



Development of intelligent packaging based on sol-gel fluorescent materials to monitor carbonyl compounds released by lipid oxidation

Laura Carballido

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Laura Carballido. Development of intelligent packaging based on sol-gel fluorescent materials to monitor carbonyl compounds released by lipid oxidation. Other. Université Bourgogne Franche-Comté, 2023. English. <NNT : 2023UBFCK035>. <tel-05381822>

HAL Id: tel-05381822

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DOCTORAL DISSERTATION OF THE UNIVERSITY OF BURGUNDY FRANCHE-COMTE

PREPARED AT L'INSTITUT AGRO DIJON

Doctoral school n° 554

« Environnements – Santé »

PhD in agri-food biotechnologies

By

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**Development of intelligent packaging based on sol-gel fluorescent materials
to monitor carbonyl compounds released by lipid oxidation.**

Thesis presented and defended in Dijon on July 6, 2023

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La thèse n'est pas un sprint, c'est marathon. Enfin, un marathon couru par Usain Bolt.

REMERCIEMENTS

Je remercie tout d'abord M. Laurent BENEY, directeur de l'Unité Mixte de Recherche « Procédés Alimentaires et Microbiologiques » de m'avoir accueillie au sein de son unité. Je remercie également le programme « Investissements d'Avenir » du dispositif ISITE-BFC pour le financement de cette thèse.

J'exprime mon immense gratitude envers M. Thomas KARBOWIAK, directeur de l'équipe « Physico-Chimie des Aliments et du Vin » pour m'avoir accordé sa confiance en me recrutant sur ce projet de thèse. Mais avant tout, merci d'avoir décroché le téléphone en cet après-midi du vendredi 7 juin 2019, sans quoi cette aventure commune n'aurait jamais débuté. Avec toi, pas de place pour le syndrome de l'imposteur, tu m'as toujours considérée comme une chercheuse et je devais être à la hauteur. Merci pour ton exigence afin de tirer le meilleur de ce travail. Je n'oublierai jamais l'immense satisfaction que j'ai lue sur ton visage lorsque nous avons appris que notre revue avait été acceptée dans *Chem*.

Pour M. Elias BOU-MAROUN, mon directeur de thèse, ni les cinq lettres du mot merci ni toutes les lettres de l'alphabet ne suffiraient à exprimer à quel point je le remercie d'avoir encadré cette thèse au quotidien et d'avoir toujours été à mes côtés dans les moments de joie comme dans les moments de doute. Merci de m'avoir épaulée tout au long de ce parcours. Ta gentillesse, ta bienveillance et ta sérénité en toute circonstance m'ont permis de faire face à tous les obstacles. Ce fut un vrai bonheur de travailler avec toi.

Merci à M. Philippe CAYOT et M. Nicolas SOK pour votre accueil en thèse. Philippe, merci de m'avoir fourni les clés nécessaires pour appréhender le vaste domaine de l'agroalimentaire à mon arrivée.

Je remercie également tous les membres du jury Mmes Sandra DOMENEK, Christine GOZE et Anne BOOS ainsi que MM. Piersandro PALLAVICINI et Eric MARCHIONI, d'avoir accepté d'évaluer ce travail, et qui, j'en suis sûre, ne pourront que l'enrichir de leurs précieuses réflexions. Mmes Christine GOZE et Anne BOOS, merci d'avoir fait partie de mon comité de suivi de thèse. Vos retours, toujours bienveillants, m'ont beaucoup apporté.

Je tiens à remercier tous ceux que j'ai pu côtoyer au sein de l'université au cours de ces années de thèse. Merci notamment à tous les membres permanents de l'équipe, Camille LOUPIAC, Adrien LEBRET, Ali ASSIFAOU, Samuel LUBBERS, Dominique CHAMPION, Gaëlle ROUDAUT, Nathalie CAYOT, Régis GOUGEON, Rémi SAUREL, Agathe PISIS, Anne-Sophie VIAUX et toutes autres personnes dont j'ai pu croiser le chemin. Céline LAFARGE, merci pour ta gentillesse, ta disponibilité, ton temps et ton expertise en chromatographie. Pascale WINKLER, je te remercie pour ta disponibilité et ton aide en spectroscopie de fluorescence. Igor BEZVERKHYY et Guy WEBER, quelle chance d'avoir eu l'occasion de travailler avec vous. Merci pour le temps que vous avez passé avec moi sur ce projet pour des expériences ou à me former dans des domaines que je ne connaissais pas. Merci pour votre accueil toujours chaleureux et votre disponibilité, j'ai adoré travailler avec vous et j'ai énormément appris grâce à vous. Bernadette ROLLIN, à côté de toi, tous les ingénieurs ingénieux font pâle figure ! Merci pour l'aide précieuse que tu m'as apportée quand il s'agissait de monter une nouvelle manip. Si ce labo ne devait avoir qu'une âme, ce serait la tienne sans hésiter ! Alexandra DA SILVA, merci pour ta disponibilité et ton investissement pour que les thèses se déroulent au mieux. Emilie BOURGEOIS, merci de ta gentillesse et ton accompagnement pour le dépôt du brevet et la candidature au concours i-PhD.

Virginie GUERLET, Lucie RICHETER, vous avez été des stagiaires exceptionnelles et au-delà du travail remarquable que vous avez fourni, j'ai été ravie de partager ces périodes avec vous. Vous avez toutes deux contribué à la réussite de cette thèse.

Merci à tous mes collègues doctorants ou contractuels Tiffany, Gisela, Mehrsa, Gayane, Mathieu, Sandy, Léo, Maria, Truc, Taisiia, Thierry, Marie, Manon, Fanny, Marie-Sarah, Camille, Maelys, Boni, Franck, Fatima et toutes les personnes que je n'ai pas citées qui ont permis de faire de ces années de thèse une aventure humaine. Pierre, je ne m'attendais pas à commencer ma thèse exactement le même jour que toi. Quelle joie cela a été de partager ce quotidien de thésard avec toi, de prendre le thé et de se lamenter sur nos expériences pas toujours couronnées de succès. Julie, merci de m'avoir si bien vendu la ville de Dijon et la vie au labo, te parler lors de mon entretien n'a fait que renforcer ma certitude que ma place était à vos côtés. Et s'en est suivie une belle amitié. Max, tu es le soleil italien de ce labo. Estelle, merci pour ta bonne humeur au quotidien et tes incroyables Murder Party. Mélissa, merci

pour ton franc-parler qui illumine la vie au bureau. Jonathan, je suis ravie d'avoir rencontré un ami amateur de musées. Aurélie, merci pour ta joie communicative.

Merci à tous les professeurs qui m'ont tant appris au cours de ces innombrables années passées sur les bancs de l'école, une partie de chacun de vous m'a guidée jusqu'ici. La liste serait longue pour citer toutes les personnes à qui je dois ma réussite aujourd'hui, mais je souhaitais tout de même accorder une place particulière à M. BOUGNOL – alias BouBou – mon professeur de mathématique de première année de prépa. Steve CUTAYAR, merci d'avoir pris soin de ma santé physique et mentale avec tes fabuleux cours de Pilates.

Evidemment, mes plus profondes pensées vont à ma famille, en particulier ma petite sœur adorée et mes parents, à qui je dois tout et qui m'ont permis de me tenir là aujourd'hui. J'espère que ce titre et ce travail les rendra fiers de ce que je suis devenue. Tatïe, tonton, ça y est, 10 ans après avoir passé mon bac, je peux enfin vous dire que j'ai terminé mes études ! Minouche, tu mérites que je te remercie pour ton soutien dans la rédaction de ce manuscrit. S'il y a des fautes de frappe, ce sera parce que tu as marché sur le clavier.

Ker gi, merci n'est pas à la hauteur de ce que j'ai à te dire après nos 9 années de vie commune. Tu fais partie des piliers de cette réussite. Si tu ne m'avais pas donné un coup de pouce en prépa, qui sait si le parcours aurait été le même ? Merci de m'avoir épaulée chaque jour de cette thèse comme tu l'as toujours fait, de m'avoir écoutée répéter des milliers de fois pour mes congrès, et de m'avoir laissé t'expliquer mes résultats inattendus. Ta curiosité m'a bien aiguillée.

Enfin, mes amis de Strasbourg, de Rennes et d'ailleurs, beaucoup sont devenus docteurs, plusieurs le deviendront encore, à mon tour de célébrer ce diplôme avec vous !

LIST OF PUBLICATIONS AND COMMUNICATIONS

POSTER COMMUNICATION

1. Laura CARBALLIDO, Elias BOU-MAROUN, Philippe CAYOT, Nicolas SOK, Thomas KARBOWIAK. Intelligent packaging to reduce food waste. **Forum Jeunes Chercheurs (FJC) by the doctoral school “Environment & Health”, UBFC**. Online (June 2021).

ORAL COMMUNICATIONS

1. Laura CARBALLIDO, Elias BOU-MAROUN, Philippe CAYOT, Nicolas SOK, Thomas KARBOWIAK. Development of intelligent packaging to monitor food degradation. **Forum Jeunes Chercheurs (FJC) by the doctoral school “Environment & Health”, UBFC**. Dijon, France (June 2022). Price of best oral communication.
2. Laura CARBALLIDO, Elias BOU-MAROUN, Philippe CAYOT, Nicolas SOK, Thomas KARBOWIAK. Développement d’un emballage intelligent permettant de suivre la dégradation des aliments et réduire le gaspillage. **1ères Journées Scientifiques SCF-GFP (Société Chimique de France – Groupe Français des Polymères) Grand-Est**. Besançon, France (July 2022).
3. Laura CARBALLIDO, Elias BOU-MAROUN, Philippe CAYOT, Nicolas SOK, Thomas KARBOWIAK. Développement d’un emballage intelligent permettant de suivre la dégradation des aliments et réduire le gaspillage. **Conférence Internationale Matériaux**. Lille, France (October 2022).
4. Laura CARBALLIDO, Elias BOU-MAROUN, Philippe CAYOT, Nicolas SOK, Thomas KARBOWIAK. Development of intelligent packaging to monitor food degradation and reduce food waste. **36th EFFoST (European Federation of Food Science and Technology) International Conference 2022**. Dublin, Ireland (November 2022).
5. Laura CARBALLIDO, Elias BOU-MAROUN, Philippe CAYOT, Nicolas SOK, Thomas KARBOWIAK. Développement d’un emballage intelligent permettant de suivre la dégradation des aliments. **Journée des Doctorants (JDD) by the UMR “Food and Microbiological Processes”, Institut Agro Dijon**. Dijon, France (November 2022).

6. Laura CARBALLIDO, Elias BOU-MAROUN, Philippe CAYOT, Nicolas SOK, Thomas KARBOWIAK. Développement d'un emballage intelligent permettant de suivre la dégradation des aliments. **Deuxièmes Rencontres Capteurs de l'Université Bourgogne Franche-Comté (RCUBFC)**. Dijon, France (November 2022).

PUBLICATIONS

1. Pending patent n° FR2113403, December 13th, 2021.
PCT international extension n° PCT/EP2022/085460, December 12th, 2022.
2. Carballido, L. *et al.* Applications of molecularly imprinted polymers and perspectives for their use as food quality trackers. *Chem* **8**, 2330–2341 (2022), 10.1016/j.chempr.2022.05.021.

COMPETITIONS

1. Regional finalist of the competition “**Ma Thèse en 180 secondes**” 2022 (Burgundy Franche-Comte).
2. Winner of the **i-PhD** competition 2023 (BPI France).

ABSTRACT

Reducing food waste while ensuring food safety is a major challenge facing the agri-food industry today. Lipid oxidation is the main chemical degradation process involved during food spoilage. In addition to a loss of nutritional value, lipid oxidation generates unpleasant off-flavors contributing to the consumer rejection of food products. It also leads to the production of compounds harmful to human health. With the sole information provided by the expiration dates, a significant amount of food is wasted at the distributor and consumer levels because of excessive precautions taken to ensure food safety. The agri-food industry lacks a rapid and easy-to-use tool to control food freshness in real time throughout the entire shelf-life of the product. Intelligent materials, intended to monitor the evolution of packaged food and to provide information to the user, offer a perfect user-friendly solution. This PhD thesis reports on the development of fluorescent materials to track lipid oxidation. More specifically, the fluorescence of the designed materials was rapidly quenched (less than 1 min) upon interaction with carbonyl compounds, a family of molecules which concentration increases during lipid oxidation. Their real-time monitoring was made possible since the fluorescence loss correlates with the concentration of carbonyls. Several synthesis strategies were tested. Acrylate-based materials, synthesized by radical polymerization, were compared to silicate-based materials, obtained by the sol-gel process. The toxicity of the so-produced materials was systematically assessed. A sol-gel material, based on aniline, was particularly promising and was thus more deeply investigated. Its performance was validated in direct contact with carbonyls, using different liquid phases as food simulant, as well as in indirect contact, by exposure of the material to a gas phase containing carbonyl vapors. The fluorescent sensor covered a very broad range of carbonyl concentrations (from 0.5 mmol/L to 0.5 mol/L), allowing to track the entire shelf-life of a food product. Finally, this new sensor was successfully applied to monitor the accelerated oxidation of a commercial fish oil. All of these results demonstrated the possibility of using this new intelligent sol-gel material instead of classical methods for the fast, simple, and sensitive determination of the lipid oxidation level of lipid-rich products, with little sample preparation and without use of any toxic reactant. This new fluorescent sensor appears thus promising for a wide range of applications.

