans cette étude, la technique de fabrication des films va être réalisée en plusieurs étapes. Dans un premier temps, un polymère commercial EVOH est dissout dans une solution alcool (propanol/eau) à 50°C. Les huiles essentielles vont ensuite être ajoutées (concentration de 5 à 10%) à cette solution sous agitation magnétique. Le mélange obtenu est alors vaporisé sur un film polypropylène, puis passé sous un chauffage pendant environ 10 minutes. Cette technique employée par Muriel-Galet et al (2012) pourrait également rentré dans la gamme des designs présentée dans la partie C) 1) c) qui représente la création d'une couche fine sur un matériau constitutif des emballages finaux.

E. S. Park et al (2003) [152] ont étudié la fabrication de matériaux polymères avec une introduction de 3 acides actifs (acide salicylique, acide amino-benzoique et acide hydroxybenzoique) par simple mélange dans un EVOH. Ceci a permis d'ancrer chimiquement les acides sur la matrice par des liaisons hydrogène créant des sites actifs sur les polymères qui doivent permettre une action antimicrobienne. Une particularité intéressante avancée par ces chercheurs est le choix porté sur le poly(éthylène-co-vinyle alcool). D'après eux, l'EVOH a été employé en tant que matrice, pas

seulement du fait qu'il possède des groupements hydroxyles, mais également du fait qu'il présenterait certaines activités antimicrobiennes naturelles. Cette particularité pourrait permettre de considérer l'EVOH comme un matériau pouvant rentrer dans la dernière partie descriptive des différents designs (Partie C) 1) e)). Il pourrait ainsi être intrinsèquement antimicrobien et utilisable directement en tant qu'emballage, même si ce point n'a pas encore été démontré dans ces travaux de Park et al (2003). [152]

C-1-c- Revêtement ajouté post-fabrication de l'emballage (protection de zones par une couche protectrice)

Cette technique permet de constituer une fine couche antimicrobienne qui va venir s'intercaler entre l'extérieur de l'emballage et les aliments comme l'illustre la figure 4.
Il tout d'abord possible d'évoquer l'exemple précédemment mentionné dans la partie C) 1) b) sur la modification d'un EVOH par des huiles essentielles. Muriel-Galet et al (2012) [151] ont démontré la possibilité de déposer une fine couche d'EVOH modifié avec des sites actifs d'huiles essentielles pour former une couche protectrice sur un polypropylène destiné à être utilisé en tant qu'emballage. Ces recherches mettent aussi un aspect intéressant en évidence, la possibilité d'utiliser plusieurs types de designs pour créer un emballage actif.

C. H. Lee et al (2003) [49, 153] ont démontré l'efficacité d'un agent actif couramment utilisé dans ce type d'emballage ; la Nisine. Ce composé permet d'éviter la croissance de Gram positif (L. monocytogenes) et Gram négatif (E. Coli). Le principe de l'enrobage repose sur la capacité de relargage des actifs ainsi que leur migration dans l'aliment. Ils ont élaboré leurs revêtements par voie solvant (éthanol ou acide acétique). Ils ont mélangé un copolymère d'acétate-éthylène avec 3% de Chitosane ou de Nisine en tant qu'agent antimicrobien pour obtenir un mélange liquide qu'ils ont finalement déposé sur une surface papier à protéger. Une ultime étape de séchage a alors permis d'obtenir une couche protectrice antimicrobienne d'environ 3mm d'épaisseur.

Egalement cités dans la partie précédente, Han et al (2000) [128] ont étudié l'incorporation d'agents dans les emballages actifs qui vont ensuite migrer au sein des aliments. Ceci par diffusion des agents à travers la matrice. Le fait d'utiliser une couche revêtement permet de traiter des zones bien précises des emballages, limiter les quantités d'agents antimicrobiens ajoutés et avoir une libération plus rapide. En effet, la capacité de migration des actifs est importante dans ce type de design et dépend de paramètres extérieurs, comme la température, la nature des aliments ou encore du type de matériau qui compose la matrice.

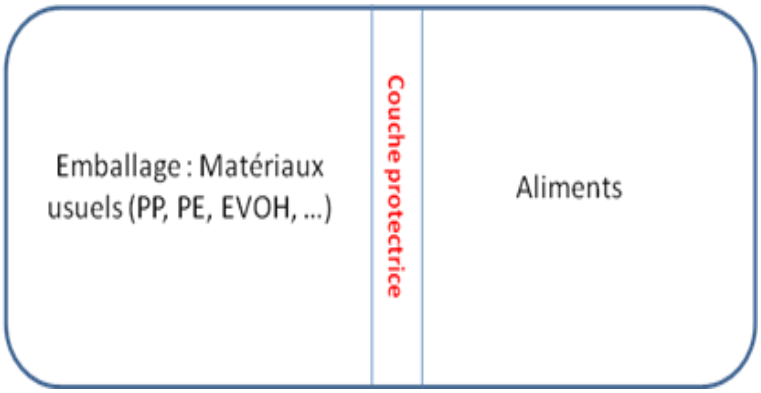


Figure 4 : Représentation schématique d'un emballage avec ajout d'un revêtement protecteur qui va venir traiter des zones spécifiques

Une autre façon d'introduire une protection antimicrobienne est de fabriquer un film polymère par technique de co-extrusion ou extrusion afin de venir introduire les antimicrobiens dans le matériau. Le principal inconvénient de cette technologie est qu'une fois introduit, l'antimicrobien ne présente plus la totalité de son activité. Cette perte d'activité est principalement provoquée par la forte chaleur d'extrusion ou d'injection qui génère des dégradations importantes des antimicrobiens [131, 132].

Les zones de recouvrement peuvent être réduites à des parties spécifiques de l'emballage qui ont réellement besoin d'une action antimicrobienne (les zones de contacts directs entre les aliments et le consommateur ou les zones exposés à l'extérieur). Cette approche permet ainsi de minimiser les zones antimicrobiennes des emballages ce qui va réduire la quantité nécessaire d'agents actifs, l'activité antimicrobienne nécessaire, et les coûts de fabrication. De plus, le fait de diminuer ces zones actives permet de réduire la quantité d'agents qui va diffuser dans les aliments et seraient susceptibles d'entraîner des allergies chez certains consommateurs. Il est également important que les cinétiques de libération des antimicrobiens soient maitrisées pour maintenir la concentration des agents aux valeurs limites d'inhibition autorisées. [129]

Une autre technologie permet la création d'un emballage multicouche. C'est l'application d'un revêtement ou dépôt par vaporisation d'une solution liquide qui va subir par la suite une étape de polymérisation (radicalaire, en chaîne, cationique, …) sous effet de chaleur ou encore d'irradiation (UV, gamma, ozone, …). La photo-polymérisation est une technologie précédemment évoquée visant à créer des solutions plus ou moins visqueuses qui vont ensuite être irradiées sous une lampe UV. Cette étape d'irradiation en présence d'un photo-amorceur va entraîner la création de radicaux qui vont réagir avec le monomère pour former un réseau. L'objectif dans ce cas est d'introduire les agents antimicrobiens dans les solutions pour les piéger dans la matrice polymère. [154]

Il est également possible de venir diluer ces résines en présence d'eau pour former des hydrogels. On peut alors contrôler plus ou moins le gonflement du réseau, ce qui peut permettre d'accroitre les zones réactives et donc augmenter les capacités de greffage ou d'incorporation des actifs. Les hydrogels photo-polymérisés représentent une piste intéressante pour certaines applications dans le domaine médical.

C-1-d- Utilisation de capsules dans lesquelles se trouve l'agent antimicrobien et qui seront ajoutées ultérieurement à la matrice polymère

Il s'agit ici d'une catégorie d'emballages antimicrobiens particulière. En effet, les actifs qui sont généralement employés ne sont pas, à la base, destinés à ce genre d'applications mais le plus souvent utilisés en tant que générateurs de CO_2, absorbeurs d'oxygène ou d'humidité (figure 5).

Il est possible de considérer les recherches de Bako et al (2007) [142], précédemment mentionnées, et qui ont travaillé à la fabrication de capsules de polymères par photo-polymérisation à partir de monomères HEMA et PEGDMA. Ces capsules de tailles très variables, comprises entre 5nm et 500nm ont par la suite été immergées dans des solutions concentrées en chlorhexidine pendant 48h pour les charger en agents antimicrobiens. [142] Il est possible d'utiliser ces capsules pour les introduire dans un matériau destiné à constituer l'emballage final.

Une autre technique consiste à utiliser des microorganismes qui peuvent être piégés dans les matériaux. Une fois libérés, ils vont éliminer l'oxygène qui est responsable de la prolifération des bactéries (microbes dits aérobies (besoin d'air pour vivre ou survivre [155])). Ces organismes jouent alors un rôle d'absorbeur d'oxygène ce qui va laisser uniquement du dioxyde de carbone. Nielsen et al (2000) a montré que certains microbes dits aérobie ne pourront plus croitre sous une telle atmosphère. [156]

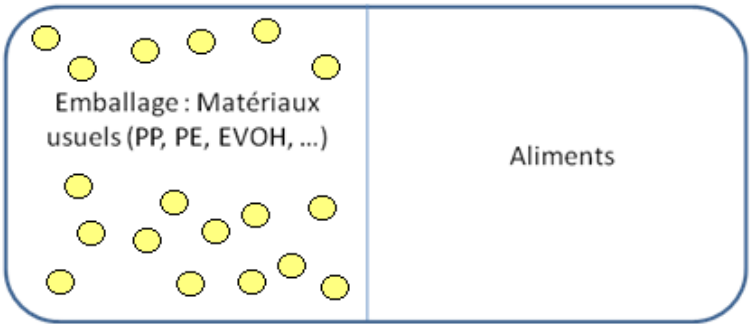


Figure 5 : Représentation schématique d'un emballage avec ajout de microcapsules (cercles) renfermant l'agent antimicrobien

Ce design d'emballage présente deux avantages majeurs. Le premier est qu'il peut ralentir une libération des antimicrobiens qui doit diffuser d'abord au travers des capsules et ensuite traverser l'emballage pour aller au contact de l'aliment. Cette spécificité est intéressante dans le cas où les industriels souhaiteraient prolonger l'action antimicrobienne.
Un autre avantage est que l'utilisation de capsules permet de protéger les antimicrobiens dont l'intégrité peut être perdue lors de leur dispersion au sein de l'emballage.

Yang Hu et al (2013) [157] ont chargé des microcapsules (de type core-shell) avec des huiles d'Artemisia Argyi (AAO) qui possèdent des vertus antimicrobiennes. Les capsules, finalement de forme sphérique avec des tailles autour de 50µm ont été fabriquées à partir d'une émulsion de type huile dans eau, chauffée à 50°C, pendant 3 heures entre des solutions aqueuses et une émulsion huile dans eau. L'agitation provoquée en présence de tous ces réactifs a permis de générer la polymérisation et donc la création des capsules. Par des calculs de modélisation, ces chercheurs ont démontré qu'il était possible d'avoir une capacité de chargement en AAO d'environ 50% avec un excellent contrôle de la libération des antimicrobiens. Yang Hu et al (2013) évoque dans leur travaux de recherches des (possibilités d'applications à un niveau industriel. En outre, les microcapsules pourraient être utilisées en tant que matériaux antimicrobiens, dans différents domaines, comme le textile, le caoutchouc ou par simple dispersion physique dans des revêtements polymères pour apporter une protection. [157]
Ravindra Reddy K et al (2010) [158] ont mis en évidence les avantages d'utiliser des microcapsules dans une couche revêtement de polymères. Entre autre, ils parlent de la possibilité de modifier et retarder une libération de composés actifs tel les antimicrobiens. C'est pour cette principale raison que la technologie de micro-encapsulation reçoit actuellement une grande attention dans la recherche et le développement. [158] Pour tenter de trouver des innovations, Ravindra et al (2010) ont fabriqué des microcapsules contenant de l'isoniazide, un antiseptique utilisé dans le secteur médical. Une technologie de gélation ionique a été employée avec l'utilisation d'alginate de sodium et de différents copolymères à base cellulose. Différentes solutions aqueuses sont préparées et mélangées pour obtenir une parfaite homogénéité. Une fois les étapes de mélange et de réaction terminées, les capsules sont récupérées par décantation avant une dernière étape de nettoyage dans l'eau. Ces microcapsules de forme sphérique avec des tailles moyennes de 920µm ont finalement des capacités d'encapsulation qui vont varier entre 80% et 54%. [158]

Malheureusement, malgré les nombreux avantages apportés par ce design de matériaux, celui-ci présente également un inconvénient, celui d'augmenter le nombre d'étape de mise en œuvre. En effet, deux étapes sont nécessaires : la fabrication des capsules et la dispersion au sein de l'emballage. Cet inconvénient peut s'avérer gênant pour des applications industrielles en termes de coût en augmentant

le nombre d'étapes. Enfin, un dernier inconvénient de cette technologie est que l'incorporation de microcapsules peut générer, selon leurs tailles, l'apparition de zone de fragilité au sein de l'emballage.

C-1-e- Utilisation d'une matrice polymère possédant naturellement des propriétés antimicrobiennes

Certains matériaux polymères présentent des capacités antimicrobiennes naturelles et ainsi ne nécessitent pas d'incorporation d'agents actifs. Le plus connu de ces polymères utilisés dans l'application des emballages alimentaires est le chitosane. Ce composé est un polyoside issu de la distribution aléatoire de D-glucosamine et de N-acétyl-D-glucosamine. Cette matière première est déminéralisée par un traitement utilisant de l'acide chlorhydrique puis dé protéinée en présence de soude ou de potasse. Ce bio-polymère, non-toxique, possède des propriétés très intéressantes pour une utilisation dans les emballages agroalimentaires. Le chitosane est non toxique, biodégradable et présente des capacités antimicrobiennes pour éviter la croissance de microorganismes dans certains aliments.

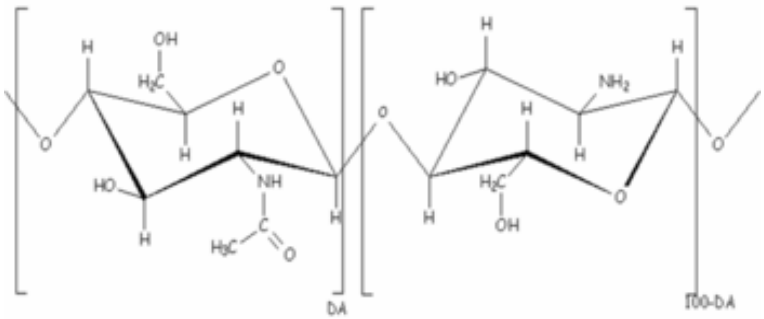


Figure 6 : Schéma moléculaire du Chitosane

Ces propriétés se dépendent de son degré de d'acétylation ainsi que de sa masse molaire. Le degré d'acétylation (DA) est un pourcentage de groupements acétylés par rapport au nombre total d'unités monomères. [159, 160, 161].

De nombreuses recherches ont été menées sur l'étude des capacités de lutte contre certains microorganismes responsables de l'apparition de maladies. Le chitosane agit de façon efficace contre des bactéries à Gram positif comme la Candida Lambica mais aussi sur certains Grams négatifs comme l'Escherichia Coli ou la Salmonella typhinurium. Malgré le fait que la capacité d'action du chitosane sur les microbes reste floue, certaines recherches ont permis d'avancer des théories. [162-165]

Cet état de l'art a permis d'apporter de nombreux éclaircissements sur les possibilités qui existent aujourd'hui pour concevoir des emballages antimicrobiens. Cette partie montre qu'une importante gamme d'emballages alimentaires existe avec des designs, des matériaux ou encore des antimicrobiens différents. Ceux-ci jouent un rôle primordial dans le maintien de la qualité [166, 167] ainsi que le contrôle des croissances de microorganismes [168]. Ces matériaux peuvent être obtenus par divers moyens de fabrications et des paramètres imposés variables. La fabrication peut se faire à basse température par polymérisation UV, par exemple. D'autres, sont réalisés à haute température, par extrusion ou encore injection.

Dans le contexte du travail présenté ici, les objectifs principaux de cette thèse sont de développer des matériaux antimicrobiens pour protéger des zones spécifiques d'emballages cosmétiques. Différentes technologies et agents vont être utilisés pour la partie conception. Dans un premier temps, l'utilisation d'une technique à température ambiante doit permettre de s'affranchir du risque de dégradation des actifs par de trop fortes sollicitations thermiques ou mécaniques imposées. [131, 132] Notre choix s'est arrêté sur un revêtement méthacrylate permettant de créer un réseau tridimensionnel. Il s'agit d'hydrogels à l'intérieur duquel l'actif sera piégé. Cette voie permettra de pouvoir réaliser une couche protectrice sur le packaging. Ces hydrogels représentent des matériaux avec de bonnes caractéristiques et c'est pour cela qu'ils sont souvent utilisés dans les secteurs médical, biomédical ou encore plus spécifiquement dans la fabrication des lentilles de contact. De plus, ce matériau dispose d'une forte capacité de biocompatibilité associée à un faible caractère toxique, ce qui le rend très sécurisant pour son utilisateur. Les hydrogels ont comme autre avantage, la possibilité de réagir sous des environnements spécifiques. Par exemple, la capacité à plus ou moins gonfler dans un milieu humide et ainsi à libérer des actifs [169-171]. Pour ce qui est des adaptations industrielles, ce matériau présente, là encore, un avantage certain puisque différentes techniques de mise en œuvre existent. Ceci offre de larges perspectives pour adapter ces derniers à une application finale dans une chaine process.

Un autre point important constaté lors de l'étude bibliographique est qu'une grande variété d'antimicrobiens existe. Cependant, les études menées sur les agents sont généralement partielles, réalisées sur une famille donée dans des conditions variables (concentrations ajoutées, nature des polymères, tests antimicrobiens, …). Se pose alors la question de savoir quels sont les meilleurs antimicrobiens lorsque ceux-ci sont incorporés dans des mêmes conditions de fabrication. Cette première partie à basse température aura un autre objectif, celui de permettre une comparaison finale de l'efficacité antimicrobienne du système matrice avec différents agents actifs sélectionnés. Il sera alors possible de choisir les agents les plus adaptés pour l'application industrielle. Une fois cette

sélection faite, la suite devra permettre d'évaluer les effects de leur concentration sur les propriétés physiques, chimiques, thermiques… des matériaux préparés à température ambiante.

La seconde partie du travail présenté concernera l'élaboration de matériaux anti-bactériens par voie « chaude » qui permettra de transférer plus facilement la mise en œuvre à l'échelle industrielle, étant adaptable à des procédés d'extrusion ou d'injection. Dans un premier temps, il sera intéressant de voir s'il est possible de conserver une activité antimicrobienne malgré des sollicitations thermiques et mécaniques imposées par le mélange des produits. L'EVOH a été choisi comme matrice polymère car [172] c'est un matériau souvent utilisé dans le domaine médical, le secteur des emballages ou dans la fabrication de polymères antimicrobiens [150, 152, 173-178]. Une étude similaire sur le contrôle de l'activité des plusieurs anti-microbiens et sur les modifications apportées par l'ajout des antimicrobiens sera réalisée sur ce copolymère à haute température. L'introduction des actifs pouvant jouer sur les propriétés physico-chimiques, mécaniques, thermiques,… il est intéressant de savoir comment celles-ci peuvent évoluer pour toute adaptation de fabrications sur une chaine de production.

Pour conclure, le premier objectif de ces travaux est de se concentrer sur la réalisation de matériaux antimicrobiens à basse température pour conserver l'intégrité et donc l'activité antimicrobienne des actifs. Cependant, les secteurs de l'emballage utilisent beaucoup plus des procédés à haute température pour la mise en forme des produits. Il est donc impératif de trouver un procédé d'élaboration de matériau anti-antimicrobien par voie chaude.

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AVANT PROPOS

Une fois la démarche bibliographique effectuée, plusieurs points ont été éclaircis concernant la bactériologie et les matériaux antimicrobiens. Il a été constaté d'une part que les secteurs les plus ciblés pour les matériaux antimicrobiens étaient l'alimentaire (packaging), le médical et le biomédical. Il n'existe que très peu de recherches sur les packagings cosmétiques. Généralement, les études sont très axées sur les modifications de formule pour conférer des propriétés spécifiques (antimicrobiennes, viscosités, aspects, …) et beaucoup moins sur l'emballage.
Cependant, il existe actuellement une volonté grandissante du consommateur de réduire les quantités d'agents conservateurs directement introduits dans les formules. Notamment à cause des parabènes, molécules très sujettes à controverses en raison de leur possible toxicité.
D'autre part, on constate l'existence d'une très large gamme d'agents possiblement antimicrobiens.

La première problématique s'est posée sur le choix des meilleurs antimicrobiens pour les applications industrielles. En effet, différentes familles d'agents existent. On retrouve ainsi, des acides, amines, alcools, terpènes, antiseptiques, agents inorganiques, métalliques, oxydes,… Un choix très, voire trop vaste. De plus, les chercheurs s'intéressent généralement à une famille ou un agent en particulier d'où une impossibilité de réaliser une comparaison directe en matière d'efficacité.
L'objectif initial était de trouver le système (matrice avec agent antimicrobien) le plus performant en termes d'activité antimicrobienne et qui puisse s'adapter à une application industrielle.

Nous avons fait une sélection d'antimicrobiens par famille et nous les avons incorporés dans une matrice référence pour effectuer une comparaison directe et un possible classement.
L'autre point important a été de trouver un système matériau qui puisse facilement intégrer ces différents agents et de plus, être adaptable à un procédé industriel. Une autre contrainte est également survenue : la non dégradation des agents antimicrobiens sous une sollicitation thermique trop extrême. Cette problématique s'est posée lors d'un entretien avec un des partenaires industriels (STRAND Cosmetic). Il était important de trouver un polymère qui puisse être mis en œuvre à une température respectable. Le choix s'est finalement arrêté sur une matrice acrylate ou multi-acrylate polymérisée sous irradiations UV afin de former un réseau radicalaire. Ce choix est justifié pour plusieurs raisons :

- Matériaux initialement sous une forme liquide d'où une dispersion des antimicrobiens possibles. De plus, ce système peut être adapté à un procédé industriel tel le spray coating, dépôt en couche mince… ce qui peut être intéressant en vue de venir traiter certaines zones d'un emballage.

- L'utilisation des acrylates ou multi-acrylate permet de former des hydrogels avec leurs avantages. Premièrement, une possibilité de gonfler dans des milieux spécifiques et ainsi

permettre une libération d'actif qui va venir protéger les formules. Deuxièmement, la possibilité de réaliser des copolymérisations et de former du greffage de sites actifs en masse. Ces sites seront à l'intérieur même du réseau et éviteront les relargages. L'idée ici est d'avoir une activité antimicrobienne par contact direct en attirant les bactéries sur ces sites actifs pour y être neutralisées.

Cette première partie de thèse a consisté à découvrir le sujet. Ensuite, il a fallu choisir un système qui puisse renfermer selon les mêmes critères de synthèses, des antimicrobiens de diverses familles et pouvoir effectuer une comparaison directe de l'activité.

C'est cette partie qui doit permettre de trouver les meilleurs agents antimicrobiens et va conditionner la suite des travaux. En effet, après cette sélection, une étude plus approfondie sera faite sur les impacts que peuvent avoir l'incorporation d'actifs sur les propriétés des matériaux polymères fabriqués soit à température ambiante, soit à haute température.

CHAPITRE 2 :

Ce chapitre est rédigé sous forme d'une publication qui a été soumise au journal « Polymer Engineering and Science ».

Cet article est consacré à l'introduction de différentes natures d'antimicrobiens dans un matériau référence, toujours fabriqué dans les mêmes conditions.
L'objectif est d'établir une comparaison directe des activités antimicrobiennes de chaque matériau réalisé, cela pour permettre une classification et sélectionner un ou plusieurs actifs pour une application industrielle.
Concernant la fabrication des matériaux, c'est finalement une technologie de polymérisation radicalaire sous irradiations UV qui a été retenue. Cela consiste à réaliser un pré-mélange liquide de monomères méthacrylates et multi-méthacrylates auxquels est ajouté l'actif. Cette solution est par la suite mise sous irradiation UV de deux manières : soit en couche mince (100µm) sur un substrat de nature polyoléfine afin de se rapprocher des conditions de fabrications industrielles. Soit dans un moule téflon pour permettre de créer des éprouvettes rectangulaires avec des dimensions supérieures et plus facilement manipulables lors des étapes de caractérisations matériaux.

Dans cette publication, une première partie bibliographique fournit une brève description des matériaux antimicrobiens, ainsi, que différents types de polymères existants.

La partie « matériel et méthodes » décrit le mode de préparation des matériaux ainsi que les caractérisations effectuées.

Dans la partie « Résultats et discussions », les résultats se présentent sous deux grandes parties. Une première est consacrée à la fabrication d'une matrice polymère référence, sans ajout d'agent antimicrobien. Le principal objectif est de sélectionner le polymère référence dans lequel seront incorporés les antimicrobiens retenus par l'étude bibliographique.
La deuxième partie des résultats traite plutôt de l'aspect antimicrobien des polymères fabriqués avec différents agents. Tout d'abord, les propriétés thermiques par DSC des matrices (100µm) en fonction de la nature de l'agent antimicrobien sont présentées, puis l'évaluation de la libération des actifs. Enfin, l'activité antimicrobienne de chaque matériau est testée selon deux techniques. D'une part, une activité par contact direct en milieu gélosé est faite entre les matériaux polymères et trois microorganismes différents. D'autre part, une étude d'immersion dans des suspensions microbiennes a été réalisée pour évaluer les variations de concentrations afin de voir si certaines solutions peuvent réduire la population de germes. Les conclusions principales de cette étude peuvent être résumées comme suit :

- Les agents potentiellement antimicrobiens se présentent sous différentes formes initialement (solide, liquide, coloration…). Ces divers paramètres provoquent des aspects de matériaux finaux très variés avec différents caractères de transparence ou de coloration.

- Les propriétés thermiques des matériaux méthacrylates sont fortement influencées par les natures des agents antimicrobiens. Ces modifications sont liées à la capacité réactionnelle des actifs au moment de la polymérisation. Certains agents ne vont pas réagir avec le réseau et jouer un simple rôle de charges. D'autres agents, vont présenter des doubles liaisons réactives et pouvoir générer des copolymères avec les monomères. Ces réactions vont jouer activement sur les températures de transitions vitreuses des matériaux.

- Les propriétés de libérations en milieu aqueux sont donc également conditionnées par la réactivité des agents avec les réseaux. Les différentes natures vont provoquer l'apparition des capacités de libérations variables. Ainsi, des matériaux copolymères où les actifs ont réagi, ne vont pas beaucoup libérer, voir pas du tout tandis que les simples charges non réactives, vont pouvoir plus relarguer. Ce paramètre est également lié à la capacité de diffusion des charges dans le polymère (généralement lié aux propriétés physico-chimiques des agents), mais aussi à l'affinité entre les actifs et le milieu environnant.

- Les activités antimicrobiennes finales sont variables selon les différents polymères mis en œuvre. Cette propriété est liée aux différents paramètres précédemment évoqués. Grâce à cette étude comparative, on ressort 5 actifs, sur une quinzaine d'antimicrobiens testés, particulièrement intéressants pour l'application industrielle. Il s'agit de deux antiseptiques, la chlorhexidine digluconate, l'acétate de chlorhexidine et de trois sels métalliques, l'acétate d'argent, le nitrate d'argent et l'acétate de cuivre (II).

Antimicrobial resins for cosmetic packaging

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2-1- Abstract

To reduce the quantity of antimicrobial (AM) agents contained in consumer products, several AM were directly incorporated in a hydrogel coat. The effects of the addition of the AM on the properties of different coat materials tailored for this use were first studied and optimized.

Then, their antimicrobial effects were analyzed using first “a static test” in which the inhibition zones of cylindrical samples placed at the surface of agar culture media were measured, then via immersion tests.

The antimicrobial effects were directly compared or analyzed using antimicrobial effect at equivalent concentration (EEC) calculated using antimicrobial activity and their diffusion.

2-2- Introduction

Cosmetic formulations include antimicrobials to eliminate or minimize contamination. This should prevent the growth of pathogenic microorganisms that may be harmful to humans. Usually, these compounds are introduced directly into the formulation, as in creams or shampoos for instance. To reduce the quantity of these compounds in the formulation, it was envisaged to introduce a part in the packaging allowing thus an antimicrobial activity by contact or diffusion.

In the literature, there are several recent examples of synthesis of antimicrobial matrices containing a large range of different antimicrobial additives. However, these materials have been designed mainly for other sectors, such as the food or medical industries [1-8].

Presently, there are comparatively few studies on modifications of materials destined to packaging design in the cosmetic sector. The works found were more specifically focused on formulations (agents called preservatives) and less on the packaging itself. [9-13]

Nevertheless, the target microbes (bacteria and yeasts) being often the same for both uses, it is highly probable that the antimicrobial agents used in food and biomedical sectors should be efficient in cosmetics as well.

The effect of several AM agents has been well described in the literature. The antibacterial efficiency of different families, such as acids [14-18], amino acids [19, 6], alcohols, terpenes [3, 20-21], peptides, proteins [22-25], polysaccharides [8, 26], antiseptics [27-33], metal oxides [34-36] and metal salts [25, 37-42] are reported in relation with their concentrations or the nature of the target pathogens.

The action mechanism of these antimicrobial agents is described in more specialized journals and only few references to these studies will be made here, whenever necessary.

In antimicrobial packaging, a component can be incorporated in several ways depending on the required life time of the package. For a short activity time and a surface contamination, it seems reasonable to immobilize the active agent or to incorporate it in a thin film on the package's surface. If a long time of activity is required, it may more be interesting to introduce the biocide agent in the material and control the release properties (time, released quantities…) [43-44].

Active agents were therefore introduced directly during processing [45, 9] or as masterbatch [46].

The immobilization of the active agent either at the surface or in the bulk can be more efficient when covalently linked to the polymer matrix, as did Anthierens and al., grafting quaternary phosphonium on a poly(butylene adipate) matrix, thus reducing the proliferation of E. coli. [2]

Although antimicrobial food packaging research is rich in the literature, it cannot be denied that the used matrix, the active agent concentration and incorporation, the process implementation, the target

nuclei and the antimicrobial activity evaluation are quite different, making a reasonable comparison and the choice of an antimicrobial agent hazardous.

Actually, a problem in the literature is that the parameters are highly variable and different techniques of synthesis of antimicrobial materials co-exist (nature of the antimicrobial concentrations, size of matrices, processes, protocols, bacteriological tests…). However, to our knowledge, no study compared the activity of different types of the active additives listed above under the same conditions and in the same polymer matrix. Consequently, it is impossible to make a direct comparison between the different active agents and make a final choice.

That is why, in this study dedicated to the choice of antimicrobial for gel-coats in cosmetic packaging, it is proposed to maintain these parameters constant and to change only the antimicrobial agent for each specimen. Besides, a hydrogel varnish was tailored to facilitate the release of the active agent as well as the contact of the antimicrobial agent with the bacteria. The addition of active additives, liquid or solid, in polymer matrices can result in a change in physico-chemical or mechanical properties of the obtained materials. Therefore, it seemed important to evaluate the potential impact of these inclusions on some final properties of the synthesized coatings. In the first part of this study, the influence of the introduction of the antimicrobial agent on the properties of the material will be analyzed. Then, the evaluations of the bactericidal effect will be investigated. Finally, it will possible to comparatively assess antimicrobial efficacy and evaluate the best antimicrobials that should be most effective in the conditions of final production. [47-51]

2-3- Materials and methods

All the reagents, photo initiators and antibacterial agents were purchased from Sigma Aldrich France. The tertiary amine used (CN381) was offered by Sartomer.
All reagents were used as received without further purification.

- *Synthesis of acrylates films*

The used photo-initiating system was composed of a homogeneous solution consisting of 60wt% CN381, 20wt% Benzophenone BP and 20wt% 2, 2 dietoxy acétophénone DEAP. The acrylate solutions, also called UV resins, were composed of 5 wt% of the photo-initiating system in two monomers, 2-hydroxyethyl methacrylate (HEMA) and poly(ethylene glycol) dimethacrylate (PEGDMA) (50/50 wt%).
For the antimicrobial materials preparation, the antimicrobial agent was dispersed or solubilized in the monomer mixture prior to the photo-initiating system addition.
It was used to produce thin films (100 μm) using a Bar Coater (elcometer 4340 applicator) or placed in Teflon molds to produce thicker materials (70x10x1.5-2mm).

- *UV light Banc Fusion UV system F300S (fusion system)*

The system is a UV light associated with a conveyor belt. The speed of this device is adjustable allowing the samples' translation at a fixed rate. The UV lamp can irradiate with wavelength between 220nm and 300nm.The wavelengths and dose measurements are assessed by a system named Power puck UV radiation (UV Power puck II from EIT Company).This irradiation was repeated 5 times for an optimal curing of the monomer solution.
For the fabrication of thin layers, the acrylate solutions are placed on a polymeric support with a mechanical system, a Bar Coater (elcometer 4340 applicator), just before UV irradiation.

- *Characterizations*

- *Thermogravimetric Analysis by TGA*

A thermogravimetric analyzer (TGA) from Mettler Toledo, TGA/DSC 1, was used. Thermal degradation experiments were made under nitrogen flow with a rate of 80 $mL \cdot min^{-1}$ for all the

experiments. Samples ranging from 15 to 20 mg were heated from 30°C to 500°C with a heating rate of 10°C·min^{-1}.

- *Thermal analysis by DSC*

Differential scanning calorimetry (DSC) measurements were carried out with a Q10 calorimeter from TA Instruments. Samples were transferred to hermetic pans, sealed, and analyzed from -80°C to 270°C with a heating rate of 20°C/min. The glass transition temperatures were measured at the inflection points.

- *DMTA measurement by a rectangular torsion mode rheology*

The tests were performed using a Rheometric Scientific ARES N2 rheometer. The dimensions of the specimens were measured then saved in the software. All the samples were subjected to a sinusoidal deformation in tension mode analysis at a frequency of 1.0 rad/s and strain amplitude of 1% in the linear domains from -75°C to 100°C with a heating rate of 5°C min-^{1}.

- *DMTA measurements by tension mode rheology:*

The dynamic mechanical thermal analyses of the PEGDMA/HEMA network films were also performed with a Rheometrics Solids Analyzer (RSA II, TA-Instruments) to obtain tensile dynamic mechanical spectra. Test samples were cut with a predetermined shape (40mm x 10mm x 100µm) from the acrylate samples. For dynamic tensile measurements, a nominal strain of 0.1% was adapted, with an applied frequency of 1Hz. Storage modulus E', loss modulus E", and loss factor tan δ were determined as a function of temperature. Data were taken from -50°C to 100°C using a heating rate of 3°C·min^{-1}. Each sample was equilibrated in the same chamber under dry nitrogen at the starting temperature prior to running the test. The main objective was to have a direct comparison between two processes of characterization (with two solicitation modes) with different dimensional characteristics of samples.

- *Density measurement*

Analyzes of the densities of acrylate matrices were carried out with a glass pycnometer (water).The main objective of these tests was to evaluate the impact of variations in the ratios of monomers and in particular if a modification could change physical properties.

- *Swelling measurement*

The tests consisted in immersing rectangular samples (100mg) in a fixed volume of water during a period of 48 hours and under environmental conditions. All the samples were retrieved and dried before the weight measurement with a filter to remove all traces of external moisture. The catch rates of weight q were calculated using equation 1:

$$q\% = (mt - mi)/mi * 100 \quad (1)$$

mt and mi represent the weights of samples, t : time of one measure and i represents the initial time.

- *Analysis of the conversion rate by UV Spectroscopy*

The release kinetics of the residual monomers from an immerged piece of PEGDMA: HEMA matrix in reference water was followed for several hours by UV spectroscopy (Agilent Technologies Cary Series 100 UV-VIS Spectrophotometer) at room temperature and under magnetic stirring. Since the methacrylate matrices only contained small quantities of residual monomers and photo-initiator, the matrix had to be immersed in the reference water for several days to release these reactants.
The solution was then analyzed by UV spectroscopy before comparing these results with standard curves. Knowing the quantities introduced, it was possible to determine the concentrations of residual compounds. By calculating a ratio between monomer compounds used for films synthesis and residual monomers, it was possible to know the conversion rate approximately.

- *Adhesion capacities on LD-PE slub substrates*

This study is mainly qualitative and opens up an opportunity to check the ability of synthesized films to adhere on different polymeric materials destined to the final applications of packaging.

- *Releases of antimicrobial agents were monitored by HPLC*

The chromatographic experiments were carried out using an HPLC system after 72 and 168 hours immersion. For the data acquisition, the same reagents (used for the antimicrobial analyses by the protocol 2) were used to determine the final concentrations after the two immersion times. All the results were expressed in ppm.

- *bacteriological measures: antimicrobial tests*

Protocol 1: Antimicrobial evaluation in an agar environment was performed. A microbial suspension (200 to 500 µl of which the bacterial concentration is between 10^4 and 10^5 cfu/ml) was deposited on the surface of the different coats. For the evaluation of the antimicrobial effectiveness, the samples with cylindrical forms were put in contact with bacteria in a culture medium favorable to their growth. With the potential release of antimicrobial agents, the inhibition properties should prevent any microbial growth. This property should result in formations of inhibition zones, the diameters of which will demonstrate the capacity of the final coating. The evaluation of different diameters will indicate the presence of antimicrobial activity according to the size of the inhibition zone.
To estimate the different inhibitions, a simple calculation was done. The equation 2 was used to calculate a percentage of inhibition.

$$\mathbf{I = (D2 – D1) / (D1) * 100 \quad (2)}$$

Where I represents the percentage of inhibition, D1 is the diameter of the sample tested and D2 the diameter of sample with the addition of the inhibition zone.

A reference matrix without antimicrobial additive revealed no antimicrobial activity with final inhibition zones equal to the initial samples diameters. These observations were similar for each type of strain tested (S. Aureus, P. Aeruginosa and A. Brasiliensis).

Protocol 2: the antimicrobial Evaluation was based on an ISO normal test 22196.
Antimicrobial activity of EVOH films was evaluated by an immersion test. The antimicrobial activity of the matrix was evaluated for different formulations. For this, three different strains were used: two bacteria (S. Aureus and P. Aeruginosa) and a yeast (A. Brasiliensis). For each sample, an EVOH was immersed in a liquid solution, with a fixed concentration of microbes. Then, the samples were incubated in a specific environment favorable to a growth of microbial germs during a period set to 24 hours. After this interval, the concentrations of the liquid solutions were measured to assess the evolution (increase or reduction) and the final antimicrobial activity.
The neat EVOH copolymers have revealed no antimicrobial activity for each strain used for the tests.

2-4- Results and discussion

2-4-1- Influence of the methacrylate ratios on the properties of the matrix without antimicrobial agent

To assess the unique effect of an antimicrobial agent with a view to comparing different antimicrobials, it is necessary that the parameter affecting the support material remain constant. That is why a standard formulation based on methacrylate and dimethacrylate monomers was chosen. This part was aiming at evaluating the modification of the properties of materials with different ratios between two monomers. The coating must have suitable properties to offer a good final antimicrobial activity (hydrophilicity, wettability, surface contact adapted, or capacity of diffusion of the active agents...). With these characterizations, it will be possible to find a system that is loaded with different antimicrobial agents.
The synthesis of a hydrogel matrix by UV polymerization was chosen because it can be easily scaled-up for industrial use.

- *Evaluation of thermal stability by TGA measurements*

A thermal stability study was done on matrices with different monomers and cross-linker formulations. Results are depicted in Table 1.

TABLE 1. Thermal damage associated with HEMA part (a) and PEGDMA (b) part of the photo-polymerized acrylate hydrogels.

Matrice HEMA/PEGDMA (wt%)	a) Total weight loss	a) Midpoint (°C)	b) Total weight loss	b) Midpoint (°C)
Matrice 30/70	29.3%	349	68.2%	402
Matrice 50/50	50.9%	359	46.6%	408
Matrice 70/30	70.1%	370	28.5%	418

Degradation occurs systematically at these temperatures and stresses for each sample analyzed.
These results clearly show that the hydroxyethyl methacrylate degradation occurred first, immediately followed by the degradation of the polyethylene glycol dimethacrylate (PEGDMA) in 2 well-separated zones with midpoints respectively around 360 and 410 °C.

Figure S1 in supporting data shows the derivative of weight measurement for a sample of HEMA/PEGDMA with a weight ratio of 50/50. The figure reveals two peaks of degradation situated approximately at 360°C and 410°C.

A coating with 30 wt% HEMA (theoretical value) has a weight loss equal to 29.3% in the first zone, while the material with 70%wt HEMA shows a weight loss to 70.1 % in the second zone. (Table 1)

- *Evaluation of thermal properties - DSC measurement*

DSC measurements were first carried out on two sets of HEMA/PEGDMA films. The measurement of Tg is given in supporting data S2 and compared to those calculated by the Fox equation (3) [52]

$$\mathbf{1 / Tg = w1 / Tg1 + w2 / Tg2 \quad (3)}$$

w1, w2 and Tg1, Tg2 are respectively the weight fractions and the glass transition temperatures of the two copolymerized homopolymers.

Generally and when this approximation can be applied to a system, it reveals very good precision to determine glass transitions for polymer blends or in the case of random copolymers. [46]
The measures show that a neat PEGDMA has a glass transition temperature of -20 ° C. For the neat p-HEMA, radical polymerization under UV radiation proved an impossibility of synthesis. Referential values were used to compare with the Fox equation.
This work has demonstrated that the thermal properties (Tg) of materials vary greatly by modification in the initial monomer solution. Thus, a matrix with a weight of 70% HEMA has a final glass transition equal to 46 ° C. A good agreement between experimental results and those calculated from the Fox equation could mean that there is no phase separation in the obtained material. More information on the morphology of these materials will be given in the following part.

- *Thermomechanical properties*

Stability tests over time were performed to ensure that changes in thermomechanical properties of the studied materials would not due entirely or partly to structural evolution of the material. As shown in supporting data S3, G' of several materials stayed constant for 1 hour at 100°C indicating a good stability of the materials.
The results showed constant curves with a modulus approximately equal to 7.8 MPa for a concentration of 30 weight% in HEMA. These values decreased when the amount of HEMA in the ratio increased. For a concentration of 70wt% of HEMA, the storage modulus was equal to a value of 2.07 MPa.

Also as expected, the storage moduli values constantly increased when the dimethacrylate wt% increased.

Temperature sweep tests were then made for different compositions of the hydrogels. First the comparison between the technologies of DMTA in tension and torsion modes (respectively for thin coating (100µm) and rectangular samples with a thickness around 1.5mm) was carried out. Figures S4 a) and S4 b) in supporting data represent the direct comparison of the tan(δ) measured between the two processes of characterization.

This test allows the characterization of thin coatings, more difficult to handle but more representative of the final applications. The results are depicted in supporting data S5. A superposition can be observed between the different curves of tan (δ) as a function of the temperature. The relaxations are similar regardless of the variable parameters, the nature of the test, the temperature rate or the dimensions of samples. These tests demonstrate that the parameters have no influence on the results of the final properties for identical materials.

For the evaluation of the modification of methacrylate ratios, the torsion mode was used. Fig. 1 a) and 1 b) represent the curves of tan(δ) and storage modulus G' as a function of temperature for all the samples.

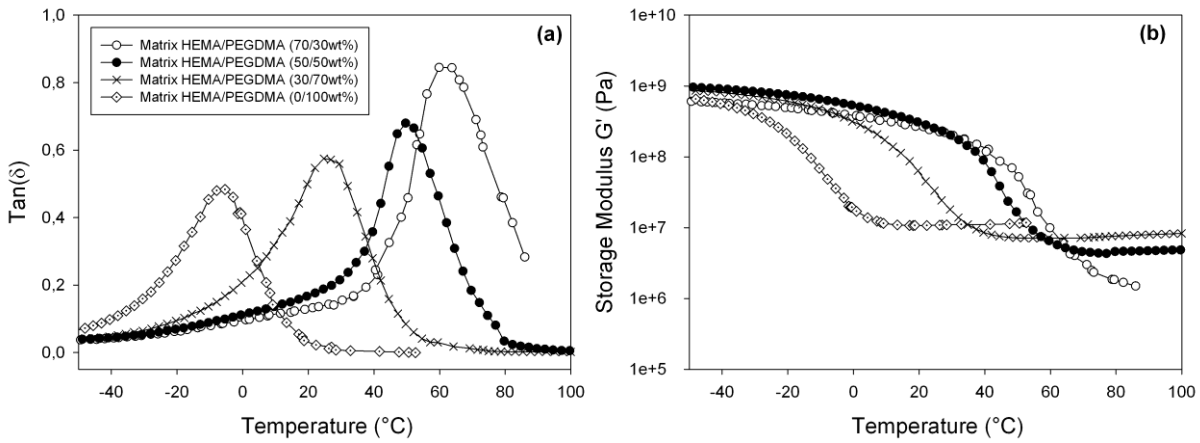

FIG. 1. a) Curves of tan(δ) as a function of temperature analyzed by rectangular torsion rheology for matrices with different weight ratios between HEMA and PEGDMA; b) storage modulus as a function of temperature analyzed by rectangular torsion rheology for matrices with different weight ratios between HEMA and PEGDMA.

First, it is important to note that only one tan(δ) maximum, corresponding to an α transition peak, was observed for each test. This confirmed that the HEMA and the dimethacrylate copolymerized correctly, leading to homogeneous single phase materials.
Comparing results from DSC and DMTA analyses, Tg and the Tgδ maximum (summarized in table 2) changed with the composition of matrices in the same direction. They both decreased when the PEG dimethacrylate concentration increased. A matrix with 70 wt% of HEMA has a Tα equal to 64 ° C against -2.2 ° C for the neat PEGDMA. A similar increase of 66 ° C was also observed by DSC analysis. Thus, it is possible to make assumptions about molecular network modifications of the different coatings. These variations could be associated to modifications in weights between the nodes of crosslinks constitutive of the final networks. It is possible that a high ratio of PEGDMA caused the presence in the network of larger chain lengths making it easier for them to move under mechanical stresses. This could explain the formation of more flexible materials with lower glass transitions.