Keywords: food waste, food shelf-life, intelligent packaging, lipid oxidation, sol-gel material, fluorescent sensor

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LIST OF ABBREVIATIONS

2-VA	2-vinylaniline
3-VA	3-vinylaniline
4-TMSA	4-trimethoxysilylaniline
4-VA	4-vinylaniline
AIBN	2,2'-azobis(2-methylpropionitrile)
ALA	α -linolenic acid
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
CAR	carboxen
CD233	conjugated dienes
CI	chemical ionization
CTAB	hexadecyltrimethylazanium bromide
DHA	docosahexaenoic acid
DMPAP	2,2-dimethoxy-1,2-diphenylethanone
DNA	deoxyribonucleic acid
DSC	differential scanning calorimetry
DVB	divinylbenzene
EA	elemental analysis
EC	European Commission
EFSA	European Food Safety Authority
EGDMA	ethylene glycol dimethacrylate
EI	electronic ionization
EPA	eicosapentaenoic acid
EU	European Union
FA	fatty acid
FAO	Food and Agriculture Organization
FRET	Förster resonance energy transfer

FTIR	Fourier transform infrared spectroscopy
GC	gas chromatography
HPLC	high performance liquid chromatography
LA	linoleic acid
LC-PUFA	long-chain polyunsaturated fatty acid
LOD	limit of detection
MDA	malondialdehyde
MIP	molecularly imprinted polymers
MIS	molecularly imprinted silica
MOF	metal-organic framework
MS	mass spectroscopy
NFC	near field communication
NIRS	near infrared spectroscopy
NMR	nuclear magnetic resonance
O/W	oil in water
OECD	Organization for Economic Co-operation and Development
OLA	oleic acid
PCT	Patent Cooperative Treaty
PDMS	polydimethylsiloxane
PE	polyethylene
PET	polyethylene terephthalate
PLA	polylactic acid
PP	polypropylene
ppb	parts-per-billion
ppm	parts-per-million
PTFE	polytetrafluoroethylene
PV	peroxyde value
Q	quadrupole

QD	quantum dot
RFID	radio frequency identification
ROS	reactive oxygen species
rpm	rotation per minute
SEM	scanning electron microscopy
SPME	solid phase micro extraction
TBARS	thiobarbituric acid reactive substances
TEOS	tetraethyl orthosilicate
TGA	thermal gravimetric analysis
TMOS	tetramethyl orthosilicate
TOF	time-of-flight
TVB-N	total volatile base nitrogen
TVC	total viable count
UV	ultra-violet
wt%	weight percent

RESUME DE LA THESE EN FRANCAIS

RESUME DE LA THESE EN FRANCAIS

Développement d’emballages intelligents à base de matériaux sol-gel fluorescents permettant le suivi des composés carbonylés générés par l'oxydation des lipides.

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LISTE DES ABREVIATIONS

CE	Commission Européenne
DDM	dates de durabilité minimale
DLC	dates limites de consommation
GC-MS	chromatographie en phase gazeuse couplée à de la spectrométrie de masse
PLA	acide polylactique
PV	indice de peroxyde
RFID	identification par radiofréquence
SPME	microextraction en phase solide
TBARS	substances réactives à l'acide thiobarbiturique
UV	ultra-violet

1. INTRODUCTION

Les lipides sont essentiels au bon fonctionnement de l'organisme. Leur oxydation représente néanmoins le premier mécanisme de dégradation chimique des aliments. L'oxydation implique une perte de qualité nutritionnelle et organoleptique des produits, avec notamment le développement d'odeurs rances^{1,2} qui constituent le premier critère de rejet d'un produit par le consommateur³. De plus, la consommation de produits oxydés peut avoir un impact négatif sur la santé, notamment du fait de la présence de radicaux libres, identifiés comme responsables de certaines maladies dégénératives comme Alzheimer^{4,5}. Afin d'assurer la sécurité et la qualité des aliments, les industriels sont légalement tenus d'indiquer des dates de péremption sur la plupart des produits alimentaires⁶. Cependant, une fois emballés, la fraîcheur des aliments ne peut plus être évaluée en temps réel. Dans les pays développés, cela engendre un gaspillage important tant au niveau des distributeurs que des consommateurs. Un tiers de la production alimentaire mondiale est ainsi gaspillée chaque année tout au long de la chaîne alimentaire⁷ : il s'agit d'un désastre environnemental, économique et social. Des techniques analytiques de référence existent afin d'évaluer le niveau d'oxydation d'un produit. Elles sont cependant longues, coûteuses, difficiles à mettre en œuvre et nécessitent l'utilisation de réactifs dangereux pour l'homme et l'environnement. L'industrie agroalimentaire manque donc d'un outil rapide et facile d'utilisation afin de déterminer la fraîcheur des aliments emballés en temps réel. Une solution prometteuse réside dans l'utilisation d'emballages intelligents, destinés à suivre l'état des aliments emballés et à fournir des informations à son utilisateur⁸. La mise au point d'un tel outil pourrait contribuer à réduire le gaspillage alimentaire tout en garantissant la sécurité et la qualité des aliments. Certains emballages intelligents ont déjà été conçus pour suivre la dégradation des aliments, mais aucun n'a encore été appliqué au suivi de l'oxydation des lipides.

Cette thèse de doctorat est consacrée au développement d'emballages intelligents pour le suivi en temps réel de l'oxydation des lipides. Plus précisément, elle fait état de la synthèse et de l'évaluation des performances de différents matériaux fluorescents pour le suivi des carbonyles. Elle a été réalisée au sein de l'équipe « Physico-Chimie des Aliments et du Vin » (PCAV) de l'Unité Mixte de Recherche « Procédés Alimentaires et Microbiologiques » (UMR PAM), Institut Agro Dijon, Université de Bourgogne Franche-Comté. Elle s'est déroulée sous la direction de MCF Elias BOU-MAROUN, MCF Nicolas SOK et Pr Thomas KARBOWIAK dans le

cadre du programme français « Investissements d'Avenir », ISITE-BFC (contrat ANR-15-IDEX-0003). Le projet est né d'une idée originale combinant l'expertise de MM. Thomas KARBOWIAK et Elias BOU-MAROUN, respectivement spécialistes des emballages et des capteurs (en particulier les polymères à empreintes moléculaires). Ce nouveau thème de recherche a été abordé pour la première fois au sein de l'UMR PAM au travers de cette thèse.

2. ETAT DE L'ART

2.1. CONTEXTE SOCIAL

Réduire le gaspillage alimentaire tout en garantissant la sécurité sanitaire des denrées alimentaires est un défi majeur auquel fait face l'industrie agroalimentaire aujourd'hui.

Le gaspillage alimentaire est défini comme la perte d'aliments initialement destinés à la consommation humaine à tout stade de la chaîne d'approvisionnement⁷. En France, cela représente 29 kg de nourriture gaspillée par an et par habitant, dont 7 kg sont encore emballés⁹. Plusieurs scandales sanitaires ont ébranlé le monde ces dernières décennies, certains ayant entraîné des conséquences majeures sur la santé des consommateurs. L'industrie agroalimentaire est donc constamment à la recherche de nouvelles méthodes analytiques permettant de garantir la sécurité de ses produits¹⁰.

Cependant, une fois l'aliment emballé, la seule indication sur son état de fraîcheur est sa date de péremption. En France, deux types de dates coexistent selon le type de produit alimentaire : les « dates limites de consommation » (DLC) et les « dates de durabilité minimale » (DDM)¹¹. L'apposition de DLC et de DDM est obligatoire sur presque tous les produits⁶. Cependant, il n'existe aucune loi qui détermine les dates théoriques à appliquer. Cette évaluation est placée sous la responsabilité du fabricant.

2.2. DEGRADATION DES ALIMENTS

De nombreux mécanismes de dégradation, qu'ils soient chimiques, microbiologiques ou enzymatiques, sont impliqués dans la péremption des aliments. L'oxydation des lipides constitue le principal mécanisme de dégradation chimique. Les acides gras polyinsaturés (oméga 3, 6 et 9) jouent un rôle clé dans le bon fonctionnement de l'organisme mais sont très sensibles à l'oxydation¹². Ce processus de dégradation suit un mécanisme radicalaire pouvant être initié par la lumière, la chaleur ou encore la présence de métaux tels que le fer, de

pigments tels que la chlorophylle ou d'enzymes¹³. L'oxydation des lipides génère de nombreux composés, certains étant volatils². Parmi eux, les carbonyles ont été identifiés comme produits majoritairement générés lors de l'oxydation et contributeurs principaux de la détérioration de l'odeur des aliments^{1,2}. En ce sens, ils participent directement au gaspillage alimentaire. C'est pour ces raisons qu'ils ont été choisis comme traceurs de l'oxydation dans le cadre de ce projet.

2.3. EMBALLAGES ALIMENTAIRES

Afin de ralentir l'oxydation des lipides, la mise sous vide, sous atmosphère modifiée, l'utilisation d'emballages barrières à l'oxygène ou opaques ainsi que l'ajout d'antioxydants directement dans le produit alimentaire ou dans l'emballage sont des techniques couramment utilisées¹⁴⁻¹⁶. Récemment, le développement d'emballages actifs pouvant interagir avec les aliments ou leur environnement par le biais d'une sorption ou d'une libération contrôlée de composés a permis de prolonger leur durée de conservation⁸. Ces solutions permettent d'améliorer la durée de vie des produits mais pas de contrôler leur état de fraîcheur en temps réel. Ceci est rendu possible par les emballages intelligents, capables de contrôler l'état d'aliments emballés et transmettre cette donnée à son utilisateur⁸. Il en existe trois types : les systèmes RFID (radio frequency identification), les indicateurs et les capteurs.

2.4. MATERIAUX INTELLIGENTS POUR LE SUIVI DES CARBONYLES

Dans le cadre de cette thèse, deux types de matériaux intelligents ont été synthétisés : des matériaux acrylates, synthétisés par polymérisation radicalaire et des matériaux silicates générés par voie sol-gel. La synthèse par voie sol-gel repose sur l'hydrolyse et la condensation de dérivés tetraalkylorthosilicates, usuellement dans un mélange alcool/eau sous catalyse acide ou basique¹⁷. Les matériaux sol-gel ont fait l'objet d'études plus approfondies dans le cadre de ce projet car ils présentent de nombreux avantages : faible toxicité, résistance mécanique élevée, grande porosité, synthèse verte à température ambiante^{18,19}.

2.5. FLUORESCENCE COMME MODE DE TRANSDUCTION DU SIGNAL

Initialement à l'état fondamental, une molécule fluorescente est excitée par l'absorption d'un photon. La molécule reste dans un état excité pendant une très courte période avant de retourner à l'état fondamental soit par un processus radiatif (avec émission d'un photon), soit par un processus non radiatif (sans émission de photon). Un fluorophore peut donc être défini comme un composé capable d'émettre un photon lorsqu'il est excité²⁰. Des capteurs chimiques

fluorescents ont été développés car ils fournissent généralement une réponse plus rapide et une sensibilité plus élevée que leurs homologues colorimétriques et ne nécessitent pas de mesure de référence²¹⁻²³.

2.6. REACTIVITE AMINE-CARBONYLE

Dans le cadre de ce projet, il a été proposé de développer des matériaux intelligents à base de dérivés aminés afin de promouvoir l'interaction entre les matériaux et les composés carbonylés ciblés. Les amines primaires sont connues pour réagir avec les aldéhydes et les cétones pour former des imines, également appelées « base de Schiff », selon un mécanisme d'addition/élimination²⁴.

2.7. AXES DE RECHERCHE DE LA THESE

Comme indiqué en **Figure 1**, cette thèse s'est déroulée en plusieurs étapes. L'objectif de ce travail était de développer des emballages intelligents pour le suivi de la dégradation alimentaire en temps réel afin d'aider à réduire le gaspillage alimentaire tout en garantissant la sécurité sanitaire des aliments. Au début du projet, une réflexion a été menée afin d'identifier les catégories de produits alimentaires qui pourraient bénéficier d'une telle innovation. Les produits emballés, de grande valeur, qui sont souvent gaspillés et dont la durée de conservation est soit courte soit très longue se sont avérés être des cibles de choix. Répondant à ces critères, les poissons et les fruits de mer, viandes, produits laitiers et huiles comestibles ont donc été ciblés. Ces produits sont riches en lipides. Parmi les mécanismes de dégradation des aliments, il a donc été décidé de se concentrer sur l'oxydation des lipides. L'impact de ce processus sur ces matrices alimentaires a donc été étudié. L'idée étant de proposer une méthode non destructive de suivi de l'oxydation des lipides, il était d'intérêt de développer un système de détection de composés volatils. Or, au cours de l'oxydation des lipides, des volatils constituant les "produits secondaires d'oxydation" sont générés. Une étude bibliographique de ces composés a montré qu'ils sont principalement composés d'aldéhydes, de cétones et d'alcools. Afin d'appliquer la technologie développée à différents types de produits alimentaires, il était judicieux d'employer une stratégie « non ciblée ». Cela signifie que le capteur ne serait pas conçu pour détecter un seul analyte, mais plutôt une famille de molécules que l'on trouve couramment dans différentes matrices alimentaires oxydées. En particulier, il a été décidé de se concentrer sur la famille des carbonyles pour plusieurs raisons. Tout d'abord, les carbonyles

ont été identifiés comme responsables des odeurs désagréables générées pendant la dégradation du produit. De plus, l'odeur s'est avérée être le premier critère utilisé par les consommateurs pour rejeter un aliment. Ces composés contribuent donc directement au gaspillage alimentaire. De plus, les carbonyles ne sont présents qu'à de faibles concentrations, voire pas du tout, dans les produits frais et sont principalement générés par le processus d'oxydation des lipides au fil du temps. Un nouveau capteur pour la détection des composés carbonylés a donc été conçu. Fournir au consommateur une telle innovation aurait pu s'avérer contre-productif en cas de mauvaise utilisation, conduisant à une augmentation du gaspillage alimentaire. Un outil destiné aux distributeurs a donc été privilégié. C'est pourquoi la fluorescence a été choisie comme mode de transduction du signal. Des stratégies ont ensuite été étudiées pour synthétiser de nouveaux matériaux de détection pour le suivi des composés carbonylés à base de fluorescence. Deux catégories principales de matériaux ont été envisagées : les matériaux à base d'acrylates et les matériaux à base de silicates. Dans les deux cas, les critères de développement étaient de pouvoir produire les matériaux à faible coût, avec des réactifs disponibles commercialement à des prix raisonnables, en utilisant une procédure de synthèse rapide et facile à mettre en œuvre. Afin d'inscrire ce projet dans une démarche de chimie verte, la toxicité et le nombre de réactifs ont également été pris en compte. Dans un premier temps, il a été décidé de synthétiser des matériaux à base d'un seul monomère présentant toutes les caractéristiques nécessaires au développement du capteur, à savoir pouvoir interagir avec les carbonyles et présenter une modification de ses propriétés de fluorescence en cas d'interaction. Il a été proposé de baser l'interaction chimique du capteur avec les cibles sur la réactivité des amines primaires avec les carbonyles. Des monomères portant donc à la fois un groupement amine et un noyau fluorescent ont été sélectionnés. À base de ces monomères, des matériaux ont été synthétisés soit par polymérisation radicalaire, soit par procédé sol-gel. Les performances des matériaux intelligents ont ensuite été évaluées. Tout d'abord, l'interaction entre les matériaux et les carbonyles a été vérifiée en déterminant la capacité d'adsorption des matériaux par chromatographie en phase gazeuse couplée à de la spectrométrie de masse (GC-MS). Ensuite, les modifications des propriétés de fluorescence du capteur après interaction avec les carbonyles ont été démontrées. Enfin, la toxicité des matériaux a été estimée afin de les appliquer à l'étude de produits alimentaires. Un matériau en particulier, synthétisé à base d'aniline par voie sol-gel, était particulièrement prometteur et a fait l'objet d'une étude plus approfondie. Après une caractérisation systématique, ses

performances ont été étudiées après contact direct entre le matériau et les carbonyles dans des milieux liquides modèles (éthanol et mélange éthanol/eau) et des matrices alimentaires liquides réelles (huile de colza et huile de poisson). Le suivi de l'oxydation accélérée de l'huile de poisson à l'aide du matériau intelligent à base d'aniline a été réalisé. Afin d'envisager une analyse non destructive des échantillons, ses performances ont également été évaluées après l'avoir exposé à une phase gazeuse contenant des vapeurs de carbonyles. Pour cette application, le matériau a été intégré à un polymère d'emballage à base d'acide polylactique (PLA). Enfin, pour améliorer certaines caractéristiques du capteur et notamment décaler son émission de fluorescence dans la gamme de longueur d'onde visible, de nouvelles stratégies de synthèse de matériaux sol-gel ont été étudiées. La première nouvelle stratégie reposait sur l'utilisation de deux monomères différents, l'un portant un groupement amine, l'autre portant un noyau fluorescent. L'offre commerciale en monomère à base de silice étant restreinte, une seconde stratégie permettant d'utiliser des fluorophores communs a été envisagée. Elle s'inspire de la stratégie « silicas doppés » et repose sur la solubilisation d'un fluorophore au sein de micelles et la copolymérisation d'un réticulant et d'un monomère à base de silice portant un groupement amine autour des micelles préformées.

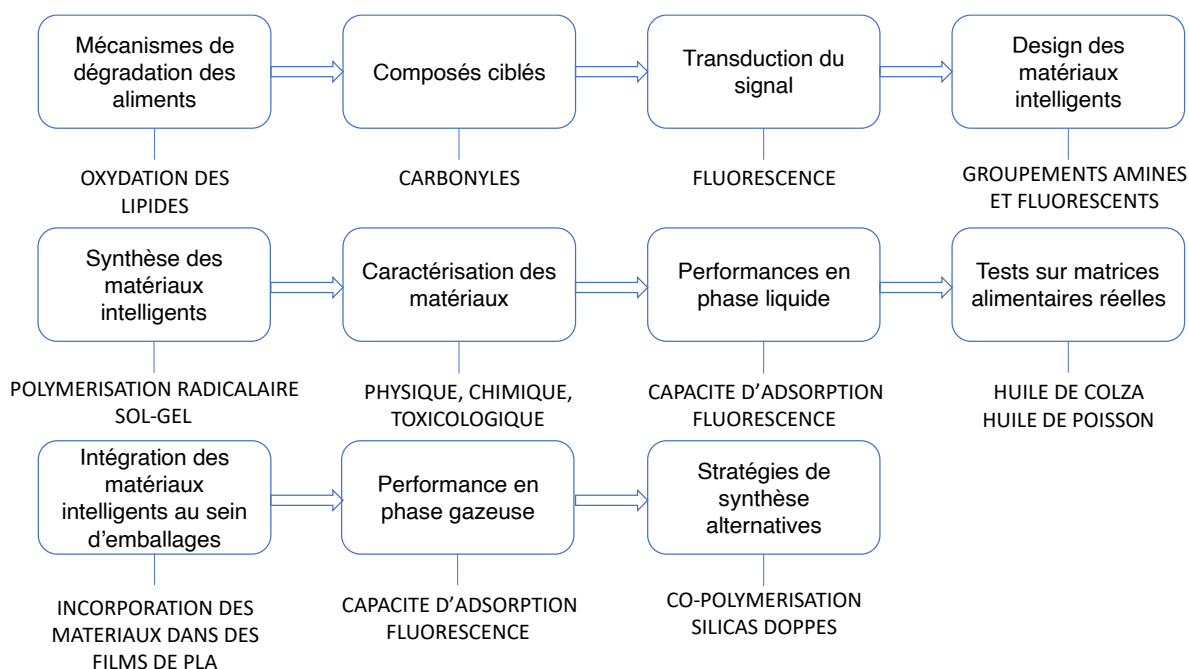


Figure 1 : Etapes du projet de thèse.

3. MATERIELS ET METHODES

3.1. STRATEGIES DE SYNTHESE

Trois stratégies de synthèse de matériaux intelligents fluorescents ont été développées dans le cadre de ce projet de thèse (voir **Figure 2**). La première repose sur un seul monomère portant un groupement amine et un noyau fluorescent. Via cette stratégie, des matériaux ont été synthétisés par polymérisation radicalaire et par voie sol-gel. Dans la seconde stratégie, deux monomères fonctionnels ont été utilisés, l'un portant un groupement amine et l'autre portant un noyau fluorescent. La troisième stratégie impliquait la solubilisation d'un fluorophore au sein de micelles et la polymérisation autour de ces micelles d'une structure de silice comportant des groupements amines par voie sol-gel.

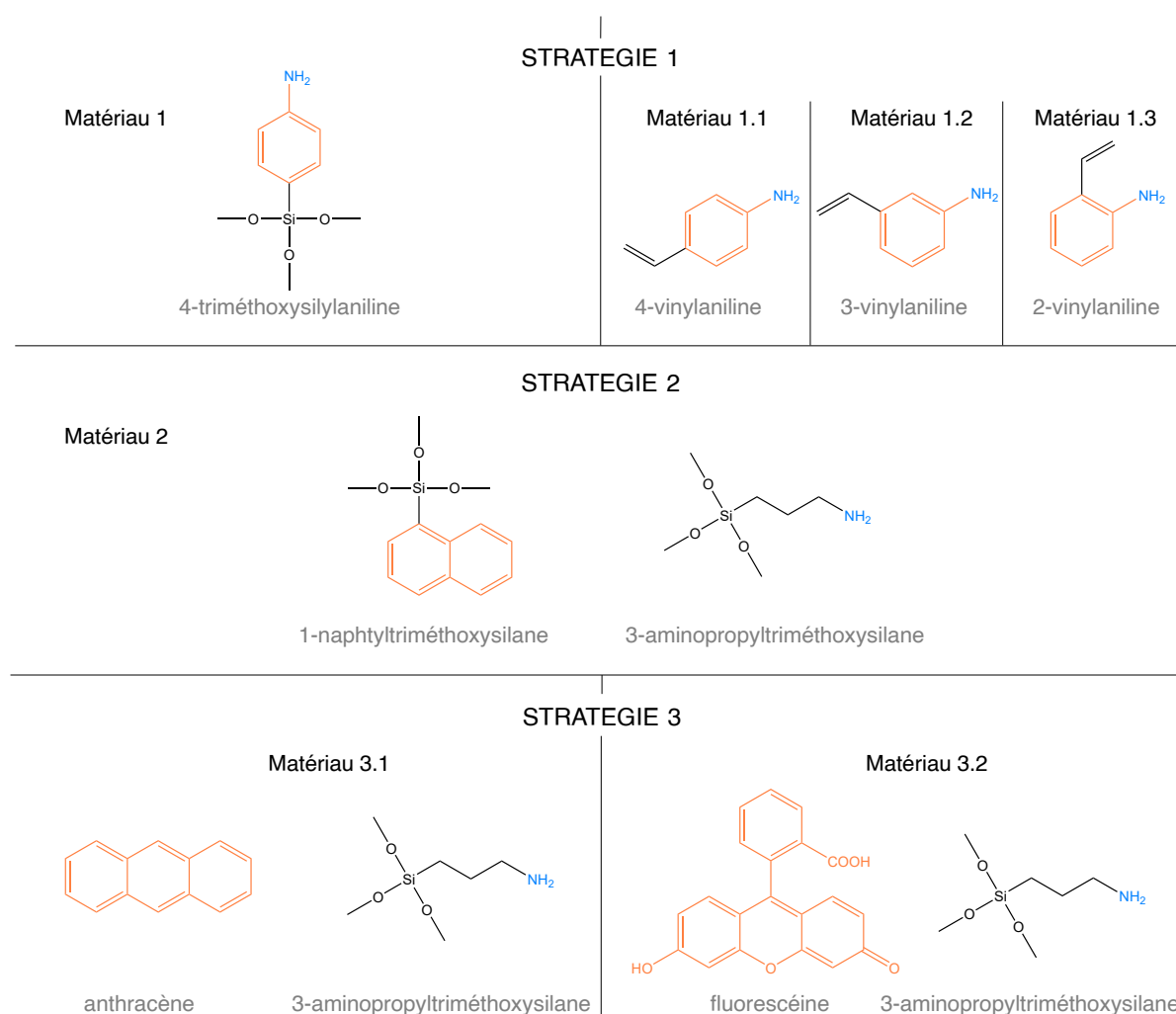


Figure 2 : Principaux réactifs utilisés au cours de la synthèse des différents matériaux intelligents selon les trois stratégies développées dans le cadre de cette thèse. En orange sont représentés les noyaux fluorescents et en bleu les groupements fonctionnels amines responsables de l'interaction avec les carbonyles.

3.2. CARACTERISATIONS DES MATERIAUX

Les matériaux synthétisés ont été caractérisés chimiquement (résonance magnétique nucléaire du solide, analyse thermogravimétrique, analyse élémentaire, spectroscopie infrarouge) et physiquement (adsorption de vapeur d'eau, microscopie électronique à balayage, granulométrie laser, adsorption d'azote). Leur toxicité a également été évaluée (test de Ames sur deux souches bactériennes). L'impact du vieillissement du matériau sur ses performances et sa toxicité a également été considéré.

3.3. CAPACITE D'ADSORPTION DES CARBONYLES

La capacité d'adsorption correspond dans cette étude à la masse d'un carbonyle adsorbée par 1 g de matériau sous air, à 22 °C et 30 % d'humidité relative.

Lors de l'interaction entre le matériau intelligent et un composé carbonylé en phase liquide, la capacité d'adsorption du carbonyle par le matériau a été calculée selon l'équation suivante :

$$\text{Capacité d'adsorption en phase liquide} = \frac{(C_i - C_f) \times V_{liq.}}{m} \quad (1)$$

avec C_i la concentration initiale de carbonyle dans la phase liquide en contact avec le matériau, C_f la concentration de carbonyle restant dans la phase liquide à l'équilibre, $V_{liq.}$ le volume de la solution de carbonyle et m la masse de matériau. C_i et C_f ont été déterminées par GC-MS. Le schéma expérimental utilisé est présenté en **Figure 3**.

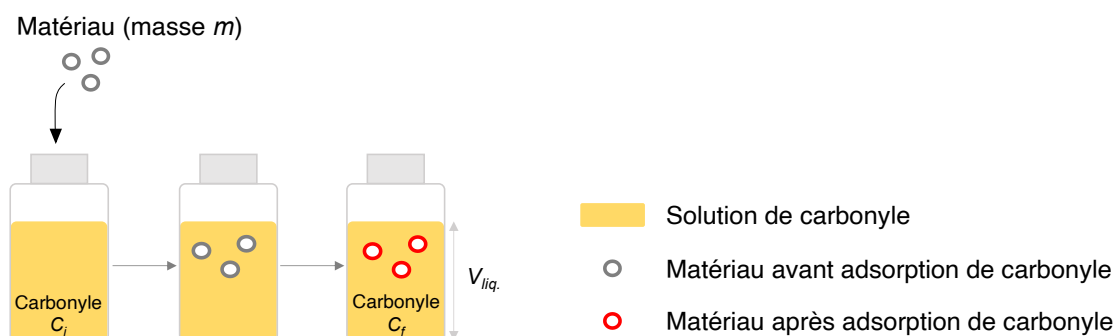


Figure 3 : Représentation schématique du dispositif expérimental utilisé pour la détermination de la capacité d'adsorption des carbonyles par le matériau en phase liquide.

Lors d'une interaction en phase gazeuse, l'équation est modifiée selon :

$$\text{Capacité d'adsorption en phase gazeuse} = \frac{(C_i - C_f) \times V_{\text{gaz.}}}{m} \quad (2)$$

avec C_i la concentration initiale de carbonyle dans l'espace de tête du flacon avant contact avec le matériau, C_f la concentration de carbonyle restant dans l'espace de tête à l'équilibre, $V_{\text{gaz.}}$ le volume de l'espace de tête du flacon et m la masse de matériau. C_i et C_f ont été déterminés par GC-MS en utilisant le procédé de microextraction en phase solide (SPME). Le schéma expérimental utilisé est présenté en **Figure 4**.