Another information concerns the variations observed in table 2 between the Tg obtained by DSC and rheology analysis. Important differences appear according to technical analysis (DSC or rheology). A matrix of 50wt% of HEMA has a Tg of 23°C by DSC analysis against 50°C with a DMTA analysis. Hypotheses could explain these shifts. The main reason is that the test conditions are different; DSC is performed in a static solicitation while rheology is dynamic. In this case the acrylate material is a three-dimensional network in which the chains would relax in different ways under the application of mechanical stresses and in the presence of a thermal solicitation. The Tα taken at the Tg δ maximum correspond to only relaxation.
Finally, all these results could help to choose a final matrix with suitable characteristics for industrial application. It is important to have a matrix which can adapt to specific areas of packaging, so it would be interesting to have a Tg closest to room temperature. It would be flexible enough to adapt the matrix to the specific forms of the cosmetic packaging. However, it is also important that it isn't too hard to avoid fractures.

- *Calculation of molar mass between cross-links by evaluation of densities*

The calculation of molar mass between cross-links was carried out for each formulation via the use of the equation (4) summarized below. In the past, researchers have used this equation to determine the measurements of densities by means of rheological measurements (elastic G ' modulus). It is important that certain conditions be met for this equation to be applicable to a polymer system. The techniques directly derived from the theories of Flory and constitute two aspects of an effect that is equivalent to the entropic elasticity of cross-linked polymers. It is necessary to take the values of elastic modulus in the rubbery state.

$$G = RT\rho\nu \quad (4)$$

G represents the shear modulus (Pa), R the molar gas constant (R = 8.314 J / mol / K), T the temperature (K), ρ the density of the material and ν is the concentration of elastically active chains (in mol / kg)

Measurements of rheology and density films will allow the evaluation of the final value of elastically active chains' concentrations. These values will be used to obtain the molar mass between crosslinks of nodes:

$$\nu = 1 / Mc \quad (5)$$

Mc is the molar mass between crosslinks of the material in kg / mol

The determination of densities and so the molar mass in the different networks were obtained and summarized in Table 2.

TABLE 2. Values of glass transitions Tg (DSC), Tα (DMTA) and of molecular weights between crosslink nodes of the synthesized networks.

Matrix HEMA/PEGDMA (wt%)	Tg (°C) (DSC)	Tα (°C) (DMTA)	G' (Pa)*10^{-6}	Density (kg/m^3)	Mc (Kg/mol)
70/30	46	64	1.5	1171	1,9
50/50	23	50	4.5	1204	0.66
30/70	0.7	27	8.3	1273	0.38
0/100	-20	-2.2	/	/	/

It appears from the results that a modification of monomer formulation changes the final densities. It appears that the increase in concentration of PEGDMA causes an increase of material densities. The density of a matrix formed by a weight ratio of monomer HEMA / PEGDMA fixed to 70/30 is equal to 1171 kg/mL. By contrast for a matrix with a ratio equal to 30/70, the measured density is equal to 1273 kg/mL. The modification of the monomers' ratios could change the molecular weights of the molecular chain networks. It was also observed in table 2 that a matrix with 30 wt% HEMA has a Mc value equal to 0.38 kg/mol against 1.92kg/mol when this same concentration is increased to 70%. Measurements show that modifying the weight ratios of monomer formulations causes changes in the final properties of the synthesized networks.

Concerning the variations of HEMA / PEGDMA ratios, an assumption could be a modification of the molar weights of the different molecular chains which form different final cross-linked networks.
This will impact the physical properties of matrices related to other properties, such as thermal, or viscoelastic characteristics.

- *Swelling measurements*

For the analysis, matrices with different weight ratios were immersed in a solution of water during a period of 48 hours and under environmental conditions. At periodic time, masses were measured to evaluate the impact of modifications. Figure S6 in supporting data represents the evolution of weight over time.
We could observe, in particular, very small variations in the degree of swelling. Generally, the final materials had a significant increase in weight over a very short time. After 2 hours of immersion, the sample's weight had increased by approximately 17% and continued to grow up to 36-37% after an immersion time of 20h. In the end, the weight of our samples revealed swelling values around 35%.
These first results show that all matrices have equivalent swelling behaviors. But there are weak differences associated with the modifications of formulations. The results show a greater and more rapid swelling when the concentration of PEGDMA is high. A matrix HEMA/PEGDMA (70/30) swells by about 35.5%, against 37.6% for an opposite ratio of 30/70. It could be that formulation modifications responsible for changes in the cross-linked network cause variations of the swelling comportments.

- *Analysis of the conversion rate by UV Spectroscopy*

The measurements showed that acrylate monomer compounds have a peak of UV absorbance value to a wavelength equal to 207nm. The results concerning matrices without the addition of antimicrobial ingredient have demonstrated a release mainly caused by a certain amount of UV initiator. A high conversion rate means a lower presence in residual monomers. This characteristic is important for the toxicity of the final materials. Finally, it appears that the synthesized materials are safe for consumers.
By a simple calculation with the calibration equations, it was possible to determine the molar amount of un-reacted monomers in the UV irradiation process. Finally, the percentage of monomers which have not participated in the UV polymerization is equal to approximately 0.75% which corresponds to a final conversion degree of approximately **99%.**

- *Adhesion tests*

For these tests, the polyethylene and polypropylene were flamed. To check the good adhesion of these films, the scraping of surfaces with a metal spatula was carried out.
Then, the PE materials used being flexible enough, it was possible to make torsion strains in order to check if this stress did not cause a detachment of the antimicrobial films.
All these tests showed a good adhesion of coatings on polyolefin with no separation effect.
In conclusion, this part of the study has a high interest because it demonstrates the possibility of adhesion for antimicrobial surfaces on different substrates modified with a flame treatment. This final characteristic is very important for industrial applications and in particular for cosmetic packaging (Protection of specific zones).

The main objective was to have a final material which could be covered in specific areas with an antimicrobial film. For this, a choice was made of an acrylate matrix with many advantages (robustness, capacity release). Furthermore, another advantage of this polymer is that it has a liquid form; therefore it is very easy to introduce additive compounds inside it. Finally, UV irradiation will allow the formation of the network within the antimicrobial agent which will be trapped (to be released by a swelling capacity of the hydrogel). This last point is interesting because the industrial adjustment seems very simple, and the absence of thermal stress (high temperature) must be able to conserve the integrity of various additive agents and so of their antimicrobial properties.
Finally, it is a formulation consisting of 50 wt% HEMA and 50 wt% of PEGDMA which was selected for the second part of this work. The choice of this formula was made for several reasons such as a simple adaptation to an industrial system. However, the main reason concerns the glass transition of the final material synthesized. It is around a value of 20°C. So, this feature allows us to obtain at the end a flexible matrix at room temperature. Thus, this allows having a final material which can be easily adapted to a particular form of cosmetic packaging and protect specific areas against bacterial growth. Then, this compromise of glass transition produces a film with a good integrity, good mechanical properties and a clean external visual appearance.

2-4-2- Influence of antimicrobial agents on the properties of the acrylate matrices

- *Impact of different agents on the thermal properties*

The results of Tg measurements are presented in Fig. 2 and the modifications of glass transition as a function of each antimicrobial agent introduced are compared.

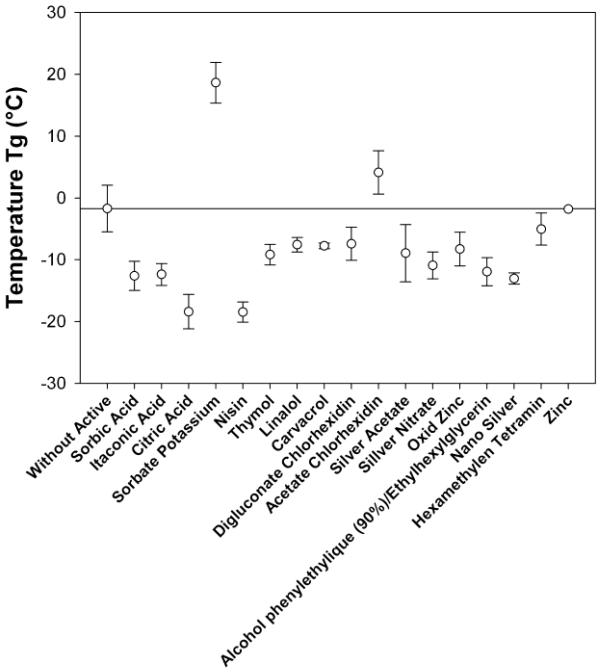

FIG. 2. The variations of Glass temperature for HEMA/PEGDMA (50/50 wt%) Matrices with different antimicrobials introduced (the black line represents the Tg of the referential matrix).

The first observation concerns the variation of glass transition temperatures measured for a same formulation of monomers but polymerized in two different dimensions (Comparison between thin film of 100µm thickness and rectangular samples with a higher thickness around 1.5mm).

In the previous part, DSC characterization was performed on materials with high thickness (1.5mm). But for this part of the evaluation, the matrices were polymerized in thin coatings and deposited on a polymer substrate to approach the final industrial application. The DSC was made on theses coatings.

This dimensional variation causes glass transitions modifications for a HEMA / PEGDMA matrix (50/50 wt %) without antimicrobial agent, because the sample with a large thickness (1.5mm) has a Tg of 23 ° C. By comparison, the same formulation deposited in a thin layer has a final Tg reduced to -1.7 ° C. Several equivalent results were reported in the literature [53-55]. The main reason for this evolution was attributed to a more important effect in the case of reduced thickness of a rubber layer to

the air / polymer interface (corresponding to external surface). In addition, the tacticity (isotactic or syndiotactic) of the polymer can change, leading to an evolution of Tg.

The results in Fig. 2 show that the introduction of different agents cause decreases of the Tg. This evolution could have different causes.

It could be firstly that there are chemical reactions during the polymerization induced by UV irradiation between acrylate monomers and fillers (possible in each case but could not be confirmed without testing).

There are different causes, such as the presence of antimicrobial additives which could inhibit UV curing. Another element which could inhibit UV curing is the presence of agents that would prevent the reaction of free radicals and so the network formation. Actually, specific compounds have groups that can capture these free radicals. This effect prevents polymerization. Another possibility would be the presence of residual compounds which would react during the polymerization and thus modify the final properties (thermal polymerization of the residual monomers, UV reduction ...). In particular, it is possible to cite metal salts. These specific compounds have the possibility to react under different stresses (thermal, radiation…). However, the best theory to explain this decrease of Tg is the possibility of reaction between the acrylates solutions and the antimicrobial agent during UV polymerization. There could be a small reactivity which could explain the observed evolutions in Tg by DSC analysis. Several explanations can be made regarding changing values of Tg of final matrices.
There are two types of antimicrobial agents that have been used in this work and thus these two main phenomena previously seen affect the properties of the acrylate matrix.
There are reagents with double bonds, include sorbic acid, Itaconic acid, Nisin, or Linalol.
The other type is transfer agents; there are citric acids, Thymol, Carvacrol, Chlorhexidine, Nano-silver, Hexametylen tetramin, phenylethanol or ethylhexyl glycerin. Other types of antimicrobial agents exist and are described more precisely in the next part of this study.

The first category concerns antimicrobial agents with the presence of reactive sites (double bonds) which will react with the liquid monomers to generate copolymers. Concerning the polymerization, these additives will be able to cause modifications on the network (by the presence of double bonds with a number more or less important (vinyl, acrylic, allyl, diene, conjugated diene ...)). These reactive bonds will participate in the free radical polymerization of monomers under UV irradiation. These additives will allow copolymerization which will participate to the formation of films with different thermal properties compared to the neat matrix (Antimicrobial agents with reactive double bonds such as Sorbic Acid, Itaconic Acid, Nisin, and Linalol …).

The second type represents the antimicrobial agents with no reactive site. During polymerization, there will be transfer reactions. These agents will not react with the monomers under UV irradiation. This will then stop the reaction resulting in the appearance of free channels, not related to the network. The radical polymerization will then start on another monomer group (HEMA / PEGDMA) and thus participate to the creation of free channels in the polymer. Finally, it will have a lower density and the properties will be changed with the reduction of Tg values (Antimicrobial agents with no reactive bonds such as Citric Acid, Thymol, Carvacrol, Chlorhexidin, Zinc, Silver, Phenyl ethanol …).

Then, this part represents the particular case of antimicrobial agents. Some additives exist which possess the two capacities of reaction mentioned previously, because these have the two types of groups, reactive and non-reactive sites. That is the reason why the final plasticized effect represents a combination of the two phenomena (Copolymerization and transfer reaction).

The sorbic acid will have two impacts: a plasticizing effect during radical polymerization, leading to a decrease in glass transition, and the presence of double bonds which are able to react with the network and thus form a different final material. Another example is the citric acid: it does not have double bonds but will also act in two ways during the synthesis of materials. The first action is a plasticizing effect of the active agent during radical polymerization. The second effect is different comparatively to the copolymerization seen previously. The acid will act as transfer agent and because it does not have double bonds, it will cause chain ruptures.

Two other antimicrobial agents used represent particular cases: Nisin and Linalol. For Nisin, the final glass transition is equal to -18.5°C and for the Linalol, this same value is equal to -7.6°C. In these two cases, there is a reduction effect of Tg for the methacrylate matrix.

However, the results have also shown another specific nature of antimicrobial agents, in particular, Potassium Sorbate, which is a salt of sorbic acid. The particularity of Potassium sorbate is that it has a permanent ion which will be able to form ionic bonds. These ionic compounds could be responsible for the creation of supramolecular bonds. These additional bonds created inside the polymer network will compensate the plasticizing effect resulted by the introduction of fillers. That is why the glass transition obtained at the end is equal to 18.7°C.

- *Releasing of the different Antimicrobial agents*

Antimicrobial agents can act in two different ways. Either by diffusing out of the material and act on bacteria, or by remaining fixed to the material and act by direct contact with bacteria. It can also act at the same time by diffusion and contact with bacteria to maximize these possibilities of action. The

material was tailored as a hydrogel allowing the diffusion of water and microbes into the gels and also the diffusion of antimicrobial agents outside of the gels.

A diffusion study was first made by HPLC. The results were depicted in Fig. 3 a) for 72h of immersion and Fig. 3 b) for 168h.

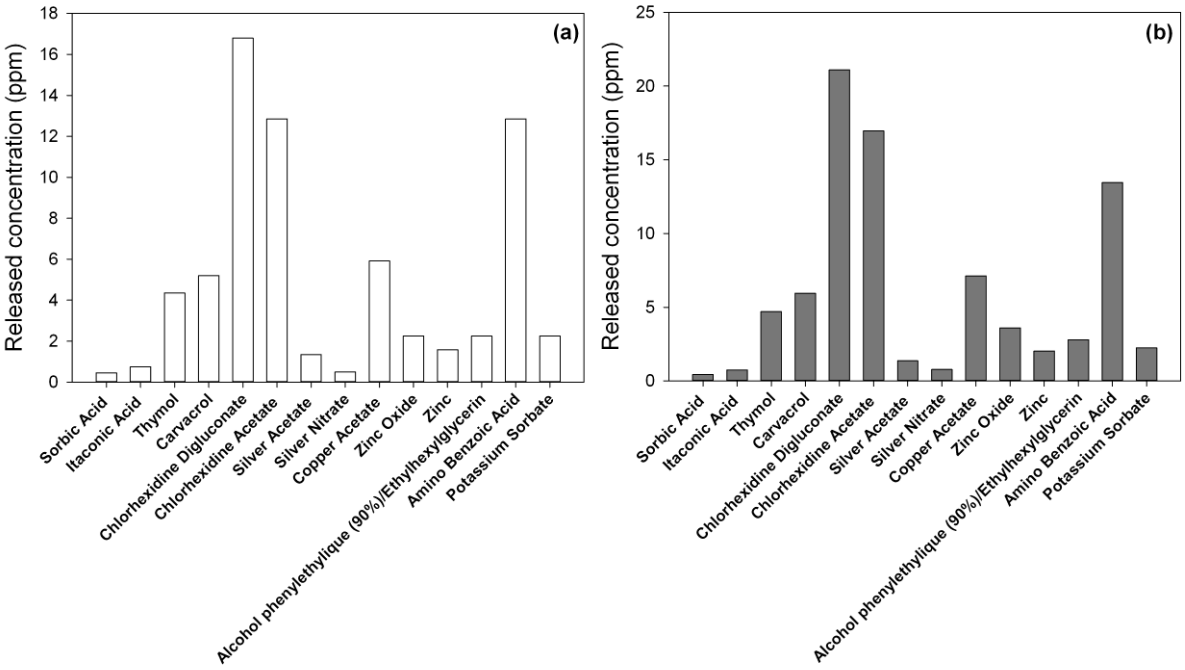

FIG. 3. (a) Releasing behaviors in ppm for each antimicrobial film after a test period of 72h; (b) Releasing behaviors in ppm for each antimicrobial film after a test period of 168h.

Theses analyses have demonstrated some capacities of diffusion by the final materials with variable results in function of the type of antimicrobial agent used.

Actually, as seen in the previous part of the analysis of thermal properties by DSC, some active agents have the possibility to react with the acrylate network during polymerization. The material synthesized is a copolymer formed by just one network. This capacity to form the material is caused by the presence inside the antimicrobial agents of reactive sites which can participate to the reaction under UV irradiation. The diffusion of these antimicrobial agents is very low.

On the contrary, other additives used in this work do not react with the polymer. These agents are not attached to the network and can therefore move inside the polymer and diffuse outside to obtain an antimicrobial activity.

Concerning the results, generally, it is possible to see that the diffusion is concordant with the different types of antimicrobial agents (reactive or not). The results show that some non-reactive agents do not diffuse into the aqueous environment. Finally, the antimicrobial agents will stay preferably in the hydrogel.

For organic agents, some of them have reactive double bonds that copolymerize with the other monomers during the polymerization under UV irradiation. This was the case of Sorbic and Itaconic Acids. As expected, the final amount released was less than 0.8 ppm after 7 days.

For the additives without double bonds, the releasing behavior is conditioned by an equilibrium state between their solubility in the aqueous environment and their interaction affinity with the matrix. Chlorhexidine and amino Benzoic Acid have a high solubility in water and so explain the important amount released equal respectively to 21ppm and 14 ppm after 7 days. On the contrary, the less soluble agents such as thymol, carvacrol, or blends composed of 90wt% of phenylethylic alcohol and 10wt% of ethylhexylglycerin have finally a lower diffusion capacity.

Concerning the inorganic agents, such as Silver Acetate, Silver Nitrate, Copper (II) Acetate, Zinc and Zinc oxide, their solubility in water is weak. For this reason, the results have shown a weak release from all these antimicrobial agents.

- *Antimicrobial activities of coatings by direct contact with the microbial strains*

Depending on their structure and their reactions or interactions with the matrix, some antimicrobial agents will rather have more contact activity while others will be released from the matrix and act directly in the external environment. This justifies the existence of two types of tests, each of them being more adapted to these two extreme behaviors.

Experiments were made to keep as well as possible all the parameters constant and varying only the nature of the antimicrobial agent.

These first tests represent a comparison of the biological activities of various agents listed introduced within the same matrix. Results of antimicrobial activities in an agar environment (contact) for the microorganisms P. Aeruginosa (Gram-), S. Aureus (Gram+) and A. Brasiliensis (Yeast) are depicted in Fig. 4 a); Fig. 5 a) and Fig. 6 a).

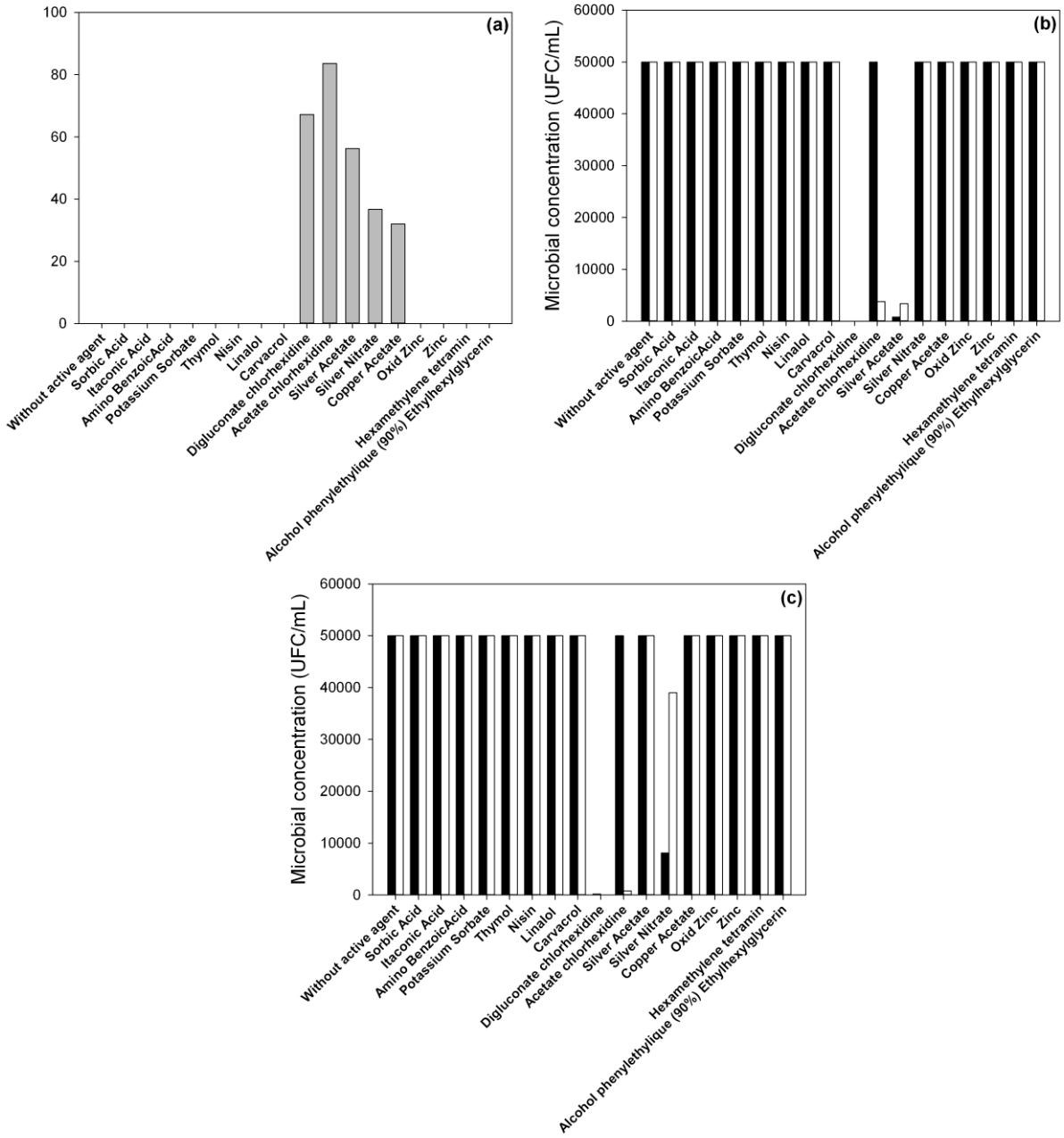

FIG. 4. a) Antimicrobial evaluation of Inhibition zone diameters (%) measured after a time of 72h of incubation in an agar environment: P. Aeruginosa (Gram-); b) Bacterial concentrations in suspensions for P. Aeruginosa RUN 1 (C0 = 2800UFC/mL): 24h of immersion (black); 72h of immersion (white); c) Bacterial concentrations in suspensions for P. Aeruginosa RUN 2 (C0 = 5500UFC/mL) 24h of immersion (black); 72h of immersion (white).

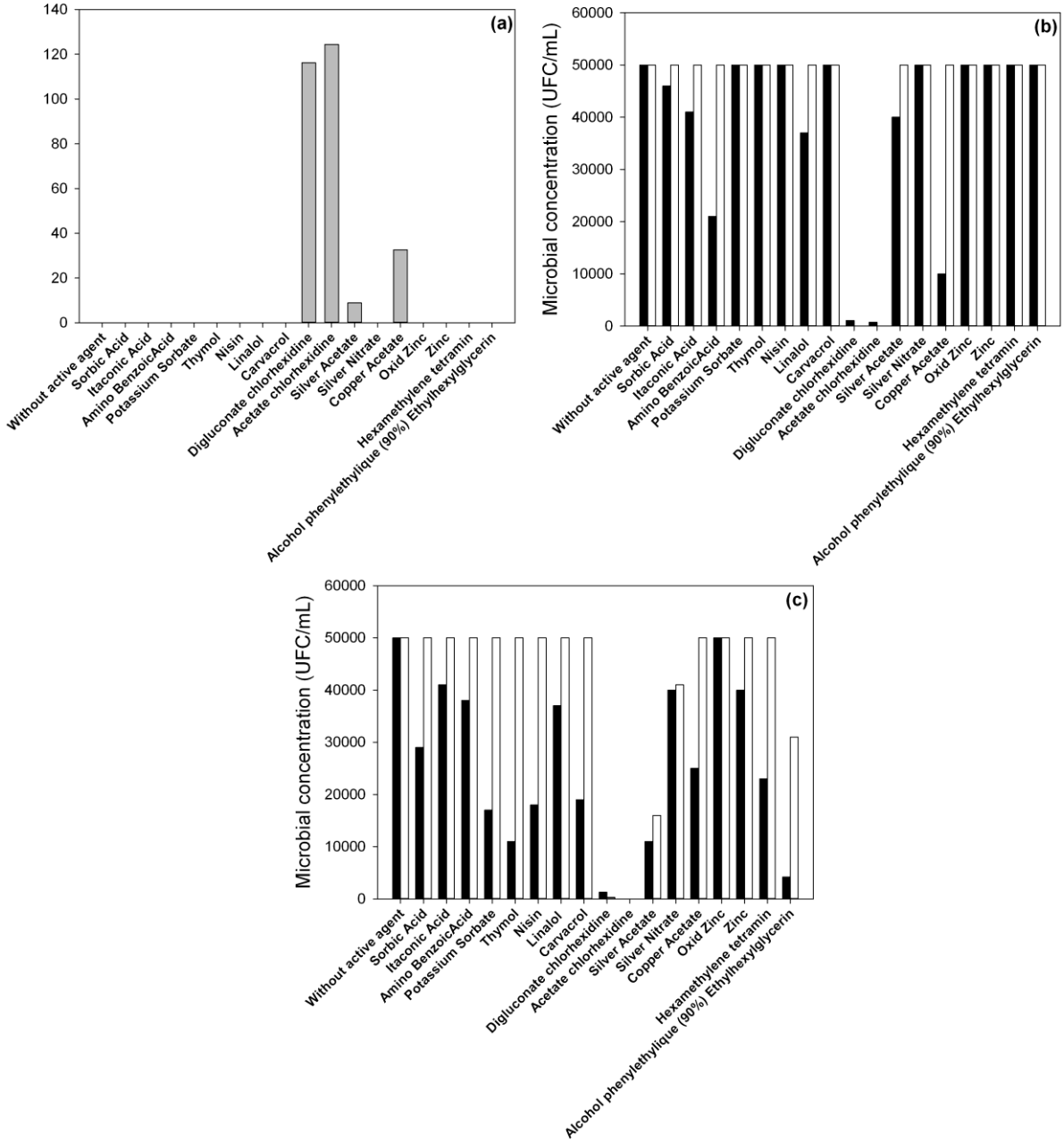

FIG. 5. a) Antimicrobial evaluation of Inhibition zone diameters (%) measured after a time of 72h of incubation in an agar environment: S. Aureus (Gram+); b) Bacterial concentrations in suspensions for S. Aureus RUN 1 (C0 = 5200UFC/mL): 24h of immersion (black); 72h of immersion (white); c) Bacterial concentrations in suspensions for S. Aureus RUN 2 (C0 = 3700 UFC/mL): 24h of immersion (black); 72h of immersion (white).

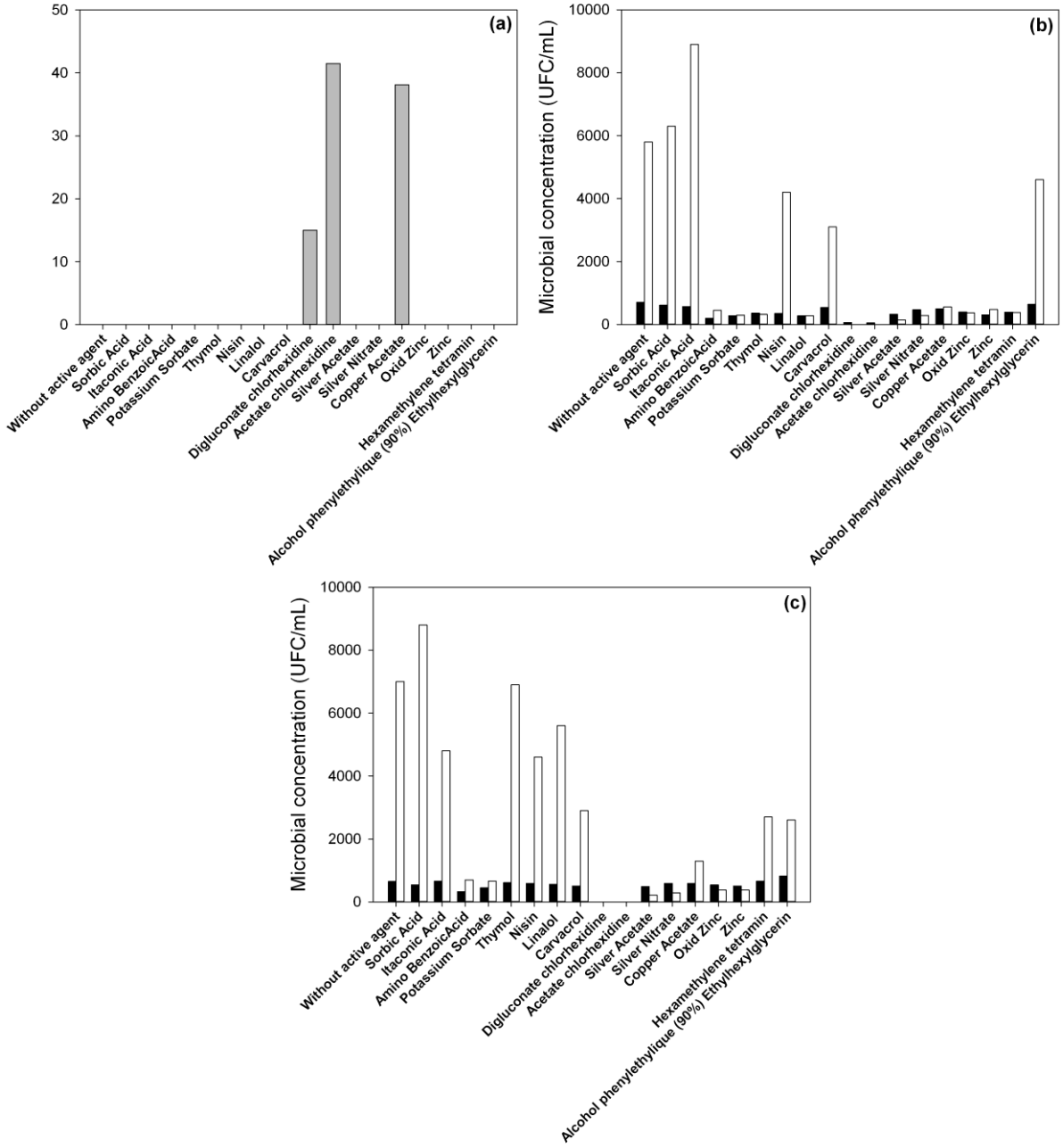

FIG. 6. a) Antimicrobial evaluation of Inhibition zone diameters (%) measured after a time of 72h of incubation in an agar environment: A. Brasiliensis; b) Yeast concentrations in suspensions for A. Brasiliensis RUN 1 (C0 = 280 UFC/mL): 72h of immersion (black); 168h of immersion (white); c) Yeast concentrations in suspensions for A. Brasiliensis RUN 2 (C0 = 580UFC/mL): 72h of immersion (black); 168h of immersion (white).

First, it is important to note that the HEMA/PEGDMA matrix without additive has no antimicrobial activity with any presence of inhibition zone. The result confirms the possibility to use this material as reference matrix.

Few ingredients have interesting results, such as Copper (II) Acetate or Chlorhexidine (digluconate and Acetate).

Concerning copper acetate, Fig. 4 a) shows antimicrobial activity with the appearance of inhibition zones for some strains. For example, a contact of the coating with P. aeruginosa causes an inhibition percentage of approximately 60%.

For the other example, chlorhexidine proves to be the most effective antimicrobial agent tested with the appearance of important values of inhibition zones. On Fig. 4 a) and Fig. 5 a), the results show a strong capacity of action with the appearance of inhibition zones for P. aeruginosa and S. aureus. The example of S. aureus shows an inhibition of approximately 100% after contact, and the zone of inhibition corresponds to a final diameter of 20.3mm (Initial diameter → 10mm).

These tests have identified several effectively active agents in a same initial matrix (Referential formulation). Four agents seem to be very active: chlorhexidine acetate, chlorhexidine digluconate, copper acetate, silver acetate and silver Nitrate. In comparison, all the other ingredients used showed no activity with results equivalent to the acrylate matrix reference (without antimicrobial agent).

- *Evaluation of the antimicrobial activities of the coatings by immersion in bacterial suspensions with different concentrations*

Antimicrobial evaluation was done in a humid environment, namely bathing medium (Aqueous solution charged with NaCl compound), which will allow the diffusion of the active ingredients through the matrices. The test was associated with the releasing analysis and consisted of an immersion of the samples in a liquid suspension containing a fixed concentration of pathogenic microorganisms. The coatings were left in the solutions for a fixed time and then the bacterial strains concentrations were again evaluated to see if there was a reduction or an increase (comparatively to a reference). The first objective was to evaluate the antimicrobial activity by immersion (aqueous environment) for each antimicrobial agent used. Another objective was to check if these activities were linked to the diffusion capability. All the results are depicted by the bar charts on Fig. 4 b) c); Fig. 5 b) c) and Fig. 6 b) c).

The different strains tested have specific particularities. For example, Microbial germ S. Aureus is one that is the most representative of the targeted application (protection of packaging) after the first direct contact between the consumer and the material. It is a human bacteria and represents the total range of

Gram(+) microbes. The other germs, P. Aeruginosa and A. Brasiliensis are more virulent and present in other sectors, as medical or biomedical (Medicine, hospital…).

From the tests, certain agents have revealed an antimicrobial activity. Several metallic salts as the copper (II) Acetate, the silver acetate or the silver nitrate have slowed the microbial growth (P. Aeruginosa, S. Aureus and A. Brasiliensis) comparatively to other antimicrobials used.
Then, certain acids and terpens have antimicrobial capacities against S. Aureus but no systematically.
However, these tests seem demonstrated that the Chlorhexidines represent the best antimicrobial agents. For each strain, the matrices with 5wt% of chlorhexidine digluconate and 5wt% of Chlorhexidine acetate cause a complete reduction of the microbial concentration (72h of immersion for S. Aureus and P. Aeruginosa and 7 days for A. Brasiliensis).
Then, the tests have shown that the acid agents improve the growth of A. Brasiliensis (Fig. 6 b) and Fig. 6 c)) with final concentrations higher than the reference material. It seems that the yeasts in contact with an acidic environment have better opportunities to proliferate.

As in protocol 1, this second test has identified the particular efficiency of the same five antimicrobial agents listed, the two Chlorhexidines (Chlorhexidine digluconate, and Chlorhexidine acetate), copper (II) acetate, silver acetate and silver Nitrate. By contrast, all other agents tested were less effective and the results demonstrate similar activities to an acrylate coating without antimicrobial agents. These observations are important for the final choice.

- *Determination of antimicrobial effects at equivalent concentration (EEC)*

By HPLC tests, it appears that the higher releases are for CHX (digluconate and acetate). This shows that this antiseptic acts as filler and does not react with the final radical matrix. This may explain the important antimicrobial activity observed. However, the previous part has also demonstrated an antimicrobial activity of others agents, in particular metallic salts. But the releasing tests have shown that this type of additives do not release important quantities in the external environment. For this reason, it seemed necessary to establish a rating scale to correlate antimicrobial efficacy and diffusion capacity of the different agents. Therefore, two rating scales have been developed for each test protocol.
For protocol 1, the antimicrobial effect at equivalent concentration (EEC) corresponds to a ratio between the result of inhibition percentage (%) and the quantity released after 72h and measured by the HPLC analysis (ppm).

Concerning protocol 2, the EEC represents a ratio between the values of concentrations (UFC/mL) of bacterial germs (Difference between the sample value measured and the maximum contamination of the liquid suspension) and the quantity released after 72h and measured by the HPLC analysis (ppm).
A specific notification is necessary for the strain A. Brasiliensis. For this, the maximum value of contamination selected is the final concentration of the referential matrix without additive.
The bar chart on Fig. 7 and Fig. 8 give an approximation on the EEC.

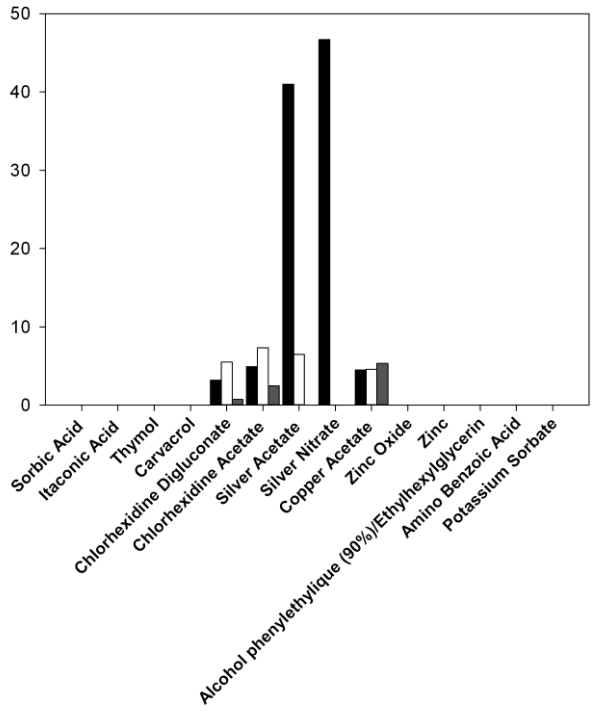

FIG. 7. Representation of the EEC for each antimicrobial agent (Protocol 1): P. Aeruginosa (Black); S. Aureus (white); A. Brasiliensis (Grey).

These approximations give new conclusions on the real antimicrobial effectiveness. Actually, the results on Fig. 7 for the first protocol demonstrate that antimicrobial activity is not nessecary associated with a important release behavior.
It is particularly true for Silver Acetate and Silver Nitrate. In these cases, they reveal a more important antimicrobial activity against P. Aeruginosa comparatively with Chlorhexidine. It is in contrast with the previous results which have shown that Chlorhexidine was the most effective antimicrobial agent.

Then, the results for the immersion tests are summurized on Fig. 8 a), Fig. 8 b) and Fig. 8 c).

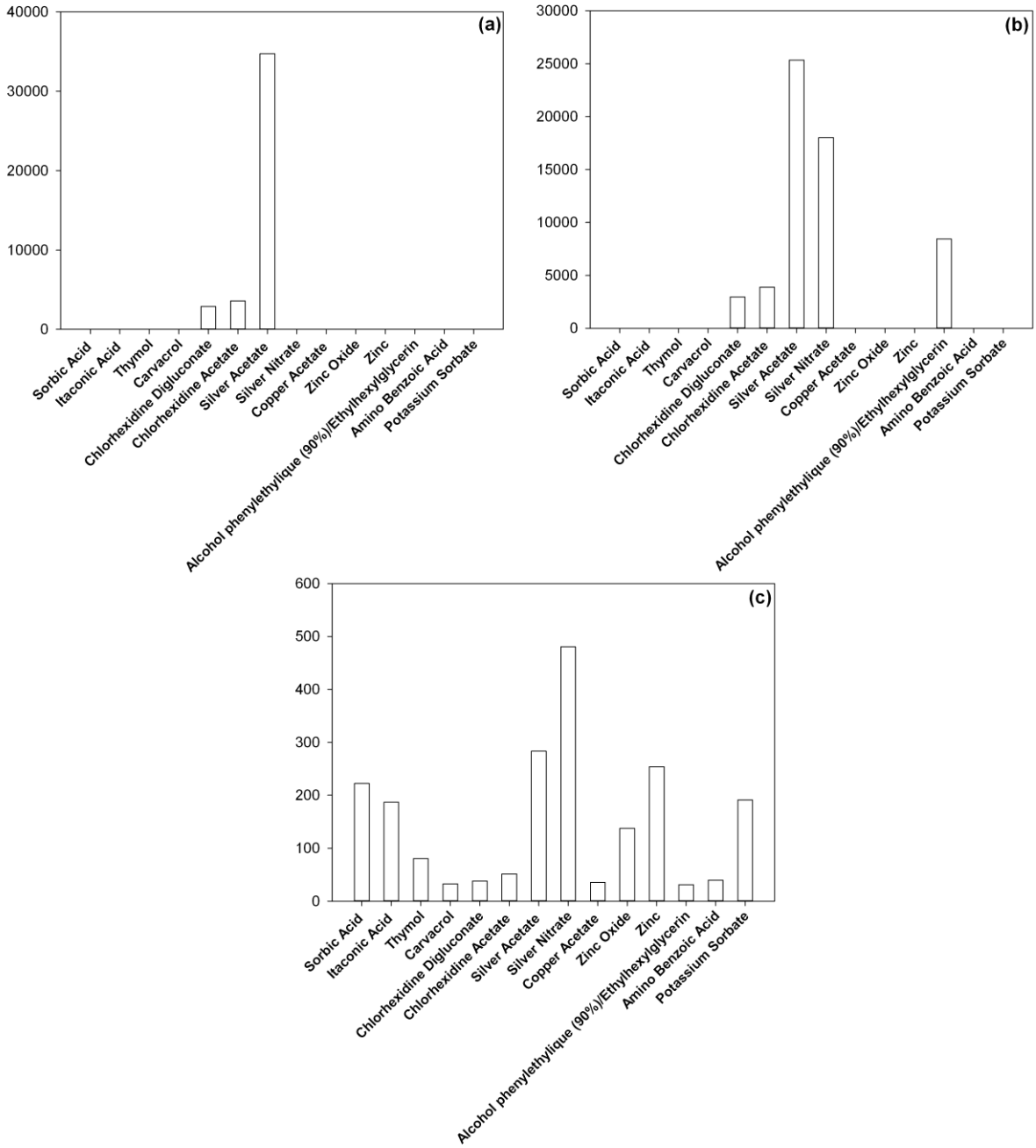

FIG. 8. a) representation of the EEC for each antimicrobial agent ***(Protocol 2)*** *after an immersion of 72h: P. Aeruginosa; b) representation of the EEC for each antimicrobial agent* ***(Protocol 2)*** *after an immersion of 72h: S. Aureus; c) representation of the EEC for each antimicrobial agent* ***(Protocol 2)*** *after an immersion of 72h: A. brasiliensis.*

The figure confirms the previous observations on the fact that the antimicrobial activity is not necessarily associated with a important release behavior. With this calculation of EEC, it seems that

the most effective antimicrobial agents are silver Nitrate and Silver Acetate. It is true for the two bacterial germs P. Aeruginosa and S. Aureus.

Concerning A. Brasiliensis, there are similar results for the two silver salts; however, it is also possible to mention two other antimicrobial agents: zinc and potassium Sorbate.

The results of these two particular additives show a high EEC and so the correlation between antimicrobial activity and release behavior.

This part has a high interest because it provides new essential information. In particular, it seems very important to differentiate between the antimicrobial activity of the matrix without taking into account the release parameter and the same activity but with a correlation between antimicrobial activity and diffusion. In the first case, the results have shown previously that the two most effective agents are chlorhexidine digluconate and chlorhexidine acetate.

However, these new results have demonstrated that other agents seem more effective. Especially, Silver acetate and Silver Nitrate seem to be the most effective antimicrobial compounds. These results will be an important criterion of selection for the final application.

2-5- Conclusion

First, the objective of this work was to identify different antimicrobial agents likely to be used in the cosmetics industry. It was necessary to find a coating system that could be added on specific areas of industrial applications of Packaging to be protected. One of these objectives was to develop manufacturing and characterization protocols to find a really interesting system for the study and industrial applications. Once these steps were done, the choice of polymer coating could be performed. The material selected was a photo-curable coat which is destined to adhere to areas of packaging requiring antimicrobial protection. Then, different active additives selected by a literature study have been introduced in the referential materials. Characterization tests were performed to have direct comparison in the same synthesis conditions. These tests have been used to characterize different properties of final materials. The additives have different impacts on thermal properties of these matrixes with a reduction of glass transition temperature as a general rule.

Regarding antimicrobial properties, this is related to the ability to release the active agents outside hydrogels. Finally, the final antimicrobial coatings have different capacities.

It appears that the release is variable, which may lead to different reaction or diffusion phenomena. Some active agents can react or not with the matrix during polymerization. That is why antimicrobial agents have different diffusion capacities in the external environment.

However, a high diffusion is not systematically necessary to obtain a material with antimicrobial properties. A calculation of the antimicrobial effect at equivalent concentrations has shown that certain antimicrobial agents have an activity for a weak amount released in the external environment.

So, this study has shown that the releasing behavior and antimicrobial activity of all the additives are very important to observe. It is particularly true for an industrial application where other characteristics have a real interest, such as the cost of materials and adaptability to the industrial process.

Finally, concerning the antimicrobial agents tested, several showed an interesting activity, in particular chlorhexidine acetate, chlorhexidine digluconate, silver acetate, silver nitrate and copper acetate.

Acknowledgement

This work was supported through a research program titled STABIPACK II devoted to the development of antimicrobial materials for adaptation to cosmetic packaging. The research program was funded thanks to financial aids provided by a FUI (Fonds Uniques Interministeriels) in the clusters COSMETIC VALLEY and PLASTIPOLIS.