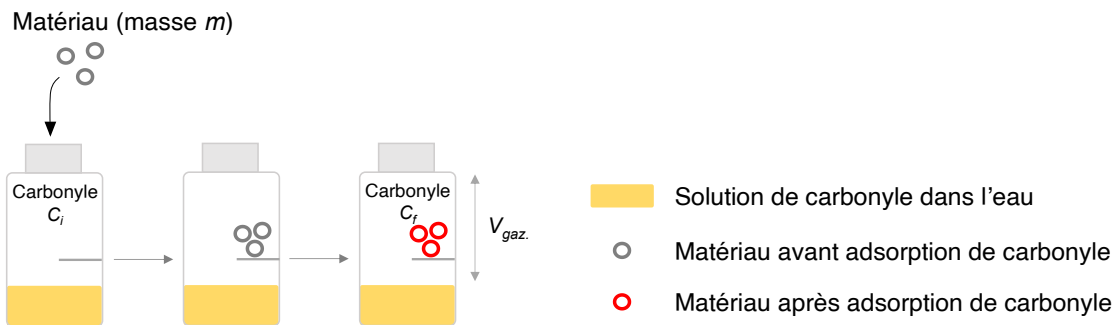


Figure 4 : Représentation schématique du dispositif expérimental utilisé pour la détermination de la capacité d'adsorption des carbonyles par le matériau en phase gazeuse.

3.4. FLUORESCENCE DES MATERIAUX

La modification des propriétés de fluorescence des matériaux lors du contact direct entre le matériau intelligent et les carbonyles en phase liquide a été mesurée en mettant en suspension le matériau dans une solution de carbonyle (voir **Figure 5**). Le spectre d'émission de fluorescence du matériau a été mesuré avant et après contact avec les carbonyles dans la phase liquide. Le pourcentage d'extinction de fluorescence du matériau après interaction avec un carbonyle a été déterminé comme suit :

$$Q = \frac{I_0 - I}{I_0} \times 100 \quad (3)$$

avec I_0 l'intensité de fluorescence du matériau à son maximum d'émission avant l'addition du carbonyle et I son intensité après exposition au carbonyle à une concentration donnée pendant un temps donné.

L'impact de différents paramètres sur la fluorescence du matériau a été étudié : temps de contact entre le matériau et le carbonyle ; nature, structure et concentration de carbonyle

(propanal, hexanal, nonanal, hexan-2-one, 1-penten-3-one, 1-octen-3-one) ; nature, polarité et pH de la phase liquide (éthanol, éthanol/eau (50/50, v/v), éthanol/eau (90/10, v/v), huile de colza, huile de menhaden).

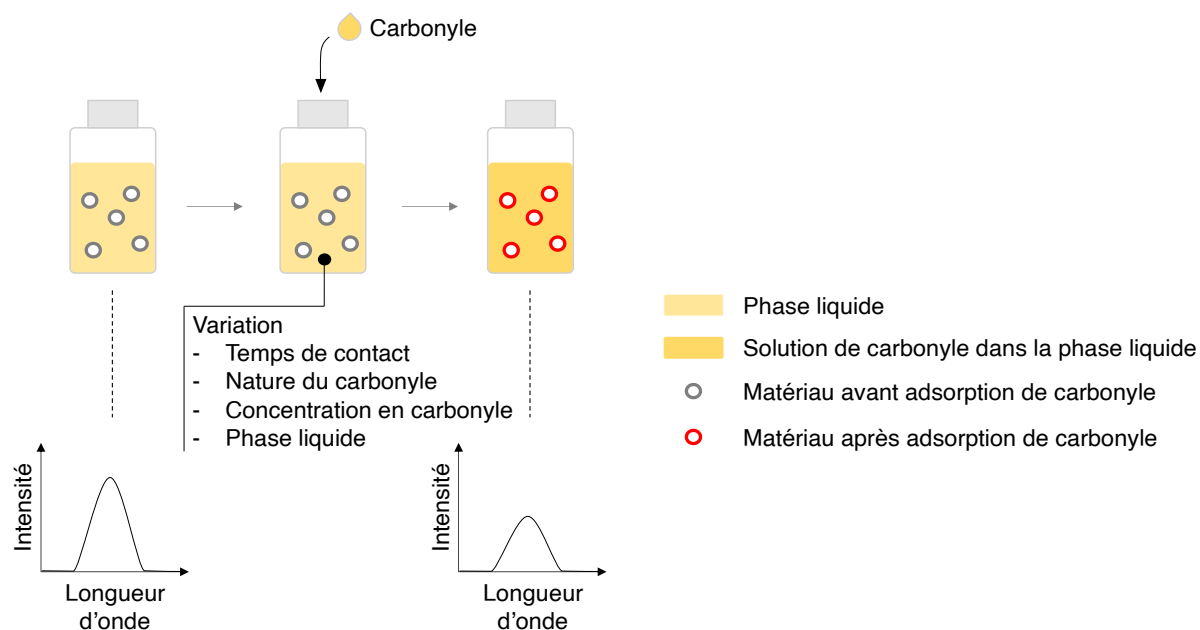


Figure 5 : Représentation schématique du dispositif expérimental utilisé pour la mesure du spectre d'émission de fluorescence du matériau en suspension dans une phase liquide avant et après interaction avec des carbonyles.

Pour déterminer la modification des propriétés de fluorescence des matériaux intelligents après interaction avec des composés carbonylés en phase gazeuse, le matériau a été incorporé dans un film de PLA puis exposé à des vapeurs de carbonyles (voir **Figure 6**). Le temps de contact, la nature et la concentration de carbonyles sont les paramètres ayant été étudiés.

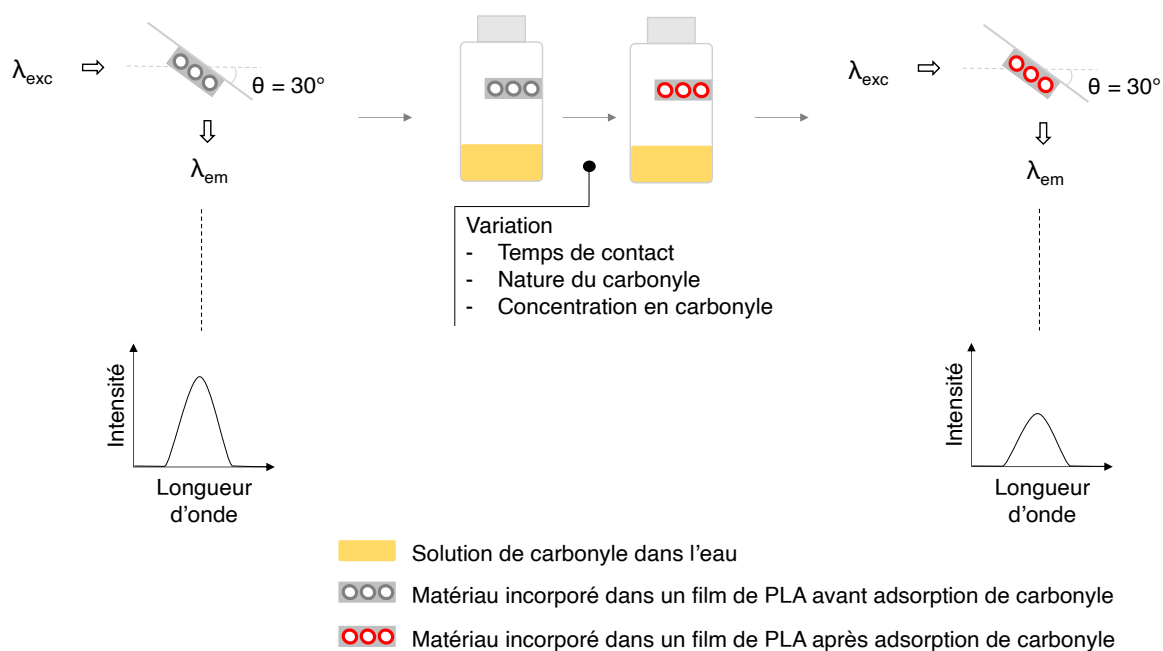


Figure 6 : Représentation schématique du dispositif expérimental utilisé pour la mesure du spectre d'émission de fluorescence du matériau incorporé dans un film de PLA avant et après avoir été exposé à des vapeurs de carbonyles.

3.5. SUIVI DE L'OXYDATION ACCELEREE D'UNE HUILE DE POISSON COMMERCIALE

Le suivi de l'oxydation accélérée d'une huile de menhaden commerciale a été réalisé. L'huile a été oxydée de manière accélérée à 60 °C. La fluorescence du matériau intelligent a été mesurée au cours du temps. En parallèle, le niveau d'oxydation de l'huile a été évalué à l'aide d'indices classiques : indice de peroxydes (PV), dosage des substances réactives à l'acide thiobarbiturique (TBARS) et suivi des composés volatils par SPME-GC-MS.

4. RESULTATS ET DISCUSSION

4.1. SYNTHÈSE DE MATÉRIAUX FLUORESCENTS POUR LE SUIVI DES CARBONYLES

Dans un premier temps, différents matériaux intelligents pour la détection fluorescente de composés carbonylés ont été synthétisés. Plusieurs paramètres de synthèse ont été optimisés afin de maximiser le rendement de synthèse, la capacité d'adsorption des matériaux et leur intensité de fluorescence initiale. Le type de polymérisation (radicalaire ou sol-gel), le type d'initiation de polymérisation radicalaire (UV ou thermique), la nature du monomère fonctionnel (4-vinylaniline, 3-vinylaniline, 2-vinylaniline, 4-triméthoxysilylaniline) et le ratio

molaire entre les réactifs de synthèse (monomère, réticulant, initiateur, solvant) ont été examinés.

Le matériau synthétisé par voie sol-gel à base de 4-triméthoxysilylaniline s'est avéré être le plus prometteur des candidats testés pour une application en tant que matériau intelligent pour la détection de composés carbonylés par fluorescence. En effet, comparé aux matériaux acrylates, il présentait une bonne reproductibilité de synthèse ainsi qu'une capacité d'adsorption et une intensité de fluorescence plus élevées. De plus, ses performances étaient stables dans le temps, contrairement aux matériaux synthétisés par polymérisation radicalaire. Ce matériau a donc été étudié plus en détail.

4.2. CARACTERISATION ET PERFORMANCES D'UN MATERIAU SOL-GEL POUR LA DETECTION DES CARBONYLES EN PHASE LIQUIDE

Le matériau sol-gel à base d'aniline sélectionné au chapitre précédent a été caractérisé d'un point de vue chimique, physique et toxicologique. Les analyses en spectroscopie infrarouge ont permis de confirmer la présence de groupements anilines au sein de la structure du matériau. L'azote provenant du noyau aniline représente 2 % de la masse du matériau. Il est composé de particules de taille micrométrique de forme irrégulière. Sa porosité est importante (surface spécifique : $452 \text{ m}^2 \cdot \text{g}^{-1}$), provenant de la présence à la fois de micropores et de mésopores. Il n'est pas mutagène d'après le test de Ames. Sa capacité d'adsorption est élevée, $83 \pm 8 \text{ mg} \cdot \text{g}^{-1}$ au minimum. Sa structure et son principe de fonctionnement sont donnés en **Figure 7**. Des phénomènes d'interactions chimiques et physiques pourraient être à l'origine de l'extinction de la fluorescence observée en présence de carbonyles. Des processus d'extinction à la fois statiques et dynamiques sont impliqués, pour lesquels plusieurs mécanismes peuvent être considérés dans chaque cas.

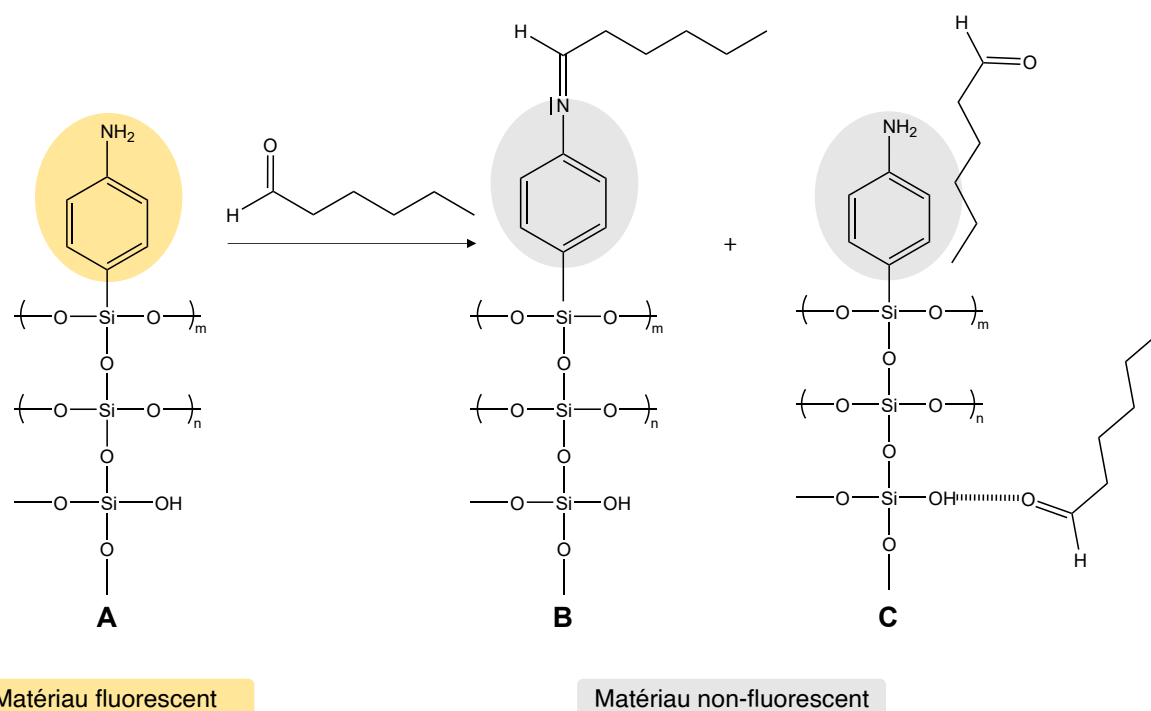


Figure 7 : Structure et principe de fonctionnement du matériau sol-gel fluorescent permettant la détection des carbonyles (exemple de l'hexanal). (A) : matériau fluorescent avant contact avec les carbonyles. (B), (C) : matériau non fluorescent après interaction avec les carbonyles (B, réaction chimique ; C, interactions physiques). m et n sont les rapports molaires respectifs du monomère fonctionnel et du réticulant utilisés pour la synthèse du matériau sol-gel fonctionnalisé.