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SUPPORTING INFORMATION

S1. Representation of the first derivative of weight loss by TGA analysis between 30°C and 500°C for a HEMA/PEGDMA (50/50 wt%) matrix.

S2. Comparison between theoretical and experimental values of glass transition of different HEMA / PEGDMA acrylate matrices.

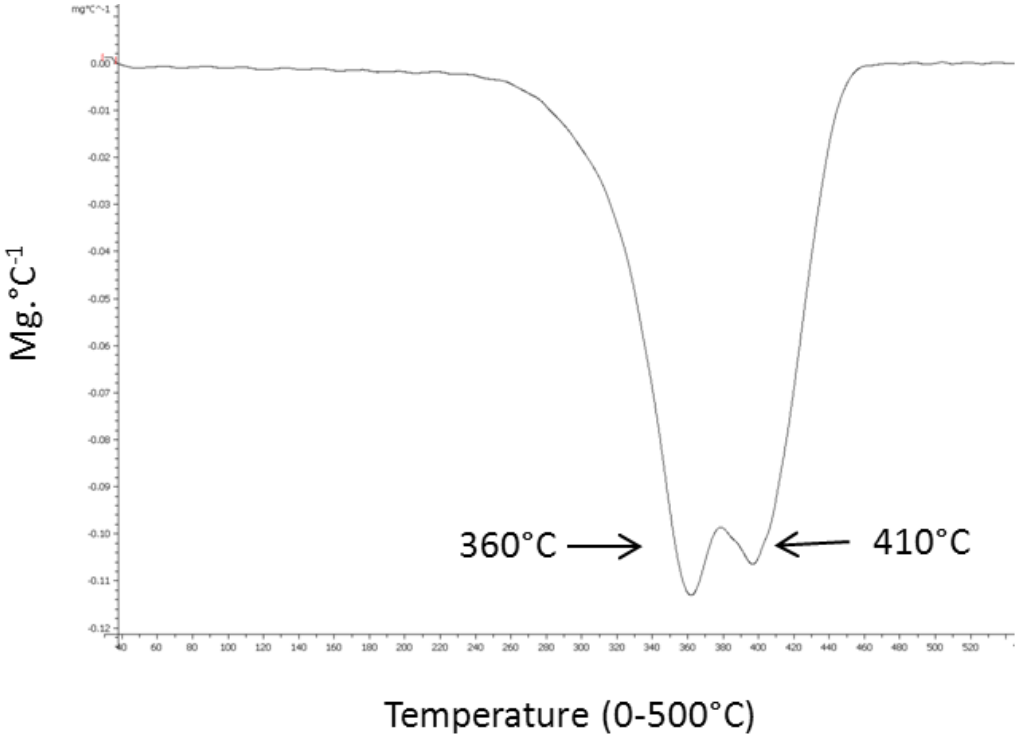
S3. Curves of the storage modulus G' as a function of time measured by rheology in torsion rectangular mode in the rubbery state of materials at 100°C.

S4. Comparative results between two technologies of measurements and different sample sizes→ Rectangular torsion rheology and DMA voltage (thin film).

S5. Comparison of results obtained by two different techniques.

S6. Degree of swelling (%) of acrylate matrix vs time in hours.

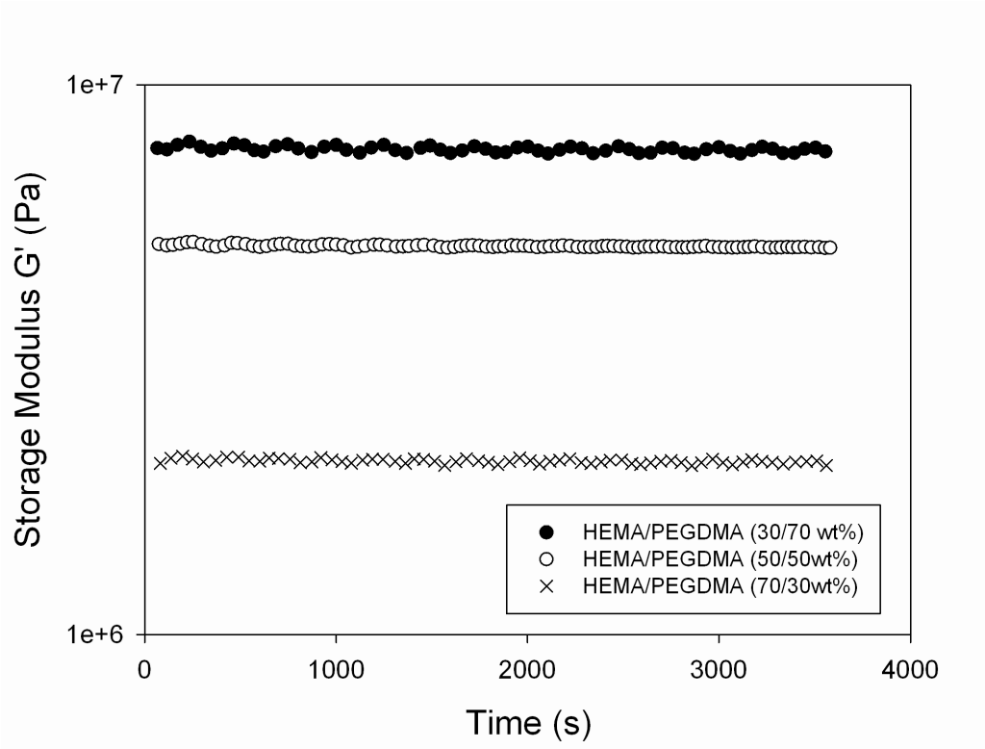
S1. Representation of the first derivative of weight loss by TGA analysis between 30°C and 500°C for a matrix HEMA/PEGDMA (50/50 wt%).



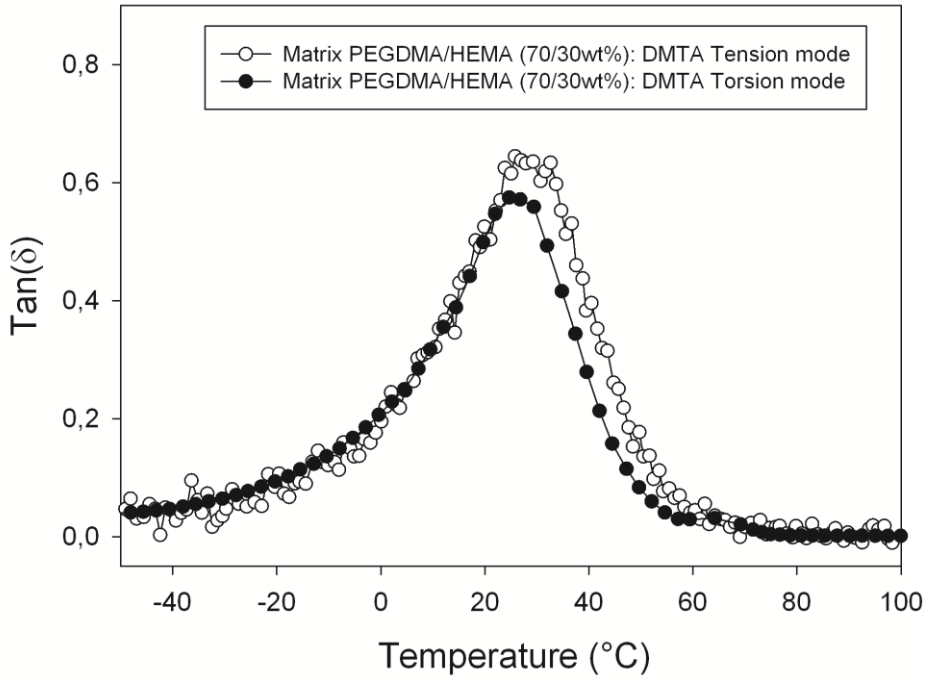
S2. Comparison between theoretical and experimental values of glass transition of different HEMA / PEGDMA acrylate matrices.

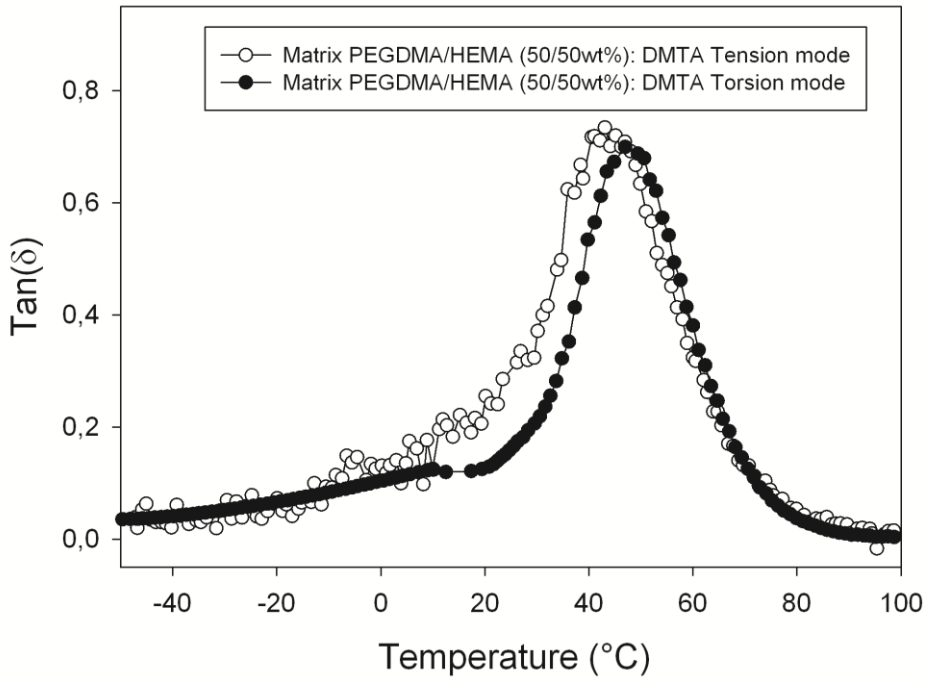
HEMA / PEGDMA (wt%)	Glass transition Tg (° C) experimental DSC	Glass transition Tg (° C) theoretical Fox Equation
100 / 0	89* [49-51]	89
70 / 30	46	47
60 / 40	31	36
50 / 50	23	25
30 / 70	0.7	5.1
0 / 100	-20	-20

S3. Curves of the storage modulus G' as a function of time measured by rheology in torsion rectangular mode in the rubbery state of materials at 100°C.



S4. Comparative results between two technologies of measurements and different sample sizes → Rectangular torsion rheology and DMA voltage (thin film).

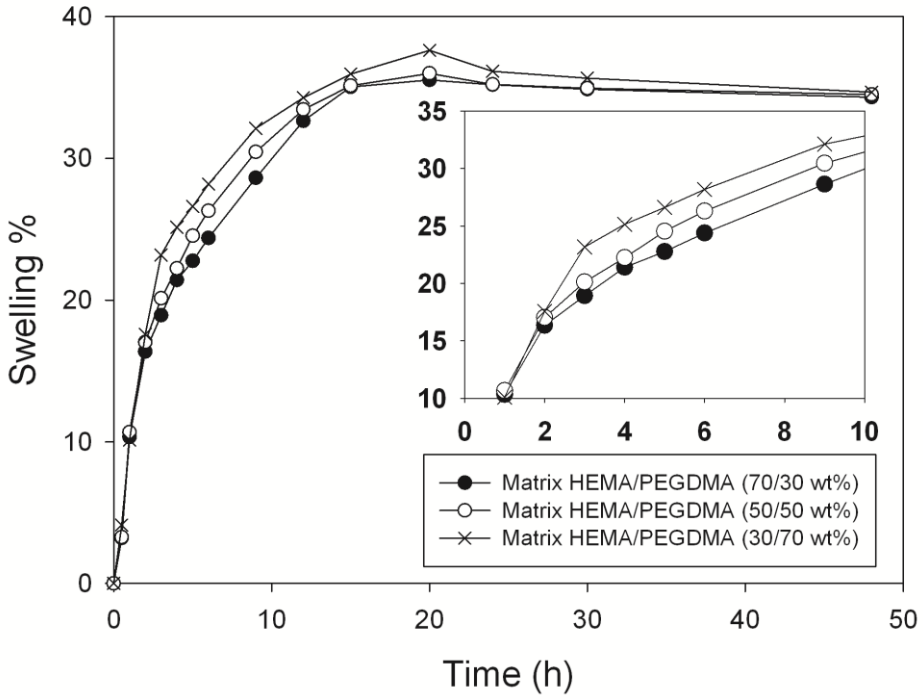




S5. Comparison of results obtained by two different techniques.

HEMA / PEGDMA (wt%)	Test type	α Relaxation (°C)
50/50	Torsion Rectangular	48°C
50/50	DMA tension	42°C
30/70	Torsion Rectangular	28°C
30/70	DMA tension	27°C

S6. Degree of swelling (%) of acrylate matrix vs time in hours.



CHAPITRE 3 :

Ce troisième chapitre est également présenté sous forme d'une publication. Cet article a été soumis dans la revue « Journal of Applied polymer Science ».

Il fait suite à la première étude de recherche sur la comparaison directe des agents antimicrobiens. Un choix de deux agents a été fait. Ce sont un acétate d'argent et un acétate de cuivre (II).

Cette partie étudie l'impact de ces sels métalliques à différentes concentrations sur le polymère méthacrylate de référence.
Pour la réalisation des matériaux, la même technologie de polymérisation radicalaire sous irradiations UV a été employée. Tous les matériaux ont été fabriqués en couche mince de 100µm sur des supports de type EVOH. Cette technique a permis par la suite de pouvoir décoller les films des supports et caractériser les propriétés thermiques, stabilité thermique, viscoélastique, morphologique, libération en milieu aqueux. L'objectif étant d'évaluer les impacts de ce type d'agents inorganiques sur les propriétés d'un polymère réticulé.
De plus, la première partie avait permis de révélé une capacité réactionnelle de ces sels métalliques utilisés à une certaine température, pouvant être causée par un phénomène de décomposition. C'est cela qui permet généralement la formation de certains composés ou particules élémentaires pouvant prendre des formes sphériques avec des tailles nanométriques.

La partie introductive présente le contexte de cet article, avec des informations sur les matériaux utilisés. Celle-ci apporte également des informations sur le fait qu'actuellement, et à notre connaissance, pas ou très peu d'études portent sur l'introduction de composés inorganiques de tailles micrométriques dans des polymères réticulés.

Une partie « Matériel et méthodes » décrit les techniques de fabrications et de caractérisations des matériaux antimicrobiens.

Dans la partie « Résultats et discussions », différents résultats sont présentés. Cela commence par une présentation de la stabilité thermique des polymères par analyses ATG. Ensuite des analyses thermiques et thermomécaniques sont décrites en fonction de la nature des actifs, mais également de leurs concentrations dans les réseaux. Ensuite, une étude morphologique par microscopie électronique à balayage est également présentée, afin de vérifier l'homogénéité de dispersion des charges au sein des matériaux. Dans cet article est également présentée la libération des sels en milieu aqueux selon la concentration. Les principales conclusions sont :

- Les sels métalliques d'argent et de cuivre ont été sélectionnés suite à la première étude qui a démontré une certaine activité antimicrobienne.

- L'introduction des agents antimicrobiens améliore les modules de conservation dans l'état vitreux et caoutchoutique des réseaux méthacrylates. Cette introduction provoque également une diminution de la température de transition vitreuse finale.

- Tous ces paramètres sont associés à la concentration introduite qui amplifie les phénomènes précédemment cités.

- La densité des matériaux est également modifiée suite aux différents ajouts d'agents inorganiques. Les valeurs de densités sont augmentées par les introductions ce qui réduit du coup les valeurs moyennes de masses molaires entre nœuds de réticulation. Ce changement de densité est lié à la réactivité des agents avec les matériaux méthacrylates, modifiant du coup les réseaux finaux. Ces densités variables vont également jouer sur les possibilités de diffusions des antimicrobiens et donc les libérations de ces derniers dans un environnement aqueux.

- Un dernier point important de cette étude est l'apparition d'une séparation de phase dans les réseaux méthacrylates avec certaines zones fortement chargées en sels métalliques.

Effects of antimicrobial agents on the thermal and mechanical properties of acrylate hydrogel matrices

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3-1- Abstract

Polymer materials with antimicrobial activity are prepared by UV polymerization of acrylate and methacrylate mixture at room temperature. The antimicrobials are silver acetate and copper acetate, used without pre-treatment. Their chemical stability in the acrylate matrix and their effect on the thermal and mechanical properties of the polymer matrix are investigated as a function of their concentration up to 15 wt%. Physico-chemical, thermal, rheological and morphological analyses as well as the surveillance of metal salts release in aqueous medium are conducted. A significant decrease in the thermal stability of the salts, introduced into the acrylate matrix after UV treatment, is observed. The metal salts also have significant effects on the properties of the matrix. A plasticization and densification of the material associated with an aggregation of salts up to the phase separation at the highest concentration are highlighted. At equal concentrations, the effects are more pronounced in the presence of copper salts. The latter was released more slowly than silver salts from acrylate material.

3-2- Introduction

Acrylate hydrogels are materials with many advantages which explains their particularly frequent use in medical and biomedical domains, or more specifically for contact lenses. They are biocompatible with a low toxicity, so they represent a safe material for any user or consumer. Another important advantage is their high swelling capacity in particular environments, for example with aqueous solutions with high humidity levels. [1-4]

Hydrogels may be synthesized according to different polymerization methods (gamma ray, UV light, heat application) to finally obtain the formation of networks. For this reason, these materials can be easily integrated in an industrial process. [5-6]

The antimicrobial activity of hydrogel materials can be obtained relatively easily by dispersing the agent in acrylate monomer solutions [7-9] or after polymerization, by immersing the hydrogel in an aqueous solution with an active agent.[10-12]Another possibility is to conduct a reaction between monomers and an active agent to synthesize active copolymers. [13-15]Two types of antimicrobial materials may be obtained. The first type is a material where the antimicrobial agent diffuses to be directly in contact with the bacteria and act against their proliferation. The second possibility is to elaborate a material with active sites grafted in the polymer matrix. In this case, the pathogen germs will come in contact with the active sites which will have a bactericidal action. [16-19]

Different antimicrobial agents have been used in the past such as antiseptics (fluorescein, ciprofloxacin, Chlorhexidine, diacetate, Gatifloxacin ...), [20-23] oxides (zinc oxide), [24-25] peptides (maximin lipopeptide -4), [9, 26] antimicrobial polymers (chitosan, Polyvinylpyrrolidone ...) [27] or metal compounds (nanometric particles or silver nitrate). [28-31]

In our previous study, antimicrobial activity of acrylate hydrogels with copper acetate and silver acetate as agent, introduced before polymerization, has been demonstrated and compared with seventeen other molecules. [32] These metallic salts obtained one of the best activities. Several families of pathogens have been tested: P. Aeruginosa, S. Aureus or A. Brasiliensis. These different microbial compounds represent germs susceptible to proliferate in cosmetics products.

The antimicrobial activity of metal particles, such as silver or copper, is known but their action mechanism is not yet fully understood. Very probably, the metal acts as active agent under its ionic form (Ag^+ for silver, for example) in moist environment. [33-34] These ionic compounds will come into contact with the cell walls of microbes and will act on their integrity to prevent proliferation. Presently, metallic salts are mainly used as ionic tanks to generate elementary nanometric particles before their dispersion in materials and to obtain antiseptic effectiveness. The direct dispersion of salts in materials prevent any contact between metallic nanoparticles and the human body and could be a safe alternative for antimicrobial materials elaboration. Moreover, a partial redox reaction between

cationic silver or cationic copper and anionic acetate groups may occur allowing the formation in situ of neutral solid filler.

The objective of this work is to evaluate the chemical stability of copper acetate and silver acetate in acrylate matrix after UV polymerization and the impact of salts on the acrylate matrix properties. In particular, the effects on the thermal properties, visco-mechanical characteristics, morphological aspect and release behavior of the two salts and their respective concentrations will be studied. To the best of our knowledge, very few studies have focused on the effect of antimicrobial agents on these polymer matrix properties. Our materials will be used to protect strategic areas of cosmetic packaging by the treatment of a thin film coating.

3-3- Materials and methods

- *Materials*

2-hydroxyethyl methacrylate (HEMA), silver and copper acetates and polyethylene glycol dimethacrylate (PEGDMA), benzophenone (BP) and 2,2-diethoxyacetophenone (DEAP) were purchased from Aldrich and used as received without further purification. The tertiary amine was an experimental product (CN381) and kindly delivered by Sartomer.

- *Synthesis of acrylate films*

The photo initiating system was a homogeneous solution composed of 60wt% tertiary amine (CN381), 20wt% Benzophenone (BP) and 20wt% 2,2-diethoxyacetophenone (DEAP). The two monomers, poly (ethylene glycol) dimethacrylate (PEGDMA) and 2-hydroxyethyl methacrylate (HEMA) were mixed with a mass ratio of 70:30, respectively. To this blend, an equivalent of 5 wt% of the photoinitiating system was added. The antimicrobial agent was dispersed in the monomer mixture with different mass concentrations equal to 5wt%, 10wt% and 15wt % prior to the photoinitiating system addition (% relative to total blend).
The irradiation system was a U.V. light (Banc Fusion UV system F300S (fusion system)) associated with a conveyor belt to pass the sample under the U.V. lamp. with wavelengths between 220 nm and 300 nm The wavelengths and dose measurements were assessed by a system named Power puck UV radiation (UV Power puck II from EIT Company). This irradiation step was repeated 5 times for an optimal curing of the monomer solution and finally to obtain the network. Thus, the resulting system is a polyacrylate coat with a thickness of around one hundred micrometers.

- *Characterizations*

- *Thermal analysis by DSC*

Differential scanning calorimetry (DSC) measurements were carried out with a Q10 calorimeter from TA Instruments. The samples with masses between 3 and 4 mg were transferred to hermetic pans, sealed, and analyzed from -80°C to 200°C with a heating rate of 10°C.min^{-1} (2 cycles). The glass transition temperatures were collected by identifying the inflection points.

- *Thermogravimetric analysis by TGA*

A thermogravimetric analyzer (TGA) from Mettler Toledo, TGA/DSC 1, was used. Thermal degradation experiments were done under nitrogen purge with a flow rate of 80 $mL \cdot min^{-1}$ for all experiments. Samples ranging from 15 to 20 mg were heated from 30°C to 500°C with a heating rate of $10^{o}C \cdot min^{-1}$.

- *DMTA measurements*

The dynamic mechanical thermal analyses of the PEGDMA/HEMA network films were performed with Rheometrics Solids Analyzer (RSA II, TA-Instruments) to obtain tensile dynamic mechanical spectra. The samples were cut with a predetermined shape (40mm x 10mm x 100µm) from the synthesized samples. For dynamic tensile measurements, a nominal strain at 0.1% was fixed, with an applied frequency of 1 Hz. Storage modulus E', loss modulus E" and loss factor tan δ were determined as a function of temperature. Data were taken from -50°C to 100°C using a heating rate of $3^{\circ}C \cdot min^{-1}$. Each sample was equilibrated in the same chamber under dry nitrogen at the starting temperature prior to running the test.

- *Observation of morphologies by Scanning electronic microscopy*

SEM (HIROX SH 4000M Scanning electron microscope) was used to observe the morphology of the different surfaces of the films. The surface of each sample was covered with a gold-palladium layer prior to analysis (DENTON VACUUM DESK V: 40mA/30s).). The size of the dispersed phase was evaluated with "Image J" software.

- *Release of antimicrobial agents*

The release kinetics of the antimicrobial agents from an immerged piece of PEGDMA: HEMA (wt% ratio fixed at 70:30) matrix in 10g ± 0.02g of reference water (Distilled Water used for standard curves) was followed for several hours by UV spectroscopy (Agilent Technologies Cary Series 100 UV-VIS Spectrophotometer) at room temperature and under magnetic stirring. The volume of each sample was equaled to approximately 18.2 ± 4.8 mm^3 and with masses between 19 and 32mg.
The methacrylate matrices presented very small quantities of residual monomers and photo-initiator but according to our calibration curves carried out in the same conditions, they absorbed 5 to 10 times more than the antimicrobial agents. So it was necessary to correct the measured absorbance at 224nm. This correction allowed the evaluation of metallic salts released by minimizing masking effects of the photo-initiator and the monomer.

- *Density measurements*

The determination of densities of the methacrylate matrices was done by measuring the dimensions of each sample associated with a measure of weight. Dimensions were measured with a digital caliper 0-150mm of reference “otelo”. The thicknesses were measured with an elcometer. Weight was obtained with a precision of 0.1mg.

3-4- Results and discussion

The metallic salts used as antimicrobial agents in this work are powders. Characteristics of these powders have been analyzed and reported before the study on the impact of the salts dispersion on the physico-chemical, thermal, rheological and releasing properties of an acrylate matrix elaborated from blend PEGDMA/HEMA (70/30).

3-4-1- Characteristics of the antimicrobial agents

The silver acetate powder was mostly needle-shaped with a shape factor of about 12 (average length=30 μm and average width = 2.5μm). The copper (II) acetate powder had a shape factor closer to 1 with a size of around 5.1 μm but highly agglomerated in large grains with an approximate dimension of 76μm (Figure 1). The densities of these powders were respectively equal to 3.26g.cm^{-3} and 1.88g.cm^{-3} for silver and copper. These values were higher than the density of the neat acrylate matrix (1.29g.cm^{-3}).

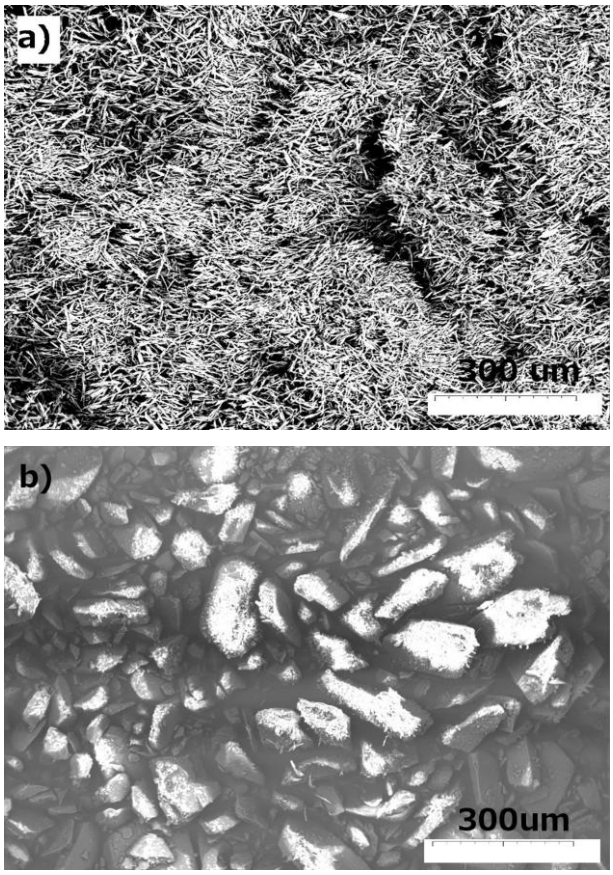


FIGURE 1: ***a)*** *SEM images* ***(X100)*** *silver Acetate powder;* ***b)*** *SEM images* ***(X100)*** *Copper (II) Acetate powder*

The thermal stability of the metallic salts was verified by TGA analysis. The results are summarized in table 1.

TABLE 1: Thermal stability of silver Acetate and copper (II) Acetate (Pure compounds)

Powder	Degradation steps number [30-500°C]	Degradation temperature (°C) (inflexion point)	Weight loss Δm (%)
Silver Acetate	1	293	35
Copper (II) Acetate	3	273	25
		288	23
		301	21
			69

The loss of weight of two salts was initiated around 180°C and was terminated at 290-300°C but it was not total. For the silver acetate, only one step of thermal degradation was observed with a weight loss equal to 35%. By contrast, 3 steps of decomposition around 270-300°C were observed for copper acetate and for each step, the compound lost approximately 23 wt%. The total decomposition of acetate groups can be considered and verified from the calculation of the relative mass proportion of acetate group in the salts, which is equal to 35 % in silver salt and 65% in copper salt. The weight losses determined from TGA analysis correspond fairly well to the initial relative proportion of acetate in the salts.

These results are in agreement with those in the litterature. [36, 38-40] According to Logvinenko et al. [36] an intramolecular reduction of silver ions in smaller metallic particles ($Ag^{+} \rightarrow Ag^{0}$) occurs with a release of CO, H_20, CO_2, CH_3COOH and small quantities of radicals during the decomposition process. Beyond 300°C, a recrystallization of fine metallic particles with a porous structure and the formation of oxides were recently characterized by XRD, FE-SEM, TEM and XPS techniques [37]. The particles' size of the Ag nano-crystals was in the range of 10–35 nm. The decomposition of copper salts is more complex ($Cu^{2+} \rightarrow Cu^{+} \rightarrow Cu^{0}$) and poorly studied. [39-40]

3-4-2- Effect of the metallic salts on the thermal stability of acrylate hydrogels

The thermal stability of acrylate films with different concentrations of metallic salts was also verified by TGA analysis. Figure 2 depicts the weight losses of samples under thermal treatment without or with 5% of metallic salts.

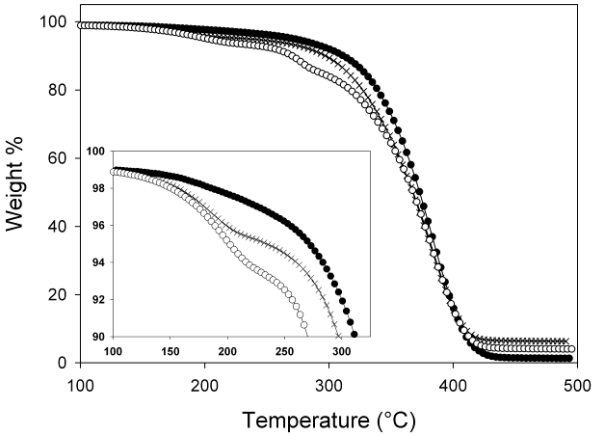


FIGURE 2: TGA curves of PEGDMA/HEMA/ (70/30) without antimicrobial agent (black dots); with 5wt% of Silver Acetate (cross dots); with 5wt% of Copper (II) Acetate (white dots)

Figure 2 shows that an introduction of metallic salts in an acrylate matrix prevents the total degradation of materials with the presence of residual components at the highest temperatures while the neat matrix undergoes a complete degradatation. Two to three steps of degradation were observed which can be attributed to the filler for the first steps and to the acrylate matrix for the last step. A range of about 100-110°C was noted between the first steps and the last. The weight losses at the first step represent about 80% of the quantity of salts introduced in the matrix. Figure 3 highlights the impact of the matrix on the salts' thermal stability and conversely, the impact of the salts' presence on the thermal stability of the acrylate film. A weakening of the salts' thermal stability in the matrix was observed. The neat salts are decomposed around 270-290°C, while in acrylate films the decomposition temperature is decreased in the 210-220°C range. On the other hand, the decomposition temperatures of the polymer matrix, which correspond to the last step, are not significantly modified by the presence of metallic salts. So, metallic salts did not seem to interfere with the polymerization and crosslinking between the two monomers. An intermolecular interaction metallic salt-acrylate matrix such as the coupling force and hydrogen bonding between the Cu ($CuSO_4$) and the hydroxyl groups of HEMA observed by Liu et al [41] is not probable in our acrylate films.

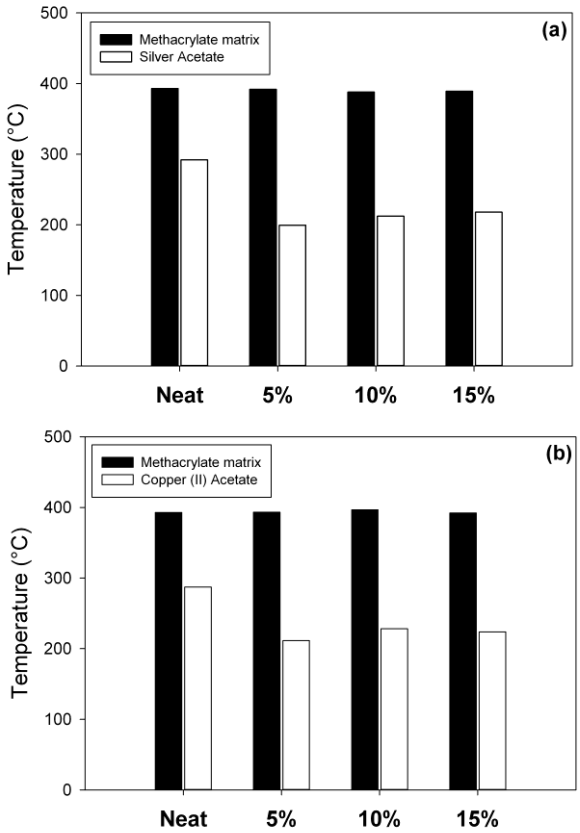


FIGURE 3: Decomposition temperatures of the metallic salts and the methacrylate matrices under their neat form and in blend with 5,10 and 15%w of metallic salts: Silver acetate ***(a)****; Copper (II) Acetate* ***(b)***

The thermal stability decrease of salts in an acrylate matrix can be explained on the one hand by a lowering of ionic interations between acetate and silver ions when they are dispersed in methacrylate matrix, and on the other hand by the reactivity of metallic salts under U.V. irradiation applied during the process of fabrication. Previous works have already shown the ability of these salts to be reduced in metallic nanoparticles under such conditions [42-43]. In our elaboration process, the salts were dispersed before U.V. polymerization. Their characteristic change of color was observed after treatment.

3-4-3- Effect of the metallic salts on thermal properties

The thermal properties of the matrix with different concentrations of metallic salts, determined by DSC analysis, are summarized in tables 2 and 3.

TABLE 2: Glass transition temperature of PEGDMA/HEMA (70/30) matrix with different mass concentrations of Silver acetate

Silver salt concentration (%w)	Tg heating Cycle 1 (°C)	Tg heating Cycle 2 (°C)	Delta Tg Heating cycles 1 and 2 (°C)
0	-7.2	-14.8	-7.3
5	-14.3	-24.1	-9.8
10	-11.9	-27.7	-15.7
15	-10.8	-31.5	-20.7

TABLE 3: Glass transition temperature of PEGDMA/HEMA (70/30) matrix with different mass concentrations of Copper acetate

Copper salt concentration (%w)	Tg heating Cycle 1 (°C)	Tg heating Cycle 2 (°C)	Delta Tg Heating Cycles 1 and 2 (°C)
0	-7.2	-14.8	-7.3
5	-8.4	-21.8	-13.5
10	-12.9	-35.1	-22.2
15	-16.8	-43.8	-27.1

Glass transition temperatures are much lower than polymerization temperatures under U.V. irradiation. Without salts, the glass temperature of PEGDMA/HEMA (70/30) matrix is observed at -7 °C for the first heating and -14°C for the second heating. In the presence of metallic salts, these glass transition temperatures decrease. This effect is more pronounced after a first heating cycle and depends on salt concentrations. For the copper salt, the correlation between glass transition temperature Tg and concentration was clearly observed for each cycle. At its highest concentration, the Tg lowering reaches -29°C. The difference of Tg observed between the two cycles is probably caused on the one hand by a slight degradation of the polymer matrix (note the thin thickness of formed films) and on the other hand by a partial reduction of the metallic salts under thermal treatment around 200°C, as mentioned before.

It is known that the shape, size, toughness and covalent or no-covalent surface interactions of fillers might bring about changes in the Tg, both an increase and a decrease of polymer matrix Tg as a function of filler loading [44]. The change depends strongly on surface effects; the matrix Tg can decrease with the flexibility of the polymer-filler interface when the filler is soft, or polymer-filler interactions are weak. [45] The Tg changes can also be related to interface and surface energies [46, 47], a reduction of cross-linking density polymer matrix [48], an aggregation of filler [49], or even residual solvents [50].

In this study, the polymerization was carried out without solvent; very small quantities of residual monomers may be present but the reduction of cross-linking density is not probable because, according to TGA results, the thermal stability of the polymer matrix is conserved in the presence of salt. However, the conversion of metallic salts into metallic silver or copper can cause changes in the filler/polymer interface, especially with higher concentrations. As our study focuses on thin acrylate films, we can consider that the effects are even more pronounced. In addition, exchange reactions between small molecules formed during thermal reduction and acrylate matrix are probable causes.

3-4-4- Effect of the metallic salts on thermo-mechanical properties

The thermo-mechanical properties of acrylate materials with 5wt% and 10wt% of silver acetate and copper (II) acetate were determined by DMTA analysis (tensile mode). Figures 4 a) and 4 b) describe the storage moduli measured as a function of the temperature between -40°C and 100 °C for the two samples' series. The curves of storage modulus show an increasing of values E' at glass state (<20°C) and rubbery state (>40°C). This change was lower at the glass state than at the rubbery state and especially in the presence of copper (II) acetate.

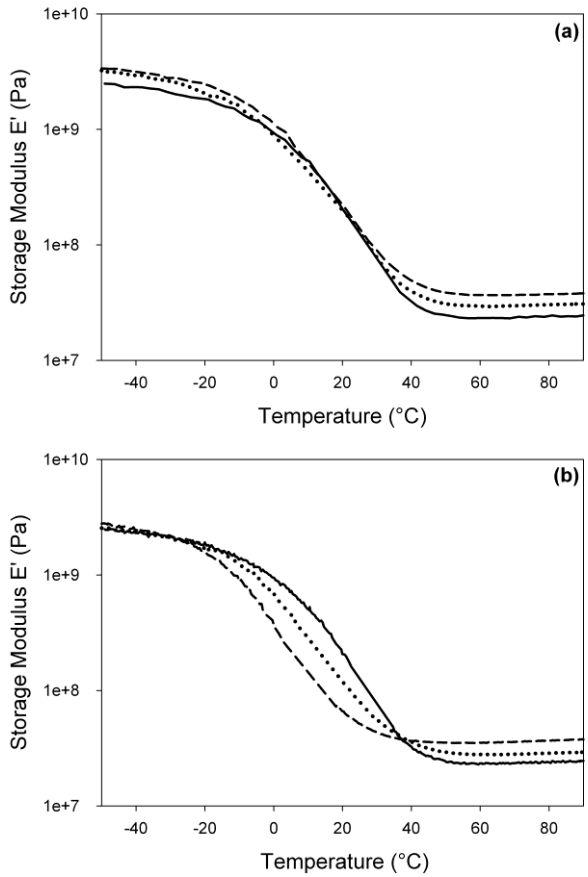


FIGURE 4 : ***(a)*** *Storage Modulus E' of PEGDMA/HEMA (70/30) without antimicrobial agent (full line); with 5wt% (dotted line) and 10wt% of Silver Acetate (dash line) as a function of temperature;*

(b) *Storage Modulus E' of PEGDMA/HEMA (70/30) without antimicrobial agent (full line); with 5wt% (dotted line) and 10wt% of Copper (II) Acetate (dash line) as a function of temperature*

Tα was determined from tan δ and the values are reported as a function of temperature for the two samples' series (Figures 5 a) and 5 b)). Their values are also presented in tables 4 and 5. Figures 5 a) and 5 b) clearly reveal the lowering and the widening of tan(δ) peak when the concentrations of metal salts increase. This trend is particularly pronounced with the copper salts.

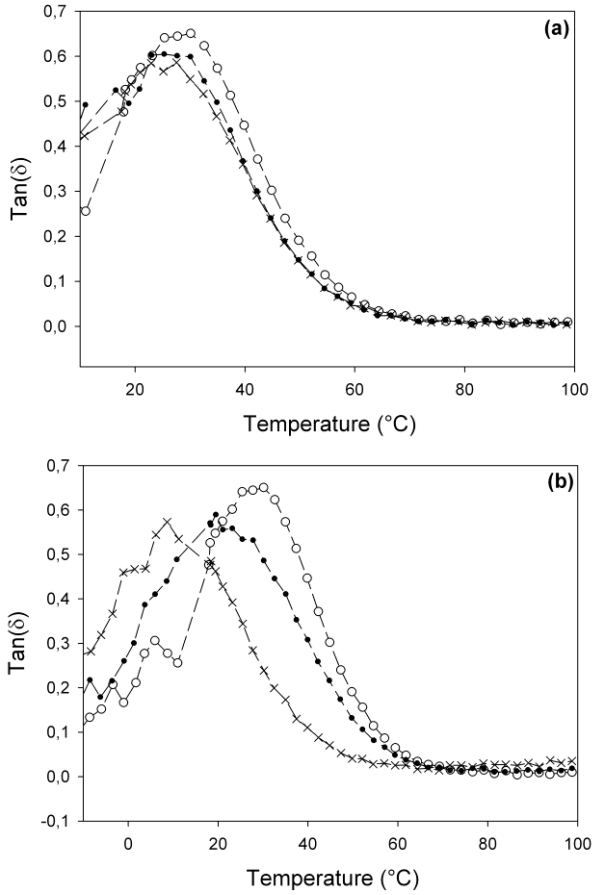


FIGURE 5 : ***(a)*** *Tan (δ) of PEGDMA/HEMA (70/30) without antimicrobial agent (white dots); with 5wt% (black dots) and 10wt% of Silver Acetate (cross dots) as a function of temperature;* ***(b)*** *Tan (δ) of PEGDMA/HEMA (70/30) without antimicrobial agent (white dots); with 5wt% (black dots) and 10wt% of Copper (II) Acetate (cross dots) as a function of temperature*

So, the Tα value (tables 4, 5) of filled matrices decreases by 5 to 20°C compared to that of the initial acrylate matrix. The highest reduction was also observed with copper (II) acetate. These DMTA results are in good agreement with DSC results.

TABLE 4: Tα and storage modulus (in glass and rubbery states) of PEGDMA/HEMA (70/30) in the presence of silver acetate

wt% Silver salt in PEGDMA/HEMA (70/30 %wt)	Tα (°C)*	Storage Modulus (Pa) Glass state (-50°C)	Storage Modulus (Pa) Rubbery state (95°C)
0	28.7	$2.49.10^9$	$2.50.10^7$
5	24.7	$3.26.10^9$	$3.12.10^7$
10	23.8	$3.39.10^9$	$3.82.10^7$

* Maximum of peak of tan (δ)

TABLE 5: Tα and storage modulus (In glass and rubbery states) of PEGDMA/HEMA (70/30) in the presence of Copper (II) acetate

wt% Copper salt in PEGDMA/HEMA (70/30 %wt)	Tα (°C)*	Storage Modulus (Pa) Glass state (-50°C)	Storage Modulus (Pa) Rubbery state (95°C)
0	28.7	$2.49.10^9$	$2.50.10^7$
5	18.2	$2.62.10^9$	$2.97.10^7$
10	7.4	$2.75.10^9$	$3.82. .10^7$

* Maximum of tan (δ) peak

In fact, the filled matrices have a higher hardness associated with an enhancement of the damping capacities. These results have demonstrated plasticizing effects (reduction of relaxation temperatures) with better mechanical properties (higher storage modulus for each state of acrylate materials). Therefore, why does the matrix loaded with metallic salts exhibit lower Tg (or Tα) and higher E' values in the rubbery and glassy regions than those of the neat methacrylate networks? Regarding the increase of the storage modulus, this effect is caused by the introduction of fillers, which generally generates the improvement of this parameter in the rubbery state. In figures 5 a) and 5 b), a little shoulder of tan(δ) peaks towards lower temperature and what is more, a decrease of intensities and a broadening of tan(δ) peaks appear when the quantity of metallic salts increases. As for the offset of Tα, this phenomenon is more important concerning the copper (II) Acetate and can be explained by a strong aggregation of salts.

The literature is poor on the subject but some works have mentionned the question, in particular Cho and al. in 2006 [51] who studied the impact of inorganic molecules on the viscoelastic behavior of different systems (acrylate/methacrylate-based polymers or epoxy-based polymers). First, the authors have interpreted this behavior as an increase of the density of the polymer network with the formation of more flexible siloxane nodes. Suriati et al [49] have observed a Tg decrease with storage modulus increase and an anomalous increase of the coefficient of thermal expansion after Tg in silver-filled epoxy-composites which it has been explained by an aggregation of silver at higher concentrations, especially for silver nanoparticles, which have higher surface energy than silver flakes. However, this

phenomenon remains difficult to explain, especially in our case, because we cannot ignore the fact that the matricial polymers can react between each other, for instance by transesterification exchanges between hydroxyl groups of HEMA of a chain with the ester group of HEMA of another chain, and that they may consequently participate to modulus increase.

3-4-5- Determination of densities and average molar mass between crosslinks

The filler-polymer matrices' densities can be calculated from the theory of blends by equation (1) [35]. The acrylate films' densities were measured and then compared to these theoretical values (Tables 6 and 7).

$$\rho_{pm} = \frac{\rho ms * \rho np}{\rho np * mms + \rho ms * (1 - mms)} \quad (1)$$

Where ρ_{pm}, ρms and ρnp are the densities of the polymer matrix, metallic salt and neat polymer, respectively, and mms is the mass fraction of metallic salts.

TABLE 6: Densities for PEGDMA/HEMA (70/30) *films with Silver Acetate*

Silver salt (wt%)	Density ($kg.m^{-3}$) (experimental)	Density ($kg.m^{-3}$) (theoretical)
0	1291	1291
5	1294	1331
10	1443	1374

TABLE 7: Densities for PEGDMA/HEMA (70/30) *films with Copper (II) Acetate*

Copper salt (wt%)	Density ($kg.m^{-3}$) (experimental)	Density ($kg.m^{-3}$) (theoretical)
0	1291	1291
5	1490	1312
10	1601	1333

Since the salts are denser than the acrylate matrix, experimental densities of blends are expected to be higher than that of the original matrix and to increase with the salts' concentration. In fact, they are higher overall than theoretical values. The largest differences are observed for acrylate films with copper acetate at the highest concentration. These results are in agreement with the storage moduli increase highlighted from DMTA results. In filler-polymer composites, a positive difference between the experimental densities and expected densities is often attributable to the nucleation of filler crystals in the loaded material. [35]

3-4-6- Effect of the metallic salts' concentration on polymer system morphology

The morphologies of acrylate materials loaded with several concentrations of silver acetate and copper (II) acetate (5, 10 and 15 wt %) were verified by SEM analysis. Images in Figure 6 and 7 show the filled film surface for each sample.

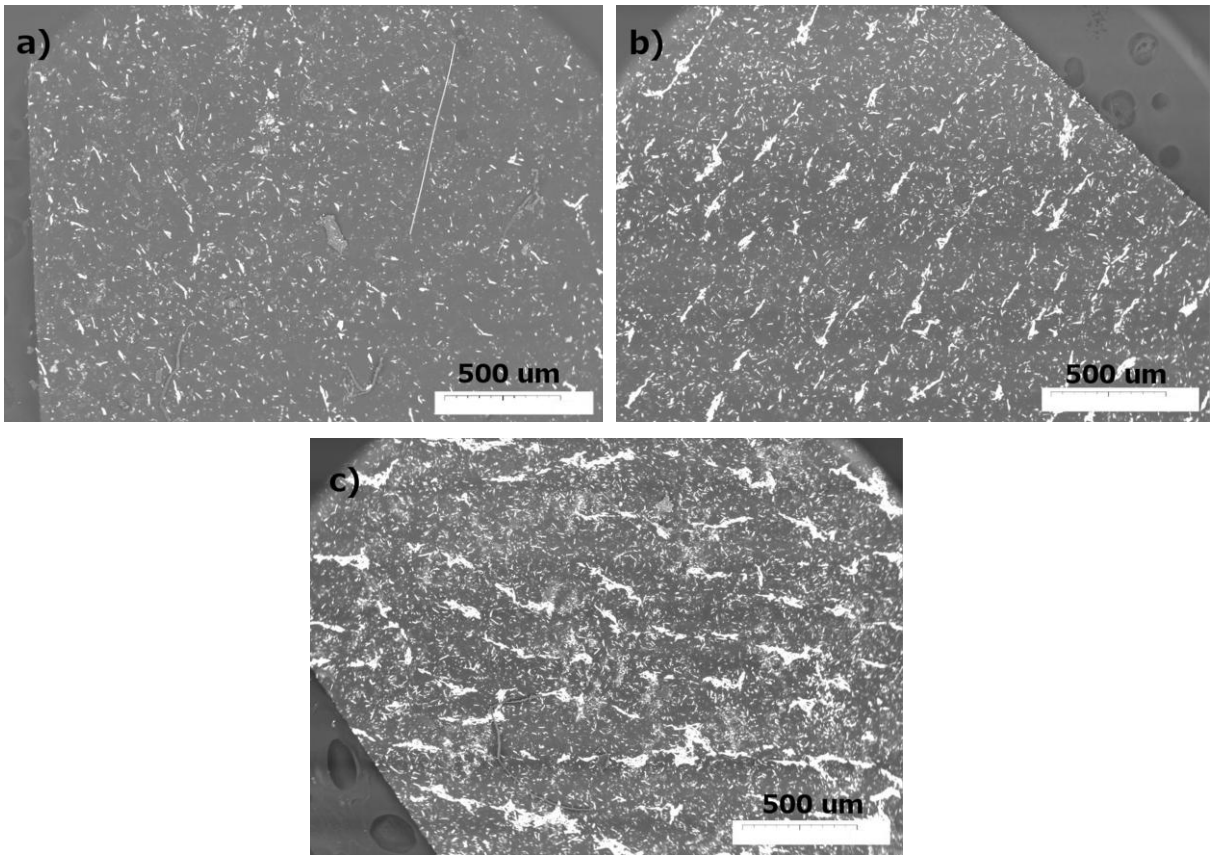
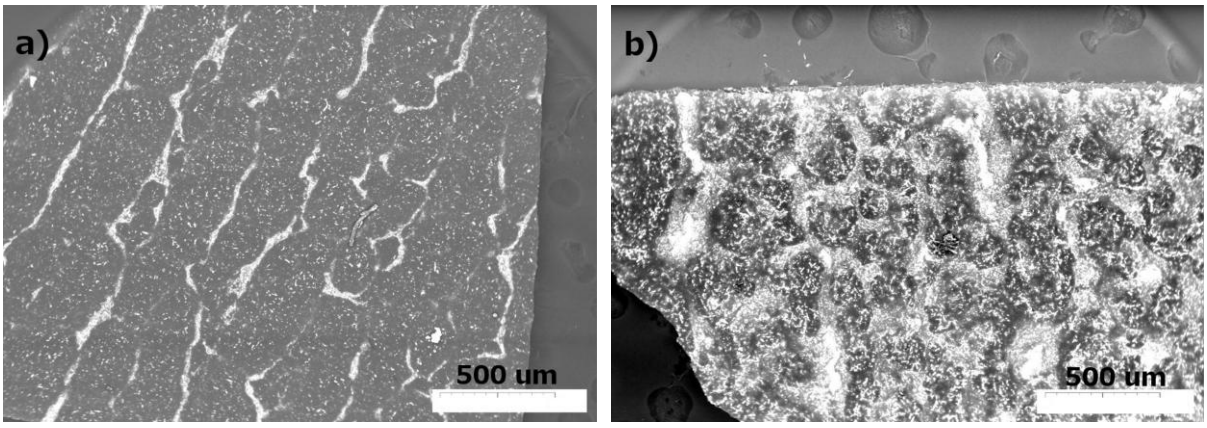


FIGURE 6: SEM images of polymer PEGDMA /HEMA (70/30) with silver acetate (concentration of 5 ***(a)****, 10* ***(b)*** *and 15wt %* ***(c))*** ***(X 50)***



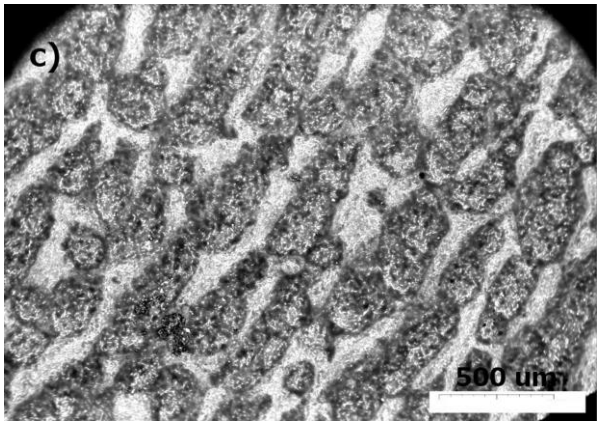


FIGURE 7: SEM images of polymer PEGDMA /HEMA (70/30) with copper (II) acetate (concentration of 5 ***(a)****, 10* ***(b)*** *and 15wt %* ***(c))*** ***(X 50)***

As depicted on figure 6 and on figure 7, salt particles (white stain) appear dispersed or aggregated in all blends except for acrylate matrix with 5% of silver acetate. It seems that one of the phases is rich in polymer with metallic salt particles well dispersed and the other is rich in metallic salt aggregates and poor in polymer. For the blend with 5% of silver acetate, a homogenous dispersion of needle particles is obtained without significant change of form and size. The particles have a shape factor of 13.4 with similar dimensions, very close to that of initial crystals. For blends with 10 and 15% of silver acetate, the formation of needle aggregates growing in a specific direction with the concentration can be observed; at 10%, length = 177 μm and width = 39 μm and at 15wt%, length = 300 μm and width = 57 μm.

All acrylate films with copper salt contain dispersed particles in the polymer matrix and concentrated aggregates; these aggregates grow in a preferred direction with salt concentration and seem to tend towards percolation. The shape of copper acetate particles does not seem to change in the different blends, with a shape factor of about 1 and a size of approximately 9-10μm.

We can conclude that morphologies of the two series of acrylate blends are similar. The most highlighted difference between these series seems to be the level of percolation threshold. In the presence of copper acetate, it appears for a concentration close to 15% in the presence of copper salt while this phenomenon is much less marked at the same concentration in silver salt.

These observations are consistent with the DMTA and densities results; they confirm the aggregation effects on the Tg and the storage modulus. It is possible that the aggregates act like noncovalent crosslinks and generate the formation of a network with a more important density. [51, 59-62]

<u>3-4-7- Release of metallic salts from acrylate blends</u>

The objective of the test is to compare the release of metallic salts from acrylate matrix as a function of salt and of its concentration in an aqueous environment at room temperature. It has also a good interest for the final industrial application of antimicrobial packaging.

Figure 8 depicts U.V. absorbance after correction as a function of time for the different acrylate materials with silver acetate and with copper (II) acetate.

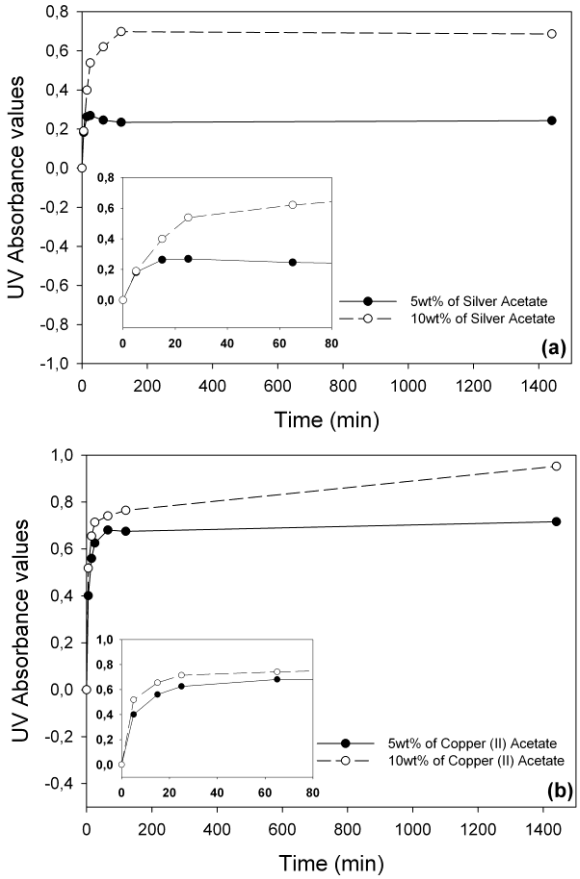


*FIGURE 8: absorbance values of released metallic salts from matrices with **(a)** 5wt% and 10wt% of silver acetate; **(b)** 5 and 10wt% of copper (II) Acetate*

In the two series, the releasing of salts was very short for no evolution of absorbance was observed after an immersion in aqueous solution for 60 minutes. Therefore a first conclusion is that these materials are more suitable to industrial applications with a necessity of rapid action.

Table 8 presents the rates of released agents calculated from mass ratios of released quantity upon initially introduced quantity.

TABLE 8: Rate of salts release in aqueous medium at room temperature

PEGDMA/HEMA (70/30)	Rate of release (%)
5wt% of Silver Acetate	100%
10wt% of Silver Acetate	100%
5wt% of Copper (II) Acetate	98.7%
10wt% of Copper (II) Acetate	67.6%

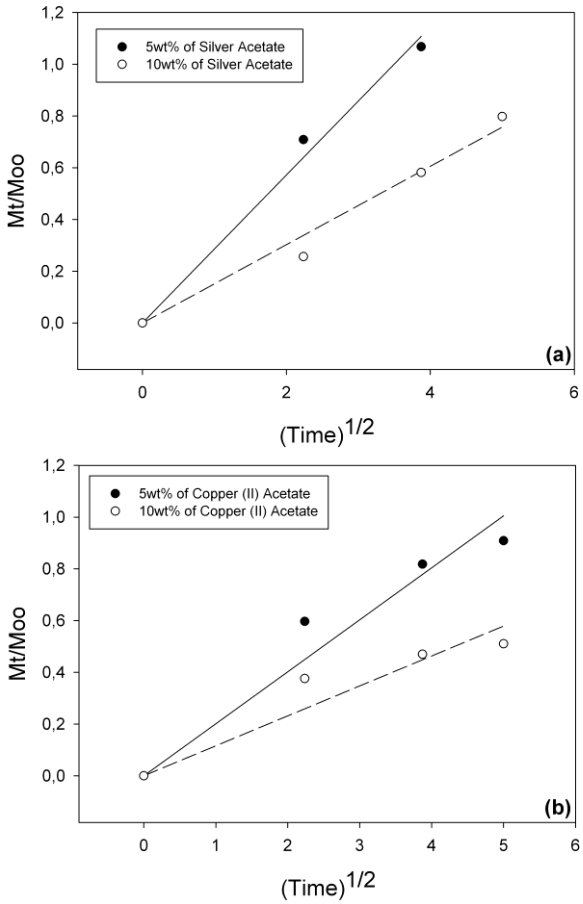


*FIGURE 9: Verification of the Fickian law for the matrix with **(a)** 5wt% and 10wt% of silver acetate; **(b)** 5 and 10wt% of copper (II) Acetate*

For the matrices with silver acetate, the totality of antimicrobial agents was delivered after 24 hours. This observation is also observed for the matrix with 5wt% of copper (II) acetate but not with 10wt% of copper (II) acetate. It appeared that the released rate after an immersion time of 24 hours was only equal to 67.6%. In our conditions, acrylate materials release silver acetate more easily than copper acetate. A verification of the Fickian law was performed and the ratio ($Mt/M\infty$) as a function of the square root of time is described on figure 9. Mt represents the mass of metallic salt at a specific time t. $M\infty$ is the mass of metallic salts at the infinity time. Here, $M\infty$ was calculated by the average of each weight of salts in water measured after 60 min (except for the matrix with 10wt% of copper (II) acetate where $M\infty$ = initial mass = 2.9 mg). The results were compared in table 9.

TABLE 9: Average total mass of metallic salt released in aqueous medium

	Average of M∞ (mg)	Standard deviation (mg)
5wt% of Silver Acetate	0.95	0.06
10wt% of Silver Acetate	2.7	0.2
5wt% of Copper (II) Acetate	1.43	0.04
10wt% of Copper (II) Acetate	2.9	/

The results on figure 9 show a linear relation between the ratio (Mt/ M∞) and the square root of time for a period of about 20 minutes. It can be concluded that the releasing of salts from the acrylate materials follow a Fickian diffusion law in this time interval.

TABLE 10: Slope in the linear domain for each sample

	slope in the linear domain
5wt% of Silver Acetate	0.278
10wt% of Silver Acetate	0.161
5wt% of Copper (II) Acetate	0.183
10wt% of Copper (II) Acetate	0.102

The slopes of the linear regression curves, given in table 10, are representative of the diffusion coefficient. The results show that the matrix with silver salt has a better diffusion capacity comparatively to copper for a similar concentration. Then, the concentrations of metallic salts also have an impact on diffusion. It decreases when concentration increases. The density of networks is usually considered as a key factor of agent release. A denser material will have a lower diffusion capacity. [41, 52-56] These release properties are closely in agreement with the experimental densities of filled acrylate films (tables 6 and 7).

3-5- Conclusion

Acrylate materials with several concentrations of silver acetate and copper (II) acetate (5-15 wt %) were elaborated by UV polymerization. These salts were used because they have previously revealed an antimicrobial activity in the same materials which is interesting for industrial applications.

The main objective of this work was to evaluate the impact of these antimicrobial agents and their concentrations on the thermal, thermo-mechanical and releasing properties of acrylate thin films. Then, morphology studies were carried out in order to better understand the variations of these properties.

The results of this study have shown that the dispersion of silver acetate or copper (II) acetate (between 5-15%w) does not modify the thermal stability but enhances the storage modulus in the glassy and rubbery states and decreases the glass temperatures Tg of the acrylate materials. However, the thermal stability of metallic salts is weakened in acrylate matrices and what is more, metallic salts undergo a partial chemical reduction during the elaboration process. The plasticizer effect could be explained by an increase of free volumes in the final matrix, which could be localized at the interface between the salts and could facilitate the mobility of the macromolecular chains at its vicinity. This effect increases with salt concentration. On the contrary, the enhancement of the storage modulus is linked to an increase of the density of materials with measured values higher than the densities predicted by the theory of blends. These density differences increase with salt concentration and can be attributed to the growth of filler crystal nucleates in the material. There are preferential interactions between metallic particles with intensities higher than that of Polymer-salt particles interaction. Finally this translated in a preferential network of the metallic particles and could explain the appearance of phase separation at higher concentrations. It appeared in this work that the density of acrylate matrix is the key factor of the releasing properties of the antimicrobial agents in an aqueous environment. The slowest release of metal salts are obtained from the densest acrylate matrices in which the salts have percolated.

Acknowledgement

This work was supported through a research program titled STABIPACK II devoted to the development of antimicrobial materials for adaptation to cosmetic packaging. The research program was funded thanks to financial aids provided by FUI (Fonds Uniques Interministeriels) in the clusters COSMETIC VALLEY and PLASTIPOLIS.

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CHAPITRE 4 :

Ce quatrième chapitre est rédigé comme les précédents, sous forme d'une publication destinée à être soumise dans une revue scientifique.

Comme dit précédemment, une sélection a été faite sur les sels métalliques, et plus précisément, l'acétate d'argent qui avait révélé une bonne activité antimicrobienne dans la première partie de la thèse. Ce chapitre aborde l'introduction de ces sels dans une matrice à haute température. Il s'agit d'utiliser des copolymères EVOH qui sont des matériaux souvent employés dans la conception des emballages.

La technique de mise en œuvre utilise une mini-extrudeuse de laboratoire bi-vis co-rotative. Toujours dans le but de s'approcher d'une application industrielle.

Une fois la mise en œuvre terminée, diverses caractérisations ont été réalisées afin d'obtenir des informations sur des propriétés thermiques, thermo-mécaniques, stabilités thermiques, morphologies, libérations, et surtout comparaisons des propriétés antimicrobiennes par deux technologies (contact direct en milieu gélosé et test normé ISO 22196 : dénombrement de colonies suite à la mise en contact d'une goutte de suspension microbienne avec les matériaux à tester). Un point important était de vérifier si l'on conservait une activité antimicrobienne suite à des sollicitations thermiques et mécaniques associées à l'extrusion.
S'est posé la question de la réactivité des acétates d'argent par la forte sollicitation thermique et mécanique d'extrusion. En effet, la précédente partie avait permis de montrer que les sels métalliques étaient susceptibles de réagir par des phénomènes de décomposition thermiques.
Pour tenter de reproduire ce type de réactions des sels, certains paramètres d'extrusion ont été modifiés, comme la température ainsi que le temps de séjour. Ces modifications doivent permettre de favoriser les réactions des antimicrobiens et de pouvoir comparer les propriétés finales des matériaux à l'aide des mêmes protocoles de caractérisations.

Les conclusions principales de cette étude peuvent sont les suivantes :

- Il est possible d'introduire les sels dans un copolymère EVOH par une technologie d'extrusion dite réactive. L'introduction est faite par un pré-mélange sans solvant avant ajout des préparations en extrudeuse.

- L'introduction des acétates d'argent modifie certaines propriétés des copolymères EVOH. Ces variations sont liées à la concentration en antimicrobiens mais également à une capacité de

réaction chimique qui a lieu entre les sels et les EVOH au moment de la polymérisation. Cette réactivité a été découverte suite à une étude d'ATG couplée à une analyse FTIR.

- Lors de la mise en œuvre, une réaction chimique se produit entre les acétates et les copolymères EVOH ce qui forme de l'acide acétique. Cette génération d'acide pourrait favoriser une réduction des ions argent Ag^+ en leur forme élémentaire Ag^0. Il est de plus possible que simultanément à cette réaction de réduction ionique, une autre réaction de thermo-oxydation de l'EVOH puisse se produire et être catalysée par la présence de donneurs libres, à savoir des oxydes d'argent AgOC. Finalement, une oxydoréduction des Ag^+ ainsi que des alcools pourrait être obtenue, créant finalement des argents élémentaires, des carbonyles ainsi que des produits dihydrogènes. Par d'autres caractérisations, il a été découvert que ces réactions ne sont que partielles sous certains paramètres d'extrusion et qu'il est nécessaire d'augmenter les contraintes pour pouvoir les compléter. Cela a amené à modifier certaines caractéristiques, tels, le temps de séjour et la température d'extrusion pour essayer d'obtenir une réaction complète.

- Une analyse de DRX a permis de prouver et confirmer la production d'argent élémentaire d'où une réaction de réduction des Ag^+ généré lors d'une sollicitation thermique.

- Suite aux modifications de mise en œuvre (température d'extrusion et temps de séjour), une comparaison des propriétés finales a montré que plus la réaction est avancée, plus un effet d'inhibition de cristallisation sur les EVOH, révélée par DSC et DMTA, est important avec des pertes de propriétés thermiques et viscoélastiques. En effet, il apparait que la réaction provoque une forte réduction des modules dans les domaines vitreux et caoutchoutique.

- Les propriétés de libération des antimicrobiens par les copolymères EVOH sont également liées à la cristallinité du matériau final. Un matériau plus amorphe va avoir des capacités de gonflement plus grandes en milieu aqueux et donc diffuser plus facilement des actifs dans le milieu extérieur. Les résultats de libération des agents s'avèrent cohérents avec les pertes de cristallinités liées aux introductions des sels métalliques. Enfin, l'utilisation d'une modélisation mathématique a démontré que la libération des antimicrobiens était contrôlée par une diffusion purement Fickienne.

- Les caractérisations bactériologiques ont démontré une capacité antimicrobienne finale des EVOH avec des acétates d'argent. L'activité est liée à deux paramètres. D'une part, la concentration initiale, qui lorsque celle-ci est augmentée permet d'obtenir un matériau plus

antimicrobien. Enfin, la réaction précédemment citée a également un impact puisque les résultats montrent qu'à même concentration, des matrices dont la réaction entre EVOH et acétates est plus avancée (sollicitations thermiques et mécaniques lors de l'extrusion plus importantes), possèdent une meilleure capacité antimicrobienne.

Synthetic polymer matrices EVOH with introductions at high temperature of metal compounds for antimicrobial applications (Silver acetate)

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4-1- Abstract

Polymer materials with antimicrobial activity were carried out by extrusion of EVOH, at high temperature. The antimicrobial agents were silver acetates, used without pre-treatment. Their thermal reaction in the material and their effect on the thermal and mechanical properties of the polymer were investigated as a function of their concentration. Physico-chemical, thermal, rheological and morphological analyses as well as metal salt release measurements in an aqueous medium were conducted. A significant thermal reaction of the salts, introduced into the EVOH, was observed. This reaction of metallic salts was partial when the extrusion temperature was fixed at 190°C and complete at 230°C. The antimicrobial agents also had significant effects on the properties of the matrix. Reductions of glass temperature and storage modulus were observed by the analyses. All the variations were dependant on the concentration of silver acetate, but also on the extrusion parameters.

The antimicrobial activity was studied and demonstrated a possibility to create, with this process, an antimicrobial material in a one-step extrusion method, without solvent.

4-2- Introduction

Over the past few years, the introduction of additives in packaging has been a possible solution to prevent microbial growth just after opening the package. At the moment, research is focused on reducing the quantity of those antimicrobials contained in consumer products, particularly for food or cosmetic applications.

The industrial market also tends to reduce the process steps to facilitate the preparation of final materials. Intelligent packaging is mainly used for food or in the medical sector but much less for cosmetics although it is a good opportunity to reduce preservatives directly in the formulations. Therefore, the objective is to introduce the agents in the matrix in order to satisfy the future consumers and extend the shelf life of cosmetic products as well as quality and safety and to prevent a recontamination with pathogen organisms. [1-5]

In antimicrobial packaging, a component can be incorporated in several ways. The antimicrobial agent can be introduced during the process directly in the bulk with the raw material or by an immobilization on the package's surface. [6-11]

Several kinds of antimicrobial molecules from different families have been used, such as acids [12-16], amino acids [11, 17], alcohols, terpenes [18-20], peptides, proteins [21-24], polysaccharides [25-26] antiseptics [27-33], metal oxides [34-36], metal salts [37-42]. Many additives have been tested to obtain the best antimicrobial activity and to create an optimal material. A previous study has demonstrated the antimicrobial activity of acrylate hydrogels in which silver acetate was introduced [43]. Several natures of pathogens have been tested: P. aeruginosa, S. aureus or A. brasiliensis. These different microbial compounds globally represent all germs susceptible to proliferate in cosmetics. It has been shown that metals, such as silver, have the capability to prevent microbial growth [44-46]. Silver under different forms (elementary nanoparticle or salt) has been used to control microbial growth, for different sectors such as medical applications, dentistry and catheters [47-50]. New applications have since emerged and it is now possible to cite other sectors: textile clothing, home water, food protection, deodorants [51-56]. Silver or copper have specific abilities for antimicrobial action but, actually, the mechanisms are not yet fully understood. Several studies try to give assumptions, among which the fact that metal reaction may produce ionic entities. These ionic compounds come into contact with the cell wall of microbes and act on their integrity to prevent proliferation [57]. However, the metallic salts are mainly used to generate elementary nanometric particles [58-63]. These particles are then used as ionic tanks and dispersed in a large variety of materials to obtain an antiseptic effectiveness. Besides, this kind of agent represents a possibility of biocide activity susceptible to resist high thermal solicitations.

Presently, the choice of antimicrobial agents is often limited by their low thermal stability during high thermal and shear polymer processing, as for extrusion and injection molding [64-65]. Thus, several antimicrobial agents may not conserve their activities and specifically after a high temperature process

when they are dispersed in a polymer matrix in the molten state. Silver seems like a good candidate because as a metallic compound, it can resist these constraints and keep its antimicrobial action.

To our knowledge, very few studies have focused on the direct introduction of these metallic salts in EVOH [46-47]. This material is a semi-crystalline polymer, widely used in food packaging for its gas barrier properties. Salts have high thermal resistance and fast crystallization kinetics as well as good optical characteristics. The hydroxyl groups in EVOH are responsible for its high cohesive energy. [66-67] EVOH in contact with moisture is severely plasticized, increasing its permeability and swelling properties [68-73]. This last modification could have a good interest to the final release of biocide agents.

This works aims to evaluate the impact of silver acetate on EVOH materials according to the chosen process, in particular the effect of metal decomposition into salts on the thermal and thermo-mechanical properties, morphological aspect, and the ability to release antimicrobial agents. The last aim of this work is to evaluate the antimicrobial activity against different germs and its possible applications in active cosmetic packaging. Presently, several studies have evaluated the impact of additives on these antimicrobial properties and particularly for EVOH materials. [72, 74-84]

4-3- Materials and Methods

The EVOH E105B was purchased from EVAL with a melting point (mp) equal to 165°C, a glass transition temperature (Tg) around 55°C and an ethylene content of 44 mol%. Silver acetate, used as antimicrobial agent, was purchased from Sigma Aldrich and used as received without purification. The metallic salts are in a powder form. The particles have been observed by SEM with a growth ×100. Silver acetate needle shapes were characterized at about 31µm in length and 2.5µm in width.

- *Synthesis of EVOH matrices*

The batches were made in a glass beaker. These blends were composed of a powder of EVOH to which were subsequently added the antimicrobial compounds. When the correct quantities were present in the container, a stirring was done to obtain a homogeneous powder mixture. Different ratios were prepared: 5, 7.5, 15, 20 and 35 weight percent (wt %) in silver acetate.
Extrusions were carried out on a MiniLab II Haake rheomix CTW5 co-rotating mini twin-screw extruder. The extruder screws are conical. The screw diameter is 5 mm and its length 109.5 mm. Reactants (typically 7g) were introduced through the extruder hopper. The screw speed was set at 100 rpm, the barrel temperature was set at 190 or 230°C and the mini-extruder closed cycle mode was chosen. Once the mixing time of 4 minutes (190°C) or 8 minutes (230°C) was completed, the cycle was opened to get the polymer matrices into rectangular shapes.

- *Characterizations*

- *Thermogravimetric Analysis by TGA-FTIR*

A thermogravimetric analyzer (TGA) from Mettler Toledo, TGA/DSC 1 was used. Thermal degradation experiments were done under nitrogen purge with a flow rate of 80 $mL \cdot min^{-1}$ for all experiments. Samples ranging from 10 to 20 mg were heated from 30°C to 500°C with a heating rate of $10^{o}C \cdot min^{-1}$. EVOH samples were characterized using simultaneous thermogravimetric and FITR analyses. Data were collected on a Perkin-Elmer TGA 4000 thermogravimetric analyzer coupled with spectrum RX 100 spectrometer with a 1 m TL 800 EGA transfer line. The transfer line and FTIR cell temperature were both kept at 215°C.

- *X-Ray analysis by XRD*

An Oxford Xcalibur Mova diffractometer was used with a 50kV and 0.8mA supply source. The wavelength corresponds to the specific value of Molybdenum component (Mo).
X-Ray measurement was carried out on EVOH solid samples with different quantities of silver acetate. The data were measured in the range of diffraction angle 2θ = 3-30°.

- *Thermal Analysis by DSC*

Differential scanning calorimetry (DSC) measurements were carried out with a Q10 calorimeter from TA Instruments. Samples were transferred to hermetically sealed pans, and analyzed from -80°C to 200°C with heating and cooling rates of 10°C/min (2 cycles).

- *Observation of morphologies by Scanning electronic microscopy*

SEM (HIROX SH 4000M Scanning electron microscope) was used to observe the morphology of film surfaces. Each sample was covered by a gold-palladium layer prior to analysis (DENTON VACUUM DESK V: 40mA/30s).

- *Rheology in solid state by rectangular torsion*

The dynamic mechanical thermal analyses of the EVOH materials were performed with a Rheometrics Scientific ARES N2 by rectangular torsion. Test samples were cut with a predetermined shape from the EVOH samples. For dynamic torsion measurements, a nominal strain of 0.3% was adapted, with an applied frequency of 1Hz. Storage modulus G' and loss factor tan δ were determined as a function of temperature. Data were taken from -75°C to 150°C using a heating rate at $5^{\circ}C \cdot min^{-1}$. Each sample was equilibrated in the same chamber under dry nitrogen at the starting temperature.

- *Release test*

The release kinetics of the antimicrobial agents was followed by U.V. spectroscopy (Agilent Technologies Cary Series 100 UV-VIS) at room temperature with a magnetic stirring. Each sample was placed in 10 g of distilled water. The volume of each sample was equal to approximately 88.4 mm^3 +/- 1.9 mm^3 with masses at 97mg +/- 2 mg.
The measure of EVOH with silver acetate was performed at 209 nm by U.V. to evaluate the kinetics of silver acetate release. It was possible to determine the mass of silver acetate released by comparison with a previous master curve. The measurements were only done on EVOH extruded at 190°C during 4 minutes because the different materials created at 230°C during 8 minutes had revealed no presence

of UV peak because all the silver acetates had reacted by extrusion. Consequently, there was no detectable presence of silver acetate in the EVOH which explained the absence of apparition of UV peaks for all the materials made at 230°C. These thermal reactions of silver acetates in EVOH under extrusion will be explained precisely in these works.

- *antimicrobial tests*

Protocol 1: An antimicrobial evaluation in an agar environment was done. Microbial suspensions (200 to 500 µl) with a bacterial concentration between 10^4 and 10^5 CFU/ml was deposited at the surface of the different prepared EVOH in cylindrical shape. The evaluation of the antimicrobial effectiveness was performed on samples that were put in contact with the microbial strains in a culture medium favorable to their growth. This activity by contact resulted in formations of inhibition zones the diameters of which demonstrated the capacity of the final coating.
Reference matrix, a neat EVOH without antimicrobial agent had revealed no activity with final inhibition zones identical to the initial sample diameter. These observations were similar for each type of strain tested (S. Aureus, P. Aeruginosa and A. Brasiliensis).

Protocol 2: the antimicrobial evaluation was based on an ISO normal test 22196.
Three different strains were employed: two bacteria (S. Aureus and P. Aeruginosa) and one yeast (A. Brasiliensis). The samples were prepared with particular dimensions specific to the antimicrobial test. Suspensions with target microbial concentrations were prepared and used at the test inoculums.
The surface to be analyzed was then was put in contact with the different microbial suspensions and covered by a film to keep the humidity. Each specimen prepared was then placed in a sterile Petri dish, favorable to the growth of microbial germs. These Petri dishes were incubated at a temperature of 35°C and a relative humidity of not less than 90% during 24 hours. After this period of time, the proliferation of microbial germs was stopped. The microbial suspensions previously in contact with each sample were recuperated, diluted and placed on other sterile Petri dishes at 35°C during 40h. After incubation, the number of colonies was counted. Each concentration was given in UFC/ml to quantify the evolution and the final antimicrobial activities. A neat EVOH revealed no microbial activity for each strain.

4-4- Results and Discussion

It was earlier explained that silver acetate can be efficient as an antimicrobial agent but its behavior is not completely understood. In particular, silver acetate can be reduced into Ag^0 or it can be dissociated into cationic Ag^+ and anionic acetate. This distinction is not clearly established in the literature. Because this additive was used at high temperature by extrusion with EVOH, its behavior was first logically examined by model analyses.

4-4-1- Thermal stability of EVOH and silver acetate

The thermal stabilities of silver acetate and a pure EVOH were studied by TGA analysis and observed on Figure 1.

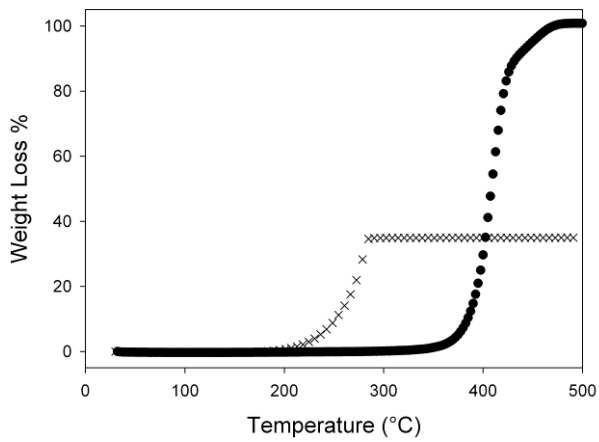

Figure 1: TGA curves: Weight loss percentage of the neat compounds, pure EVOH copolymer (●)and pure silver acetate (×) by a ramp at 10 °C/min

The TGA analysis (Figure 1) revealed an important difference between the decomposition temperatures of EVOH and the antimicrobial. A reduction of silver salt was initiated at around 200°C. A higher temperature was necessary to decompose the EVOH at 380°C with a final reduction of 100%.

More precisely regarding silver acetate, the weight loss of salts began at around 180°C (Figure 1) and was not finished at 290-300°C. One step of this thermal degradation was observed between 180 and 290°C with a weight loss equal to 35%. This percentage exactly corresponded to the mass proportion of acetate group in the silver acetate. These results are in agreement with the litterature [65, 85, 87-88]. According to Logvinenko et al. [85], an intramolecular reduction of cationic Ag^+ silver into neutral Ag^0 occured with releases of CO, H_2O, CO_2, and small quantities of radicals during the decomposition

process. Beyond 300°C, a recrystallization of fine metallic particles with the formation of oxides was recently characterized by XRD, FE-SEM, TEM and XPS [86]. The particles' size of the Ag crystals was in the range of 10–35 nm [87-88]. Thus, the observations had shown that the silver acetates had the possibility to react thermally around 200°C.

The same TGA characterizations were carried out for the next part where silver acetate was mixed at different concentrations in EVOH.

4-4-2- Thermal stability at different concentrations of silver acetate in EVOH matrix

There was an important difference between the decomposition temperature of the EVOH and the pure silver acetate. The results on Figure 1 confirm that EVOH copolymers may resist at a temperature of 200°C without thermal degradation. The TGA of EVOH samples with different concentrations of silver acetate prepared by mini-extrusion at 190°C were carried out and the results are presented on Figure 2.

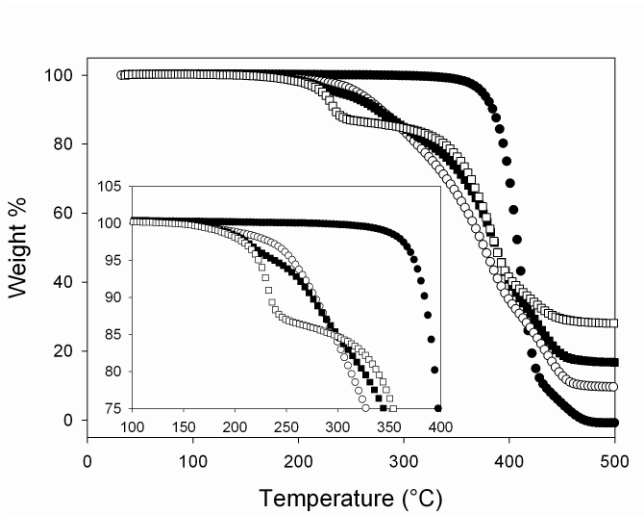

Figure 2: TGA curves of EVOH with different weight concentrations of silver acetate: ● Neat EVOH ○ 7.5wt% ■ 15wt% □ 35wt% by a ramp at 10 °C/min

For the highest silver acetate content, 35 wt%, a thermal degradation occured at 230°C with a weight loss that reached 15%. This first important decrease may be attributed to the silver acetate. A second weight loss occured at a higher temperature and became important above 300°C. It was also unambiguous that this second degradation step could be attributed to the EVOH matrix. It was remarkable that the matrix degradation was accelerated by the presence of silver acetate. This interaction between the fillers and the polymer was confirmed by the other samples with 15 and 7.5 wt%: in these cases the second weight loss occured at a higher temperature and was less prononced

than with a silver acetate content of 35 wt%, (Figure 2). The total weight loss of blended copolymers cannot reach 100% at 500°C with the silver acetate increase for metal silver always remains.

4-4-3- Specific thermal decomposition of silver acetate

To go further, the previous TGA analyses (Figure 2) were presented dividing the weight loss by mass fraction of the silver acetate (Figure 3) and the thermal degradation was represented beween 100°C and 300°C in a range where only the silver acetate was degraded and the EVOH conserved their integrity (Figure 1). Figure 3 presents a normalization of the thermal degradation of only silver acetates introduced in EVOH but also under their pure form.

This new presentation on Figure 3 clearly revealed that all the experiments showed a weight loss that converged to the same value of 35% meaning that the silver acetate always lost the same weight fraction whatever its content in the blend.

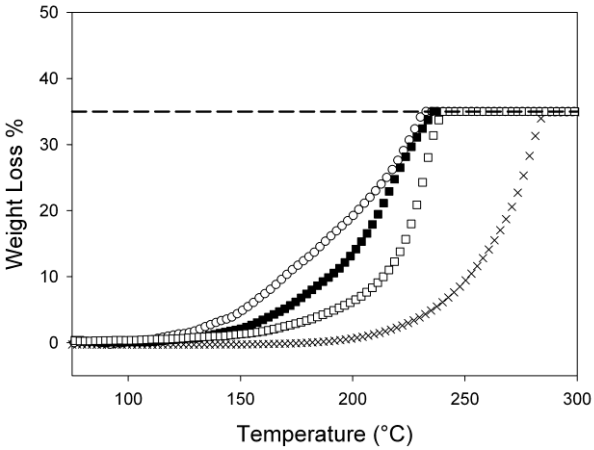

Figure 3: Normalization of TGA curves of EVOH copolymers with different concentrations of silver acetate: Representation of the weight loss of the neat silver acetate introduced in the EVOH and under their pure form: × Pure Silver Acetate ○ 7.5wt% in EVOH ■ 15wt% in EVOH □ 35wt% in EVOH by a ramp at 10 °C/min

The silver acetate introduced in the EVOH was decomposed at lower temperatures by comparison with the neat compound (Figure 3). Previous observations had revealed that pure silver acetate decomposition was initiated at 180-190°C. However, when the silver salt was blended with an EVOH at 180°C, its decomposition was activated at lower temperatures in relation with its content. The decomposition could be initiated at 120-160°C when its content was at the minimum in the chosen range (7.5wt% introduced into EVOH). The weight losses confirmed that the acetate part of silver salts was well eliminated. Having looked this, a TGA-FTIR analysis should allow the determination of the nature of the vapor formation.

Two EVOH samples were characterized with 15wt% and 35wt% of silver acetate respectively. The decomposition part of the silver acetate was isolated at 225°C from the decomposition of EVOH and analyzed with the Fourier-Transform Infrared Spectroscopy. Figure 4 compares vapor phase FTIR spectra of each EVOH sample.

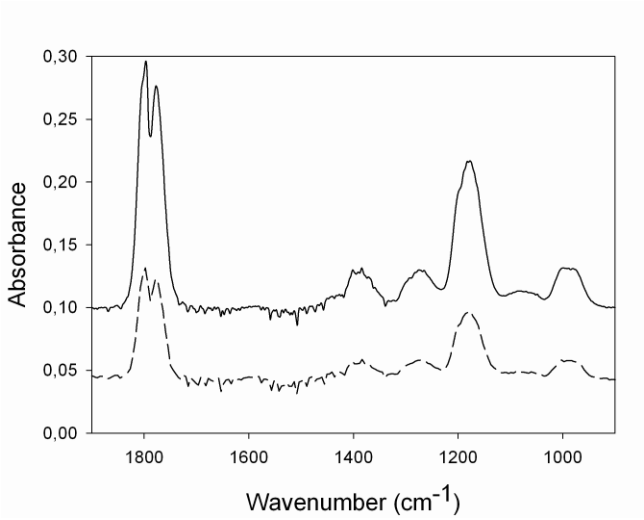

Figure 4 : IFTR spectra of EVOH with 15wt% (- - -) and 35wt% (—) of Silver Acetate : Spectra were arbitrarily shifted on the absorbance axis for better clarity

Figure 4 has shown a double absorbance at 1790cm^{-1} and 1814cm^{-1} specific to the C=O group of an acid. The two spectra were similar for each sample, so the same compound was destroyed at this temperature. The only variation was the intensities of the peaks, more important for the EVOH with 35wt% of silver acetate. It seemed that each spectrum was representative of a neat acetic acid. These results were confirmed by the study of N. S. Nhlapo and al (2012) [89], who worked on the TGA-FTIR analyses of the vapors released by triethylamine-acetic acid mixtures. Their spectra analyses had revealed identical peak absorptions.

4-4-4- Analysis of silver in the EVOH copolymers by X-Ray crystallography

TGA-FTIR tests proved that acetic acid was formed by a thermal effect when EVOH were in contact with silver acetates. According to the literature, [89] an intramolecular reduction of silver ions in smaller metallic silver particles occurs during the decomposition process. To verify which conditions were required to generate the silver acetate reduction, two extrusion processes were tested at 190°C and 230°C with respectively 4 or 8 minutes of blending. 190°C was first chosen because it represents a usual temperature for the EVOH melting [83]. However, this temperature was probably not sufficient to reduce all the silver acetate totally and rapidly within these short heating times of 4 minutes. In particular, the TGA-FTIR analyses (Figure 4) had shown that the formation of acetic acid, probably

associated to the reduction of silver ions in smaller metallic particles ($Ag^{+} \rightarrow Ag^{0}$), was carried out at 225°C.

Consequently, samples were prepared with a similar extrusion process at 230°C with a mixing time of 8 minutes to show if the temperature had an effect on the reactions of these metallic salts.

X-Ray crystallography was carried out with three EVOH (Figure 5) without and with silver acetate (15 wt %) extruded at 190 and 230°C.

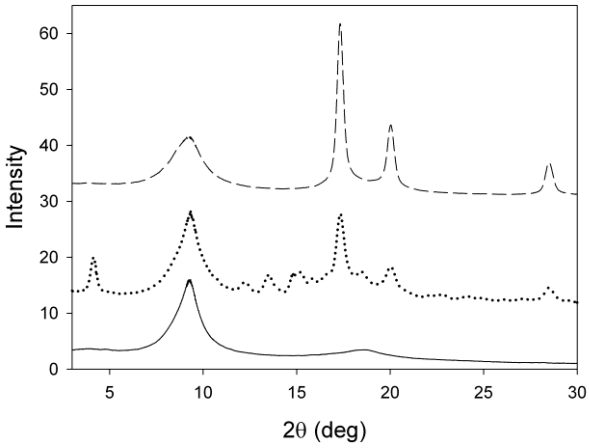

Figure 5: X-Ray spectrum of Neat EVOH copolymer (–)*; EVOH copolymer with 15wt% of silver acetate (190°C and 4 minutes)* (⋯)*; EVOH copolymer with 15wt% of silver acetate (230°C and 8 minutes)* (---): (*Spectra were arbitrarily shifted on the intensity axis for better clarity)*

The X-ray spectrum of neat EVOH without silver acetate was observed on Figure 5. Two characteristic peaks at 9.3° and 18.7° corresponded to the EVOH crystallinity. With this reference, a possibility to distinguish the peaks of the polymer and the silver compounds became possible.

EVOH with 15wt% of silver acetate prepared at 230°C just showed the presence of pure silver in the polymer. The results showed the existence of only three peaks of 2θ values equal to 17.4°, 20.1° and 28.6°, corresponding to pure silver Ag^{0}. This analysis confirmed that 230°C and 8 minutes of extrusion were sufficient to generate a complete thermal reduction of silver acetate with acetic acid elimination as previously demonstrated by FTIR.

Then, the X-Ray of EVOH with 15 wt% of silver acetate, blended at 190°C during 4 minutes, revealed the existence of several new components in the material but also the presence of pure silver, confirmed by the existence of the three peaks with 2θ values of 17.4°, 20.1° and 28.6° (figures 5). However, this spectrum confirmed a non-complete reduction of the silver acetates, as expected after the previous observations. Several peaks remained, which were associated to other non-identified

products. These peaks proved the non-complete reaction at 190°C for 4 minutes of blending by a presence of residual reagents in the EVOH copolymers.

Finally, all the results revealed a possibility of reaction of the silver acetate in contact with an EVOH copolymer during the extrusion process. This reaction had finally two simultaneous effects: the formation of acetic acid and also a reduction of silver acetate to silver Ag^0 . In addition to the thermal reduction of Ag^+, a redox reduction of Ag^+ and the EVOH alcohol can occur and create elementary silver Ag, carbonyl, and dihydrogen.

In addition, a dehydration of EVOH could lead to the formation of double bonds in the EVOH backbone. All these reactions induced by the presence of EVOH can explain the premature degradation/transformation compared to both pure AgOAc and EVOH induced by the presence of AgOAc.