La réponse du matériau intelligent est très rapide. Il montre en effet une diminution significative de sa fluorescence en moins d'une minute (**Figure 8a**). De plus, seulement 10 mg de matériau permettent de couvrir une très large gamme de détection ($0,5 \text{ mmol}\cdot\text{L}^{-1}$ à $0,5 \text{ mol}\cdot\text{L}^{-1}$) (**Figure 8b**). Le suivi en temps réel est possible puisque la perte de fluorescence est corrélée à la concentration de carbonyle détectée.

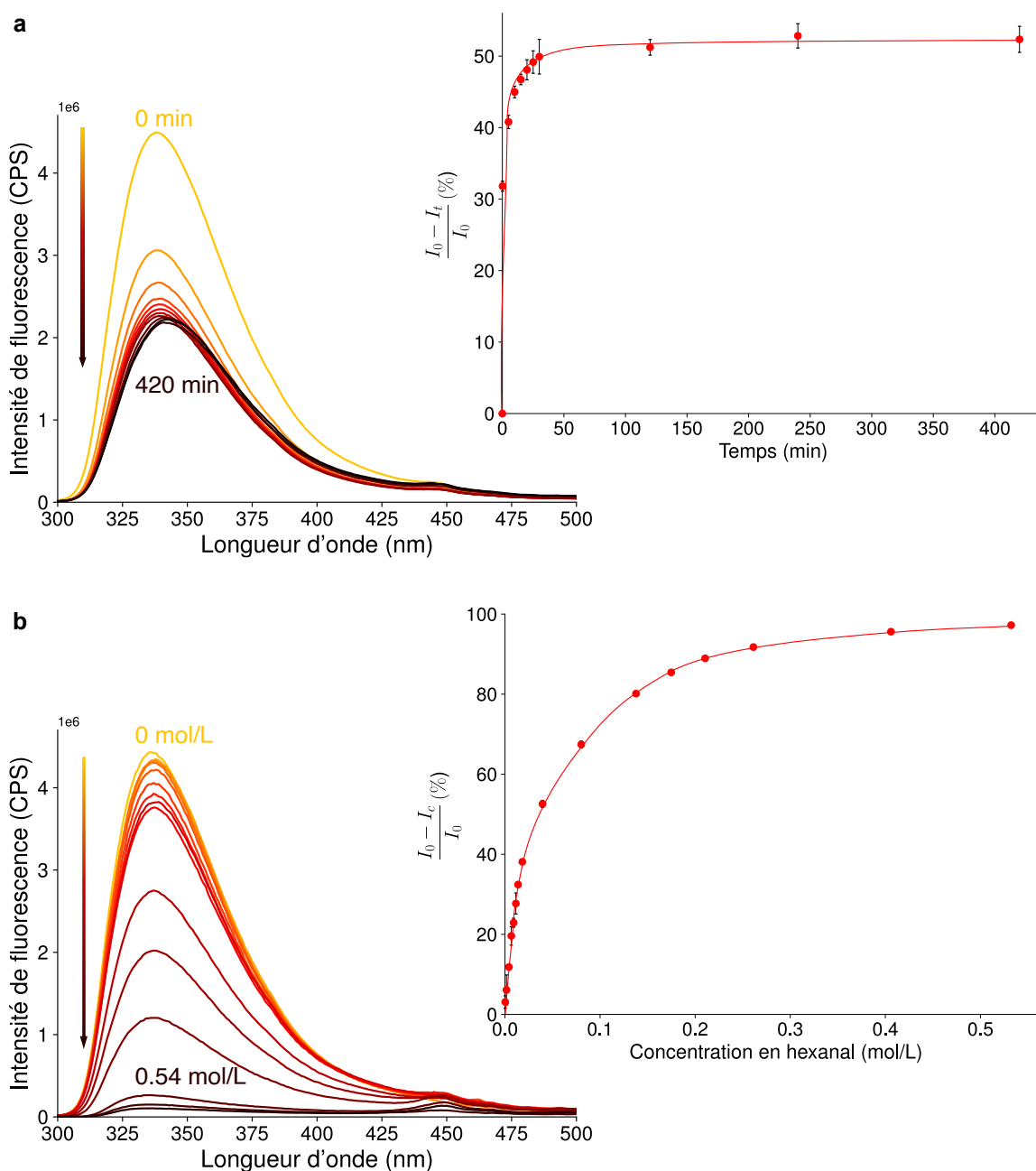


Figure 8 : Evolution du spectre d'émission de fluorescence du matériau après interaction avec l'hexanal dans l'éthanol.

a. Evolution du spectre d'émission de fluorescence du matériau au cours du temps (concentration : $9,98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) et cinétique d'extinction de fluorescence du matériau correspondante. La courbe jaune correspond à la fluorescence initiale du matériau, les autres courbes décrivent l'émission de fluorescence du matériau au fil du temps après l'ajout d'hexanal dans la phase liquide.

b. Evolution du spectre d'émission de fluorescence du matériau selon la concentration d'hexanal dans la phase liquide (temps de contact 10 min) et pourcentage d'extinction de la fluorescence en fonction de la concentration correspondante. La courbe jaune correspond au spectre d'émission du matériau avant ajout d'hexanal dans la phase liquide, les autres courbes décrivent l'émission de fluorescence du matériau en présence

d'une concentration croissante d'hexanal dans la phase liquide. Les points rouges sont des données expérimentales, la ligne continue est un guide de lecture. Les barres d'erreur représentent l'écart-type obtenu pour 3 répétitions. I_0 correspond à l'intensité de fluorescence du matériau à 337 nm avant interaction avec l'hexanal. I_t et I_c correspondent à l'intensité de fluorescence du matériau à 337 nm après un temps t d'interaction avec l'hexanal ou en présence d'une concentration c d'hexanal.

4.3. INFLUENCE DE L'ANALYTE ET DE LA PHASE LIQUIDE SUR LA PERFORMANCE DU MATERIAU INTELLIGENT

Le matériau a ensuite été testé dans diverses conditions afin de déterminer son champ d'application. Le matériau intelligent étant développé pour cibler l'ensemble de la famille des carbonyles, l'impact de la nature et de la structure du carbonyle (propanal, hexanal, nonanal, hexan-2-one, 1-penten-3-one, 1-octen-3-one) sur ses propriétés de fluorescence a été étudié. La longueur de chaîne et l'hydrophobicité de la cible semblaient être les paramètres déterminant de la cinétique, du pourcentage d'extinction de fluorescence ainsi que de la limite de détection mesurée.

L'influence de la nature, la polarité et le pH de la phase liquide ont également été évaluées. Les premiers essais ont été réalisés dans l'éthanol utilisé comme milieu modèle. Celui-ci a ensuite été remplacé par un mélange éthanol/eau (10/90, v/v), utilisé comme simulant alimentaire. L'intensité de fluorescence du matériau diminue quand la polarité du solvant augmente, du fait de la formation de liaisons hydrogènes entre le matériau et le solvant, qui stabilise l'état fondamental du matériau. Le pH optimal d'utilisation du matériau intelligent est compris entre 7 et 9, ce qui correspond à la forme moléculaire de l'aniline, conférant au matériau à la fois une fluorescence élevée et une grande réactivité vis-à-vis des carbonyles. Le matériau intelligent s'est donc avéré efficace dans des simulants alimentaires, à un pH correspondant à la majorité des aliments riches en lipides, avec une plage de détection permettant le suivi de l'oxydation des lipides tout au long de la durée de conservation d'un produit.

4.4. APPLICATION DU MATERIAU INTELLIGENT AU SUIVI DE L'OXYDATION D'UNE HUILE DE POISSON COMMERCIALE

Le matériau intelligent a été appliqué avec succès au suivi de l'oxydation d'une huile de menhaden commerciale. Il a été possible de corrélér positivement le pourcentage de perte d'intensité de fluorescence du matériau intelligent aux résultats de trois méthodes de suivi

classiques du niveau d'oxydation : PV, TBARS et suivi des composés volatils par SPME-GC-MS. La nouvelle méthode analytique proposée s'est révélée être 225 fois plus sensible que la méthode TBARS. Cette étude a démontré la possibilité d'utiliser ce nouveau matériau intelligent en remplacement des méthodes classiques pour la détermination rapide, simple et sensible du niveau d'oxydation des lipides dans les produits alimentaires, avec peu de préparation de l'échantillon et sans manipulation d'un quelconque réactif toxique.

4.5. SUIVI DES CARBONYLES EN PHASE GAZEUSE

Afin d'utiliser le capteur synthétisé pour développer un emballage intelligent, le matériau a été incorporé à un film de PLA. Il a ensuite été exposé à des composés carbonylés en phase gazeuse afin d'évaluer la possibilité de l'exploiter dans le cadre d'une analyse non destructive. De même qu'en phase liquide, l'intensité de fluorescence du matériau diminuait rapidement (moins de cinq min) en présence de carbonyles en phase gazeuse et une large gamme de concentration pouvait être couverte ($0,5 \text{ mmol}\cdot\text{L}^{-1}$ à $25 \text{ mmol}\cdot\text{L}^{-1}$).

4.6. STRATEGIES ALTERNATIVES DE SYNTHÈSE DE MATERIAUX FLUORESCENTS POUR LE SUIVI DES CARBONYLES

Le matériau sol-gel identifié comme prometteur au début du projet s'est en effet révélé adapté au suivi par fluorescence en temps réel des composés carbonylés générés par l'oxydation des lipides dans les produits alimentaires. Cependant, une des rares limites pouvant lui être reprochée est sa longueur d'onde d'émission de fluorescence dans l'UV. Un système de détection est donc nécessaire pour percevoir les changements spectraux du matériau induits par la présence de carbonyles. De plus, de nombreux polymères d'emballages sont fluorescents dans l'UV, limitant le nombre de plastiques dans lequel le matériau intelligent pourrait être intégré. Pour pallier cela, et principalement décaler l'émission de fluorescence dans le visible, des stratégies de synthèse alternatives ont été investiguées. L'offre commerciale en monomère fonctionnels à base de silice possédant à la fois des groupements amines et fluorescents, la première stratégie alternative consistait à synthétiser un matériau par voie sol-gel à base de deux monomères fonctionnels, l'un possédant un groupement amine (3-aminopropyltriméthoxysilane) et l'autre un noyau fluorescent (1-naphtyltriméthoxysilylaniline). Afin d'utiliser des fluorophores classiques, une autre stratégie a également été testée reposant sur la solubilisation d'un fluorophore (anthracène ou

fluorescéine) au sein de micelles et la copolymérisation d'un réticulant avec un monomère à base de silice portant un groupement amine autour des micelles préformées.

Le matériau à base de deux monomères était non mutagène mais les modifications de fluorescence induites par la présence de carbonyles étaient moins intenses que dans le cas du matériau à base d'un seul monomère, du fait de la distance entre le site amine de détection et le noyau fluorescent.

Bien que les matériaux obtenus en suivant la dernière stratégie aient été 1000 fois plus fluorescents que les matériaux des deux autres stratégies, ils présentaient un relargage de tensioactifs et de fluorophores, les rendant mutagènes d'après le test de Ames et donc inappropriés pour une application agro-alimentaire.

5. CONCLUSION ET PERSPECTIVES

L'objectif de cette thèse était de développer des emballages intelligents pour suivre en temps réel la dégradation des aliments, et plus précisément l'oxydation des lipides, afin de réduire le gaspillage tout en assurant la sécurité sanitaire des denrées. Elle portait sur la conception, la synthèse et l'évaluation des performances de matériaux fluorescents pour la détection des carbonyles. Des matériaux à base d'acrylates, synthétisés par polymérisation radicalaire, et des matériaux à base de silicates, obtenus par le procédé sol-gel, ont été produits. Tous les matériaux ont été conçus selon le même principe : ils devaient posséder un groupement amine, pour la réaction avec les composés carbonylés, et un noyau fluorescent, dont les propriétés devaient être modifiées par l'interaction avec les analytes. L'un des matériaux, synthétisé par un procédé sol-gel à base d'aniline, s'est avéré particulièrement adapté et a donc été étudié plus en détail. Le capteur étant destiné à la détection de plusieurs analytes, la réponse de fluorescence du matériau après interaction avec divers composés carbonylés, qualifiés de traceurs de l'oxydation des lipides (propanal, hexanal, nonanal, hexan-2-one, 1-penten-3-one, 1-octen-3-one), a été analysée. Son intensité de fluorescence était rapidement diminuée (moins d'une minute) par les carbonyles. Le suivi en temps réel était possible par la corrélation du pourcentage de perte d'intensité de fluorescence à la concentration de carbonyles. Le matériau intelligent permet de couvrir une large gamme de détection ($0,5 \text{ mmol}\cdot\text{L}^{-1}$ à $0,5 \text{ mol}\cdot\text{L}^{-1}$), ce qui permet de suivre l'entière durée de vie d'un produit alimentaire. Les performances du matériau ont été principalement évaluées en phase

liquide, en utilisant une solution modèle (éthanol), un milieu simulant un aliment (mélange éthanol/eau) et des aliments réels (huile de colza et huile de poisson). L'influence de la nature de la phase liquide et de son pH a révélé que le capteur développé pouvait être appliqué à une grande variété de produits alimentaires, avec une plage de pH optimale (7 – 9) correspondant au pH de la majorité des produits riches en lipides. Le capteur a été employé avec succès au suivi de l'oxydation d'une huile de poisson commerciale. Il a en effet été possible de corrélérer la réponse de fluorescence avec les résultats fournis par les méthodes classiques utilisées pour déterminer le niveau d'oxydation des aliments (PV, TBARS et SPME-GC-MS). Cette étude a démontré la possibilité d'utiliser ce nouveau capteur à la place des méthodes classiques pour la détermination rapide et simple du niveau d'oxydation des lipides dans les produits alimentaires. Intégré dans des films PLA, le matériau a en outre été utilisé pour la détection de carbonyles en phase gazeuse. Il pourrait ainsi être mis en contact direct avec l'aliment, soit être intégré dans un emballage alimentaire habituel pour suivre les modifications de l'environnement du produit. La toxicité du matériau a été évaluée afin d'exploiter ce capteur dans le secteur agroalimentaire. Le matériau n'était pas mutagène selon le test d'Ames ni sous forme de poudre ni intégré à un film de PLA. Enfin, afin de déplacer l'émission de fluorescence du matériau dans le domaine visible et de se passer ainsi d'un dispositif de détection, de nouvelles stratégies de synthèse ont été étudiées pour élargir le choix de noyaux fluorescents. Tous les matériaux intelligents ont été synthétisés selon des procédures simples et peu coûteuses, ont fourni une réponse rapide, avec peu de préparation d'échantillons et aucune manipulation de réactifs toxiques. Ces caractéristiques avantageuses en font des outils analytiques intéressants pour une large gamme d'applications.

En perspectives, ce matériau intelligent pourrait servir à la détection de carbonyles dans d'autres domaines que l'agroalimentaire tels que la cosmétique, la pharmaceutique, l'environnement ou le biomédical. Afin de pallier le choix restreint de monomères commerciaux à base de silice répondant aux critères de sélection, il pourrait être envisagé de synthétiser des monomères fonctionnels sur mesure à base de silice portant des groupements amines et des fluorophores émettant dans le visible. Les monomères fluorescents pourraient également être remplacés par des chromophores permettant le suivi du niveau d'oxydation d'un produit par un changement de couleur.

Une analyse du cycle de vie du matériau intelligent permettrait d'estimer le bénéfice apporté par une telle innovation dans le secteur agroalimentaire. L'impact économique et environnemental du gaspillage alimentaire pourra être comparé aux conséquences de l'ajout d'un tel capteur à l'emballage. La recyclabilité et la réutilisation du matériau intelligent pourraient être envisagées du fait de la réversibilité de la réaction chimique entre le matériau et les composés carbonylés.

Pour une application industrielle de la technologie développée, l'homologation des matériaux au contact alimentaire devra être obtenue. Le test de Ames devra être complété par d'autres tests de toxicité afin que ceux-ci soient conformes au règlement CE n° 1935/2004, qui s'applique aux matériaux et objets destinés à entrer en contact avec des denrées alimentaires, ainsi qu'au règlement CE n° 450/2009, spécifique aux matériaux actifs et intelligents. Comme première étape dans l'industrialisation du procédé, un brevet a été déposé le 13 décembre 2021 (n° FR2113403). Une extension internationale PCT a également été demandée le 12 décembre 2022 (n° PCT/EP2022/085460).

Enfin, dans un contexte de fortes évolutions réglementaires favorisant les emballages alternatifs aux matériaux plastiques conventionnels, les capteurs développés pourraient être utilisés pour réévaluer la durée de vie des aliments afin de pouvoir proposer un emballage qui réponde exactement aux besoins du produit selon sa durée de conservation. Cela permettrait également de revoir la formulation des denrées alimentaires et notamment l'utilisation d'additifs. Intégrée au sein d'un équipement pilote capable de contrôler le niveau d'oxygène, la réponse du capteur pourrait ainsi être utilisée pour modéliser la durée de vie de différents produits en fonction de leurs conditions de stockage.

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INTRODUCTION

INTRODUCTION

Lipids, and especially polyunsaturated fatty acids, are essential to human diet and contribute to the daily energy intake. Although the packaging industry has developed many techniques to extend food shelf-life, food spoilage is hard to avoid. In this case, lipid oxidation is the main chemical food degradation process involved and plays a major role in consumers' rejection with the generation of off-flavors^{1,2}. Lipid oxidation does not only deteriorate the organoleptic food properties, but it also leads to a decrease in nutritional quality. With the generation of toxic compounds, lipid oxidation can even cause health issues^{3,4}. To ensure food safety and quality, producers are legally compelled to display expiration dates on most food products⁵. However, once packaged, the freshness of food can no longer be assessed in real time. In high income countries, this generates a lot of food waste at the distributor and consumer level, due to the excessive precautions taken to avoid food poisoning⁶. One third of the global food production is wasted each year all along the food chain⁶: this is an environmental, economic, and social disaster. Although many standard methods exist to assess the oxidation level of food, they are often destructive, expensive, time-consuming due to sample preparation and analysis, dedicated to highly trained professionals, and rely on the use of large amounts of solvents and reactants, some of which are highly toxic. The agri-food industry thus lacks a rapid and easy-to-use tool to determine the freshness of packaged food in real-time. A promising solution would be the use of intelligent packaging, which are intended to monitor the evolution of packaged food and provide information to the final user⁷. The development of such an intelligent packaging could help to reduce food waste while ensuring food safety and quality. Some intelligent packaging has already been designed for the monitoring of food spoilage, but they have not yet been applied to the monitoring of lipid oxidation.

This PhD thesis is dedicated to the development of a new intelligent packaging to monitor lipid oxidation in real-time. It deals with the design, synthesis, and performance assessment of fluorescent materials for the sensing of carbonyl compounds. This family of molecules represents most of the volatile products released during lipid oxidation. They are major contributors to the flavor deterioration of lipid-rich products^{1,2}. Fluorescent chemical sensors were targeted as they usually provide faster response and higher sensitivity compared to their colorimetric counterparts and do not require any reference or blank⁸⁻¹⁰. Both acrylate-

based, synthesized by radical polymerization and silicate-based materials, obtained by the sol-gel process were investigated. Sol-gel materials were more deeply investigated as they present many advantages: low toxicity, high mechanical resistance, high porosity, green synthesis at room temperature^{11,12}. In the context of this project, sol-gel materials also presented better performances in terms of carbonyls adsorption capacity and fluorescence properties. These performances were assessed both in liquid and gas phases. The developed sensors could thus either be used in direct contact with food or integrated into usual food packaging as dots or films to monitor modifications in the environment surrounding the product. The application to the monitoring of the oxidation of real food matrix was also demonstrated.

The first part of this manuscript is dedicated to a literature review of the social context of this project, the food spoilage mechanisms, the role of the packaging, the acrylate-based and silicate-based sensing materials, as well as the basic principles of fluorescence. The second part describes in detail the experimental procedure followed. In the third part, the results obtained are summarized and discussed. This last part is divided into six chapters, written as articles (except for chapter five). The first one describes the design and synthesis optimization of acrylate-based and silicate-based sensing materials suitable for a use as sensor for carbonyl monitoring based on fluorescence. The second one is dedicated to the characterization and performance assessment, in a liquid model phase, of the most promising sol-gel material identified in the first chapter. The third one describes the impact of the carbonyl structure as well as the influence of the nature and of the pH of the liquid phase on the fluorescence of the sol-gel material. The fourth one is an application of the developed sensor to the monitoring of the accelerated oxidation of a real food matrix: a commercial fish oil. The fifth one explores the response of the material after exposure to carbonyl in the gas phase. The last chapter opens on new strategies allowing to synthesize sol-gel materials for carbonyl sensing with modulated performances. The manuscript ends with a conclusive part followed by the perspectives offered by this project.

This PhD thesis was conducted within the “Physical Chemistry of Food and Wine” (PCAV) team from the Mixed Research Unit “Food and Microbiological Processes” (UMR PAM), Institut Agro Dijon, University of Burgundy Franche-Comté, under the supervision of Assoc. Prof. Elias BOU-MAROUN, Assoc. Prof. Nicolas SOK and Prof. Thomas KARBOWIAK. It was financially supported by the French “Investissements d’Avenir” program, ISITE-BFC (contract ANR-15-

IDEX-0003). The project emerged from the original idea to combine the expertise from Prof. Thomas KARBOWIAK and Assoc. Prof. Elias BOU-MAROUN, specialists of food packaging and sensing technologies (especially molecularly imprinted polymers), respectively. The complementary resources for this work were supported by PCAV team members with Assoc. Prof. Nicolas SOK, organic chemist, and Prof. Philippe CAYOT, specialized in food chemistry. The toxicological studies were conducted by the DERTTECH PackTox, from the UMR 1231 “Lipids, Nutrition, Cancer”, INSERM. This was the very first time this new theme was addressed in UMR PAM with challenging steps to be achieved in the frame of the present PhD project.

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LITERATURE REVIEW

LITERATURE REVIEW

1. SOCIAL CONTEXT

Reducing food waste while ensuring food safety are two major challenges facing the agri-food industry today.

1.1. FOOD SAFETY

Food safety relies on the insurance that products intended to be consumed are not containing any harmful bacteria, viruses, parasites, or chemical substances that may jeopardize human health. Around the world, the World Health Organization estimates that almost 1 in 10 people fall ill after eating contaminated food each year¹.

Although food safety remains a significant concern in the agri-food industry, health scandals are recurrent, affecting a wide range of products. Several notable incidents that have impacted the food safety over the past decades include Minamata disease in the 1950s, resulting from mercury-contaminated fish; mad cow disease caused by the neurodegenerative Creutzfeldt-Jakob disorder in 1986 and 1996; bird flu in 2004. Some examples are related to food fraud such as the detection of melamine in milk in 2008 and the identification of the insecticide fipronil in eggs in 2017. Chemical contamination of food by the packaging is theoretically prevented by strong regulations (EC n° 1935/2004, EU n° 10/2011) regarding materials intended to come in contact with food. The European Food Safety Authority (EFSA) consequently provides a list of authorized substances which may be used in the formulation of food packaging. If new packaging are developed, they must be approved by the EFSA before commercialization in Europe. The toxicity of the components is studied, and migration tests are performed. The used substances should not generate any gene mutation or chromosomal aberration. To estimate the toxicity of new materials, a bacterial reverse mutation assay (OECD TG 471)² and an in vitro micronucleus test (OECD TG 487)³ must be performed. If any toxicity is suspected, maximum exposure limits to the substance are established. Although the procedure to authorize the use of material in contact with food is very strict, failure can happen as exemplified by the highly mediatized scandal regarding the migration of bisphenol A from packaging, which was implicated in hormonal disruptions. In 2012 in France, the use of this substance in containers intended for children under the age of 3 was banned. The packaging is

primarily intended to prevent food from any microbiological contamination. However, contaminations can occur at the production level, as it was the case with the recent discovery of *E. coli* bacteria in pizzas.

Consequently to such scandals having major impact on the consumer health, the agri-food industry is constantly seeking improved analytical methods to guarantee the safety of its products⁴.

1.2. FOOD WASTE

Food waste refers to the loss of food initially intended for human consumption at any stage within the food supply chain⁵. The United Nations Food and Agriculture Organization (FAO) reports that each year, approximately 1.3 billion tons of food are wasted globally, accounting for one-third of the worldwide production⁵. In France, it represents 29 kg of food wasted annually per inhabitant, 7 of which are still packaged⁶. As depicted in **Figure 1**, food waste occurs all along the food supply chain, from producers to consumers.

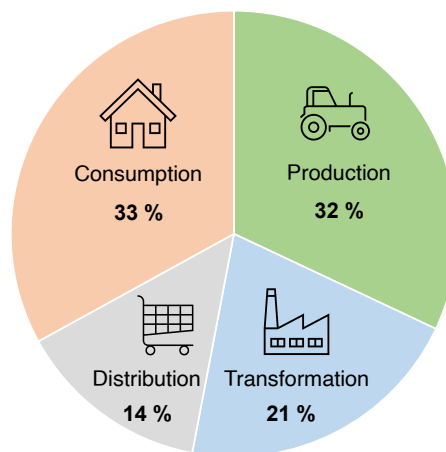


Figure 1: Percentage of food waste all along the food chain in France (figures from 2016⁶).

In developing countries, the majority of waste takes place at the beginning of the food chain, primarily due to storage and distribution constraints. In medium- and high-income countries, most food waste occurs at the end of the food chain by the consumer because of high quality standards and health precautions⁷ (see **Figure 2**).

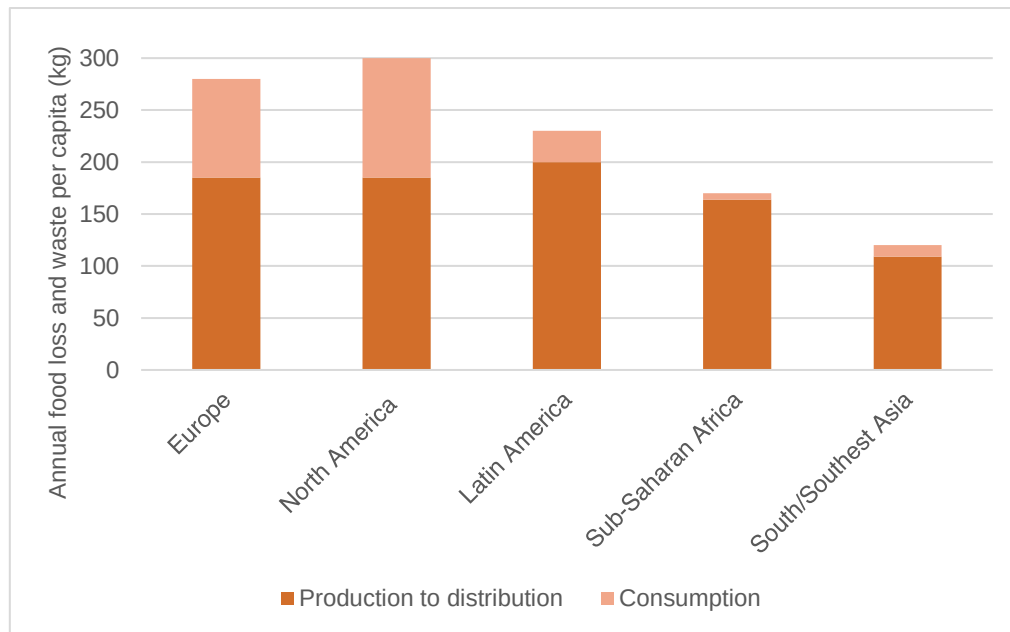


Figure 2: Distribution of the annual food waste at pre-consumptions and consumption stages in different region of the world [adapted from Gustavsson⁵].

As shown in **Figure 3**, all kind of food items is concerned by waste at some point of the supply chain. Fruits and vegetables are for examples mostly wasted at the production stage. The main reason for this is the post-harvest grading of fruit and vegetables due to the quality standards set by retailers. Important losses during fishing (around 10 %) are due to the use of unselective gears, resulting in capture of immature fish or unwanted species that are subsequently discarded. Nine percent of those fishes are thrown away at the distribution level and 11 % by the consumers, mostly because fish are prone to rapid spoilage.