Then, the resutls obtained by TGA-FTIR also showed that 190°C was not the correct temperature to reduce completely the cationic silver whereas 230°C became enough in the conditions of the chosen process.

4-4-5- Silver acetate influence on the EVOH thermal properties

The existence of a chemical reaction of silver acetate by contact with EVOH during the extrusion process has been demonstrated. This reaction could modify the thermal or viscoelastic properties of the EVOH. To evaluate these assumptions, analyses by DSC and DMTA were carried out on EVOH with different concentrations of silver acetate and extruded at two temperatures: 190°C and 230°C. For the first evaluation, the thermal properties of the matrix with different concentrations of salts were observed by thermal analysis. For the DSC, thermal characteristics of EVOH blended at 190°C (4 minutes) were measured and presented in Table 1 for two consecutive ramps (-80°C to 200°C).

*Table 1: Thermal properties of EVOH matrix blended at 190°C during 4 minutes and measured by DSC analysis. * First temperature ramp; ** Second temperature ramp*

	Tg1* (°C)	Tm1* (°C)	ΔHm1* (J/g)	Tg2** (°C)	Tm2** (°C)	ΔHm2** (J/g)
Neat EVOH	52	162	82	51	162	83
EVOH 5wt% of silver acetate	37	155	66	35	153	62
EVOH 7.5wt% of silver acetate	35	154	70	30	149	63
EVOH 15wt% of silver acetate	31	154	62	18	139	51
EVOH 35wt% of silver acetate	33	154	77	13	135	44

Thermal properties were influenced by the introduction of the silver acetate between two consecutive ramps. This variation was caused by an incomplete thermal reaction of the silver salts during the blending process by extrusion and previously observed by X-ray. These results confirmed the existence of a partial reaction of the silver salts introduced in EVOH at 190°C. It was possible that the first DSC temperature ramp up to 200°C completed the reaction between EVOH and the salts. Tests were also carried out on EVOH samples with similar concentrations, but extruded at 230°C during 8 minutes. Table 2 summarizes the comparisons of DSC measurements and the impacts on thermal properties.

*Table 2: Thermal properties of EVOH blended with two different extrusion processes and measured by DSC analysis. * First temperature ramp; ** Second temperature ramp*

	Tg1* (°C)	Tm1* (°C)	ΔHm1* (J/g)	Tg2 ** (°C)	Tm2** (°C)	ΔHm2** (J/g)
Neat EVOH	52	162	82	51	162	83
EVOH 5%wt silver acetate 190°C 4min	37	155	65	35	153	62
EVOH 5%wt silver acetate 230°C 8min	43	150	65	39	147	54
EVOH 15%wt silver acetate 190°C 4min	31	154	62	18	139	51
EVOH 15%wt silver acetate 230°C 8min	35	128	38	31	128	37

This new temperature showed that the difference between the two ramps by DSC disappeared. At 190°C, the DSC measures revealed important differences between Tg1 and Tg2 (Table 2). This shift is reduced for the materials extruded at 230°C. The 230°C temperature facilitated the reaction of the metallic salts and generated the formation of different silver particles. Finally, the process of extrusion at 230°C during 8 minutes seemed to be sufficient to get a complete chemical reaction between EVOH and silver acetate which lead to the final stabilization of the material.

Globally, all these results showed decreases of glass temperatures with the introductions of silver acetates in EVOH. The temperature also affected the melting temperature Tm and the endothermic energy ΔHm. This decrease of ΔHm is caused by an inhibition effect of crystallization due to the presence of silver acetate into the EVOH.

To explain that, it can be supposed that the temperature around 200°C is responsible for a reaction involving silver salts and the EVOH which modified the final thermal properties of EVOH. The possibility of thermo-oxidation of EVOH generated the redox reaction of Ag^+ and the alcohol but this assumption was not proved in this work. Following this assumption, intramolecular hydrogen bonds that occurred in pure EVOH and that were responsible of its crystallization should consequently

decrease, which would explain the inhibition effect, translated by the melting enthalpy reduction and the Tg decreases.

4-4-6- Silver acetate dispersion in EVOH matrix.

The morphologies of EVOH surfaces for several concentrations of silver acetate were observed by SEM. Figure 6 shows EVOH with several concentrations of salt blended at 190°C during 4 minutes.

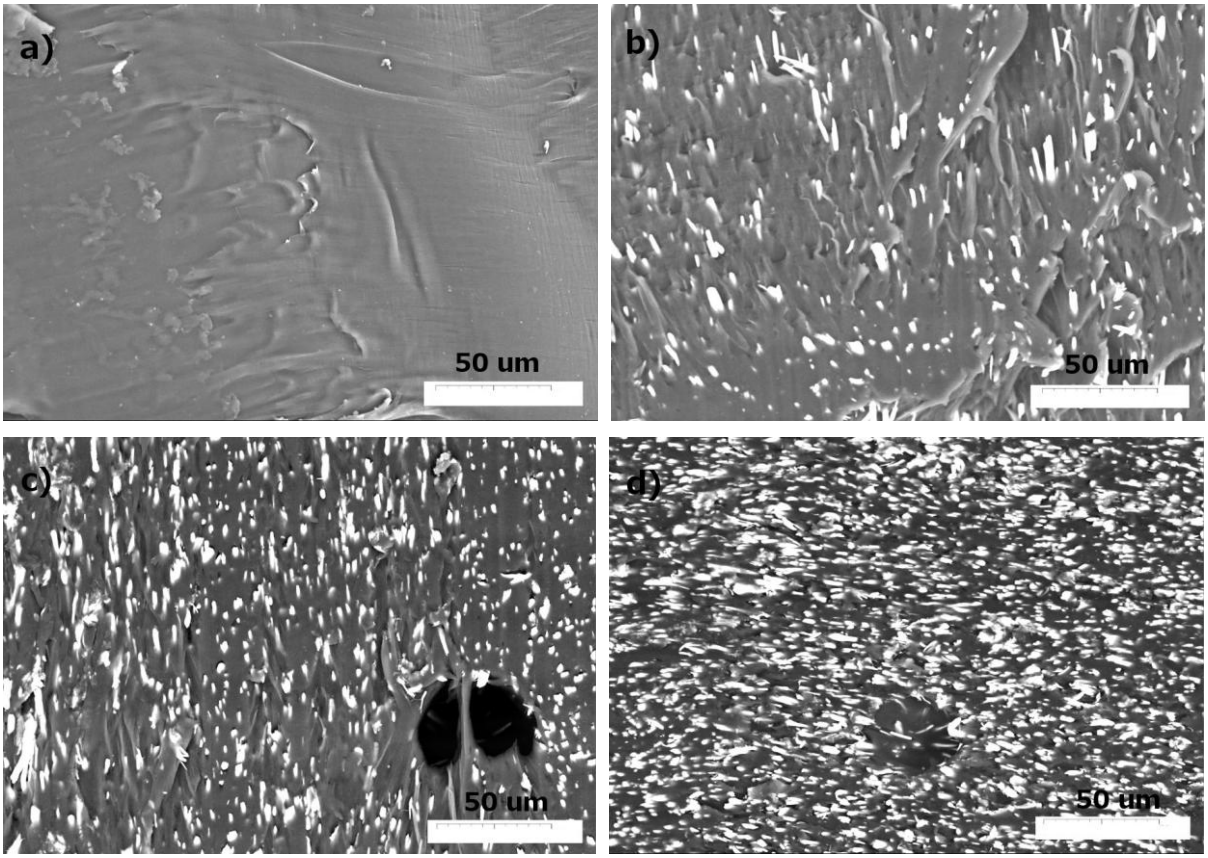

Figure 6: SEM images (BSED) ***(X500)*** *of EVOH extruded at 190°C during 4 minutes with different weight concentrations of silver acetate: a) Neat EVOH; b) 15wt%; c) 20wt%; d) 35wt%*

The density of antimicrobials increased proportionally with the concentration of silver salts. The shapes and sizes of the silver acetate fragments ranged from spherical to needle forms. The particles had micrometer dimensions with lengths of around 7µm and width close to 1.5µm, independently of the initial concentration. These images confirmed the homogeneous dispersion of the metallic particles in the materials, which was crucial to control the antimicrobial and isotropic material properties.

On the other hand, Figure 7 showed the surface of an EVOH matrix with an introduction of 5wt% of silver salt and extruded at 230°C during 8 minutes.

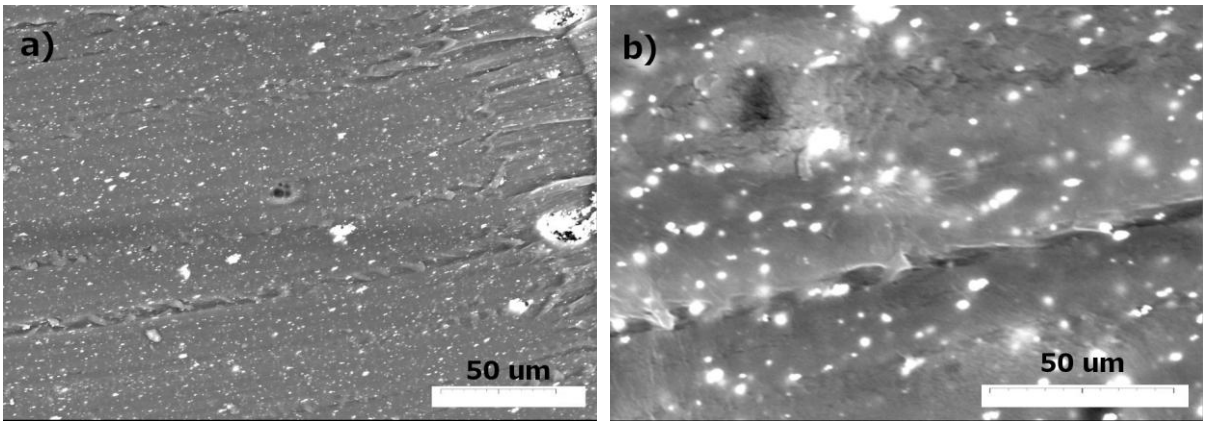

Figure 7: SEM images of an EVOH with a weight percentage of silver acetate fixed at 5wt% and extruded under a thermal solicitation of 230°C and during 8 minutes: a) ***X500****; b)* ***X4000***

These images confirmed the importance of the temperature extrusion. The SEM showed the presence of white particles with different sizes and shapes originating from the process modification. The images were consistent with the possibility to complete the thermal reaction of the silver acetate with a temperature extrusion of 230°C. These analyses also demonstrated a possibility to create particles with spherical shapes and diameter sizes of around 250-500 nm. By comparison, the extrusion at 190°C generates only white silver acetate particles with micrometric sizes and proved a partial thermal reaction, revealed by the previous SEM analyses (Figure 6).
The chosen process proved the possibility to generate nanoparticles by an in-situ process and in one step with a good homogenous dispersion of the antimicrobial agents.

4-4-7- Impact of the metallic salts on thermo-mechanical properties of EVOH

The thermo-mechanical properties of EVOH were carried out by DMTA. Table 3 shows a comparison of Tg between the first temperature cycle in DSC and only one temperature rise between -75°C and 150°C in dynamic mechanical thermal analysis.

*Table 3: (a) Comparisons between DSC and DMTA results: Glass transitions for EVOH blended at 190°C during 4 minutes with different concentrations of silver acetate. * First T°C ramp in DSC; (b) Storage Modulus G' for EVOH matrix blended at 190°C during 4 minutes with different concentrations of Copper (II) Acetate*

	(a) Tg1* (°C) DSC	**(a)** Maximum peak of tan(δ) (°C)	**(b)** G' (-45°C) * 10^9 (Pa)	**(b)** G' (100°C) * 10^7 (Pa)
Neat EVOH	51	51	1.21	4.56
EVOH 5wt % of silver acetate	37	38	1.14	2.30
EVOH 15wt % of silver acetate	31	35	0.99	2.00
EVOH 20wt % of silver acetate	31	38	/	/
EVOH 35wt % of silver acetate	33	37	0.94	2.86

As already observed, thermal characteristic properties were influenced by the introduction of silver acetates. The results showed decreases of glass temperatures with the introduction of silver acetates in EVOH. The reduction effect was observed by a glass temperature shift equal to 15°C, similar for each sample. Several works had already studied thermal variations of polymers with modifications of process (parameters, environmental conditions …) [90]. Particularly, E. Franco-Urquiza and al (2010) [90] had proved the existence of thermal changes in EVOH without filler associated simply with the modification of the morphology. Two thermal transitions with two maximum peaks on DMTA curves (tan(δ)) were observed corresponding to specific transition of the EVOH. The main thermal transition, the α peak, is associated to the glass temperature whereas the second peak represents the α' temperature of EVOH copolymer caused by its crystalline degree. This α' temperature peak has a more important value of intensity when a higher orientation of molecular chains exists in the crystal part of the EVOH [90]. This α' relaxation is not observed for a totally amorphous material.

Then, the storage modulus was also obtained and depicted in Table 3 and on Figure 8.

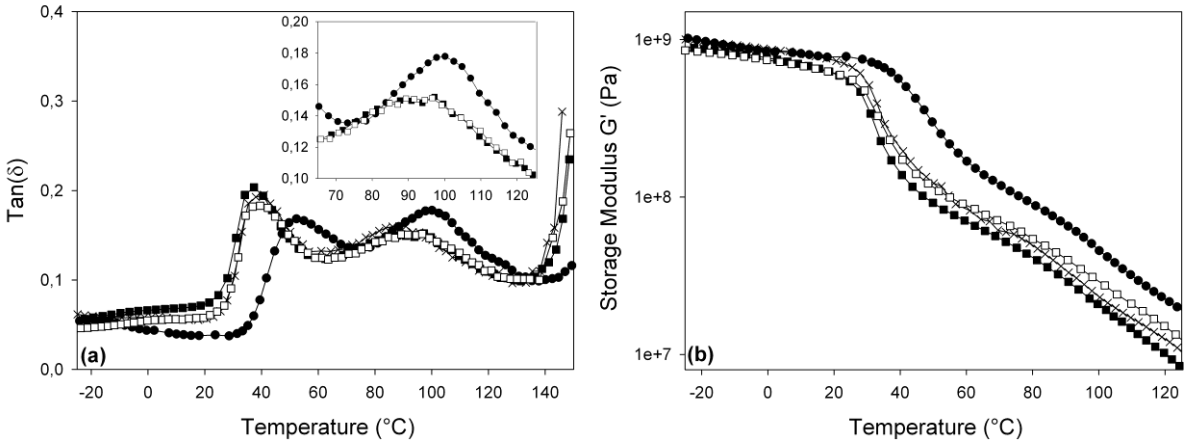

Figure 8: ***(a)*** *Tan(δ) and* ***(b)*** *Storage Modulus G' vs temperature: of EVOH blended at 190°C during 4 minutes with different concentration of silver acetate:* ● *Neat EVOH;* × *5wt%;* ■ *15wt%;* □ *35wt%*

The G' moduli decreased with the introduction of silver acetate while the filler effect was expected to improve the storage modulus. Physical models exist to predict such effect as the Krieger Dougherty model, the equations of Maron Pierce or Einstein [91-93]. However, the obtained results in DMTA revealed an opposite effect with reductions on G'. The expected filler effect predicted by all these theorethical models was probably less important and consequently masked by another phenomenon, previously observed: the reaction between EVOH and silver acetates during extrusion. This reaction generates the inhibition of crystallization, previously proved during DSC analyses and also observed on Figure 8. While unmodified EVOH is a rigid material with crystalline domains, the regular chain organization is disturbed by the presence of silver salts. This lower crystal structure in the EVOH was revealed by an intensity reduction of the tan(δ) peak generated by the α' relaxation and a decrease in Tg of copolymers compared to neat EVOH.

However, despite this intensity reduction of α' peaks, Figure 8 also showed that the EVOH with introductions of silver acetates were no completely amorphous. These peaks were not totally removed by the addition of antimicrobials and prove the presence in the EVOH of a crystalline part. This observation explained why the endothermic energies ΔHm obtained in DSC were not equal at 0 J/g and why the final EVOH were not totally amorphous.

In addition, the thermo-mechanical properties of a copolymer extruded at 230°C during 8 minutes were also carried out and are given on Figure 9 and in Table 4. These observations brought a direct comparison between extrusion at 190°C and at 230°C.

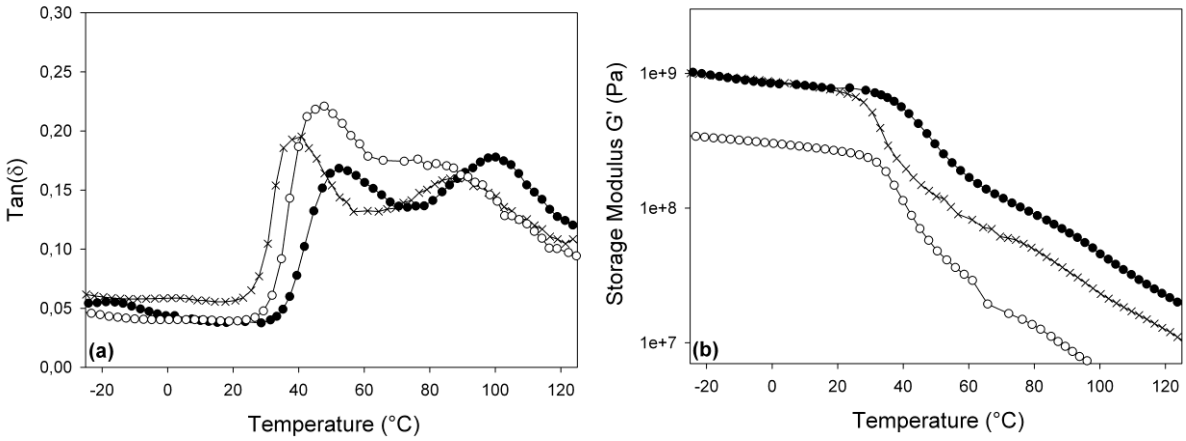

Figure 9: ***(a)*** *Tan(δ) and* ***(b)*** *Storage Modulus G'vs temperature of EVOH: Tan(δ), Storage Modulus G' vs temperature:* ● *Neat EVOH,* × *EVOH 5wt% of Silver Acetate (190°C, 4min),* ○ *EVOH 5wt% of Silver Acetate (230°C, 8 min)*

Table 4: Results of Tg, maximum peak of tan(δ) and storage modulus measured by DSC and DMTA analysis for EVOH with 5wt% of silver acetate and blended with two extrusion processes

	Tg* (°C) DSC	Maximum peak of tan(δ) (°C)	G' (-45°C) X 10^9 (Pa)	G' (100°C) X 10^7 (Pa)
Neat EVOH	51.0	51.1	1.21	4.56
EVOH 5wt % of silver acetate 190°C (4 min)	37.4	38.1	1.14	2.30
EVOH 5wt % of silver acetate 230°C (8 min)	42.6	43.9	0.39	0.64

The crystallization of the EVOH blended at 230°C was also disturbed. This sample has an amorphous final structure, translated by reductions of storage modulus and intensity of the α' peak. Comparatively to the previous extrusions at 190°C, the results indicated more important reductions for EVOH blended at 230°C. The lowest crystallinity rates for EVOH extruded at 230°C are observed by a more pronounced reduction of the storage modulus G', particularly in the glassy state of the copolymer.

A possibility concerned the thermal reaction of metallic salts. As describe previously, the extrusion at 230°C was sufficient to generate a complete chemical reaction between EVOH and silver acetates. Finally, the inhibition of crystallization was more important at 230°C with a more disturbed molecular morphology.

DMTA analyses confirmed a more amorphous morphology of synthesized copolymer in the presence of silver acetates than previously observed in DSC. This effect was reflected by a remarkable drop of

the storage modulus for the blends extruded at 190°C (4 minutes) and 230°C (8 minutes). This drop increased for the blends extruded at higher temperatures. Then, this more amorphous structure comparatively to a neat EVOH, was also observed by a reduction of the intensity of tan(δ) peak associated to the α' relaxation. Through these thermo mechanical analyses, modification of Tg and storage modulus G' caused by the introduction of silver acetates appeared for each extrusion process at 190°C and 230°C. These modifications were observed for each introduction of antimicrobial agents. For EVOH blended at 190°C during 4 minutes, the Tg were reduced by 15°C. Their storage moduli G' in the glassy state of materials were equivalent to a neat EVOH and decreased in the rubbery state from $4.5*10^7$ Pa for the neat EVOH to values equal to approximately $2*10^7$ Pa for 5, 15 and 35wt% of silver acetate introduced. All these reductions of Tg and G' were independent from the concentrations of silver acetates (5, 15 and 35wt %).

Concerning the EVOH at 230°C, the properties of Tg and G' were more reduced comparatively to the materials extruded at 190°C.

Finally, modifications of EVOH properties were systematic when an amount of silver acetates was introduced.

4-4-8- Release behavior of EVOH with dispersed silver acetate

In Figure 10, the release profiles at room temperature in water s were reported for EVOH with 7.5wt% and 15wt% of silver acetate. Firstly, a different release rate was observed in Figure 10.

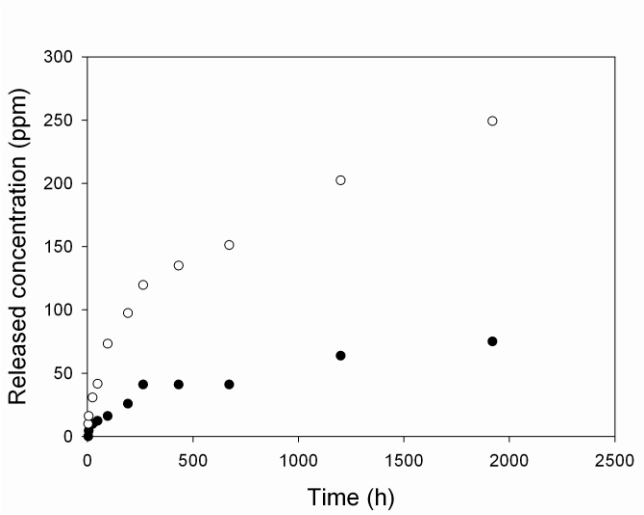

Figure 10: released kinetics of two EVOH copolymers with silver acetate 7.5wt% (●) and 15wt% (○)

For each EVOH, a main release was noted at the first 264H. After this time, the release mechanism tend to an equilibrium state with concentrations that approach stabilized values. An EVOH with

15wt% released a quantity of 250 ppm in 1920 hours of immersion in water. Comparatively, the EVOH with 7.5wt% only released 75 ppm during the same time. This better release capability from the EVOH with 15wt% was attributed to the concentration difference but may be also linked to morphology. Previously, the DSC and DMTA analyses had demonstrated the inhibition effect of crystalization by introduction of silver salts. This effect was linked to the amount of agent introduced initially because a material with a higher amount of antimicrobials in the EVOH was more amorphous. Cabedo and al (2006) [69] have studied the relation between the swelling property of an EVOH in water and its morphology. They have shown that the swelling is influenced by the crystal degree of the copolymer [69]. A more amorphous EVOH has a better swelling capability in water. This more important swelling ability can facilitate a diffusion of drugs in the polymers and their release in the surrounding environment.

- *Modeling by first Fickian law*

Release behaviours of polymer materials are generally modelled by the first Fick Law [94-96].
This law was used to determine the mechanism of drug release (Fickian or non-Fickian). In particular, the kinetics of silver acetate release at room temperature were analyzed by the semi-empirical equation $M_t/M_\infty = Kt^n$ and for M_t/M_∞ inferior to 0.6. M_t/M_∞ is the drug fraction released at time t, whereas K and n are the diffusion constant and the kinetic exponent of drug release, respectively. With the use of this equation, a calculation of the exponent n was possible and gives an indication of the release kinetics. If n = 0.5, the drug diffuses and releases out of the polymer following a Fickian diffusion [94].
For the determination of the n exponents, the first Fickian law was expressed by Equation 1

$$\mathrm{Ln}\,(M_t) - \mathrm{Ln}(M_\infty) = \mathrm{Ln}(K) + n*\mathrm{Ln}(t) \qquad \text{(Eq. 1)}$$

Thanks to this calculation, the determination of the n exponent is possible.

Figure 11 represents two plots of EVOH with respectively 7.5wt% and 15wt% of silver acetate with the linear regression.

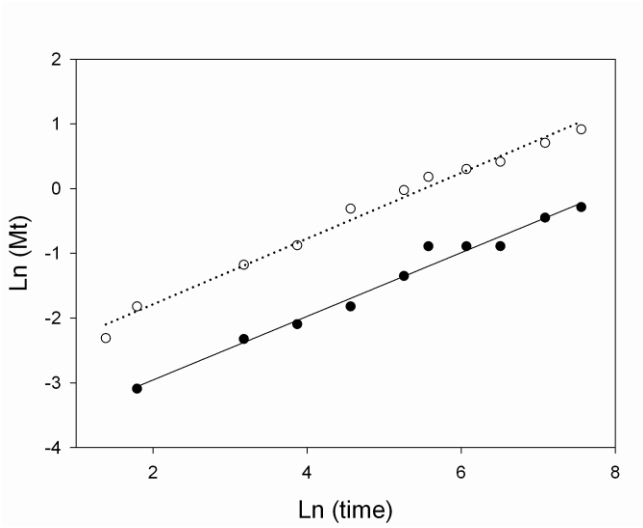

Figure 11: Representations of ln(Mt) versus ln(t) for EVOH copolymers with introductions of 7.5 (●) and 15wt% of Silver Acetate (○)

EVOH copolymers had transport exponents that approached 0.5. More precisely, each transport exponent was equal to 0.491 and 0.507 for EVOH with 7.5wt% and 15wt% of silver acetate. These results matched very well within the range of a Fickian diffusion where n = 0.5. From these tests, it appeared that this diffusion mechanism does indeed control the diffusion.

- *Application of the Fickian law to evaluate the release rate*

To confirm if the concentration of silver acetate influenced the release behaviour, the Fickian law was used and is represented on Figure 15. The calculation consisted in the M_t/M_∞ as a function of the square root of time, where M_t represents the mass of metallic salt at a specific time t. M_∞ is the mass of metallic salts at the infinity time. Since the results demonstrated that the release was not total, the M_∞ used for the calculation was the theoretical mass of introduced silver acetate.

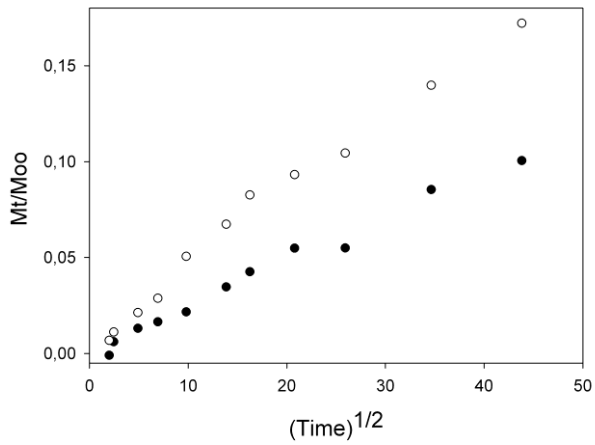

Figure 12:Representative curves of the Fickian law: M_t/ M_∞ vs square root of time *for matrix with 7.5wt% (●) and 15wt% (○) of silver acetate*

Figure 12 have shown a linearity of M_t/ M_∞ as a function of the square root of times for a period ranging from 0 to 2000h. This confirmed that the materials follow a Fickian diffusion in this time interval. The slopes of the linear regression curves were respectively 2.5×10^{-3} $h^{-1/2}$ and 3.9×10^{-3} $h^{-1/2}$. These values are representative of the diffusion coefficient and can give an idea of the release rate. The release was faster for EVOH blended with higher quantities of silver acetates. The slope of Figure 15 was higher for EVOH with 15wt% of silver acetate compared to an EVOH with 7.5wt%. The release was finally associated with two parameters. Firstly, it is linked to the silver acetate concentration but the release is also dependant on the cristallization of the EVOH.

Previous studies demonstrated the links between the swelling behaviour and the crystallization for an EVOH [69]. An EVOH less crystalline has better swelling capabilities and consequently an improved diffusion of drugs, which can facilitate their release in the surrounding media. Previous thermal and thermo-mechanical analyses by DSC and DMTA demonstrated the inhibition effect of crystallization for EVOH with introductions of silver acetates.

Finally, the results were consistent with the previous thermal analyses because the release tests have proved that an EVOH with silver acetates is more amorphous comparatively to a neat material.

4-4-9- Antimicrobial activity

- *Measure of antimicrobial activity by the protocol 1*

The antimicrobial activity of the matrix was evaluated for different formulations. The objective was to verify the possibilities of antimicrobial action on different organisms. Three different strains were used to have a global representation of all types of germs that may exist in the cosmetic sector. There were two bacteria (S. Aureus and P. Aeruginosa) and one yeast (A. Brasiliensis). For each sample, the inhibition zones were measured. The results are summarized on Figure 13.

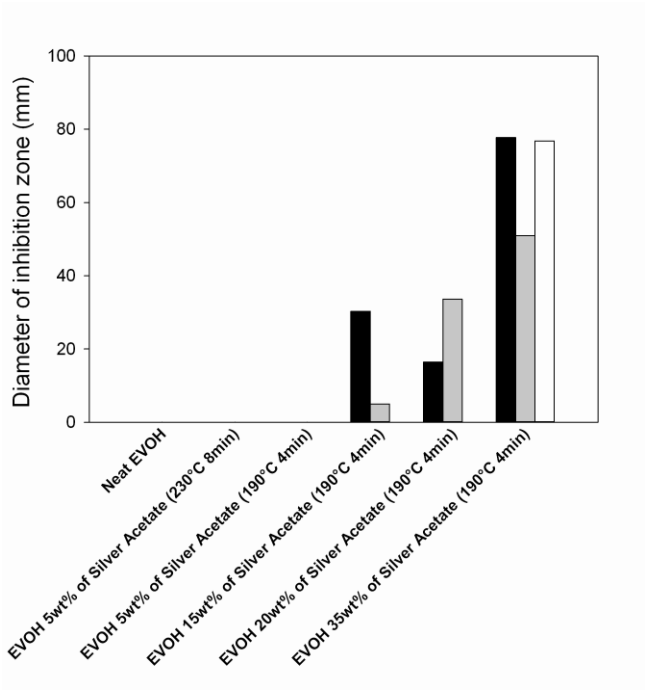

Figure 13: Antimicrobial study with protocol 1: Test by contact between polylmer materials and agar environment: Evaluation by appearance of diameter of inhibition zones for different EVOH copolymers and different microbial germs: P. Aeruginosa (Black); S. Aureus (Grey) and A. Brasiliensis (White)

Three samples have demonstrated an antimicrobial activity against S. Aureus and P. Aeruginosa: the EVOH with 15wt%, 20wt% and 35wt% of silver acetate and extruded at 190°C during 4 minutes. This activity was observed through the formation of inhibition zones where the bacterial germs did not proliferate.

For the yeast A. Brasiliensis, the results have revealed only one sample with a good antimicrobial activity: an EVOH with 35wt% of silver acetate and blended at 190°C for 4 minutes. This activity was observed through an inhibition zone of 76.8 mm. All the other EVOH have demonstrated no antimicrobial effectiveness.

- *Measure of antimicrobial activity by the protocol 2*

Antimicrobial action of EVOH films was also evaluated by a test which can approach an analysis by immersion in a liquid microbial suspension. Films with or without antimicrobial silver acetate were analyzed. This test represents an evaluation during 24 hours. For these analyses, the antimicrobial activity is linked to the ability of materials to release the antimicrobial agents in the external environment.

Neat EVOH has revealed no antimicrobial activity for each strain tested. Direct comparisons were done with this reference material. The results of concentrations for each microbial population were depicted on Figure 14.

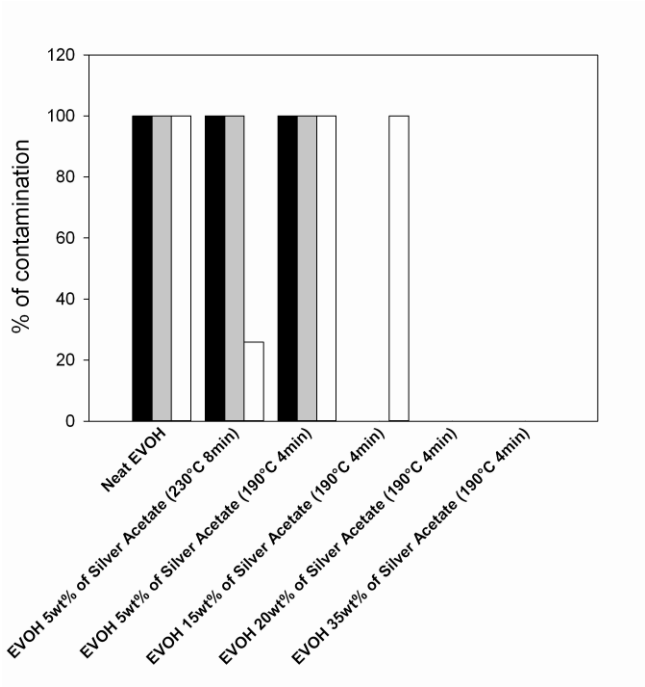

Figure 14: Antimicrobial test with protocol number 2: Test by contact between a drop of microbial suspension at fixed concentration with polymer materials during 24H: Representation by histograms of contamination percentage for different EVOH and different microbial germs after enumeration of the microbial population: P. Aeruginosa (Black); S. Aureus (Grey) and A. Brasiliensis (White)

The EVOH with 15wt%, 20wt% and 35wt% of silver acetate, extruded at 190°C for 4 minutes have revealed an antimicrobial activity against P. Aeruginosa. The final contamination was equal respectively to 0% and lower than the neat EVOH where the referential contamination was equal to 100%. The other materials had no antimicrobial activity against P. Aeruginosa.

EVOH has shown an antimicrobial activity against S. Aureus similar to the results observed for P. Aeruginosa. The most effective ones were EVOH with 15wt%, 20wt% and 35wt% of silver acetate and blended at 190°C during 4 minutes. These samples have avoided the proliferation of S. Aureus with a contamination equal at 0% after an immersion of 24 hours.

For A. Brasiliensis, differences appeared. The EVOH showed a total antimicrobial activity for EVOH with 20wt% and 35wt% of silver acetate and extruded at 190°C during 4 minutes.
Then, Figure 14 has shown a difference of antimicrobial activity between two particular EVOH. This difference concerned the EVOH with 5wt% of silver acetate, blended at 190°C (4 minutes) and 230°C

(8 minutes). These two EVOH had no antimicrobial action against S. Aureus and P. Aeruginosa. However, the results have shown a reduction of the A. Brasiliensis contamination of 26% for the EVOH blended at 230°C (8minutes).

This antimicrobial test against A. Brasiliensis has proved the importance of the process because similar formulations blended with different extrusion parameters had different antimicrobial activities. An EVOH with 5wt% of silver acetate blended at 230°C had a better antimicrobial action than the same formulation, extruded at 190°C.

Globally, these bar charts show that the EVOH blended at 190°C during 4 minutes began to have antimicrobial effectivness for concentrations of silver acetate equal to or higher than 15wt%.

The results have also proved the necessity to adapt the process of fabrication to obtain the best antimicrobial activity possible. In particular, an extrusion at 230°C during 8 minutes generated EVOH with a morphology of antimicrobial agents with a better activity against the yeast A. Brasiliensis.

4-5- Conclusion

EVOH materials with several concentrations of silver acetate were elaborated by extrusion with two different process temperatures, 190°C and 230°C. These salts were used because they have previously revealed an interesting antimicrobial activity for industrial applications. The main objective of this work was to evaluate the impact of these antimicrobial agents on thermal, thermo-mechanical and release properties of EVOH. Based on the results, the introduction causes modifications of these different properties generated by introductions of silver acetate.

Then, this work has shown the possibility of reaction of the silver acetate with EVOH under thermal and thermomechanical solicitations by extrusion. At least one chemical reaction occurs to reduce the ionic silvers Ag^+ generated in their elementary form Ag^0. These different effects cause an inhibition of EVOH crystallization revealed by DSC and DMTA. SEM images have shown two different morphologies of antimicrobial agents as a function of extrusion temperatures. Extrusion at 190°C for 4 minutes causes a partial reaction of the silver acetate and it is necessary to complete this reaction to increase the temperature and time of process up to 230°C for 8 minutes. Finally, the morphologies and the properties were variable by the application of different extrusion parameters, time and temperatures.

Finally, the EVOH copolymers have proved possibilities to reduce a microbial concentration on all three germs tested, P. Aeruginosa, S. Aureus and A. Brasiliensis. Then, the antimicrobial activity was also linked to the extrusion process. In particular, it appeared that for a same concentration of silver acetate introduced in EVOH, but blended with higher temperature and time, it had a better

antimicrobial activity. A complete reaction of metallic salts during the process seems to be the best solution.

Globally, silver acetate represents a good way to generate antimicrobial polymers.

Adknowledgement

This work was supported through a research program entitled STABIPACK. This research program was funded thanks to financial aids provided by a FUI (Fonds Uniques Interministeriels) in the clusters COSMETIC VALLEY and PLASTIPOLIS.

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CHAPITRE 5 :

Ce cinquième chapitre est également une publication et destiné à être soumis dans une revue scientifique.

Dans le chapitre précédent, l'acétate d'argent a confirmé une conservation de son activité antimicrobienne malgré des sollicitations thermiques et mécaniques plus importantes lors d'une extrusion.

Dans le second chapitre, l'introduction d'acétate de cuivre semblait intéressante. A la vue des résultats des précédents travaux, il semblait évident d'introduire ce composé à haute température dans un matériau EVOH, et ceci pour plusieurs raisons.
La première raison est que cette thèse rentre dans le cadre d'un projet collaboratif industriel FUI. Il paraissait essentiel de fournir aux différentes entreprises partenaires, plusieurs solutions et ainsi étendre les perspectives d'actions pour aboutir à la conception de produits finaux.
La seconde raison du choix de l'acétate de cuivre est liée aux résultats obtenus lors de l'étude de l'utilisation des acétates d'argent dans les EVOH (Chapitre 4). Ce chapitre avait mis en évidence des réactions chimiques lors de la mise en contact de sels métalliques et de copolymères par extrusion. Ces réactions ont modifié des propriétés des EVOH, comme la structure ou encore la capacité de diffusion et libération des antimicrobiens.
L'objectif du présent chapitre est d'introduire d'autres antimicrobiens, de nature similaire, des sels métalliques, afin de permettre une comparaison directe, entre les impacts générés sur des EVOH, par les acétates d'argent et les acétates de cuivre (II).

Nous avons testé l'introduction des acétates de cuivre (II) dans une matrice EVOH à haute température avec une mini-extrudeuse bi-vis co-rotative. Les mêmes technologies de mise en œuvre et de caractérisations ont été employées, par rapport à l'étude du chapitre n°4, et ceci pour permettre une comparaison directe des deux antimicrobiens.

Les conclusions principales de cette étude peuvent être résumées comme suit :

- Lors de la mise en œuvre, une réaction chimique se produit également entre les acétates de cuivre (II) et les copolymères EVOH. Comparativement à la réduction de l'acétate d'argent, la réaction de l'acétate de cuivre s'avère plus complexe et difficile à réaliser. Dans le chapitre 4, une réaction chimique complète du sel d'argent avait été obtenue en modifiant des paramètres d'extrusion. Ici, une simple réaction partielle s'est produite pour les acétates de cuivre (II). Cependant, malgré l'utilisation des mêmes techniques de caractérisation que dans le chapitre

4, des questions subsistent encore sur les composés obtenus suite aux réactions chimiques générées en extrusion entre les EVOH et les acétates de cuivre (II).

- Par des caractérisations de DSC et DMTA, nous avons découvert que l'introduction des acétates de cuivre (II) impacte les propriétés thermiques et viscoélastiques des EVOH de manière similaire que les acétates d'argent. Un même effet d'inhibition de cristallisation a été démontré sur la structure finale des copolymères. Il se caractérise finalement par d'importantes pertes de propriétés thermiques et viscoélastiques. Tous ces impacts sur la matière sont liés à deux points particuliers : la concentration introduite en acétate de cuivre (II) d'une part, et d'autre part les paramètres d'extrusion imposés (température et temps de séjour). Une concentration plus élevée en acétate de cuivre génère des impacts propriétés supérieurs. Il en est de même pour des températures et temps de séjour plus importants lors de l'extrusion.

- Les propriétés de libération des antimicrobiens par les copolymères EVOH avec les acétates de cuivre sont différentes de celles de l'acétate d'argent. Dans le chapitre 4, il avait été démontré une libération des antimicrobiens uniquement contrôlée par une diffusion Fickienne. Dans ce chapitre 5, la libération des acétates de cuivre (II), également impactée par la structure cristalline finale des EVOH, n'est pas gérée uniquement par une diffusion Fickienne. Il est possible que la capacité de gonflement des copolymères, liée à un caractère plus amorphe obtenu après les ajouts en acétate de cuivre, favorise la diffusion et donc la libération. Ainsi, comparativement à l'argent où une libération est uniquement contrôlée par diffusion Fickienne, la libération de l'acétate de cuivre est plus complexe.

- Les essais bactériologiques ont démontré une capacité antimicrobienne de l'acétate de cuivre finale similaire aux acétates d'argent. L'activité est également liée à deux paramètres : la concentration initiale, qui lorsqu'elle est augmentée permet l'obtention d'un matériau plus actif. La réaction chimique entre le copolymère et le sel, contrôlée par les paramètres d'extrusion a également un impact.

Synthetic polymer matrices EVOH with introductions at high temperature of metal compounds for antimicrobial applications by reactive extrusion (Copper (II) acetate)

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5-1- Abstract

EVOH materials with antimicrobial activity were produced by extrusion and Copper (II) acetate was used without pre-treatment as antimicrobial. Their thermal reaction in the material and their effect on the thermal and thermomechanical properties of the polymer were investigated as a function of their concentration. Physico-chemical, thermal and rheological analyses as well as metal salt release measurements in an aqueous medium were conducted. A thermal reaction of copper (II) acetate in the EVOH copolymers was observed. The antimicrobial agents had significant effects on the properties of the matrix, similar to a previous study dealing with the introduction of silver acetate in the same polymer material. Analyses showed a reduction of glass transition temperatures and storage modulus. Finally, the antimicrobial activity of the product was studied and demonstrated a possibility to create antimicrobial materials in one step of extrusion and without solvent.

5-2- Introduction

For a few years now, there has been an increasing interest in the development of new antimicrobial materials in various applications such as food coating, food packaging or biomedical devices.
Studies are mainly focused on food protection, in particular in preventing microbial contaminations of food products. In order to reach this goal, preservative agents are often directly introduced in the food to bring a direct antimicrobial action and to reduce microbial growth. However, consumers now want more natural food devoid of preservative agents and finally safer for them from a health point of view.
In this context, the productions of polymer packaging with antimicrobial agents introduced during the process are a potential solution to this request. Thanks to broad antimicrobial functions, these materials must prevent all the risks of food contamination and bacterial development. This new strategy of protection represents an interesting solution to preserve food without direct addition of preservatives[1-5].

Other industrial sectors such as cosmetics tend towards the same goal so as to limit the presence of preservative agents directly introduced into the products and presently used on the market, such as shampoos or creams. In this context, the introduction of antimicrobial agents directly in the packaging also seems like a good strategy. Works already exist on this issue[6]. In order to meet this goal, several additives, from different families such as acids, alcohols, terpenes, metals, or metallic salts have been introduced in polymer materials[6]. The matrices were synthesized by photopolymerization of methacrylate monomers with UV irradiations. Silver acetate has revealed good antimicrobial activity but metal oxides [8-10] or metallic salts [11-16] have also been efficient in other conditions.
Recently, a study has revealed the possibility to obtain antimicrobial activity with silver acetate dispersed in an EVOH by reactive extrusion under high shear in the molten state comparatively to the syntheses by photopolymerization[7]. This work has demonstrated the capacity of silver to prevent microbial contamination [17-19] through mechanisms of action which are not completely understood yet [20-21]. Besides, this study also revealed chemical reactions of silver acetates in synergy with EVOH during the extrusion process. These reactions generated by thermal and mechanical solicitations modified several properties of EVOH such as thermal, thermomechanical and release properties.

Copper (II) acetate is fairly similar to silver acetate. In the past, this molecule also revealed antimicrobial efficiencies [6] and it seemed evident to introduce this agent in the same EVOH, a semi-crystal copolymer, usually used for food packaging[22-29]. In this context, the first aim of the present work was to evaluate the impact of different copper (II) acetate concentrations on EVOH properties. A direct comparison between the modifications caused by copper (II) acetates and previously studied

silver acetate[6], became possible. Finally, the last objective was to test the antimicrobial activities of each copolymer extruded to determine optimal conditions to obtain antimicrobial activity.

5-3- Materials and methods

The EVOH E105B was purchased from EVAL with a melting point of 165°C, a glass transition temperature (Tg) of 55°C and an ethylene content of 44 mol%.
Copper (II) acetate was purchased from Aldrich and used as received without purification. The copper (II) acetate under particulate form of sizes around 5.1 µm was highly agglomerated in the form of large grains with sizes approximately equal to 75µm.

- *Syntheses of EVOH matrices*

The batches were made in a glass beaker. These blends were composed of an EVOH powder and the antimicrobial compounds previously mixed under stirring to get a homogeneous powder mixture. Different copper (II) acetate weight percentages (wt %) were prepared: 5, 7.5, 15, and 20.
Extrusions were carried out with a MiniLab II Haake rheomix CTW5 co-rotating mini twin-screw extruder. The screws were conical. Their diameter was 5 mm with a length of 109.5 mm. Reactants (typically 7g) were introduced through the extruder hopper. The screw speed was set at 100 rpm, the barrel temperature was set at 190 or 230°C and the mini-extruder closed cycle mode was chosen. Once the mixing time of 4 minutes (190°C) or 8 minutes (230°C) was completed, the cycle was opened to form the polymer matrices into rectangular shapes.