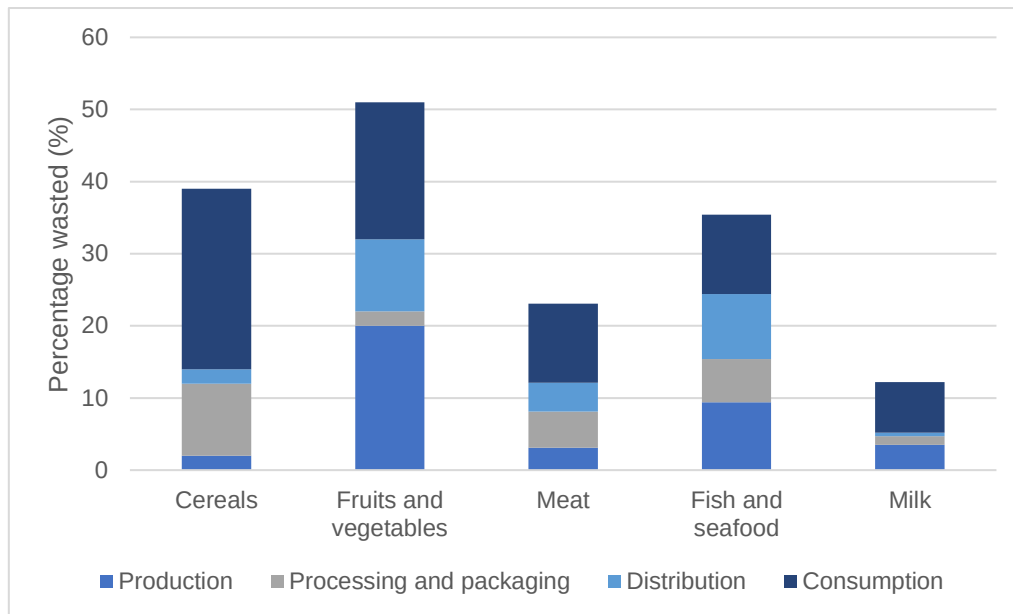


Figure 3: Waste percentages for each food category according to the food chain step (figures for Europe and Russia) [adapted from Gustavsson⁵].

Food waste is an environmental, economic, and social issue. Besides consuming natural resources in production, manufacturing, packaging, and transportation, food waste contributes a carbon footprint comparable to that of the world's third-highest polluting nation⁸. The FAO estimates the annual economic impact of food waste at \$750 billion. According to a 2019 United Nations World Food Program study, over 800 million people, i.e., 1 in 9 people worldwide, suffer from hunger today⁹. The need to feed more than 9 billion people by 2050 forces the food industry to find sustainable methods of producing and consuming. Consequently, extending product shelf-life and minimizing waste are crucial objectives.

Through its national pact against food waste, France is committed to divide by 2 the amount of wasted food between 2013 and 2025 for the catering and distribution sectors, and by 2030 for the consumption, processing, production sectors¹⁰. Numerous laws have been promulgated to this end, the latest being the AGECL law (n° 2020-105) on the fight against waste and the circular economy. With 2 years to go, it is clear that much more efforts have to be done to reach this objective. Therefore, the agri-food sector could significantly benefit from a reliable and user-friendly solution to monitor food quality throughout the chain, ensuring food safety and traceability, and increasing product shelf-life while reducing waste.

1.3. FOOD FRESHNESS

In order to develop a new tool to monitor quality all along the shelf-life of the food, it was important to first understand how food freshness is usually estimated. Once packaged, the only indications on food freshness are the expiration dates. In France, 2 different types of dates can be encountered according to the type of food product. On one hand are the “use-by” dates or “best before” dates (“Date Limite de Consommation” or DLC in French). In the other hand are the “dates of minimum durability” (“Date de Durabilité Minimale” or DDM in French)¹¹. According to the article 24 of the (EU) regulation n° 1169/2011, DLC concerns only microbiologically highly perishable foodstuffs such as fish and meat⁹. After this date, the product may present a risk to human health if consumed. Other kind of products are stamped with DDM, which could theoretically be exceeded without microbiological risk if the product was stored in proper conditions. The affixing of DLC and DDM are mandatory on almost all kind of products, except for fresh fruits and vegetables, salt, sugar, vinegar, beverages containing 10 % or more by volume of alcohol as well as bakers’ and pastry’s to be consumed within the day of manufacturing¹². However, there is no law which determine the theoretical DLC or the theoretical DDM to affix on the packaging according to the food product. This evaluation is set under the responsibility of the manufacturer. The DLC is thus defined on the basis of the microbiological development in the product. In the case of fish, the quantity of histamine has to be taken as a safety criterion according to the (EC) 2073/2005 regulation. The maximum limit is set between 200 mg·kg⁻¹ and 400 mg·kg⁻¹ due to the toxicological effects induced by the consumption of this biogenic amine¹³. The DDM is estimated by the time during which the product will keep its organoleptic, physical, nutritional and taste qualities. In most cases, whether for the estimation of the DLC or the DDM, a safety margin is incorporated. There is thus no way to estimate the real freshness state of a packaged food product in real time.

Furthermore, with the AGECL law (n° 2020-105), France aims to get rid of disposable plastic by 2040. This measure has a considerable impact on the usual packaging methods for several products, as single-use plastic is regularly used (*e.g.*, beverages bottles). The accurate estimation of the shelf-life of the products is therefore even more crucial, in order to find new appropriate packaging solutions. The packaging industry could thus also greatly benefit from tools to monitor the shelf-life of foods.

2. FOOD SPOILAGE

2.1. FOOD SPOILAGE MECHANISMS

Food spoilage can be defined as processes in which food degrades, leading to a reduction in the quality of the product until it is no longer suitable for human consumption. Food spoilage leads to changes in the visual aspect, odor, texture, or taste of the food item. During spoilage, many degradation mechanisms are involved, either chemical, microbiological, or enzymatic. Among chemical mechanisms, lipids are prone to hydrolysis, oxidation, and isomerization while proteins can be either hydrolyzed or oxidized. Microbial activity implies the growth of microorganisms (bacteria, yeasts, and molds), some of which are inherently harmful, some other generate toxic by-products. Enzymes mainly catalyze these degradation reactions. All of these degradation processes occur simultaneously during spoilage, which kinetics was modelled by Labuza¹⁴ in the 1980's in a pioneer way to predict shelf-life of food. Armenteros *et al.* demonstrated in particular that lipid and protein oxidation occur simultaneously by correlating indicators of these 2 mechanisms¹⁵. However, the prevalence of one mechanism over the others depends on the nature of the food.

2.2. FOOD QUALITY INDICATORS

Whatever the mechanism involved, food spoilage generates many by-products that can be used as quality trackers. Oxidation of proteins mainly leads to the formation of hydroperoxides, carbonyls and sulfoxide compounds¹⁶. Microbial degradation of proteins mostly leads to volatile amines (such as ammonia and trimethylamine) and biogenic amines (such as histamine, tyramine, putrescine, cadaverine, spermidine, spermine, tryptamine and methylamine), compounds that are particularly odorous. Lipid hydrolysis leads to the formation of free fatty acids. In nature, *cis*-fatty acids are mostly found. They can undergo isomerization producing *trans*-fatty acids, which consumption level should be kept as low as possible, because they are involved in higher risk of heart disease¹⁷. Lipid oxidation mainly produce hydroperoxides and volatile compounds, especially aldehydes. All these products characteristic of food spoilage can be used as food quality trackers.

Many common chemical and biological indices are commonly used at the laboratory scale to assess food quality^{18–22}. A summary of the most employed is proposed in Table 1.

Table 1: Common food quality indices.

QUALITY INDICES	MEASUREMENT	SPOILAGE PROCESS	DEGRADATION	REGULATORY LIMIT	STANDARD REFERENCE
TVB-N (total volatile base nitrogen)	Volatile amine (ammonia, dimethylamine, trimethylamine)	Bacterial degradation of proteins and hydrolysis of the proteins, catalyzed by the protease enzyme	Microbiological, chemical and enzymatic degradation	25-60 mgN/100 mg	23
Biogenic amines content	Especially histamine concentration	Decarboxylation of amino acids or amination/transamination of carbonyls due to bacterial activity	Microbiological degradation	200-400 mg/kg	24
TVC (total viable count)	Concentration in microorganisms	Microbial activity	Microbiological degradation	5×10^5 cfu/g	25
K value	Level of decomposition of ATP (adenosine triphosphate)	Microbial activity	Microbiological degradation	65 % ^{26*}	/
Free fatty acid content	Concentration in free fatty acids	Lipid hydrolysis, catalysed by the lipase enzyme	Chemical and enzymatic degradation	5 % ²⁷	28
PV (peroxyde value)	Hydroperoxydes	Primary products of lipid oxidation	Chemical degradation	20 meq/kg (olive oil) ²⁹	30
CD233 (conjugated dienes)	Conjugated dienes	Primary products of lipid oxidation	Chemical degradation	/	31
p-anisidine value	Aldehydes	Volatile secondary products of lipid oxidation	Chemical degradation	< 30	32
TBARS (thiobarbituric acid reactive substances)	Aldehydes	Volatile secondary products of lipid oxidation	Chemical degradation	< 2 ^{33*}	34

Sensory evaluation	Volatile compounds	Lipid oxidation (carbonyls, alcohols) Microbial and enzymatic degradation of proteins (sulfur and nitrogen compounds, carbonyls)	Microbiological, chemical and enzymatic degradation	/	35

* not reglementary thresholds.

This work focuses on lipid oxidation spoilage mechanism, which is detailed by the next paragraphs.

2.3. LIPID OXIDATION

2.3.1. Definition and importance of lipids

Lipids are one of the 3 main categories of macronutrients essential in human diet with proteins and carbohydrates, which contribute to the daily energy intake. Lipids are amphiphilic molecules, possessing a hydrophilic part and a large hydrophobic moiety. Sterols, phospholipids, and glycerides belong to the lipid family. Edible oils are for example composed of more than 95 % of triglycerides (also called triacylglycerol)³⁶, which are glycerol molecules esterified with 3 carboxylic acids, also called “fatty acids” in food chemistry (see **Figure 4**).

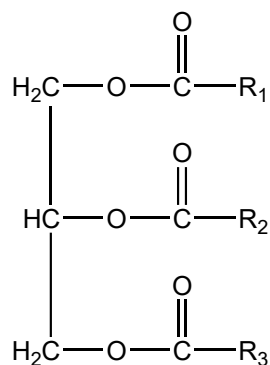


Figure 4: General form of a triglyceride molecule. R₁, R₂, R₃ are alkyl groups.

Once consumed, the triglycerides are hydrolyzed by enzymes, especially the lipases, for the metabolism of the fatty acids. However, although this does not refer to the same molecular structure, the terms lipids and fatty acids are often used interchangeably. Fatty acids (FAs) can either be saturated, monounsaturated, or polyunsaturated according to the number of double bonds they possess. A few examples of major long chain polyunsaturated fatty acids (LC-PUFAs) found in food products is given in **Table 2**. The chemical nomenclature of FAs gives the number of carbon atoms, the number of unsaturation and their position along the hydrocarbon chain,

starting the count from the carbon bearing the carboxylic acid group. In food chemistry, LC-PUFAs are classified in 3 categories: ω 3, ω 6 and ω 9, according to the position of the first unsaturation encountered in the carboxylic acid starting from the end tail.

Table 2: Structure of the most common polyunsaturated fatty acids.

USUAL NAME	SUBSTRATE	FORMULA	STRUCTURE
α -linolenic acid (ALA)	ω 3	C18:3, Δ 9, 12, 15	
eicosapentaenoic acid (EPA)	ω 3	C20:5, Δ 5, 8, 11, 14, 17	
docosahexaenoic acid (DHA)	ω 3	C22:6, Δ 4, 7, 10, 13, 16, 19	
linoleic acid (LA)	ω 6	C18:2, Δ 9, 12	
oleic acid (OLA)	ω 9	C18:1, Δ 9	

α -linolenic acid (ALA) (C18:3, ω 3), linoleic acid (LA) (C18:2, ω 6) and oleic acid (OLA) (C18:1, ω 9) are among the main fatty acids found in food products. Eicosapentaenoic acid (EPA) (C20:5) and docosahexaenoic acid (DHA) (C22:6) are ω 3-fatty acids specific from fish-derived products. ω 3-ALA, ω 6-LA are considered as “essential” fatty acids³⁷, as human being cannot synthesize them. ALA, LA, and DHA are considered as “indispensable”³⁷, which means that a minimum daily intake of each fatty acid is necessary for the proper functioning of the body. DHA does not belong to the category of “essential” fatty acids as ALA can be converted into EPA and then into DHA after consumption. However, the conversion rate from ALA to DHA is low (less than 0.5 %). This is why it is important to consume DHA-rich products in addition to ALA and LA. Lipids are commonly found in oils, oilseeds and fish. Sources of ALA are flaxseed oil, walnut oil, rapeseed oil, soybean oil while LA is mainly found in sunflower oil. DHA can be found in fatty fish like salmon, tuna, sardines, mackerel, herring, and seaweeds³⁸. ω 3 are necessary for the good functioning of the retina, the brain, and the nervous system³⁹. They also play a major role in preventing cardiovascular diseases⁴⁰ as well as in the regulation of inflammatory reactions⁴¹.

2.3.2. Lipid oxidation mechanism

LC-PUFAs are thus of high importance for human diet. However, they are way more sensitive to oxidation than saturated fatty acids, as the oxidation process involves the break of

a double bond. Therefore, the more unsaturated a fatty acid is, the more sensitive it is to oxidation. Lipid oxidation is the first cause of chemical degradation of a food product and results from the interaction of lipids with molecular oxygen. As depicted in **Figure 5**, it follows a radical mechanism⁴² composed of 3 steps: initiation, propagation and termination.