- *Characterizations*

- *Thermogravimetric Analysis by TGA*

A thermogravimetric analyzer (TGA) from Mettler Toledo, TGA/DSC 1, was used. Thermal degradation experiments were done under nitrogen purge with a flow rate of 80 $mL \cdot min^{-1}$ for all experiments. Samples ranging from 10 to 20 mg were heated from 30°C to 500°C with a heating rate of $10^{o}C \cdot min^{-1}$. EVOH samples were characterized using simultaneous thermogravimetric and FITR analyses. Data were collected on a Perkin-Elmer TGA 4000 thermogravimetric analyzer coupled with a spectrum RX 100 spectrometer with a 1 m TL 800 EGA transfer line. The transfer line and FTIR cell temperature were both kept at 215°C.

- *X-Ray analysis by XRD*

An Oxford Xcalibur Mova diffractometer was used with a 50kV and 0.8mA supply source. The wavelength corresponded to the specific value of Molybdenum component (Mo). X-Ray measurement was carried out on EVOH solid samples with different quantities of copper (II) acetate. The data were measured in the range of diffraction angle $2\theta = 3\text{-}30°$.

- *Thermal Analysis by DSC*

Differential scanning calorimetry (DSC) measurements were carried out with a Q10 calorimeter from TA Instruments. Samples were transferred to hermetically sealed pans, and analyzed from -80°C to 200°C with heating and cooling rates of 10°C/min (2 cycles).

- *Observation of morphologies by scanning electronic microscopy*

A SEM (HIROX SH 4000M Scanning electron microscope) was used to observe the morphology of copper (II) acetate. The sample was covered by a gold-palladium layer prior to analysis (DENTON VACUUM DESK V: 40mA/30s).

- *Rheology in solid state by rectangular torsion*

Dynamic mechanical thermal analyses of the EVOH materials were performed with a Rheometric Scientific ARES N2 by rectangular torsion. Samples were cut with a predetermined shape. For dynamic torsion measurements, a nominal strain of 0.3% was adapted, with an applied frequency of 1Hz. Storage modulus G' and loss factor tan δ were determined as a function of temperature. Data were taken from -75°C to 150°C using a heating rate of $5^{\circ}C \cdot min^{-1}$. Each sample was equilibrated in the same chamber under dry nitrogen at the starting temperature.

- *Release tests*

The release kinetics of the antimicrobial agents was followed by U.V. spectroscopy (Agilent Technologies Cary Series 100 UV-VIS Spectrophotometer) at room temperature with magnetic stirring. Each sample was placed in 10 g of distilled water. The volume of each sample was equal to approximately 90.6 mm^3 +/- 0.6 mm^3 with masses of 97.3mg +/- 3.2mg.
The absorptions of samples were performed at 220 nm by U.V. to evaluate the quantities of copper (II) acetate released. It was possible to determine the mass of released material by comparison with previous master curves. The release measurements were only done on EVOH extruded at 190°C during 4 minutes.

- *bacteriological measurements by antimicrobial tests*

Protocol 1: An antimicrobial evaluation in an agar environment was performed. Microbial suspensions (200 to 500 µl) with bacterial concentrations between 10^4 and 10^5 CFU/ml were deposited at the surface of the different EVOH prepared in cylindrical shapes. The evaluation of the antimicrobial effectiveness was performed on samples that were put in contact with the microbial strains in a culture medium favorable to their growth. This activity by contact will result in formations of inhibition zones around the sample, where the germs do not proliferate. The diameter of these inhibition zones demonstrates the antimicrobial capacity of the final coating. A neat EVOH without antimicrobial, used as a reference, revealed no activity with no inhibition zones whatsoever. The observations were similar for each strain tested, S. Aureus, P. Aeruginosa, A. Brasiliensis.

Protocol 2: the antimicrobial evaluation was based on an ISO normal test 22196.
Three different strains were employed: two bacteria (S. Aureus and P. Aeruginosa) and one yeast (A. Brasiliensis). The samples were prepared with particular dimensions specific to the antimicrobial test. Suspensions with target microbial concentrations were prepared and used at the test inoculums.
The surface to be analyzed was then put in contact with the different microbial suspensions and covered by a film to keep humidity. Each specimen prepared was placed in a sterile Petri dish, favorable to the growth of microbial germs. These Petri dishes were incubated at a temperature of 35°C and a relative humidity of not less than 90% during 24 hours. After this time, the proliferation of microbial germs was stopped. The microbial suspensions previously in contact with each sample were recuperated, diluted and placed on other sterile Petri dishes at 35°C during 40h. After the incubation time, the number of colonies was counted. Each concentration was given in UFC/ml to quantify the evolution and the final antimicrobial activity. A neat EVOH revealed no antimicrobial activity for all three strains tested.

5-4- Results and discussion

5-4-1- Thermal stability of EVOH and copper (II) acetate

The thermal stability of the metallic salt and a pure EVOH was studied by TGA analysis and observed on Figure 1.

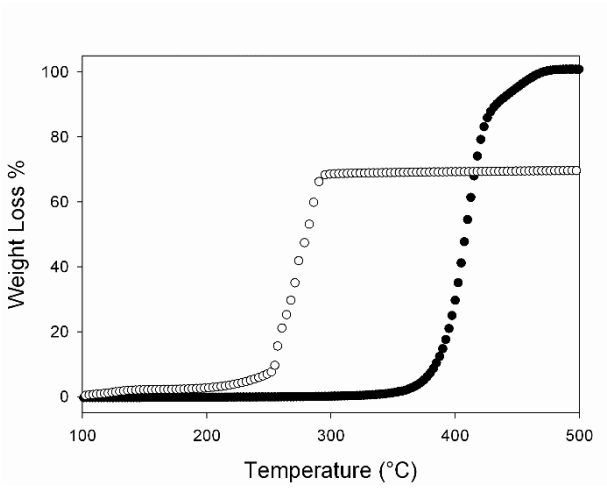

Figure 1: TGA curves: Weight loss of the neat materials: pure EVOH copolymer (●); pure copper (II) acetate (○)

There is an important difference between the decomposition temperatures of EVOH and the antimicrobial (Figure 1). Copper (II) acetate presents a reduction initiated at 250°C. A higher temperature was necessary to decompose totally the EVOH, a phenomenon which was observed at 380°C.

More specifically, the copper salt weight loss was initiated around 250°C and was not finished yet at 290-300°C. Three steps of decomposition were observed for copper (II) acetate and for each, the salt lost 22 wt%. The total decomposition of the acetate group can be considered and verified from the calculation of the relative mass proportion of acetate group in the salt which is equal to 65% in copper (II) acetate. The weight losses determined from TGA analysis corresponded well to the initial relative proportion of acetate.

Figure 1 showed possibilities of a thermal reaction for copper (II) acetate at 290°C. This result is similar to a silver acetate where an intramolecular reduction of silver ions in smaller metallic particles ($Ag^{+} \rightarrow Ag^{0}$) occurs and has been already described in the literature. [30-34] However, the decomposition of copper salt is more complex ($Cu^{2+} \rightarrow Cu^{+} \rightarrow Cu^{0}$) and poorly studied. [33-34]

The same TGA characterizations were carried out for copper (II) acetate which was already mixed with EVOH at different concentrations.

5-4-2- Thermal stability of copper (II) acetate in EVOH matrix with different concentrations

Figure 2 shows the modification of the decomposition kinetics of EVOH previously mixed with different concentrations of copper (II) acetate by extrusion at 190°C.

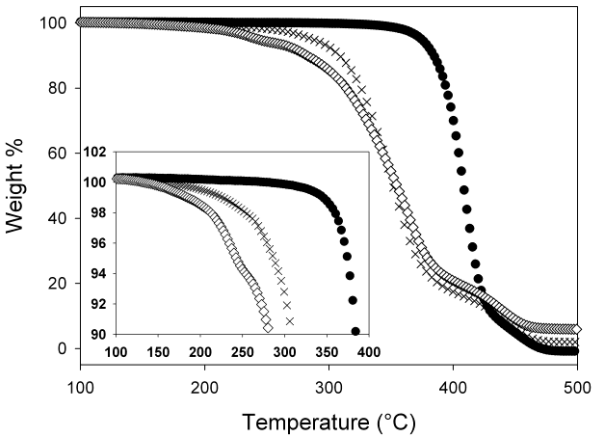

Figure 2: TGA curves of the EVOH with different concentrations of copper (II) acetate and blended at 190°C during 4 minutes: ● Neat EVOH; ×7.5wt% of copper (II) acetate; ◇ 15wt% of copper (II) acetate

For each concentration of copper (II) acetate, TGA results showed different steps of degradation that corresponded to the filler and to the EVOH. The decomposition for each compound seemed to be accelerated. These results were observed by a temperature shift at lower values. Despite these observations, the EVOH remained stable until 300°C. To observe more clearly the decomposition of only copper (II) acetate, the previous TGA analyses (Figure 2) were presented dividing the weight loss by mass fraction of the copper (II) acetate (Figure 3). The thermal degradation was represented beween 100 and 300°C in a range where only copper (II) acetate was degraded and not EVOH. The representation on Figure 3 represents a normalization of the thermal degradation of only copper (II) acetate introduced in EVOH but also under its pure form.

This new presentation on Figure 3 clearly shows that all the experiments have weight losses that converge to the same value of 65%, meaning that the copper (II) acetate always lost the same weight fraction whatever its concentration in the blend.

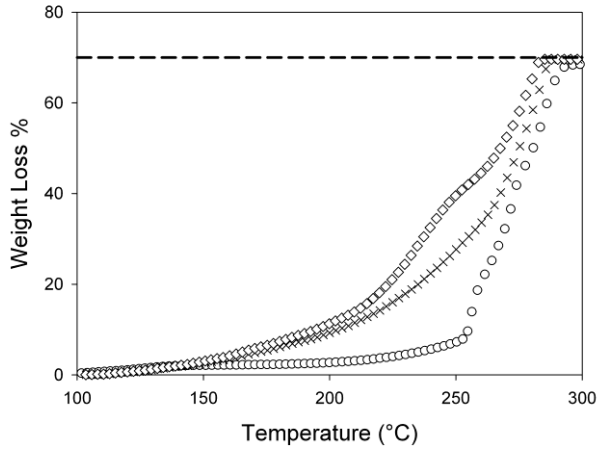

Figure 3: Normalization of TGA curves: Weight loss percentage of the neat copper (II) acetate introduced in the EVOH copolymers: ○ Pure Copper (II) Acetate; × 7.5wt% in EVOH; ◇ 15wt% in EVOH

The copper (II) acetate in the EVOH was decomposed at lower temperatures by comparison with the neat product (Figure 3). Previous observations had revealed that pure copper (II) acetate decomposition was initiated at 250°C. However, when the salt was introduced in EVOH, its decomposition was activated at lower values, around 150°C in relation with its content. Copper (II) acetate had possibilities of similar thermal decomposition comparatively to silver acetate [7]. However, the TGA also demonstrated that a more important thermal solicitation was necessary to decompose copper salts by comparison with silver acetate [7].

Finally, it is possible that an extrusion temperature at 230°C was not high enough to complete the thermal reduction of copper (II) acetate. This temperature of 230°C was optimal to have a total thermal reaction between EVOH and silver acetate. [7]

5-4-3- Analysis of copper in the EVOH copolymers by X-Ray crystallography

X-Ray crystallography was carried out with three EVOH (Figure 4) without and with copper (II) acetate (5 wt %) extruded at 190°C and 230°C.

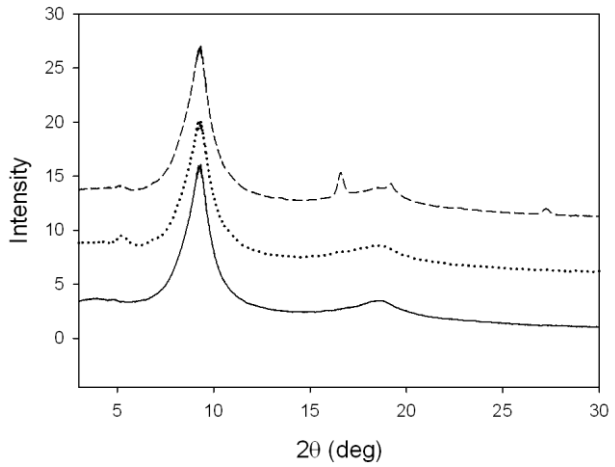

Figure 4: X-Ray spectrum of Neat EVOH copolymer (–)*; EVOH copolymer with 5wt% of copper (II) Acetate (190°C and 4 minutes)* (···)*; EVOH copolymer with 5wt% of copper (II) Acetate (230°C and 8 minutes)* (---): (*Spectra were arbitrarily shifted on the intentity axis for better clarity)*

Two characteristic peaks at 9.3° and 18.7° correspond to EVOH crystallinity. With this reference, it became possible to distinguish between the peaks of EVOH and those of copper (II) acetate. Figure 4 shows a small peak around 5.2° for the EVOH extruded at 190°C during 4 minutes. This peak was attributed to the existence of residual copper (II) acetate in the EVOH. The spectrum for this sample also shows the existence of peaks at 9.3° and 18.7°, corresponding to the polymer. The EVOH sample extruded at 230°C for 8 minutes showed the same diffraction peak at 5.2°. Comparatively to the EVOH blended at 190°C, this peak at 5.2° was reduced but new peaks were present at 16.6°, 19.2° and 27.3°. These peaks may correspond to copper (II) acetate after its partial reaction since the diffraction peak of pure copper acetate was always present. A thermal reaction between EVOH and copper salt was consequently new at 230°C, comparatively to 190°C and temperature had a great importance in order to control copper (II) acetate behavior in this process.
Actually, the reaction of copper (II) acetate during extrusion with EVOH is not really understood.

5-4-4- Copper (II) acetate's influence on the thermal properties of EVOH

The existence of a thermal reaction of copper (II) acetate by contact with EVOH during extrusion at 230°C has been demonstrated. This reaction could modify the thermal or viscoelastic properties of EVOH. For verification, analyses by DSC and DMTA were carried out with the same samples of EVOH blended at 190°C and 230°C. The thermal transitions of EVOH blended at 190°C during 4 minutes were measured and are presented in Table 1 between two consecutive ramps from -80°C to 200°C.

*Table 1: Thermal properties by DSC of EVOH matrices prepared at 190°C during 4 minutes: * First temperature ramp; ** Second temperature ramp*

	Tg1* (°C)	Tm1* (°C)	ΔHm1* (J/g)	Tg2** (°C)	Tm2** (°C)	ΔHm2** (J/g)
Neat EVOH	52	162	82	52	162	83
EVOH 5wt% of copper (II) acetate	44	160	73	41	158	74
EVOH 7.5wt% of copper (II) acetate	44	162	78	37	156	73
EVOH 15wt% of copper (II) acetate	41	156	73	28	149	62
EVOH 20wt% of copper (II) acetate	41	155	68	26	146	49

The two consecutive ramps performed for each sample clearly showed thermal evolutions during these DSC analyses. Variations were caused by an incomplete thermal reaction of the copper (II) salt during the blending process. These results confirmed the existence of a partial reaction of the copper (II) acetate introduced in EVOH blended at 190°C, and already observed by X-Ray (Figure 4). It is possible that the first temperature cycle, generated by DSC analysis, completed the characterized reaction between EVOH and the copper salt. To confirm this, tests were also carried out on EVOH with similar concentrations, but blended at 230°C during 8 minutes. Table 2 presents these thermal properties.

*Table 2: Thermal analyses of EVOH blended with different contents of copper (II) acetate and different extrusion processes (190°C, 4 minutes and 230°C, 8 minutes): * First temperature ramp; ** Second temperature ramp.*

	Tg1* (°C)	Tm1* (°C)	ΔHm1* (J/g)	Tg2** (°C)	Tm2** (°C)	ΔHm2**(J/g)
EVOH 5wt% of copper (II) acetate (190°C)	44	160	73	41	158	74
EVOH 15wt% of copper (II) acetate (190°C)	41	156	73	28	149	62
EVOH 5wt% of copper (II) acetate (230°C)	41	156	66	28	149	56
EVOH 15wt% of copper (II) acetate (230°C)	34	139	43	31	138	38

The introduction of copper (II) acetate at 230°C also generated a decrease in the glass and melting temperatures. Furthermore, they were amplified during the second rise in temperature. Through this second analysis, it was also demonstrated that an extrusion process at 230°C was not sufficient to complete thermal reaction of the copper (II) acetate as well as for materials blended at only 190°C.

In addition, all these results have shown decreases of glass temperatures between the first and second ramps, thus proving an evolution and not the expected stability of the materials under high temperatures. The processing temperature also modified the melting temperature Tm and the

endothermic energy ΔHm, translated by decreases comparatively to the neat EVOH. These modifications were generated by an inhibition effect of crystallization due to the presence of copper (II) acetate inside the EVOH.

Similarly to silver acetate and already studied by incorporation in EVOH [7], it can be hypothesized that the temperature generated a chemical reaction between copper salt with the EVOH.
Despite the fact that the reaction stayed really difficult to fully understand, analyses proved the modification of thermal properties, associated to variations of EVOH morphologies and the partial thermal reduction of copper (II) acetate for both the processing temperatures at 190°C and 230°C.

5-4-5- Impact of metallic salts on the thermo-mechanical properties of EVOH

The thermomechanical properties of EVOH were evaluated by DMTA. Table 3 shows a comparison of Tg between the first temperature cycle in DSC and the DMTA analyses.

*Table 3: Comparisons of DSC **(a)** and Dynamic Mechanical Thermal Analysis **(b)** results: Glass transition temperatures and storage modulus G' for EVOH with different concentration of copper (II) acetate: * First temperature ramp in DSC (-80°C to 200°C).*

	Tg [(a)] (°C) DSC	Tα [(b)] (°C) DMA	G' [(b)] (-45°C) * 10^9 (Pa)	G' [(b)] (100°C) * 10^7 (Pa)
Neat EVOH	51	53	1.21	4.56
EVOH 15wt % of copper (II) acetate (190°C 4 minutes)	42	44	0.95	1.86
EVOH 15wt % of copper (II) acetate (230°C 8 minutes)	34	37	0.66	0.57

As already observed by DSC, the glass transitions of EVOH with 15wt% of copper (II) acetate were reduced by the introduction of copper (II) acetate. Thermomechanical analyses in Table 3 confirmed that the EVOH Tg and Tα decreased. The reduction was observed by a 10°C shift for EVOH blended at 190°C during 4 minutes and 15°C for the material extruded at 230°C during 8 minutes. In previous works, Franco-Urquiza and al (2010) [35] proved thermal changes of EVOH caused by morphological variations. Finally, this study reveals two thermal transitions with two maximum peaks on tan(δ) curves versus temperature by DMTA. Each peak is associated to specific EVOH relaxations. The main α peak corresponds to the Tg and the second peak represents the α' temperature of EVOH copolymer caused by its crystalline degree. Finally, the α' temperature peak has a more important intensity when a higher orientation of molecular chains exists in the crystal part of the EVOH [35]. This α' peak is not observed for a completely amorphous material.

Storage modulus was also obtained by DMTA and is depicted in Table 3 and on Figure 5.

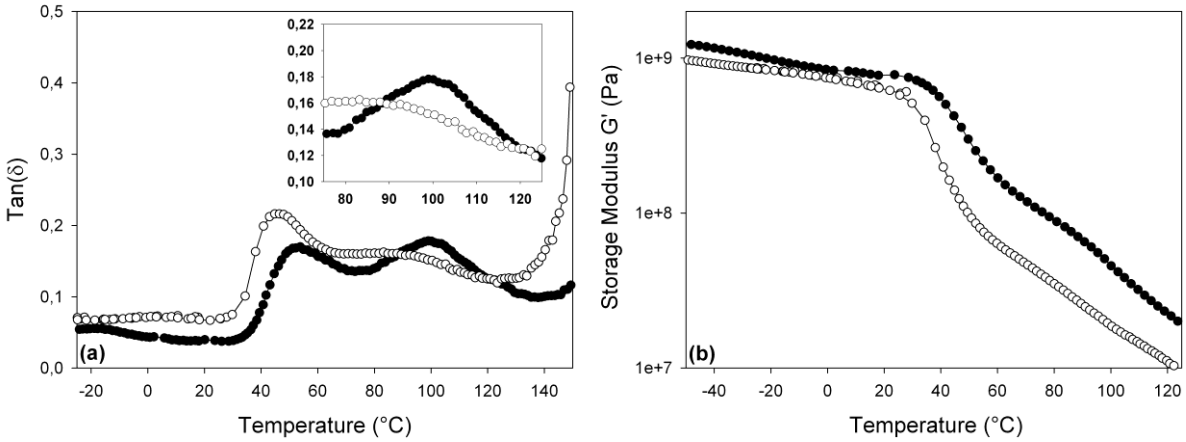

Figure 5: Rheological curves of EVOH copolymers by Dynamic Mechanical Thermal analysis: ***(a)*** *Tan(δ) and* ***(b)*** *storage modulus G'vs temperature: ● Neat EVOH; ○ EVOH with 15wt% of copper (II) acetate*

The G' moduli decreased with the introduction of copper (II) acetate while the filler effect was expected to improve the storage modulus. Physical models exist to predict such effect [36-38]. However, the results in Table 3 were the opposite of what was expected, with a reduction on G'. This expected filler effect was probably less important and consequently masked by another phenomenon, previously observed, which was the reaction between EVOH and the copper salt. This reaction generated the inhibition effect of crystallization, previously proved by DSC analyses and also observed on Figure 5. While unmodified EVOH is a rigid material possessing a crystalline domain, the regular chain organization was disturbed by the presence of copper (II) acetate. This lower crystal structure in the EVOH was revealed by an intensity reduction of the tan(δ) peak generated by the α' relaxation and a decrease in Tg of copolymers compared to neat EVOH.

In addition, thermo-mechanical properties of a copolymer extruded at 230°C with 15 wt% of copper (II) acetate during 8 minutes were also assessed. Their evolution was directly compared with the same formulation extruded at 190°C and was depicted on Figure 6.

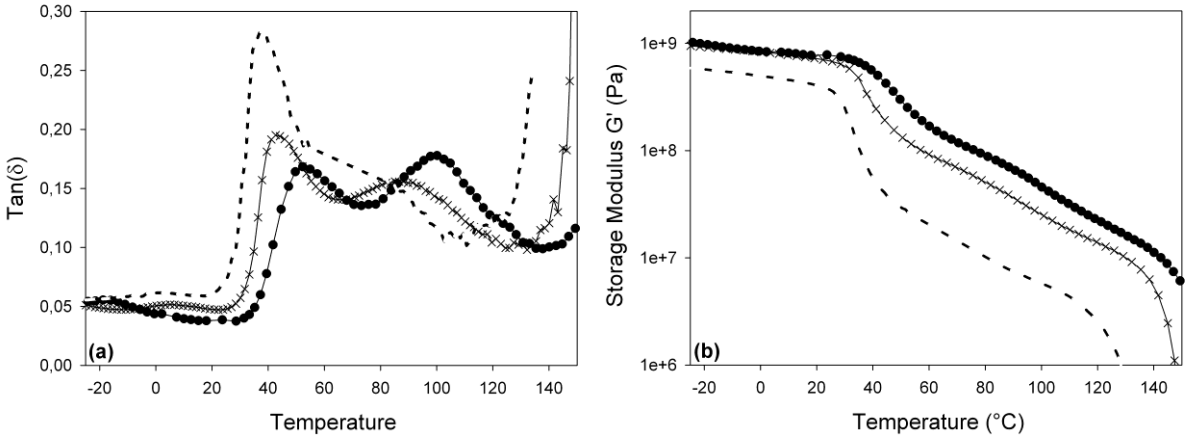

Figure 6: Rheological curves of EVOH by Dynamic Mechanical Thermal Analysis: ***(a)*** *Tan(δ) and* ***(b)*** *storage modulus G' vs temperature: (●) Neat EVOH, (×)15wt% of copper (II) acetate (190°C 4min), (- - -) 15wt% of copper (II)acetate (230°C 8 min).*

The crystallization of the EVOH blended at 230°C was also disturbed. This sample had an amorphous final structure, translated by reductions of storage modulus and intensity of the α' peak. Comparatively with the previous extrusions at 190°C, the results revealed more important reductions for EVOH blended at 230°C. The lowest crystallinity rates for EVOH extruded at 230°C were observed by a more pronounced decrease of the storage modulus G', particularly in the glassy state, and by a higher decrease of Tg.

These DMTA results were similar with the introduction of silver acetate in EVOH [7]. Silver acetate generated an inhibition of crystallization as well, further amplified by higher temperatures and times of extrusion.

5-4-6- Release behavior of EVOH with dispersed copper (II) acetate

The release profiles in water solution were reported, at room temperature (Figure 7), and for EVOH with 7.5wt% and 15wt% of copper (II) acetate.

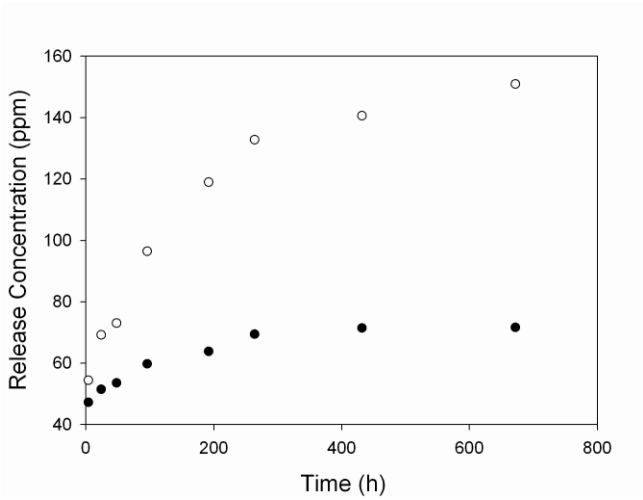

Figure 7: kinetics of copper (II) acetate release (ppm) in surrounding water vs time for extruded EVOH with copper (II) acetate: (●) *7.5wt%;* (○) *15wt%.*

For each EVOH, a sharp increase of the release was studied. After 264H, the release mechanism tended towards equilibrium. An EVOH with 15wt% of copper (II) acetate released a quantity of 150 ppm in 672 hours of immersion in water. Comparatively, the EVOH with 7.5wt% released 70 ppm during the same time. This better release capability from part of the EVOH with 15wt% can be linked to its morphology. Previously, DSC and DMTA analyses demonstrated the inhibition effect of crystalization by introduction of copper (II) acetate. This effect was linked to the amount of agent initially introduced, because a material with a higher amount of copper (II) acetate in EVOH was more amorphous. Cabedo and al (2006) [26] studied the relation between the swelling property of EVOH in water and its morphology. They have shown that the swelling is influenced by the crystal degree of the copolymer [26]. A more amorphous EVOH has a better swelling capacity in water. This more important swelling capacity can facilitate a diffusion of small molecules in the polymers and their releases in surrounding environments.

- *Modeling by first Fickian law*

The first Fick Law [39-41] is a common and simple model to express the release behaviour of polymer materials. This model was therefore tested for these studied systems. The kinetics of copper (II) acetate release at room temperature were analysed by the semi-empirical equation $M_t/M_o = Kt^n$ and for M_t/M_o inferior to 0.6. M_t/M_o is the drug fraction released at time t; K and n are a constant and the kinetic exponent of drug release, respectively. The determination of the exponent n was possible and gave an indication of the release kinetics. If n = 0.5, the drug diffuses and releases out of the polymer following a Fickian diffusion. For n > 0.5, anomalous or non-Fickian type drug diffusion occurs. If n =

0.85, a completely non-Fickian release kinetics is operative [39]. To determine the n exponents, the first Fickian law was expressed by the equation 1

$$Ln\ (M_t) - Ln(M_o) = Ln(K) + n*Ln(t) \qquad \text{(Eq. 1)}$$

By this method, a calculation of the n exponent may be possible.

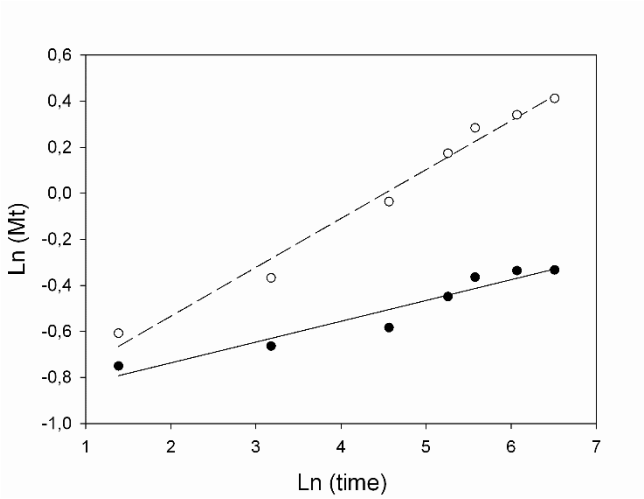

Figure 8: Representations of ln(Mt) versus ln(time) for EVOH copolymers with introductions of 7.5 (●) and 15wt% of copper (II) acetate (○)

Figure 8 represents two plots of EVOH with respectively 7.5wt% and 15wt% of copper (II) acetate with linear regressions. Transport exponents of the EVOH copolymer are different and inferior to 0.5, respectively equal to 0.21 for EVOH with 15wt% and to 0.10 for EVOH with 7.5wt%.

Other scientists had already obtained n exponents inferior to 0.5 during releases of drug introduced in polymers [39]. In these cases, they considered that the release behaviours were very complex and not only caused by the diffusion effects of drug through the polymer. The release behaviour was finally more influenced by the swelling properties of the polymers. The swelling of EVOH with introductions of copper salts are modified comparatively to neat EVOH. These characteristics contributed mainly to the apparent release rate [39] and so to n values lower than 0.5 for EVOH with 7.5wt% and 15wt% of copper (II) acetate, which proved the non-Fickian diffusion transport.

These results were different when silver acetate was dispersed in EVOH [7]. In this previous work, the mechanism controlling the release of the silver salt was purely Fickian.

5-4-7- Antimicrobial activity

- *Measure of antimicrobial activity by protocol 1*

The antimicrobial activity of each sample was first evaluated by contact tests (protocol 1). The results were depicted under histogram forms on Figure 9.

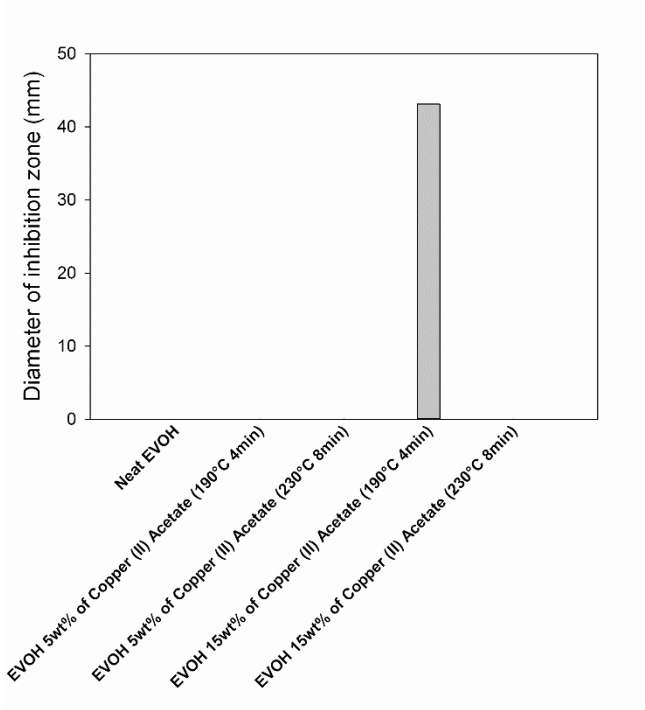

Figure 9: Antimicrobial study by protocol 1: Test by a contact between polylmer material and agar environment: Evaluation by appearance of a diameter of inhibition zones for different EVOH copolymers and different microbial germs: P. Aeruginosa (Black); S. Aureus (Grey) and A. Brasiliensis (White)

From the tests by protocol 1, one sample revealed an antimicrobial activity against S. Aureus, an EVOH with 15wt % of copper (II) acetate, blended at 190°C during 4 minutes. This activity was observed by the final appearance of inhibition zones where the bacterial strains had not proliferated. The final inhibition after 24 hours was equal to 43 mm. All the other EVOH revealed no antimicrobial activity against this strain.

For the other two strains, P. Aeruginosa and A. Brasiliensis, all the copolymers analyzed showed no antimicrobial ability.

- *Measure of antimicrobial activity by protocol 2*

The antimicrobial action of EVOH films was also evaluated via a test which may approach an analysis by immersion in a liquid microbial suspension. Films with or without antimicrobial copper (II) acetate were analyzed. This test represented an evaluation during 24 hours.

Neat EVOH revealed no antimicrobial activity for each strain. Direct comparisons were done with this referential. The concentration measurements for each microbial population were depicted on Figure 10.

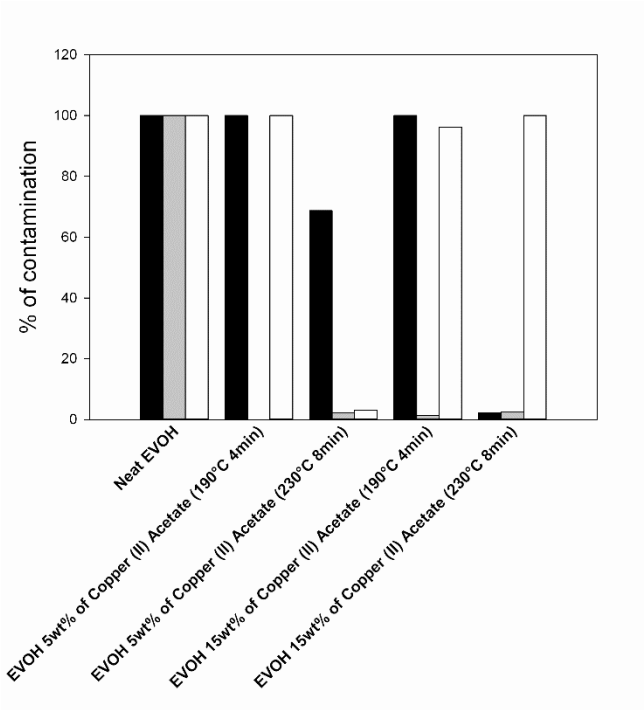

Figure 10: Antimicrobial test with protocol number 2: Test by contact between a drop of microbial suspension at fixed concentration and polymer materials during 24H: Representation by histograms of the percentage of contamination for different EVOH and different microbial germs after enumeration of the microbial population: P. Aeruginosa (Black); S. Aureus (Grey) and A. Brasiliensis (White)

The EVOH with 15wt % of copper (II) acetate and extruded at 230°C during 8 minutes revealed an antimicrobial activity against P. Aeruginosa. The final contamination was equal to 2.2% and lower than the neat EVOH where the contamination was equal to 100%. A weak antimicrobial activity was also observed for an EVOH with 5wt% and blended at 230°C during 8 minutes.

For S. Aureus, each EVOH matrix showed a more or less important antimicrobial activity. The most effective was an EVOH copolymer with 5wt% of copper (II) acetate blended at 190°C during 4 minutes. This material destroyed the totality of strains with finally no contamination. However, the other samples also demonstrated a high antimicrobial activity with final contaminations inferior to 3%. Figure 10 showed that EVOH with 5wt% of copper (II) acetate blended at 230°C during 8 minutes had a high antimicrobial activity against A. Brasiliensis. The final percentage of contamination was equal to 3.1%. All the other EVOH had any antimicrobial effectiveness against this strain.

Despite the fact that the tests demonstrated antimicrobial activities for each sample with copper salt against S. Aureus, these analyses also proved the importance of the process parameters to obtain an antimicrobial action against the others two strains, P. Aeruginosa and A, brasiliensis. This observation was confirmed by multiple results (Figure 10).
An EVOH with 5wt% of copper (II) acetate blended at 230°C had a better antimicrobial action against P. Aeruginosa and A. Brasilienis than the same formulation, extruded at 190°C. Information also brought by these tests concerned the concentration of copper (II) acetate introduced in EVOH. Indeed, a higher antimicrobial activity against A. brasiliensis were observed for EVOH prepared at 230°C with 5wt% of copper salt comparatively with an EVOH blended at the same temperature of 230°C, but with 15wt% of copper (II) acetate instead. This result demonstrated the importance of optimal copper acetate concentration to reach an antimicrobial effectiveness.

The results showed that two EVOH materials were very interesting for antimicrobial applications particularly the EVOH with 5wt% and 15wt% of copper (II) acetate and extruded at 230°C. It seemed that the materials blended under this extrusion temperature could generate EVOH with higher antimicrobial activities by comparison with the same materials blended at 190°C.

5-5- Conclusion

EVOH materials with several concentrations of copper (II) acetate were elaborated by extrusion. The main objective of this work was to evaluate the impact of this antimicrobial agent on the thermal, thermo-mechanical and release properties of EVOH. Comparatively to a previous study about the introduction of silver acetate in EVOH, this work has revealed similarities but also differences.

The possibility to generate a reaction between EVOH and copper (II) acetate under thermal solicitations in extrusion was shown. However, the reactions which occured between the reactants were more complex to understand comparativley to silver acetate. This chemical reaction between EVOH and copper (II) acetate was systematically partial for extrusion at 190°C and 230°C.
Despite the lack of evidence about these reactions, the tests proved the inhibition effect of crystalization on EVOH blended by extrusion with completely disturbed final morphologies. These modifications explained the reductions of tan(δ) intensities and the storage modulus G', observed by DSC or DMTA analyses.
As for silver acetate, its antimicrobial activity was function of the agent concentration and the release quantities of copper (II) acetate. All the copper (II) acetate in EVOH was not totally reduced and consequently was also found under its salt form after production. Finally, the release and antimicrobial

analyses showed that an EVOH with faster and higher release capability had a better antimicrobial activity. This release behaviour of copper (II) acetate was non-Fickian. Moreover, the antimicrobial activity was also linked to the extrusion process. In particular, the same concentration of copper (II) acetate introduced in EVOH, but blended with a higher temperature and time had a better antimicrobial activity.

Finally, copper (II) acetate can be used by an in-situ process of extrusion to be dispersed and without solvent to finally obtain an antimicrobial polymer.

Adknowledgement

This work was supported through a research program entitled STABIPACK. This research program was funded thanks to financial aids provided by a FUI (Fonds Uniques Interministériels) in the clusters COSMETIC VALLEY and PLASTIPOLIS.

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CHAPITRE ANNEXE :

Ce dernier chapitre est rédigé comme les précédents, sous forme d'une publication. Il s'agit d'un chapitre annexe suite à une veille bibliographique durant la thèse sur d'autres matériaux antimicrobiens plutôt destinés au secteur médical. Cette recherche a permis la découverte d'un autre actif qui a été étudié par le passé, les ammoniums quaternaires.

Cette annexe étudie principalement l'ajout d'amines de différentes natures, les amines primaires, tertiaires ou quaternaires. Les matériaux utilisés ont été des matrices méthacrylates UV dans un premier temps, avant de tendre vers l'extrusion de laboratoire et l'utilisation des copolymères EVOH. Le but était principalement de caractériser l'activité antimicrobienne, les propriétés thermiques et les propriétés viscoélastiques des polymères. Dans les précédents chapitres, ont été présentées différentes techniques de fabrication par plusieurs voies, à température ambiante et haute température. Ce sont ces technologies qui sont reprises ici pour la fabrication des matériaux à base d'amines.
Plusieurs réponses doivent être apportées dans ce chapitre. Premièrement la possibilité d'obtenir une activité antimicrobienne via l'introduction d'amines dans des matrices méthacrylates. L'étude doit permettre de vérifier si la nature de l'amine (primaire, tertiaire ou quaternaire) joue un rôle sur l'activité finale. Concernant l'utilisation des ammoniums quaternaire, une tentative d'incorporation aux EVOH par extrusion doit montrer s'il est possible de conserver une activité antimicrobienne et ce malgré des sollicitations de fabrication plus extrêmes.
Le dernier objectif concerne les impacts que peuvent avoir ces amines sur les propriétés thermiques et viscoélastiques de tous les matériaux réalisés à basse et haute température.

La partie introductive présente de manière assez générale, les matériaux hydrogels qui sont souvent utilisés pour des applications antimicrobiennes. Ce matériau est souvent chargé avec des ammoniums quaternaires pour la création de polymères destinés au secteur médical ou biomédical. De manière générale, une activité antimicrobienne est obtenue par l'utilisation des amines quaternaire, mais pas avec les deux amines primaires ou tertiaires. De plus, se pose très souvent la question de la stabilité thermique de ces ammoniums qui ne résistent pas à de trop fortes températures. C'est dans ce contexte que ce chapitre annexe présente un intérêt avec la découverte de nouvelles perspectives d'utilisations des amines.

Une partie « Matériel et méthodes » suit les chapitres précédents pour décrire les protocoles de fabrications et de mesures utilisés pour cette étude. Certains matériaux fabriqués sont constitués de monomères méthacrylates polymérisés sous UV pour former des couches minces. Les autres matériaux sont obtenus par extrusion d'un pré-mélange de copolymères EVOH et d'amines, sans utilisation de solvant. Les divers matériaux sont ensuite caractérisés par les techniques déjà utilisées

dans les précédents chapitres. Ces techniques doivent permettre d'évaluer divers paramètres : l'activité antimicrobienne, mais également certaines propriétés thermiques et viscoélastiques.

Dans la partie « Résultats et discussions », le premier point concerne les matériaux méthacrylates UV fabriqués avec toutes les natures d'amines. Pour chaque agent antimicrobien, les impacts obtenus sur les propriétés thermiques et viscoélastiques des matrices sont d'abord évalués. Ensuite, l'activité antimicrobienne des matériaux, dans une suspension microbienne est présentée.
Enfin, la deuxième partie présente la fabrication des EVOH par extrusion avec les mêmes technologies de caractérisations. Les principales conclusions tirées de ce travail sont :

- L'introduction des amines dans les polymères, joue un rôle important sur les propriétés thermiques et viscoélastiques. Ces variations sont fonctions de deux paramètres : la nature de l'amine associée aux propriétés physico-chimiques, qui vont jouer un rôle important. L'autre paramètre est la concentration ajoutée aux matériaux.

- Les matériaux méthacrylates ont présenté de manière générale, une activité antimicrobienne plus ou moins importante selon la nature de l'amine utilisée. Des résultats ont également été obtenus par l'utilisation d'amines primaires et tertiaires. Ceci est une découverte intéressante car généralement, les études bibliographiques prétendent que seuls des ammoniums quaternaires peuvent avoir une activité antimicrobienne. Ce point de conclusion offre ainsi de nouvelles perspectives d'utilisations des amines.

- Les matériaux EVOH mis en forme par extrusion et en présence de certains ammoniums quaternaires, présentent aussi des activités antimicrobiennes. Cela apporte donc un élément de réponse quant à l'utilisation des ammoniums à hautes températures, et surtout quant à leur stabilité thermique. Il semble que des sollicitations thermiques et mécaniques plus extrêmes, n'empêche pas l'utilisation des ces agents pour la fabrication de matériaux antimicrobiens. De ce point de vue, le travail effectué dans le chapitre 6 offre là encore une autre perspective pour l'utilisation des ammoniums quaternaires. Cela pourrait intéresser l'industrie qui utilise beaucoup les procédés d'injection ou d'extrusion et qui pourrait se servir des ammoniums quaternaires.

Amines in Hydrogels and reactive extrusion for antimicrobial packaging

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6-1- Abstract

Polymer hydrogels containing different amines with antibacterial activity were first prepared by UV polymerization of a methacrylate mixture. Thermoplastic EVOH copolymers containing polyacrylated or polymethacrylated quaternary ammoniums were also prepared by reactive extrusion. Thermal and rheological analyses were conducted. The antimicrobial agents' nature and concentration and the extrusion parameters had significant effects on the thermal and thermomechanical properties of the matrix. The antimicrobial activities of each sample were evaluated. The results showed a possibility to have an antimicrobial material using primary, tertiary and quaternary amines. Quaternary amines had a more important activity than other amines on certain strains.

6-2- Introduction

The use of antimicrobial agents in the field of cosmetics is common and necessary to prevent contamination. In general, they are introduced directly in the cosmetic product. To minimize their amounts, an attractive method is to introduce them partly in the package.

For this, a possible approach is mixing the antimicrobial agent in the material; in this case, the antibacterial acts by diffusion and/or by contact [1-8].

This direct method has two major downsides: first, a high diffusion in cosmetics which may alter the concentration of antibacterial.

Then, the exhaustion of the antibacterial stock in the package, which can rapidly lose its antibacterial activity.

To reduce the release rate of the antibacterial agents out of the packaging, they may be retained by covalent bonds or by interaction with the packaging matrix. These two approaches were considered in this study.

The use of amines as antimicrobial agents in medical applications, particularly quaternary ammonium methacrylates, was reported in the litterature[9-17].

Hydrogels generally have an important biocompatibility with low toxicity and they represent a safe material for humans and can be easily integrated in an industrial process [22-23]. Thanks to their high swelling capability in aqueous environments [18-21] when used as antibacterial materials matrix, they can facilitate the contact between microbes and antimicrobial agents and increases the antimicrobial effects.

In the first part of this study, hydrogels containing several amines were prepared and their properties as polymer materials as well as their antibacterial properties were evaluated.

Thereafter, the amines that had shown important antibacterial effects were polymerized in an Ethylene co-vinyl alcohol copolymer (EVOH) by reactive extrusion.

The polymers thus prepared were expected to have reduced solubility in water and in cosmetic environment. Some of them were grafted to EVOH as a result of transfer reactions.

6-3- Materials and methods

EVOH: Ethylene *co*-vinyl alcohol copolymer E105B was purchased from EVAL. The EVOH melting point was 165°C, Tg around 55°C and ethylene content 44 mol%. All other reagents **HEMA**: 2-hydroxyethyl methacrylate, **PEGDMA**: Poly(ethylene glycol) dimethacrylate, **2AEMA**: 2-aminoethyl methacrylate hydrochloride, **TEACL**: Tetraethylammonium Chloride, Luperox 101 a Thermal initiator of polymerization, **DMAEMA**: 2-(N,N-dimethylamino) ethyl methacrylate, **MADAM**: [2-(methacryloyloxy)ethyl]trimethylammonium chloride, **ADAM**: [2-(Acryloyloxy)ethyl]trimethylammonium chloride were purchased from Sigma-Aldrich and used without further purification, unless mentioned otherwise. The tertiary amine (CN381) used in the photo initiating mixture was donated by Sartomer and used without any purification.
Air free chemistry was carried out using standard Schlenk techniques.
MDAB: *N*-(2-(methacryloyloxyethyl)-*N*, *N*-dimethyldodecan-*1*-ammonium bromide was prepared according to literature procedure. ^{1}H NMR Spectroscopy was obtained on a Bruker 300 MHz (operating at 300.128) and spectra were included in the supporting information (SI 1). All peaks were reported in ppm and referenced to $CHCl_3$ peak in CDCl3.

- *Synthesis of MDAB*

7.7g of DMAEMA (49 mmol), 12.3g of Bromododecane (49 mmol) and 50ml THF were mixed inside a 100 mL round-bottomed flask equipped with magnetic stir bar, a rubber septum and a heater set at 35°C .
The solution's pH was measured and the end of the reaction was approximated at the stabilization of pH. Reaction time was typically 24 hours. The mixture was then crystallized in diethylether to obtain white needle crystals. The compounds were filtered, washed and dried under vacuum in an oven at 60°C for 24h. They were subsequently analyzed by 1H NMR. The spectrum was included in the supporting information (SI1).

- *Preparation of polyacrylate films*

The used photo initiating system was a homogeneous solution of 60wt% of tertiary amine (CN381), 20wt% of Benzophenone (BP) and 20wt% of 2, 2-diethoxyacetophenone (DEAP). The methacrylates solution was composed of 5 wt% of the photo initiating system and two monomers: poly(ethylene glycol) dimethacrylate (PEGDMA) and 2-hydroxyethyl methacrylate (HEMA). For the hydrogels, the antimicrobial agent was solubilized in the monomer mixture prior to the photo initiating system addition.

The reactive systems were placed in Teflon molds (70x10x1.5-2mm) to polymerize. Table 1 summarizes the reactive systems' composition.

For polymerizations is a UV light, a Banc Fusion UV system F300S (fusion system) associated with a conveyor belt was used. The speed of the conveyor was adjustable. The UV lamp can irradiate with wavelengths ranging from 220nm to 300nm.The wavelengths and dose measurements were assessed by a system named Power puck UV radiation (UV Power puck II from EIT Company). This irradiation was repeated 5 times on each face of the samples for an optimal curing.

Table 1: Composition of Hydrogels prepared by UV initiation

RUN	HEMA (%wt)	PEGDMA (%wt)	Amine (wt%) / Nature
1	50.0	50.0	0 / No Amine
2	45.0	50.0	5 / 2AEMA
3	40.0	50.0	10 / 2AEMA
4	37.5	50.0	12.5 / 2AEMA
5	46.0	45.9	8.1 / DMAEMA
6	40.0	40.0	20 / DMAEMA
7	45.0	45.0	10 / TEACL
8	40.0	40.0	20 / TEACL
9	47.5	47.5	5 / MADAM**
10	45.5	45.5	9.1 / MADAM**
11	47.5	47.5	5 / ADAM**
12	47.5	47.5	5 / MADAM*
13	42.5	42.5	15 / MADAM*
14	32.5	32.5	35 / MADAM*
15	47.5	47.5	5 / ADAM*
16	42.5	42.5	15 / ADAM*
17	32.5	32.5	35 / ADAM*
18	45.4	45.4	9.2 / MDAB
19	37.5	37.5	25 / MDAB

** Pure monomer * Amine as received (80wt% of amine in 20wt% of H_20)

- *Reactive extrusion of EVOH*

All the components were reduced to powder and mixed before their introduction through the extruder hopper. Different compositions were used (table 2).

Table 2: Composition of EVOH antibacterial thermoplastics prepared by reactive extrusion

RUN	EVOH weight%	Luperox*	Amine(wt%) / Nature
A	100	NO	0
B	85	NO	15 / TEACL
C	80	NO	20 / TEACL
D	85	NO	15 / MADAM**
E	65	NO	35 / MADAM**
F	85	YES	15 / MADAM**
G	65	YES	35 / MADAM**
H	85	NO	15 / MDAB
I	85	YES	15 / MDAB

* For certain EVOH, an introduction of Luperox 101 was carried out (YES in table X): Corresponding to an addition of 1mol % by comparison with the total molar quantity of others compounds; ** Powder form

Extrusions were carried out on a MiniLab II Haake rheomix CTW5 co-rotating mini twin-screw extruder. The extruder's screws were conical. The screw's diameter was 5 mm and its length 109.5 mm. Reactants (typically 7g) were introduced through the extruder hopper. The screw speed was set at 100 rpm and the barrel temperature at 190°C. A bypass, positioned at the screw's end, could force the material through a feedback channel equipped with two pressure sensors (closed cycle mode). It could also force the material out of the barrel through the extruder die (opened cycle mode). The initial time was when all the mixture had been introduced. Once the mixing time of 5 minutes was completed in a closed cycle mode, the cycle was opened to extrude the materials through a rectangular die.

- *Characterizations of matrices*

- *DSC*

Differential scanning calorimetry (DSC) measurements were carried out with a Q10 calorimeter from TA Instruments. Samples were transferred to hermetic pans, sealed, and analyzed from 80°C to 200°C with a heating rate of 10°C/min (2 cycles). The glass transition temperatures were collected by identifying the inflection points.

- *DMTA Rheology in rectangular torsion*

The dynamic mechanical thermal analyses of the samples were performed with the Rheometric Scientific ARES N2 to obtain torsion dynamic mechanical spectra. Test samples were cut with a predetermined shape. For dynamic torsion measurements, a nominal strain of 0.3% was adapted, with an applied frequency of 1Hz. Storage modulus G', loss modulus G", and loss factor tan δ were determined as a function of temperature. Data were taken from -50°C to 100°C using a heating rate of $5^{\circ}C \cdot min^{-1}$. Each sample was equilibrated in the same chamber under dry nitrogen at the starting temperature prior to running the test.

- *Antimicrobial tests*

The antimicrobial evaluation was based on an ISO normal test 22196. Three different strains were employed: two bacteria (S. Aureus and P. Aeruginosa) and one yeast (A. Brasiliensis). The samples were prepared with dimensions specific to the antimicrobial test. Suspensions with target microbial concentrations were prepared and used at the test inoculums.

The surface to be analyzed was then contacted with the different microbial suspensions and covered by a film to conserve humidity. Thanks to this protocol, the analyses could approach an immersion test in liquid solutions. Each specimen prepared was then placed in a sterile Petri dish, favorable to the growth of microbial germs. These Petri dishes were incubated at a temperature of 35°C and a relative humidity of not less than 90% during 24 hours. After this time, the proliferation of microbial germs was stopped. The microbial suspensions previously in contact with each sample were recuperated, diluted and placed on others sterile Petri dishes at 35°C during 40h. After the incubations, the number of colonies was counted. Each concentration was given in UFC/ml to quantify the evolution and the final antimicrobial activities. A neat EVOH revealed no antimicrobial activity for any of the strains.

6-4- Results and discussion

Hydrogels containing potential antimicrobial agents were first prepared by UV polymerization. Reaction compositions were given in Table 1. The thermal and thermo mechanical properties of the obtained materials are depicted in Table 3. Some Curves of tan (δ) and storage moduli G' as a function of temperature by torsion rectangular rheology are shown in Figure 1. Experiments were made with different concentrations of amines to confirm the evolution of the studied properties. Only the results obtained for one concentration of each amine was given for clarity reasons. Complete results are given as Supporting documents (SI 2)

Table 3: Values of Thermal and thermomechanical properties of polymethacrylate matrices with different Amines

RUN	Tg (°C) (DSC)	Tα (°C) (DMTA)	G' (at -45°C) (Pa) 10^{-8}	G' (at 100°C) (Pa) 10^{-6}	Damping ΔT (°C) (30% of max of tan(δ))
1	29	47	8.4	4.1	36
2	33	50	7.7	3.9	40
3	41	58	11.3	5.7	42
4	43	62	11.7	5.8	43
5	28	45	5.9	3.0	37
6	26	42	3.7	2.4	38
7	0.8	40	9.8	4.4	46
8	-1.9	28	5.8	2.3	41
9	18	58	8.6	4.9	58
10	21	72	8.5	6.6	87
11	30	58	8.9	5.4	46
12	/	52	12.1	5.4	58
13	/	/	/	/	/
14	/	/	/	/	/
15	/	50	9.1	4.2	50
16		57	/	/	86
17	/	/	/	/	/
18	29	51	11.3	4.6	34
19	33	59	7.5	2.4	39

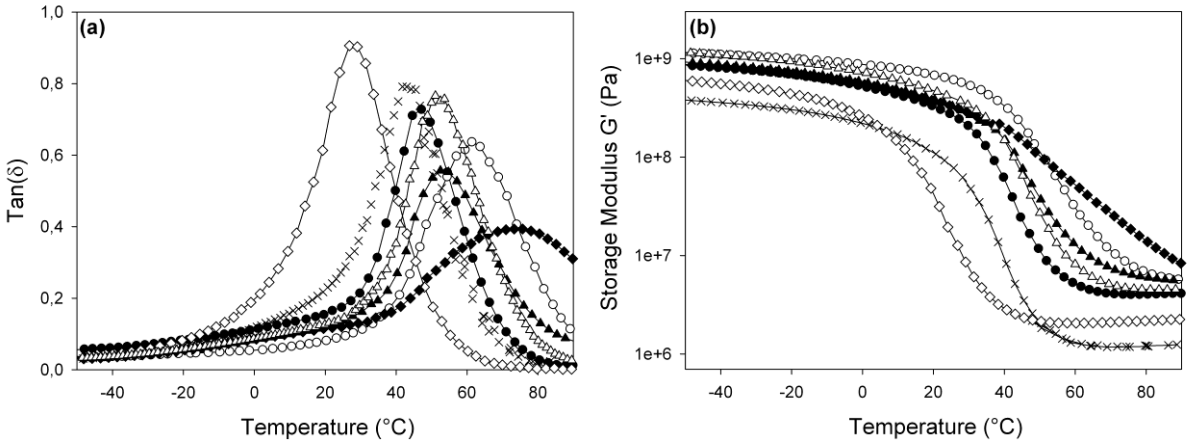

Figure 1: ***(a)*** *Tan(δ) and* ***(b)*** *storage modulus G' as a function of temperature by torsion rectangular rheology for different matrices:* ● *RUN 1;* ○ *RUN 4;* × *RUN 6;* ◇ *RUN 8;* ◆ *RUN 10;* ▲ *RUN 11;* △ *RUN 18*

6-4-1- Primary amines: polyacrylate network and 2AEMA

The thermal properties Tg of the matrix with different concentrations of 2AEMA were determined by DSC analysis and are summarized on Figure 2.

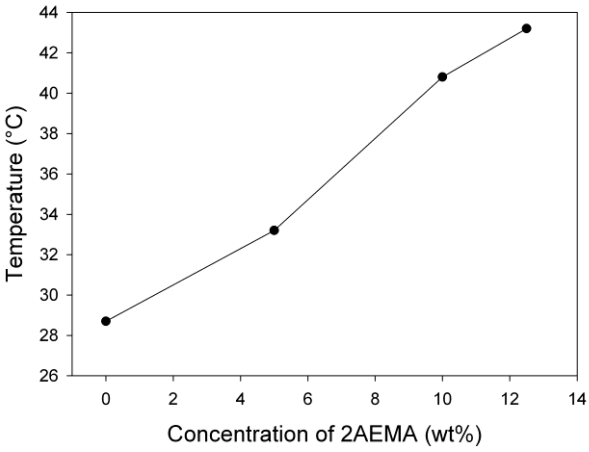

Figure 2: Tg of different matrices HEMA/PEGDMA with different concentrations of 2AEMA

The introduction of the primary amines resulted in an important and linear increase of the glass temperature, observed on Figure 2. In this case, the HEMA was partly substituted by the poly2AEMA that have a higher Tg than polyHEMA in the network. This explains the increase of Tg of the network. The thermomechanical properties were determined by DMTA analysis. The storage moduli were measured between -50°C and 100 °C. Tα was determined as the maximum of tanδ.

The curves of storage modulus show an increase of G' values upon the heating cycle at glass and rubbery states. A shift of tanδ peaks to higher temperatures when the concentrations of primary amines increase. The results have revealed a similar increase of Tα by comparison with DSC analysis. A matrix with 12.5wt% of 2AEMA has a Tα equal to 62°C. The referential material observed for these analyses had a Tα of 47°C, representing an increase of 15°C.

Information concerning the variations between the Tg obtained by DSC and DMTA can be observed in Table 3. As expected Tg and Tα are different since the test conditions are different; DSC is performed in a static solicitation while rheology is dynamic and the tanδ maximum corresponds to only one relaxation. In the next parts, similar differences were obtained.

Then, the mechanical properties evaluated by the observation of storage modulus in the rubbery and glassy states have revealed that the substitution of HEMA by 2AEMA caused an increase of these values. The poly2AEMA has probably a higher storage modulus than the polyHEMA explaining the final increase of the modulus.

Finally, to obtain the damping behavior of all the samples, the curves of tanδ as a function of temperature by DMTA were used. The calculation consists in the temperature interval for values of tan(δ) equal to 30% of the maximum peak of the α relaxation.

Figure 3 represents the damping of matrices for different concentrations of 2AEMA.

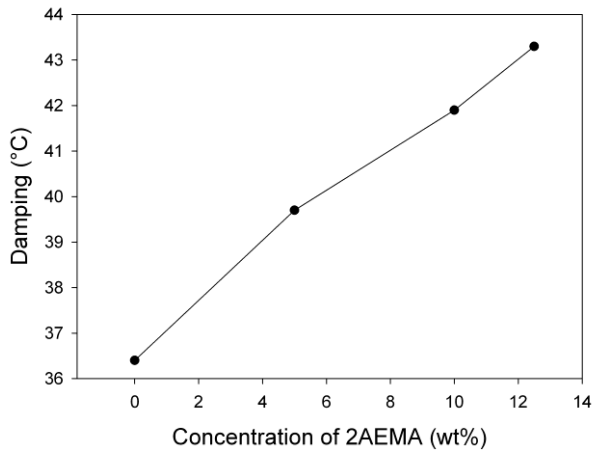

Figure 3: Damping measurements of HEMA/PEGDMA with different concentrations of 2AEMA

The results have shown an increase of the damping by substitutions with 2AEMA. These increases are linear with the weight concentration of 2AEMA. This shows that the observed networks contain a gradient of structures that have a gradient of relaxation temperatures.

Materials were also prepared using different amines. Analyses equivalent to those done with 2AEMA were performed.

6-4-2- Tertiary and quaternary amines: polyacrylate network and DMAEMA, MADAM, ADAM, TEACL and MDAB

- *Thermal and thermo mechanical properties*

The introduction of tertiary amines doesn't really modify the final glass temperatures. A material with a concentration of 20wt% of DMAEMA had a Tg reduced by 3°C comparatively to the reference. This weak reduction is probably associated with the Tg of the pure DMAEMA. The literature reveals that the pure polymer has a Tg of 19°C, which corresponds approximately to the Tg of the referential methacrylate matrix. This characteristic could explain a weak impact of this amine on the thermal properties of networks.
For the use of MDAB amine, the reason for the thermal properties' evolutions was the same as DMAEMA. The results of Tα seem consistent with the previous part of DSC analyses.

The results are different for TEACL and have shown a reduction of the Tg and Tα with the introduction in a matrix. The effect was more important when the initial weight concentration of TEACL increased. It seems that in these quaternary ammoniums, no methacrylates have a plasticizer effect. Tg decreased by 30°C and Tα decreased by 20°C. The TEACL is not a methacrylate compound and has no possibility to react with the other monomers through polymerization. It has the usual behaviour of a filler , translated by a possibility of reduction of Tg but no modification of the storage modulus in the glassy and rubbery states.
For materials with introductions of pure MADAM** and ADAM**, the tests showed no real modification of Tg (see supporting data). Materials were also prepared using different amines. Analyses equivalent to those done with 2AEMA were performed. The introduction of MADAM** and ADAM** caused important modifications on the α relaxations of materials. The results (SI) reveal important increases of Tα, associated with a high reduction of maximum peak intensities.
All these modifications were generated by a modification of the networks due to the copolymerization with MADAM and ADAM.

- *Effect of the presence of water during hydrogel preparation*

The Thermo mechanical properties of methacrylate materials with MADAM* and ADAM* (80wt% of amine in 20wt% of water) are depicted on Figure 4.

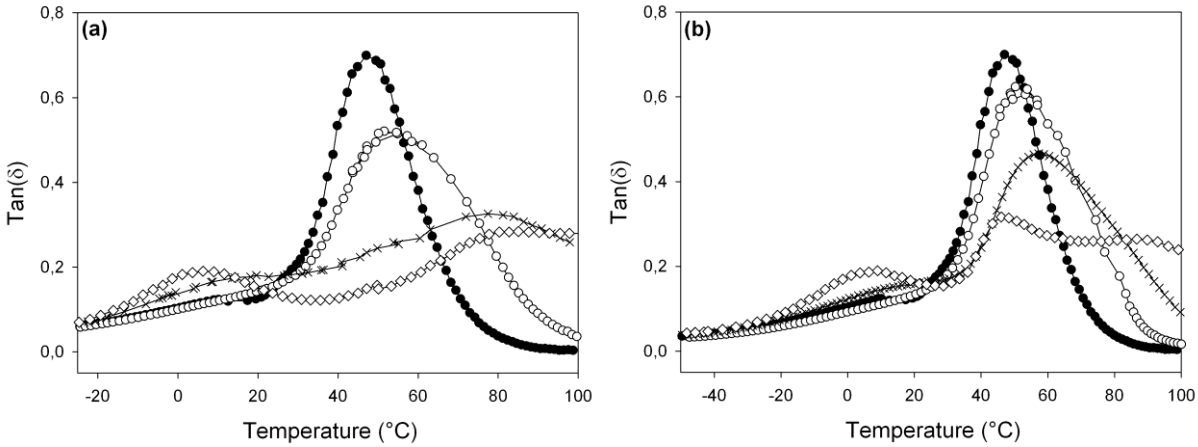

Figure 4: Tan(δ) as a function of temperature by torsion rectangular rheology for matrices HEMA/PEGDMA (50/50wt%) with different concentrations of MADAM ***(a)****:* ● *RUN 1;* ○ RUN 12*;* × *RUN 13;* ◇ *RUN 14 and ADAM* ***(b)****:* ● *RUN 1;* ○ RUN 15*;* × *RUN 16;* ◇ *RUN 17*

The tests reveal the importance of the presence of water . Compared to the previous part, the DMTA curves have shown a separation effect, observed by the appearance of two peaks of relaxation. This phenomenon is caused by the swelling of the hydrophilic part present in the networks. The effects were more important when the concentration of MADAM* and ADAM* increased and consequently the quantity of water was higher.

- *Effect of water addition to polymethacrylate network with MADAM** and ADAM***

A test was carried out on a methacrylate matrix with 5wt% of pure MADAM**. The test consisted in introducing the sample in water during a time of 45 minutes at room temperature to evaluate the final thermo mechanical behaviour. The objective was to compare this property when the water was introduced before or after polymerization of networks.

The results are summarized on Figure 5. The curves give a direct comparison of the matrices before and after immersion in water.

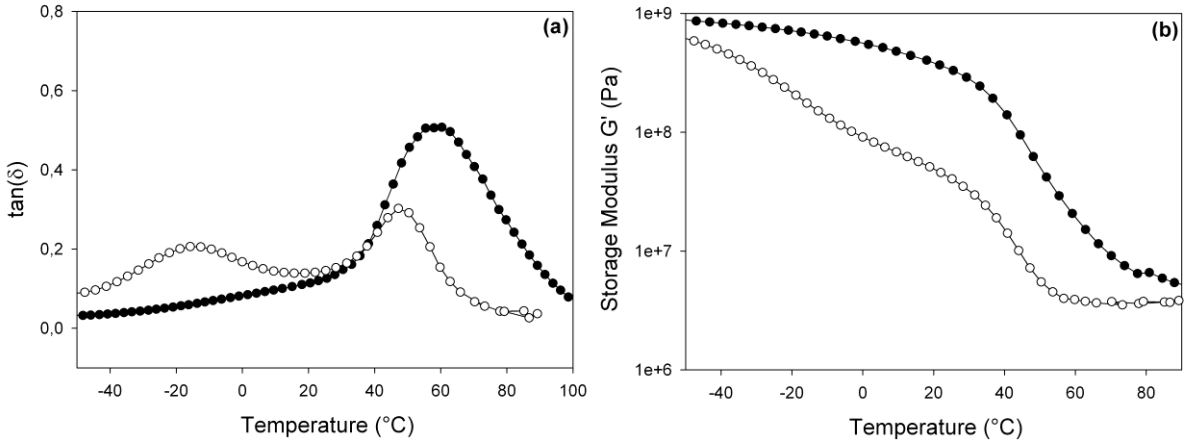

Figure 5: ***(a)*** *Tan(δ) and* ***(b)*** *storage modulus as a function of temperature by torsion rectangular rheology for different matrices:* ● *RUN 11 before immersion;* ○ *RUN 11 after immersion*

Considering these results, no separation effect appeared for the matrix without water. The thermo mechanical behaviour was totally modified after the immersion for 45 minutes in water. Figure 5 shows a separation effect, similar to that obtained using MADAM* and ADAM* at 80wt% in water. Two phases were obtained: a water rich phase with a tan(δ) around 0°C and another one with a tan(δ) at 50°C.

The results prove similar behaviours for the methacrylate matrices when the water was incorporated before or after polymerization.

- *Module evolutions*

Thermo mechanical properties of methacrylate matrices are summarized in Table 3.

The storage moduli in the glassy and rubbery states decreased when the quantities of DMAEMA increased (Runs 5, 6). A similar behaviour was also observed for TEACL (Run 8): the storage moduli in the glassy and rubbery states were reduced from 8.4 10^8 Pa to 5.8 10^8 Pa and from 4.1 10^6 Pa to 2.3 10^6 Pa respectively.

For pure MADAM** (Runs 9 and 10), the storage moduli in the glassy state were not really modified with G' at 8.5 10^8 Pa and 8.6 10^8 Pa. The storage modulus in the rubbery state increased comparatively to the referential (Run 1). G' increased from 4.1 10^6 to 4.9 10^6 Pa (Run 9) and 6.6 10^6 Pa (Run 10). A similar behaviour was obtained for the use of ADAM** (pure Run 11).

Concerning the MADAM* and ADAM* at 80wt% in water, the measures were only possible on samples with 5wt%. This impossibility was due to an instability of the storage modulus in the range of

test temperatures selected. Finally, the behaviour for these two samples was exactly similar to the materials with introduction of pure MADAM** and ADAM**. The storage moduli increased in the glassy and rubbery states.

The results concerning MDAB were different. The tests showed an increase for a concentration of 9.2wt% (Run 18) in the glassy and rubbery states. However, this evolution was inversed when the concentration of MDAB tended to a value of 25wt% (Run 19). This was attributed to a partial solubility of the antimicrobial agents.
The different modifications of G' were associated to variations of final networks' structures. Finally, the different densities of these networks should be different comparatively to the referential matrix when G' increase or reduce.

- *Damping*

The damping properties of all the polymethacrylate coatings with different amines were summarized in Table 3.

The results revealed no important modification of the damping behavior for DMAEMA, TEACL and MDAB.
Concerning MADAM and ADAM, the results were different comparatively to the referential matrix. First, it was observed that the damping behavior was correlated to the degree of water present in the networks. The thermomechanical tests by DMTA revealed that the structure of matrices with MADAM** and ADAM** (pure) was homogeneous with only one α relaxation (one peak of Tan(δ)). This behavior was modified for the uses of MADAM* and ADAM* under their liquid form at 80wt% in 20wt% in water. In this case, the final structure was heterogeneous with the creation of two peaks of tan(δ). For this reason, the calculation of damping is impossible.
Generally, the damping increased following the introduction of MADAM and ADAM. All the increases seemed amplified for the networks containing water.

- *Evaluation of the antimicrobial activity of the hydrogels*

The antimicrobial action of the modified hydrogels was evaluated with a test which can approach an analysis by immersion in a liquid microbial suspension. This test represents an evaluation during 24 hours. For these analyses, the antimicrobial activity is linked to the capacity of materials to release the antimicrobial agents in an external environment.

A neat HEMA/PEGDMA (50/50wt %) was used as referential material to estimate a final percentage of contamination. The results for the different methacrylate matrices are summarized on Figure 6.

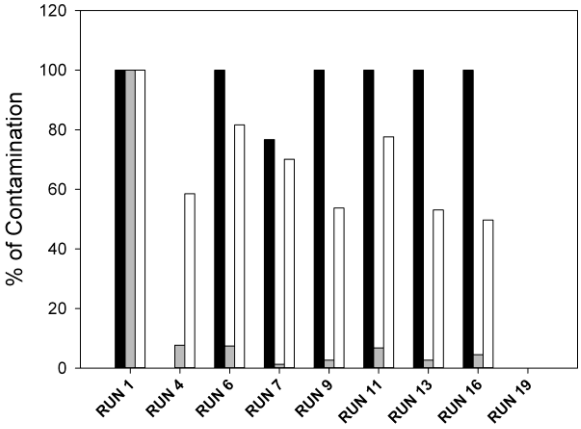

Figure 6: Microbial contamination of suspensions with an immersion of methacrylate matrices for different strains: P. Aeruginosa (black); S. Aureus (grey); A. Brasiliensis (White)

The results showed a variable inhibition effect against S. Aureus in all cases, more or less important in function of the nature of the antimicrobial agent.

A weak antimicrobial action was observed for Run 4 (2AEMA). Then, a high antimicrobial activity was observed for Runs 6 (DMAEMA) and 7 (TEACL). The antimicrobial activity against S. Aureus was also important for Runs 9, 11, 13 and 16, representing the two amines MADAM and ADAM under all their forms (pure and at 80wt% in water). The water used during the fabrication of these matrices had no influence on the antimicrobial properties. Water only had an impact on thermomechanical properties, as observed previously.

The antimicrobial activity was finally total for Run 19, a matrix with an introduction of MDAB, with an indetectable final microbial concentration.

For P. Aeruginosa, no antimicrobial activity was observed for Runs 6, 9, 11, 13 and 16. These results demonstrate no antimicrobial action for 3 amines, the tertiary amine DMAEMA and the two quaternary ammoniums, MADAM and ADAM under their two forms, pure and at 80wt% in water.

The antimicrobial activity was total for Runs 4 and 19, a matrix with respectively an introduction of 2AEMA (primary amine) and MDAB, with an indetectable final microbial concentration. These results have a great interest because they demonstrate the possibility to have a more or less important antimicrobial activity with a primary amine even if the literature tends generally to predict a possibility to have antibacterial activities only for quaternary ammoniums.

The results showed for Runs 4, 6, 7, 9, 11, 13 and 16 a weak antimicrobial action against A. Brasiliensis, observed by a reduction of the percentage of contamination comparatively with the reference material.
Finally, the antimicrobial activity was only total for Run 19, a matrix with an introduction of MDAB.

The antimicrobial results were acceptable for four amines, TEACL, MADAM, ADAM and the MDAB. For this reason, three of these amines were used in the next part, concerning the fabrication of EVOH matrices. The quaternary ammoniums selected were TEACL and MADAM that are commercially available and MDAB that was prepared for this study.
TEACL does not contain double bonds and thus cannot polymerize during extrusion. The two other amines contain double bonds and can polymerize during extrusion either spontaneously or more completely, with the use of a thermal initiator, LUPEROX 101.

6-4-3- Quaternary amines: EVOH and MADAM, TEACL and MDAB

The compositions of the systems used are summarized in Table 2 and the thermal (DSC) and thermomechanical properties in Table 4.
Examples of Tg (δ) and G' evolutions are depicted in Figure 7, representing Runs A, B, D, F and I. Complete results are given in the supporting documents (SI 3)

Table 4: Storage Modulus and relaxation temperatures of EVOH with different amines

RUN	Tg (°C) (DSC)	Tm (°C) (DSC)	ΔHm (J g^{-1}) (DSC)	Tα (°C) (DMTA)	G' (at -45°C) (Pa) 10^{-9}	G' (at 100°C) (Pa) 10^{-7}
A	51	163	83	53	1.25	4.1
B	16	134	43	31	1.07	/
C	19	122	28	27	1.26	/
D	26	141	54	36	1.23	1.42
E	19	110	24	23	1.10	/
F	30	144	46	43	1.10	1.35
G	34	117	10	41	1.02	/
H	34	157	56	41	1.09	2.73
I	31	153	56	42	1.11	2.26

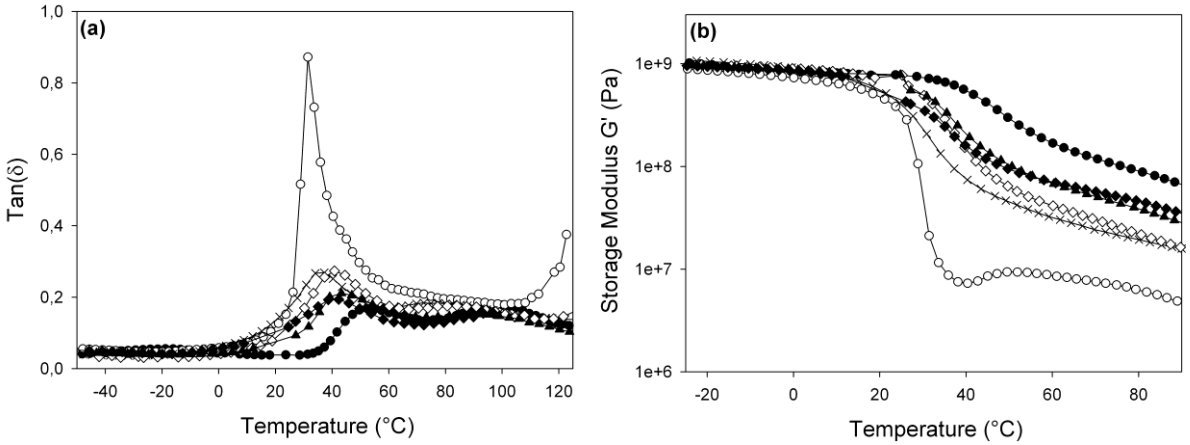

Figure 7: ***(a)*** *Tan(δ) and* ***(b)*** *storage modulus as a function of temperature by torsion rectangular rheology for different EVOH: ● RUN A; ○ RUN B; × RUN D; ◇ RUN F; ◆ RUN H; ▲ RUN I*

- *Thermal properties*

As already described in the previous part, the TEACL is a quaternary ammonium which does not contain any methacrylate function, and consequently does not polymerize. This antimicrobial is only a filler introduced in the EVOH. The results showed important reductions of thermal properties when TEACL was introduced. The reduction was more important when the quantity of TEACL increased. The addition of TEACL also caused an inhibition of the crystallization of the EVOH, observed as a reduction of □Hm values. The presence of TEACL could disturb the formation of H bonding of EVOH and explain the reduction crystallization degree.

The introduction of MADAM** causes similar reduction effects of the thermal properties, comparatively to the TEACL. The MADAM** is a quaternary ammonium methacrylate. This antimicrobial agent was introduced in EVOH without thermal initiator of polymerization and with 1mol% of Luperox 101. Finally, similar results were observed for the last quaternary ammonium MDAB. This antimicrobial agent was also introduced in EVOH without thermal initiator and with 1mol% of Luperox 101.

The tests have also shown that the thermal initiator had no real influence on the final thermal properties of the EVOH.

- *Thermomechanical properties*

Thermomechanical properties were determined by DMTA analysis. Storage moduli were measured between -50°C and 100 °C. Tα was determined as the maximum of tanδ to be compared with the measures of Tg by DSC analyses.

As previously mentionned, DMTA analyses first confirmed the reduction effect on the thermal properties. This effect is observed by a decrease of Tα after introductions of amines. This effect was observed for all the other amines, MADAM** and MDAB.

Concerning the storage modulus, the results showed no important impact of amines on the storage modulus in the glassy state of the material. However, storage moduli were really modified in the rubbery state with important reductions. These reductions were dependant on the nature of the amine and the quantity introduce in the EVOH.

Another observation on Figure 7 confirmed the previous assumption described in the DSC part. It concerns the inhibition effect of crystallization. This effect was observed by the reduction of the α' relaxation (generated by the crystal part of EVOH). Figure 7 shows the presence of only α peaks and proves the creation of a more amorphous material. The inhibition effect could explain the important reduction of mechanical properties in the rubbery states of the EVOH.

- *Evaluation of the antimicrobial activities of the EVOH*

The antimicrobial action of EVOH films was evaluated through a test which can approach an analysis by immersion in a liquid microbial suspension. This test represents an evaluation during 24 hours. For these analyses, the antimicrobial activity was linked to the capacity of materials to release the antimicrobial agents in an external environment.
A neat EVOH copolymer was used as reference to estimate a final percentage of contamination. The results for the EVOH with different ammoniums are summarized on Figure 8.

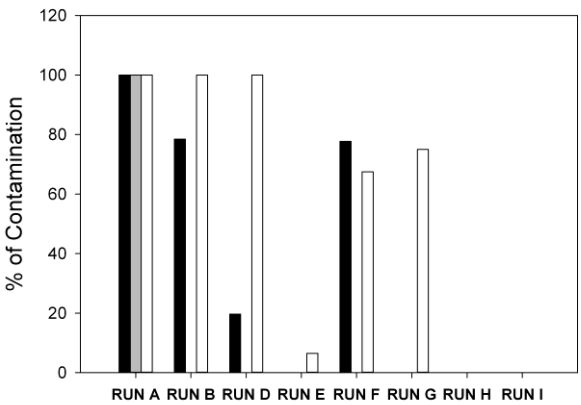

Figure 8: Microbial contamination of suspensions with an immersion of EVOH matrices for different strains: P. Aeruginosa (black); S. Aureus (grey); A. Brasiliensis (white)

The results showed inhibition effects of S. Aureus for each sample.
For P. Aeruginosa, a small concentration reduction was induced by TEACL (Run B) and by MADAM initiated by LUPEROX. An important reduction was obtained with 15wt% of MADAM without thermal initiation.
A total inhibition was obtained when MADAM was used at a higher concentration (35wt %) as well as for MDAB. For these formulations, the inhibition was total with and without the use of Luperox.

Concerning A. Brasiliensis no antimicrobial activity was observed concerning the Runs B and D which represent EVOH with introductions respectively of 15wt% of TEACL and 15wt% of MADAM without the thermal initiator LUPEROX.
A small antimicrobial action was observed in Runs F and G, EVOH with 15wt% and 35wt% of MADAM with the use of LUPEROX. An important antimicrobial activity was revealed for Run E, a similar EVOH copolymer with 35wt% of MADAM, but without the use of LUPEROX.
Finally, the microbial tests revealed a total antimicrobial activity for Runs H and I, two EVOH matrices with 15wt% of MDAB with and without the use of LUPEROX. This important activty against A. Brasiliensis was proved by a total reduction of its microbial concentration. These results are similar to the UV methacrylate hydrogel with MDAB made through a different technique at high temperature.

6-5- Conclusion

Polymer materials with different amines were carried out by UV polymerization of methacrylate, at room temperature and in EVOH copolymers by extrusion at high temperature. The main objective was to produce antimicrobial polymers for packaging applications.
The antimicrobials represented different families of amines, used without pre-treatment. The amines revealed significant effects on the thermal and thermo mechanical properties of the matrix. The effects were finally correlated to the nature of amine, but also to their concentrations.
Finally, the antimicrobial activity of each sample was tested. The results showed the possibility to have antimicrobial materials with the introduction of primary and tertiary amines. This property is interesting because in general, the literature seems to mentionne only the possibility of having an antimicrobial activity through the use of quaternary ammoniums. This work demonstrates the possibility to use other types of amines to obtain an antimicrobial polymer.
Then, regarding the introduction of quaternary ammoniums in EVOH copolymers, the main objective was to evaluate the possibility to conserve an antimicrobial activity despite a production at high temperature. From these tests, it appeared that the use of the quaternary ammoniums under this

technique to finally obtain antimicrobial materials was possible. To conclude, it seems possible to use ammoniums for the fabrication of antimicrobial materials by two different ways: under standard environmental conditions but also at extreme solicitations of temperature.

Acknowledgement

This work was supported through a research program nammed STABIPACK for a development of antimicrobial materials destined to cosmetic packaging. The research program was funded by an FUI (Fonds Uniques Interministeriels) in the clusters COSMETIC VALLEY and PLASTIPOLIS.

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SUPPORTING INFORMATION

SI 1: RMN AND FTIR OF MDAB

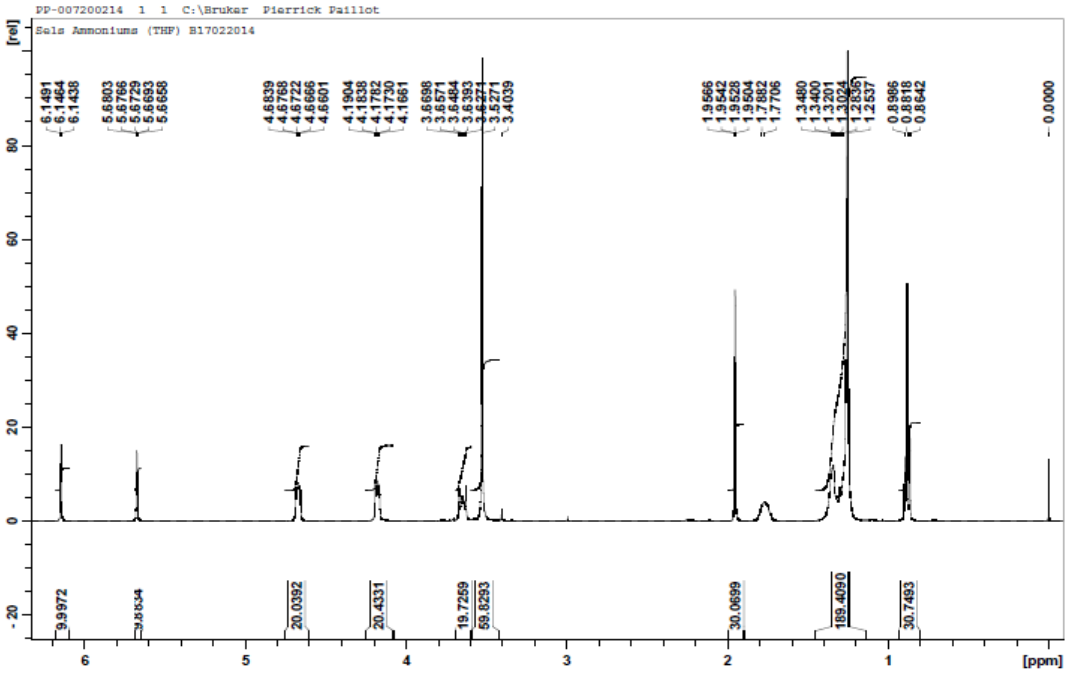


Figure 1: H RMN Spectra of the quaternary ammonium MDAB

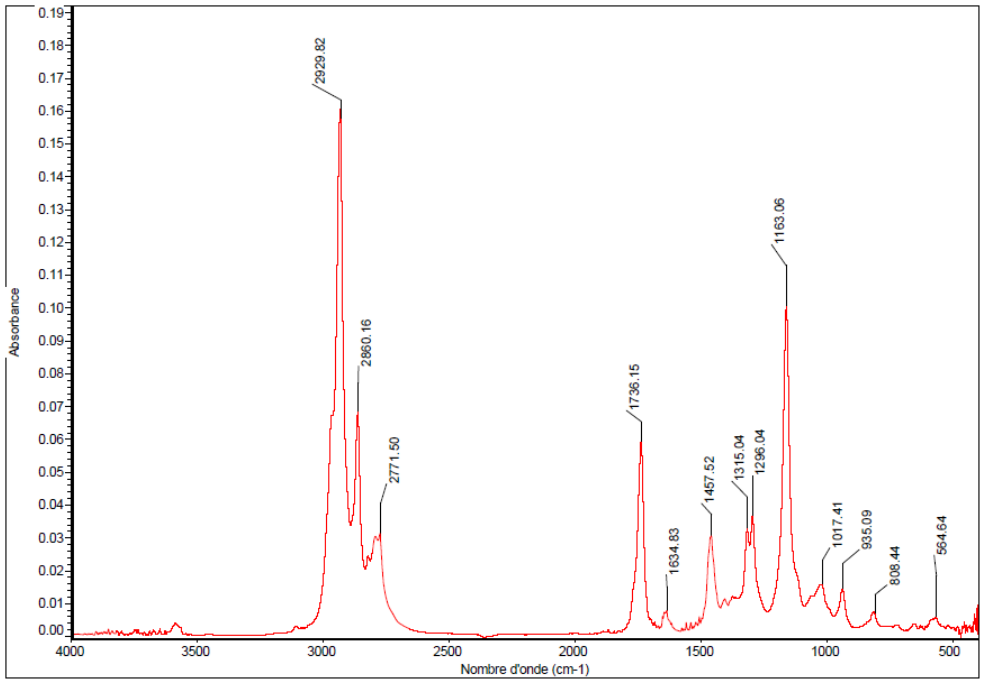


Figure 2: FT-IR spectrum of the quaternary ammonium MDAB

The structures of all products were confirmed by FTIR and H-NMR spectra. All the results were then compared with other studies which have fabricated similar compounds. In the H-NMR spectra, distinctive signals of the MDAB were observed, and means that the bromododecane have reacted with the DMAEMA to form the quaternary ammonium structure. Signals assigned to $N^{+}CH_2CH_2(CH_2)_nCH_3$ (around 3.57-3.66 ppm) confirm the formation of a pure quaternary ammonium. Then, absorption peaks around 1734cm^{-1} and 1634cm^{-1} in FTIR spectra; revealed that there were still methacrylate groups in the final product susceptible to polymerize under an UV irradiation with the others methacrylate monomers.

SI 2: THERMAL, THERMOMECHANICAL AND DAMPING PROPERTIES OF UV POLYMETHACRYLATE MATRICES

Thermal properties

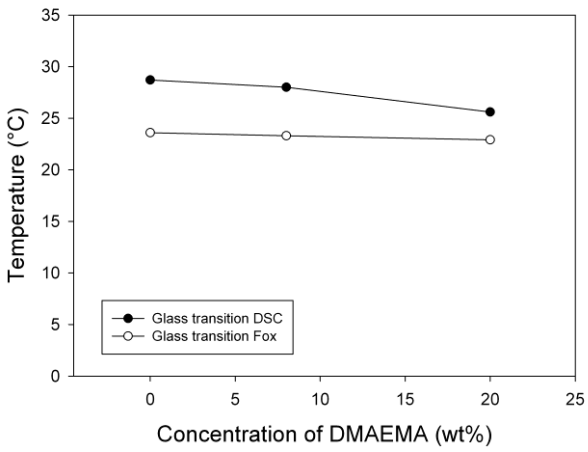


Figure 3: Curves of the glass transitions of matrices HEMA/PEGDMA (50/50wt%) with different concentration of DMAEMA: ● Tg by DSC; ○ Tg by Fox equation

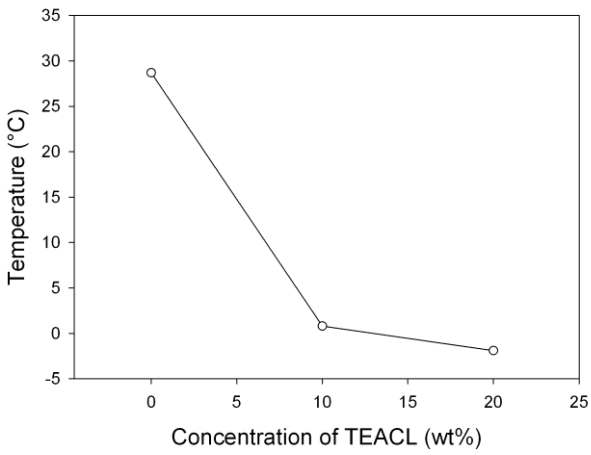


Figure 4: Curves of the glass transitions of matrices HEMA/PEGDMA (50/50wt%) with different concentration of TEACL by DSC

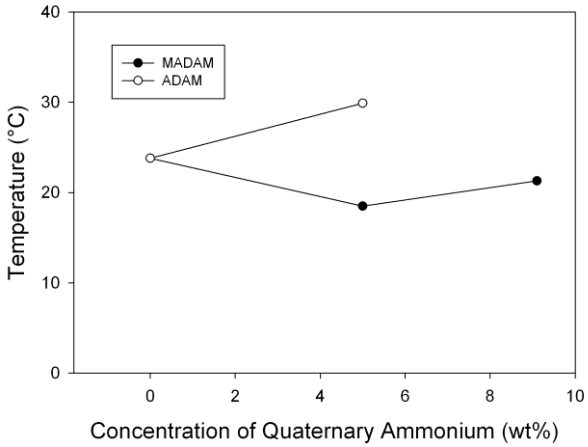


Figure 5: Curves of the glass transitions of matrices HEMA/PEGDMA (50/50wt%) with different concentration of quaternary ammoniums by DSC: ● MADAM; ○ ADAM

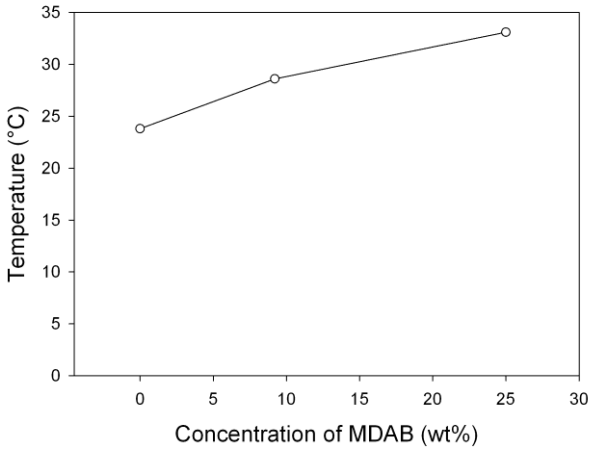


Figure 6: Curves of the glass transitions of matrices HEMA/PEGDMA (50/50wt%) with different concentration of MDAB

Thermomechanical properties

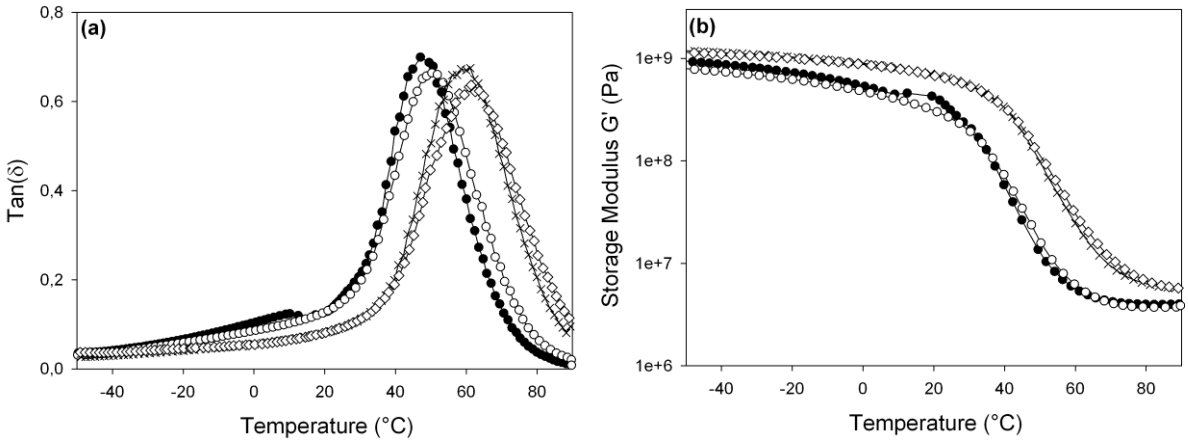


Figure 7: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for different matrices: ● HEMA/PEGDMA (50/50wt%); ○ HEMA/PEGDMA/2AEMA (45/50/5wt%); × HEMA/PEGDMA/2AEMA (40/50/10wt%); ◇ HEMA/PEGDMA/2AEMA (37.5/50/12.5wt%)

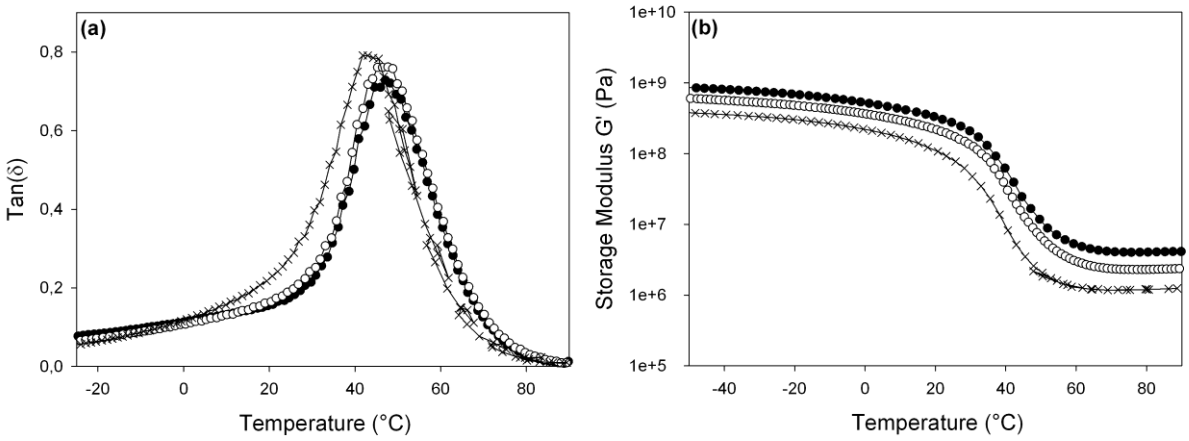


Figure 8: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices HEMA/PEGDMA (50/50wt%) with different concentration of DMAEMA: ● 0wt% DMAEMA; ○ 8wt% DMAEMA; × 20wt% DMAEMA

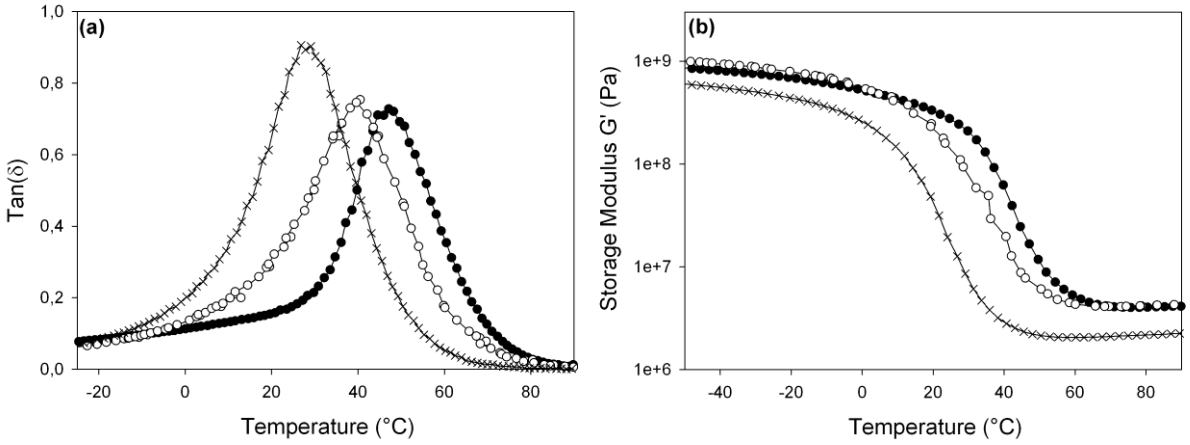


Figure 9: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices HEMA/PEGDMA (50/50wt%) with different concentration of TEACL: ● 0wt% TEACL; ○ 10wt% TEACL; × 20wt% TEACL

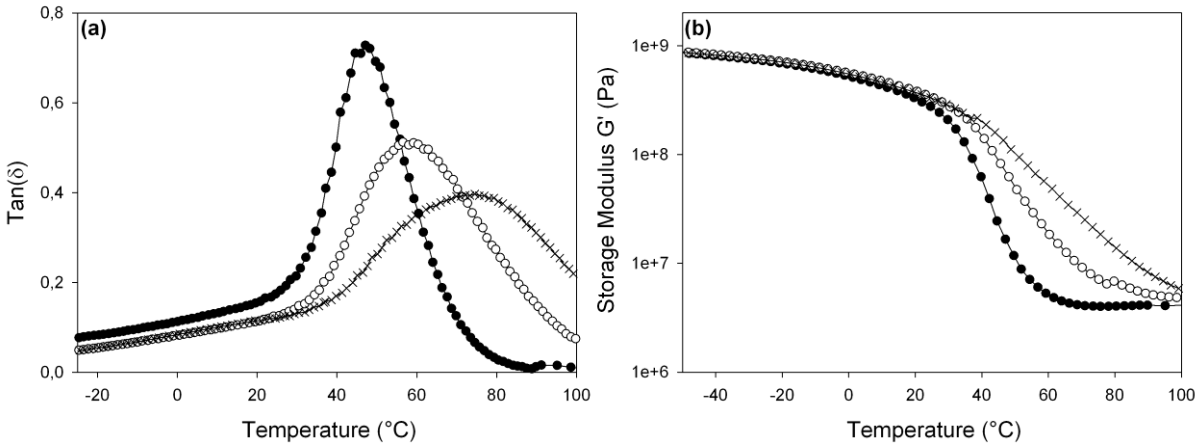


Figure 10: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices HEMA/PEGDMA (50/50wt%) with different concentration of MADAM: ● 0wt% MADAM; ○ 5wt% MADAM; × 9.1wt% MADAM

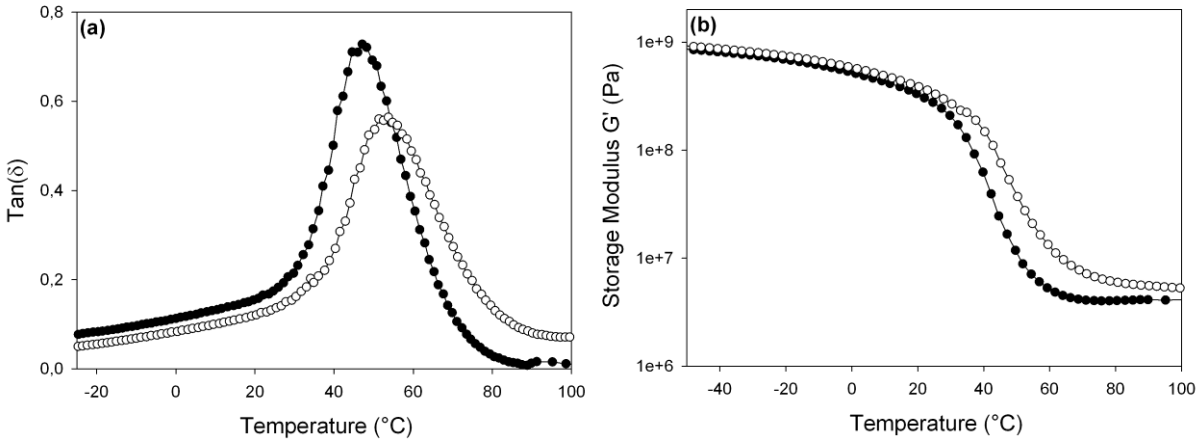


Figure 11: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices HEMA/PEGDMA (50/50wt%) with different concentration of ADAM: ● 0wt% ADAM; ○ 5wt% ADAM

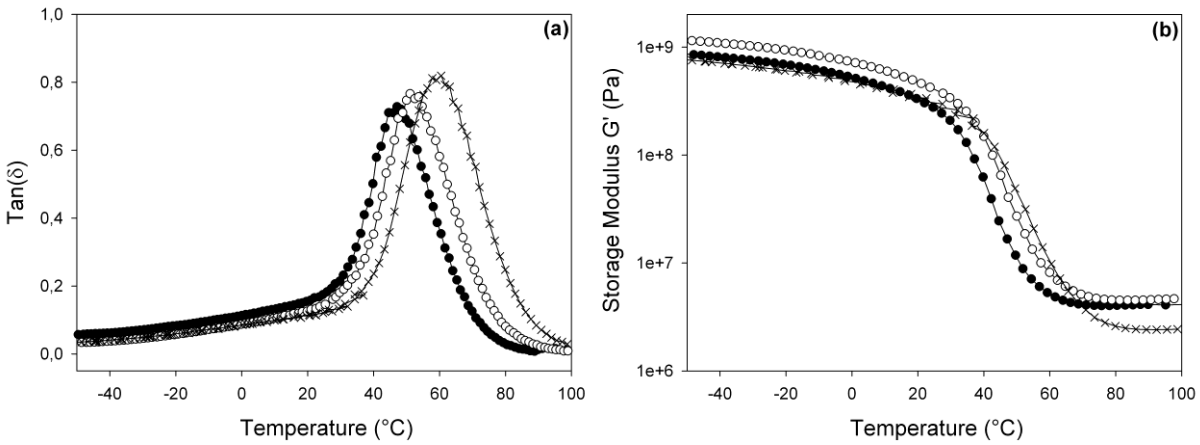


Figure 12: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices HEMA/PEGDMA (50/50wt%) with different concentration of MDAB: ● 0wt% MDAB; ○ 9.2wt% MDAB; × 25% MDAB

Damping properties

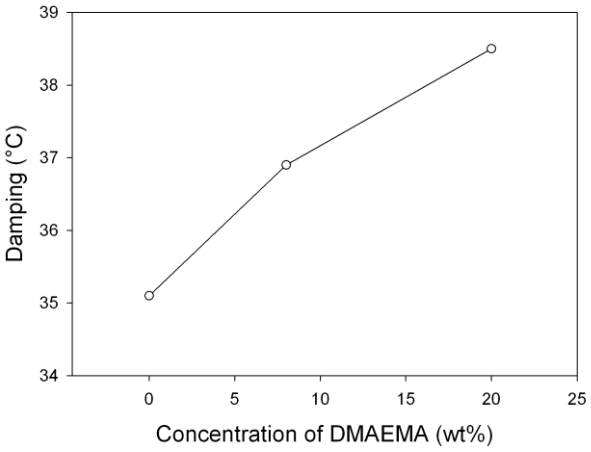


Figure 13: Curves of the damping measurements of matrices HEMA/PEGDMA (50/50wt%) with different concentration of DMAEMA

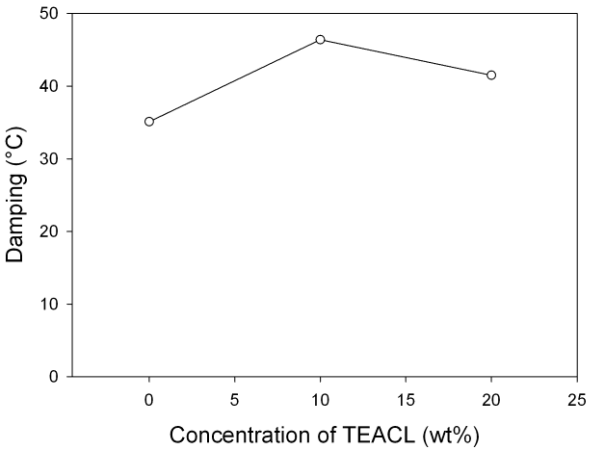


Figure 14: Curves of the damping measurements of matrices HEMA/PEGDMA (50/50wt%) with different concentration of TEACL

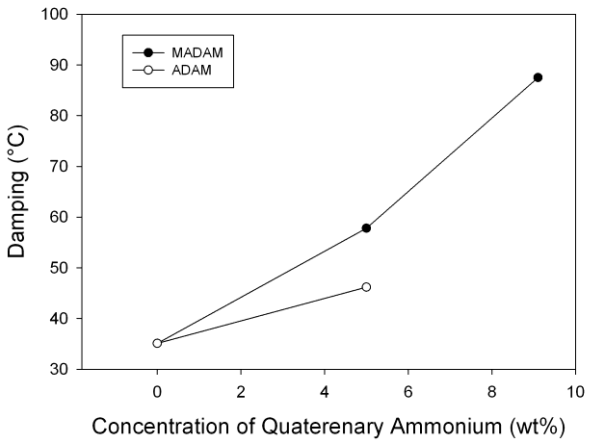


Figure 15: Curves of the damping measurements of matrices HEMA/PEGDMA (50/50wt%) with different concentration of quaternary ammoniums: ● MADAM; ○ ADAM

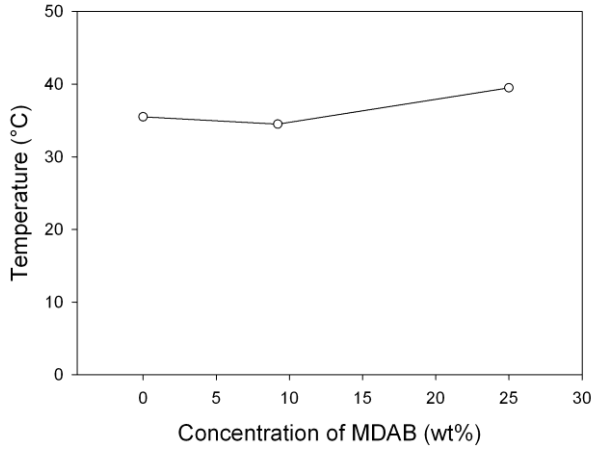


Figure 16: Curves of the damping measurements of matrices HEMA/PEGDMA (50/50wt%) with different concentration of MDAB

SI 3: THERMAL, THERMOMECHANICAL AND DAMPING PROPERTIES OF EVOH MATRICES

Thermomechanical properties

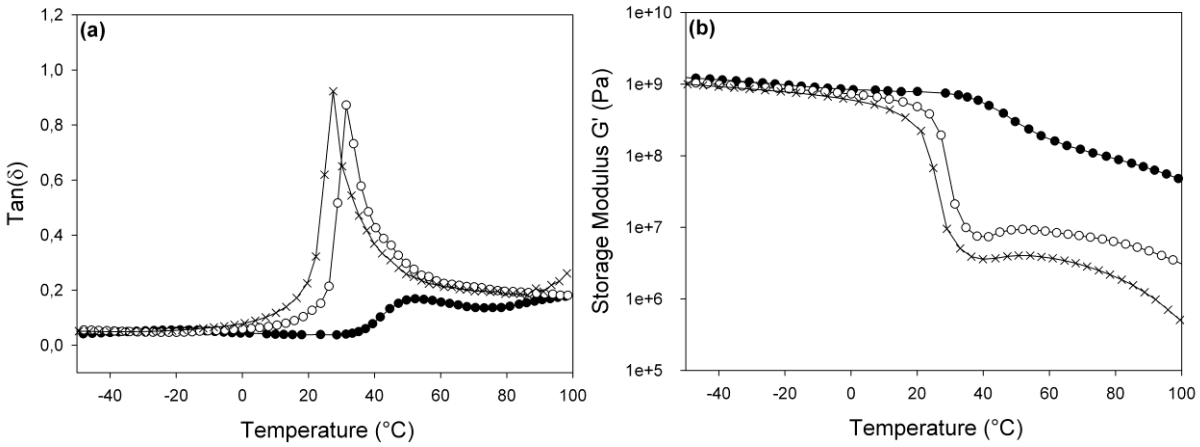


Figure 17: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices EVOH with different concentration of TEACL: ● 0wt% TEACL; ○ 15wt% TEACL; × 20wt% TEACL

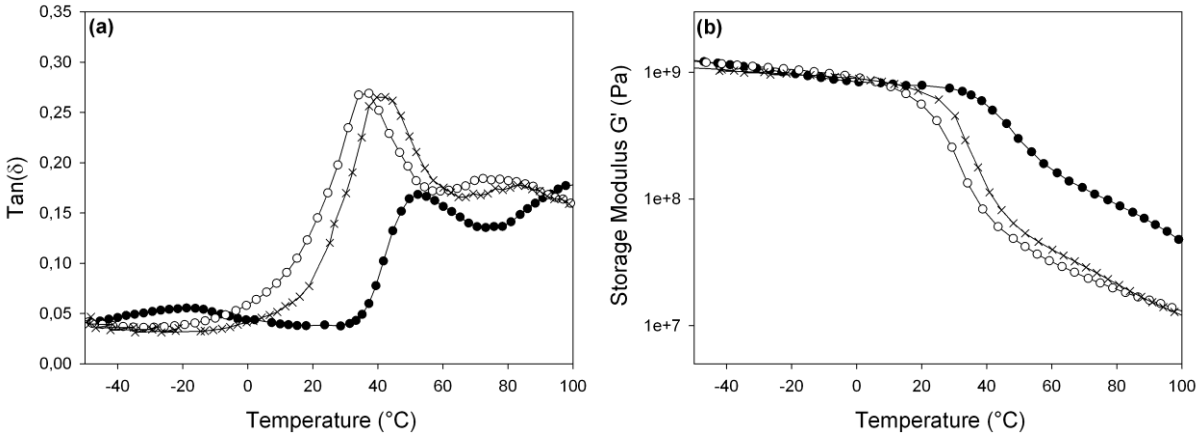


Figure 18: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices EVOH with different concentration of MADAM: ● 0wt% MADAM; ○ 15wt% MADAM without Lup101; × 15wt% MADAM with 1mol% Lup101

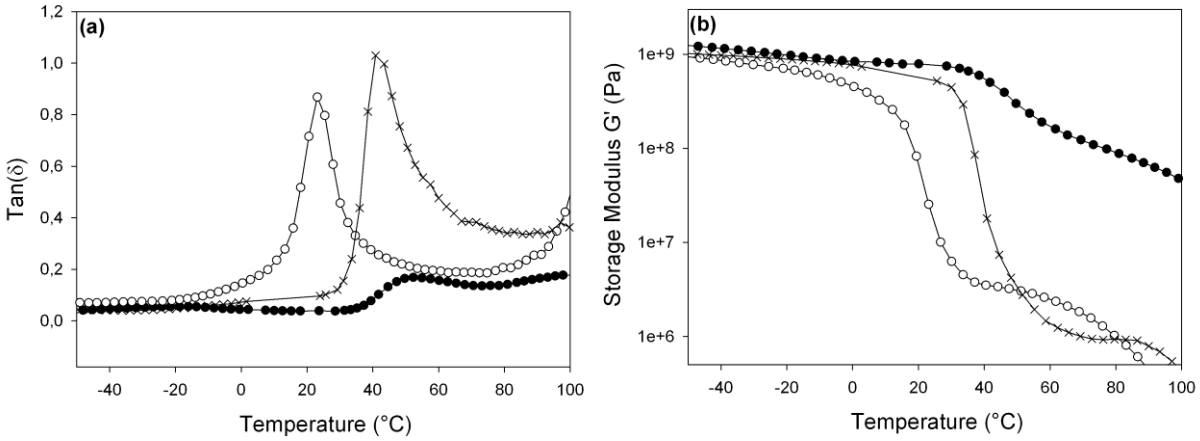


Figure 19: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices EVOH with different concentration of MADAM: ● 0wt% MADAM; ○ 35wt% MADAM without Lup101; × 35wt% MADAM with 1mol% Lup101

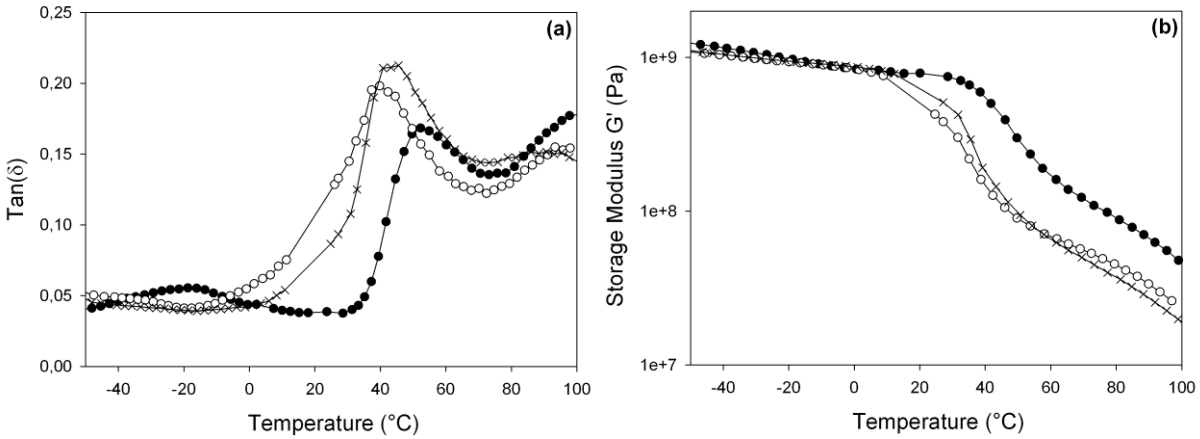


Figure 20: Curves of tan(δ) (a) and storage modulus (b) as function of temperature by torsion rectangular rheology for matrices EVOH with different concentration of MDAB: ● 0wt% MDAB; ○ 15wt% MDAB without Lup101; × 15wt% MDAB with 1mol% Lup101

CONCLUSION GENERALE

Le rôle principal d'un emballage est d'assurer une protection et une conservation du produit qu'il renferme, en termes, de qualité et d'hygiène. Cette capacité doit être effective tout au long de la « vie » du produit, de sa conception, à son utilisation par le consommateur.
Les emballages classiques permettent une protection passive mais leur efficacité est limitée. C'est pour cela, que les concepteurs de produit, en partenariat avec les fabricants d'emballages travaillent de plus en plus, sur la modification des conditionnements. Ceci pour apporter des propriétés spécifiques, et notamment une activité antimicrobienne.

Actuellement, l'allongement de la conservation grâce à l'emballage antimicrobien concerne essentiellement le secteur alimentaire. Les matériaux antimicrobiens pour la fabrication d'emballages cosmétiques sont beaucoup moins étudiés. Cependant, ce secteur évolue de plus en plus pour aller vers un packaging intelligent.
Depuis de nombreuses années, les agents conservateurs étaient plutôt incorporés aux formules cosmétiques (shampoings, crèmes de beauté, …). Mais, de plus en plus, le client exige un produit plus respectueux de sa santé mais aussi plus naturel. Ces nouvelles exigences font suite à de nombreuses polémiques. En effet, des études scientifiques mettent en garde contre l'utilisation de ces agents dits conservateurs. L'exemple des parabènes est caractéristique. Ils ont longtemps été utilisés, mais qui aujourd'hui sont soumis à controverse de part leur possible toxicité chez l'homme.
Ainsi, l'objectif des fabricants de cosmétiques aujourd'hui est de réduire au maximum la quantité des conservateurs dans leurs formulations. Le développement de systèmes d'emballage plus sophistiqués et performants en terme d'allongement de la durée de vie des produits (comme les emballages antimicrobiens) est particulièrement attendu. Ces matériaux pourraient permettre de contrôler la croissance d'organismes pathogènes pouvant proliférer dans les produits cosmétiques.
Dans la conception d'emballages actifs antimicrobiens, il est important de sélectionner des additifs efficaces contre la croissance microbienne lors de contacts répétés entre le produit qui sort de l'emballage et le consommateur.

C'est dans ce contexte que s'inscrit cette thèse portant sur la modification de polymères, par différentes méthodes afin de créer des matériaux antimicrobiens. Cette action pourra être générée par plusieurs voies, comme la libération d'actifs dans un milieu environnant, ou encore par le simple contact direct entre les microbes et des sites actifs greffés dans les matrices.

La première partie des travaux a concerné la fabrication de revêtements protecteurs à partir d'une matrice méthacrylate polymérisée sous irradiation UV. L'objectif était d'avoir un matériau sous forme liquide initialement dans lequel serait incorporé un agent antimicrobien et qui puisse facilement s'adapter à des zones bien précises de l'emballage. Plus particulièrement, il s'agit de venir protéger par

un revêtement en couche mince, des zones à fort risque de contamination, c'est-à-dire qui sont souvent au contact du consommateur.

La difficulté initiale à laquelle j'ai pu être confrontée, en tant qu'ingénieur matériaux, était une connaissance très limitée du monde de la bactériologie. En effet, je ne disposais d'aucune connaissance, sur les matériaux antimicrobiens, et plus précisément, sur les types d'agents actifs existant, ainsi que leur efficacité réelle. Je n'étais pas en mesure par rapport au système de matrice méthacrylate initialement choisit, de prédire quels seraient les meilleurs agents antimicrobiens.

Le choix d'une matrice méthacrylate polymérisée sous UV s'est imposé car j'avais la possibilité de créer un matériau à une température pas trop extrême. En effet, l'une de nos craintes initiales était la possibilité de dégrader les agents antimicrobiens par des contraintes thermiques trop élevées. On retrouve ces contraintes lors de certaines mises en œuvre, telles l'extrusion ou l'injection.

L'originalité de cette première partie de thèse repose sur l'aspect comparatif de l'efficacité antimicrobienne de différentes familles d'actifs incorporés dans un même matériau, et cela par une même technologie de mise en œuvre.

Pour ce faire, une sélection d'agents, à l'aide d'une première étude bibliographique, a été faite. Ces agents ont pu être introduits dans un matériau référence qui a ensuite été étudié.

L'évaluation des propriétés thermiques a montré que les agents, selon leur nature, agissaient de différentes manières. Selon leur possibilité de réaction avec les monomères au moment de la polymérisation. Certains agents peuvent réagir, d'autres non, ce qui provoque la formation de différents réseaux avec des propriétés variables.

Après cette étude thermique, une évaluation des propriétés antimicrobiennes, mais aussi de libération en milieux aqueux, a été réalisée. Une fois encore, nous avons démontré d'importantes différences selon les agents, en fonctions de plusieurs paramètres, diffusions, affinités avec l'environnement, propriétés physico-chimiques… Cette étude bactériologique a finalement permis de sortir quelques agents antimicrobiens, possédant une activité intéressante par rapport à l'application industrielle d'emballages. Nous avons pu établir une classification des agents antimicrobiens selon leur efficacité et faire une sélection pour la suite de nos travaux.

Dans une deuxième partie, des sels métalliques (acétates de cuivre et d'argent) ont été utilisés comme agents actifs pour l'élaboration de revêtements antimicrobiens. L'élaboration de ces films a été réalisée selon le même procédé, c'est-à-dire une polymérisation radicalaire sous UV.

Ce chapitre a surtout concerné l'impact des sels sur un réseau méthacrylate à différentes concentrations. L'objectif initial était d'évaluer l'impact que pouvait avoir une telle modification des concentrations sur diverses propriétés thermiques, viscoélastiques, morphologiques, mais aussi sur la libération des antimicrobiens dans le milieu environnant.

Les résultats ont démontré que ce paramètre concentration impactait les propriétés finales du matériau. Mais nous avons également montré une possibilité de réaction thermique des sels métalliques, facilitée par le contact avec les matrices méthacrylates.
Ce phénomène est lié à une décomposition thermique de ces sels. Ce procédé a été étudié par le passé et évoqué lors de la conception par exemple de nanoparticules métalliques.

La troisième partie de ce travail a consisté en la transition d'une technologie de mise en œuvre à température ambiante à une technique haute température. Pour cette transition, le choix de la mise en forme a été une extrudeuse bi-vis, co-rotative de laboratoire. Ceci pour dans un premier temps s'approcher des technologies souvent utilisées au niveau industriel dans la conception d'emballages.
L'idée était de conserver une activité antimicrobienne malgré de plus extrêmes sollicitations de fabrications (températures, cisaillements).
Le matériau utilisé pour cette partie était un EVOH, un copolymère semi-cristallin souvent utilisé pour la conception d'emballage. C'est l'un des critères qui a justifié le choix de ce matériau pour la présente étude. Par la suite, deux agents actifs, précédemment étudiés, l'acétate de cuivre et l'acétate d'argent ont été incorporés aux EVOH pour évaluer l'impact qu'ils peuvent avoir sur certaines propriétés thermique, viscoélastiques ou morphologiques. Nous avons pu montrer une possible réaction thermique de ces actifs. Un autre objectif a donc été de vérifier si cette réaction apparaissait in-situ, au moment de l'extrusion. Il a fallu évaluer l'impact propriété et surtout conclure sur une possibilité d'obtenir un matériau antimicrobien. Nous avons pu ainsi démontrer la possibilité d'introduire ces sels métalliques, au sein d'EVOH par extrusion, et constater que ceux-ci ne modifiaient pas de manière trop importante les propriétés de la matrice. Cela nous a montré également qu'il y avait une réaction de décomposition thermique des sels, permettant la création de particules élémentaires métalliques de taille nanométrique.
Finalement, le point le plus important a été de prouver par cette étude la possibilité de créer des matériaux polymères antimicrobiens par extrusion en une seule étape et ceci sans solvant.
Malgré tout, cette réactivité des acétates nécessiterait une étude plus approfondie dans le cas d'un passage à une échelle industrielle. En effet, cette réactivité thermique restreint de manière importante, la plage de temps de séjour en extrudeuse ainsi que les contraintes thermiques et mécaniques. D'où la nécessité d'optimiser au maximum tous ces paramètres afin d'obtenir un matériau EVOH qui pourrait venir remplacer certaines pièces des emballages commerciaux existant.

En conclusion, en se référant à l'étude réalisée, il serait possible d'utiliser toutes les solutions proposées afin de modifier les emballages cosmétiques actuels. Ces améliorations pourraient assurer une qualité et une sécurité des produits par une meilleure capacité de conservation et une moindre contamination bactérienne.

Une possibilité serait de combiner les différentes technologies proposées pour créer des matériaux hybrides, avec plusieurs types d'actions et ainsi augmenter l'efficacité antimicrobienne. L'idée serait de créer lors d'une première étape d'extrusion, certaines pièces des emballages actuels avec des granulés d'EVOH modifiés. Ensuite, la seconde étape serait de traiter ces pièces avec un revêtement protecteur par une polymérisation UV afin de réaliser une couche mince antimicrobienne. Ceci pourrait assurer une première action rapide, par la matrice UV, suivit d'une seconde action sur un temps plus long par le matériau EVOH.

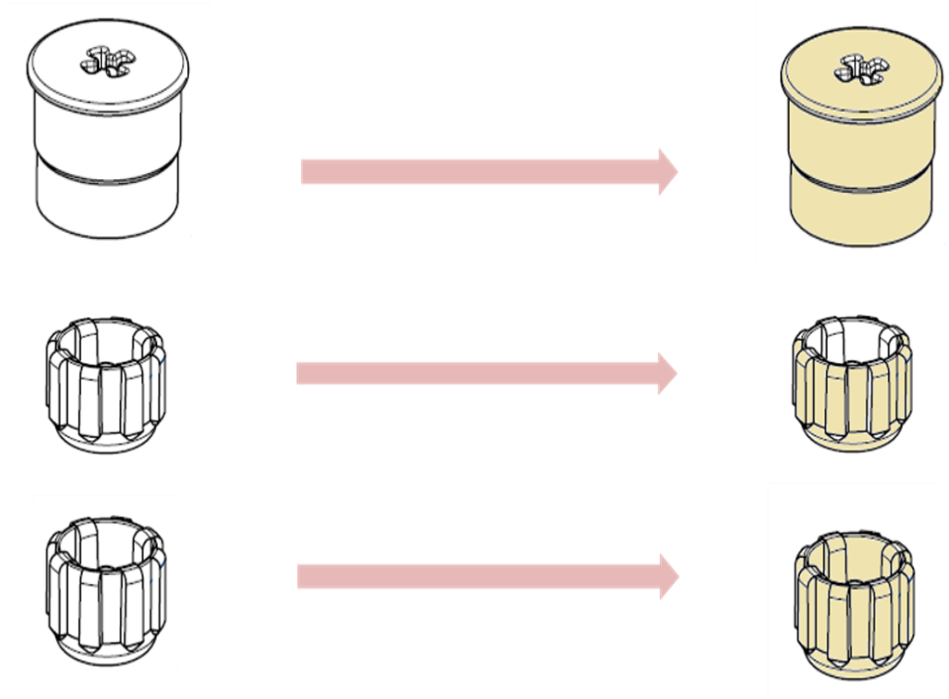
PERSPECTIVES

Cette thèse a proposé des solutions de modifications de matériaux polymères, par deux voies d'action, l'une à basse température et une autre à haute température. Ces modifications ont permis notamment de créer des polymères antimicrobiens qui pourront rentrer dans la fabrication finale d'emballages destinés au secteur de la cosmétique.
Cependant, beaucoup de choses restent à faire et en particulier la confrontation de toutes les idées proposées pour une possible adaptation à des applications commerciales.

Pour ce passage de l'étude laboratoire au monde industriel, plusieurs étapes sont nécessaires. Dans un premier temps, il sera important de réaliser des prototypes à une échelle pilote. Cette étape devra permettre d'aboutir à des essais antimicrobiens en conditions réelles d'utilisations, c'est-à-dire évaluer l'activité bactériologique de l'emballage avec un produit cosmétique conditionné à l'intérieur.
Par la suite, dans le cas ou certaines des solutions proposées s'avéreraient intéressantes, il sera envisagé de reprendre les meilleures idées pour les transposer à une échelle industrielle et concevoir des emballages antimicrobiens destinés à la vente.

Actuellement, la première étape pilote a été initiée et a permis la réalisation de prototypes d'emballages cosmétiques. Ces tentatives ont été réalisées en partenariat avec la société ALBEA. Différents prototypes d'embouts polymères ont été faits et seront adaptables à l'emballage cosmétique. Ces embouts sont constitués de deux parties, qui s'assemblent pour former le prototype final qui s'emboite sur la zone d'ouverture de l'emballage.
4 prototypes ont été crées, deux à basse température par polymérisation UV et deux autres par la méthode d'extrusion à haute température.

Concernant la voie basse température, des embouts ont été injectés à partir de granulés polypropylènes non antimicrobiens. Les deux parties (supérieure et inférieure) des embouts ont par la suite été traités avec une couche protectrice méthacrylate photo-polymérisée comme l'illustrent les schémas ci-dessous. Cela doit permettre la création de prototypes antimicrobiens et finalement la protection des formulations cosmétiques. De ces premiers essais, une fine couche méthacrylate antimicrobienne a pu être déposée sur les préformes supérieure et inférieure sans aucun problème apparent. La couche protectrice a été parfaitement polymérisée, sans défaut et un prétraitement des pièces polypropylènes par un flammage a permis d'assurer une très bonne adhésion du revêtement antimicrobien.



Traitement la zone supérieure et inférieure de l'embout prototype injecté à partir de polypropylène non antimicrobien avec une couche méthacrylate photopolymérisé sous UV

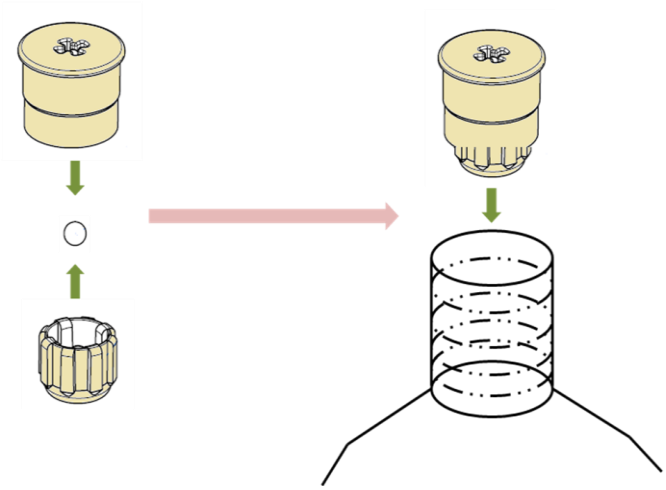
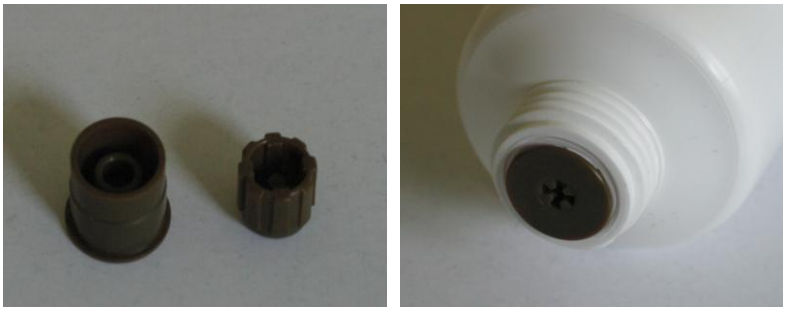


Schéma d'assemblage de l'embout prototype final avant incorporation sur la zone d'ouverture de l'emballage cosmétique

Pour la technique à haute température, une première étape a consisté à fabriquer des granulés d'EVOH antimicrobiens avec des ajouts d'acétate d'argent et d'acétate de cuivre et ceci par une technologie d'extrusion bi-vis à une échelle pilote. Plus précisément, deux formulations ont été sélectionnées, l'une avec l'argent et une autre avec le cuivre pour créer deux natures de granulés EVOH. Ce sont ces granulés qui ont par la suite été injectés pour former les embouts protecteurs et adaptables aux emballages comme l'illustrent les photos ci-dessous.



Embouts antimicrobiens injectés à partir de granulés EVOH modifiés avec des acétates d'argent

Embouts antimicrobiens injectés à partir de granulés EVOH modifiés avec des acétates de cuivre

Tous les prototypes fabriqués vont subir prochainement, des phases de tests antimicrobiens en conditions réelles. Ces évaluations doivent permettre de répondre dans un premier temps à la question, « est ce que ces embouts protègent efficacement le produit cosmétique contre toute contamination microbienne ? ».

PUBLICATIONS ET COMMUNICATIONS

PUBLICATIONS

Ce travail à donner lieu aux publications suivantes :

Antimicrobial resins for cosmetic packaging. (Soumise)
Pierrick Paillot, Frédéric Becquart, Corinne Jegat, Mohamed Taha

Effects of antimicrobial agents on the thermal and mechanical properties of acrylate hydrogel matrices. (Soumise)
Pierrick Paillot, Corinne Jegat, Frédéric Becquart, Mohamed Taha

Synthetic polymer matrices EVOH with introductions at high temperature of metal compounds for antimicrobial applications (Silver Acetate). (Prochainement Soumise)
Pierrick Paillot, Frédéric Becquart, Corinne Jegat, Mohamed Taha

Synthetic polymer matrices EVOH with introductions at high temperature of metal compounds for antimicrobial applications (Copper (II) Acetate). (Prochainement Soumise)
Pierrick Paillot, Frédéric Becquart, Corinne Jegat, Mohamed Taha

Amines in antimicrobial cosmetic packaging. (Prochainement Soumise)
Pierrick Paillot, Frédéric Becquart, Corinne Jegat, Mohamed Taha

COMMUNICATIONS

Juin 2015 European Polymer Congress – Dresde, Allemagne.
Antimicrobial materials to protect areas with risk of bacterial contamination for packaging applications.
Pierrick Paillot, Frédéric Becquart, Corinne Jegat, Mohamed Taha

Novembre 2014 GFP – Saint Malo, France. Poster Présenté.
Hydrogels antibactériens.
Pierrick Paillot, Frédéric Becquart, Corinne Jegat, Mohamed Taha

Octobre 2014 JEPO 42 – Obernai, France.
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Résumé :

Le marché de la cosmétique est l'un des marché les plus porteur actuellement dans le monde. La population française utilise énormément ces produits pour son hygiène quotidienne. Ce sont les shampoings ou autre crème de soins. Pour protéger ces produits, depuis maintenant de nombreuses années, les fabricants ajoutent des agents conservateurs pour augmenter la durée de conservation, ou encore éviter certaines contaminations microbiennes après les contacts avec la peau.
Ces dernières années ont également vu les mentalités des consommateurs évoluer et actuellement, ces derniers souhaiteraient des produits cosmétiques le plus naturel possible, sans ajouts de conservateurs. Dans ce contexte, il semble intéressant de travailler sur la protection de ces produits par d'autres moyens. En premier lieu, nous vient immédiatement à l'esprit, la protection par l'emballage. L'idée est de modifier les emballages actuels pour leur conférer des activités antimicrobiennes et ainsi les rendre protecteurs.

L'étude présentée s'intéresse à certaines possibilités de mises en œuvre et modifications de polymères pour apporter une activité antimicrobienne. Deux voies de fabrications ont été étudiées dans cette thèse. Une première a consisté en la réalisation de revêtements antimicrobiens et protecteurs à basse température. Cette technique a montré la possibilité de créer des couches antimicrobiennes par photo-polymérisation à partir de monomères méthacrylates renfermant les agents antimicrobiens. C'est cette couche finale qui va venir s'ajouter à certaines zones spécifiques des emballages finaux pour assurer la protection antimicrobienne du contenu.
La seconde voie d'action a étudié une fabrication plus industrielle à haute température. Cette technologie a permis de créer par extrusion des granulés antimicrobiens avec des introductions d'actifs de différentes natures. Ceux sont ces granulés qui sont par la suite injectés sous la forme d'emballages. Pour cette voie d'action, l'idée n'est plus de protéger l'emballage via une couche antimicrobienne, mais de substituer certaines pièces de l'emballage constituées de polymères naturellement antimicrobiens.

Ces travaux ont permis la réalisation d'une large gamme de matériaux antimicrobiens. Les différentes solutions étudiées ont également permis de réaliser des prototypes d'emballages, ceci en collaboration avec certaines entreprises partenaires du projet. Tous ces prototypes seront prochainement testés en conditions réelles d'utilisations, c'est-à-dire par des essais de mises en contact avec le consommateur d'un système complet, à savoir le produit cosmétique conditionné dans des emballages protecteurs. Ces tests devront permettre de vérifier si les solutions proposées pourraient aboutir à une adaptation sur une chaine industrielle pour une utilisation à grande échelle.

Mots clés : Emballages, cosmétiques, antimicrobiens, antibactériens, polymères, protection des formulations cosmétiques, modifications, méthacrylates, EVOH

Abstract :

Today the cosmetic market is one of the most popular in the world. French people use many these products everyday as for examples, creams or shampoos. In order to protect the cosmetic products, the manufacturers introduced during these last year additives or conservative agents in cosmetic products to increase the shelf life or avoid a microbial contamination after a direct contact with the skin of consumers. However, recent years have also revealed that the consumer mindsets evolve and now, they would like cosmetics more natural, without addition of additives.
In this context, it was interesting to work on the cosmetics protection by other ways. Firstly, we think immediately to the protection by use of packaging. The objective is to modify the current packaging to bring an antimicrobial activity and protect the cosmetics.

This work presents different technologies of fabrication and modification of polymers to get an antimicrobial activity. For this, two techniques were studied. A first technology has consisted to develop antimicrobial coating at low temperature. This way has demonstrated possibilities of creation by photo-polymerization under UV radiations. Initially, the antimicrobial agents were introduced in liquid monomers before the polymerization and the fabrication of polymer networks. The final coatings were finally destiny to be added on specific areas of packaging, generally in contact with the consumers and prevent all risks of microbial contaminations from the products.
The second technology has studied a way of polymer fabrication more industrial at high temperature. The technique has consisted to create antimicrobial pellets by extrusion with introductions of different natures of additives. The obtain pellets were injected at the end to fabricate certain pieces of the final packaging.

This work has allowed the realization of a large range of antimicrobial materials. All the studied solutions have been used to fabricate prototype packaging in collaboration with partner companies of the project. All these prototypes will be tested by antimicrobial tests in real conditions of uses. If these tests prove successful, it will be possible to envisage an industrialization step.

Keywords: Packaging, cosmetics, antimicrobials, antibacterials, polymers, protection, modifications, methacrylates, EVOH