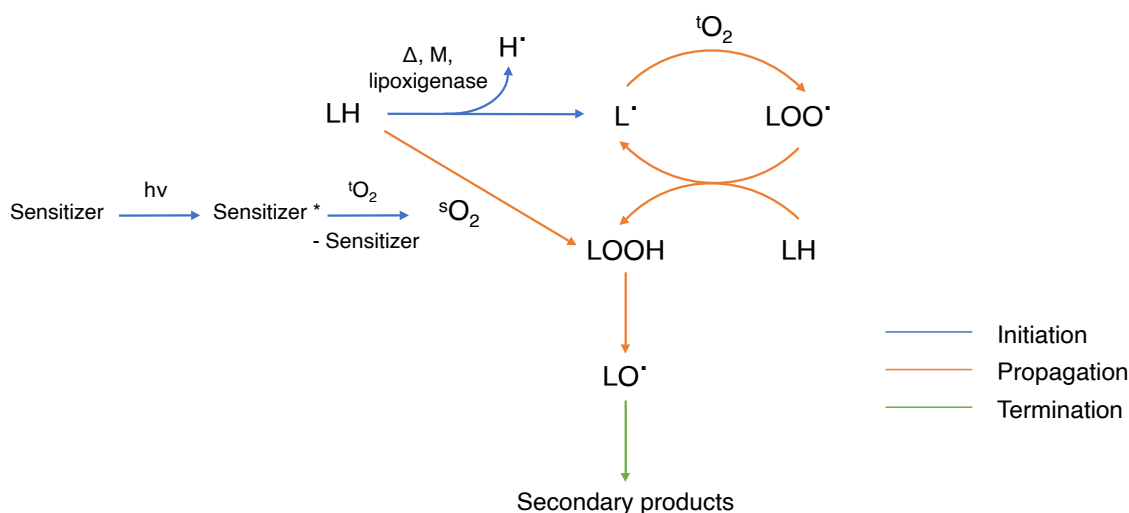


Figure 5: Schematic illustration of lipid oxidation by a radical mechanism composed of initiation, propagation and termination steps. Δ : heat, M : metal, ${}^3\text{O}_2$: oxygen in triplet electronic state, ${}^1\text{O}_2$: oxygen in singlet electronic state.

The mechanism of lipid oxidation has been debated for year, especially because several initiation paths may lead to the oxidation process⁴³. Direct reaction between oxygen and lipids is spin restricted because molecular oxygen is in triplet electronic state while double bonds of the fatty acids are in singlet state. According to the initiation path, 3 main oxidation types can be distinguished and are usually called “auto-oxidation” (initiated by heat, light or metals), “enzymatic oxidation” (catalyzed by lipoxygenase) and “photo-oxidation”. Auto-oxidation and enzymatic oxidation result in the abstraction of a hydrogen radical ($H^\bullet$) from the unsaturated lipid (**LH**). Photo-oxidation implies the excitation by light of a sensitizer molecule (*e.g.*, chlorophyll, riboflavin), which can interact with oxygen in the triplet state resulting in singlet oxygen, able to react with lipids³⁶.

The auto-oxidation mechanism is described in **Figure 6**⁴⁴. Oxidation will start with the abstraction of a hydrogen radical ($H^\bullet$) from the unsaturated lipid (**LH**) to form a highly reactive alkyl radical ($L^\bullet$). At this stage, molecular rearrangement can occur, leading to the displacement of the radical to another carbon atom of the lipid molecule, which multiplies the number of

products that can be formed by this process as a result. Propagation follows with the reaction of this radical ($L^{\cdot}$) with molecular oxygen to form a peroxy radical ($LOO^{\cdot}$). Then, the peroxy radical abstracts a hydrogen atom from another lipid to form a hydroperoxide ($LOOH$). A new lipidic radical ($L^{\cdot}$) is consequently formed and a chain reaction follows. Hydroperoxides are considered as primary products of lipid oxidation. In fact, they can be further transformed into the so-called “secondary products of lipid oxidation”⁴⁵. A homolytic cleavage of the oxygen – oxygen bond of hydroperoxides ($LOOH$) can occur under high temperature, in the presence of light or is catalyzed by metals traces (*e.g.*, copper, iron)⁴⁶. This generates an alkoxyl lipid radical ($LO^{\cdot}$), which could initiate new radical reactions but usually degrades by carbon – carbon cleavage on either side of the radical. This termination step leads to the formation of “secondary products”, some of which are volatile (aldehydes, ketones, alcohols, carboxylic acids, hydrocarbon), other are not volatile (cyclic monomers of fatty acids, epoxy fatty acids, hydroxy fatty acids, di-, tri- and tetra-polymeric forms of the fatty acids).

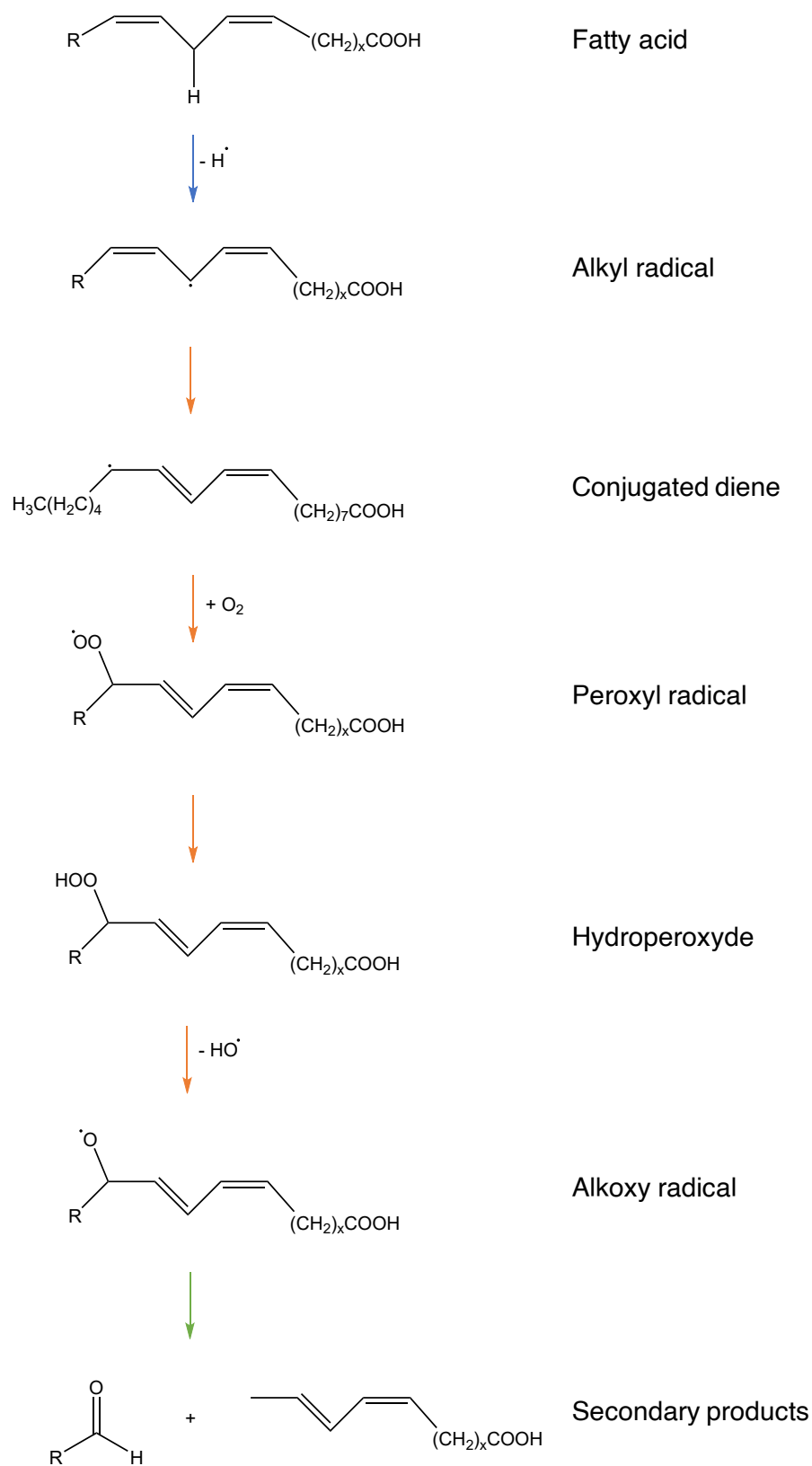


Figure 6: Schematic auto-oxidation mechanism. R = alkyl group. X ∈ [2 – 7].

As depicted in **Figure 7**, due to the transitory nature of hydroperoxides, their quantity increases and then decreases with oxidation time. Secondary products, and especially volatile compounds see their quantity increase with time.

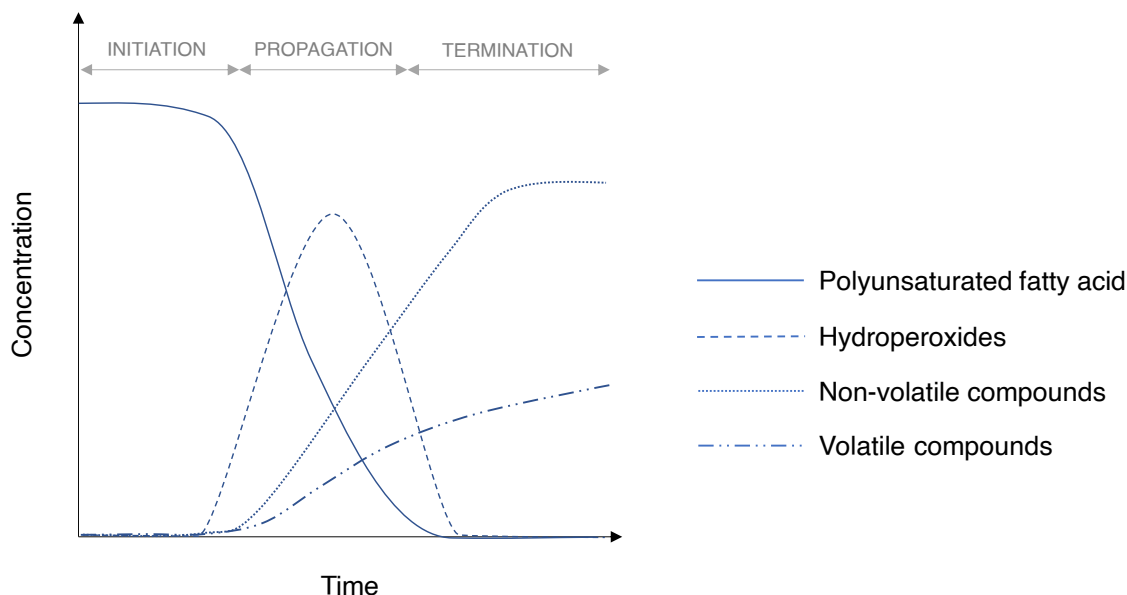


Figure 7: Evolution of the amount of lipid, primary and secondary products over the oxidation process. PUFA: polyunsaturated fatty acid [adapted from Gardner⁴⁷].

2.3.3. Volatile secondary products

In the context of this project, it was more interesting to focus on secondary products than primary products of lipid oxidation. In fact, the objective was to monitor the entire shelf-life of a product, including those having long time of storage (such as oils, mayonnaises, creams, ...), and not only detect the early stages of oxidation. Moreover, for an application of the developed technology to the monitoring of the environment surrounding the food without having necessarily contact between the food product and the intelligent material, it was more interesting to focus on volatile secondary products compared to non-volatile secondary products.

At the beginning of the thesis, a study of 40 sources was performed to identify the most common volatile secondary products released during lipid oxidation (see **Appendix A**). Various food products were targeted: meat (yak, beef, pork, turkey, chicken, Iberian ham, lamb), fish and seafood (salmon, cod, mackerel, whiting, hake, anchovy, trout, menhaden, saithe, redfish, sea bream, eel, herring, lobster, sardine, seabass, tuna, crayfish), fish oils (anchovy, sardines,

menhaden, herring, eel, seal blubber, blue crab, turbot), mayonnaise enriched with fish oil (eel), vegetable oils (soybean, sunflower, olive, palm, camellia, sunflower, perilla), beers, milk and cheese. The volatiles released during the oxidation of these products were listed and the occurrence of each compound was counted. According to the batch of paper studied, the pool of volatile compounds released during lipid oxidation was composed of 47 % of aldehydes, 24 % of ketones, 24 % of alcohols and 5 % of carboxylic acids (see **Figure 8**). Carbonyls thus represented the majority (71 %) of the volatiles.

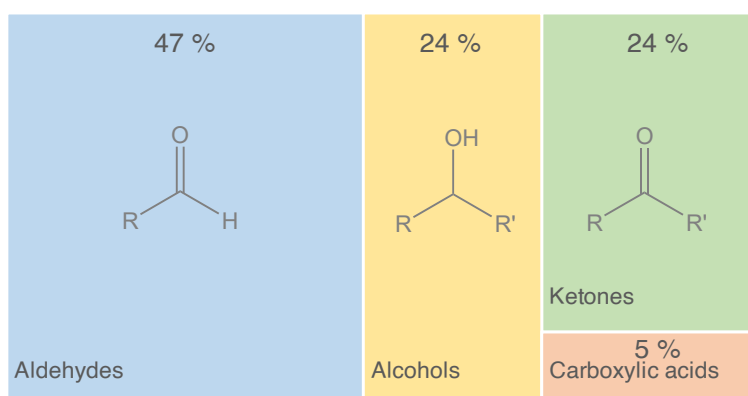


Figure 8: Distribution of the volatile compounds released during lipid oxidation according to the number of occurrences of each of these compounds found in 40 papers on the subject. R and R' are alkyl groups.

Hexanal and (*E,E*)-2,4-heptadienal were the aldehydes most often detected in the studied matrices (see **Figure 9a**). 1-penten-3-one and 1-octen-3-one were the main ketones (see **Figure 9b**). The alcohols, mostly 1-penten-3-ol and 1-octen-3-ol (see **Figure 9c**), can be partly obtained by catalytic reactions implying the alcohol dehydrogenase enzyme, which allows the interconversion of alcohols into aldehydes and ketones. This explains the structural similarity between the main ketones and the main alcohols identified⁴⁸.

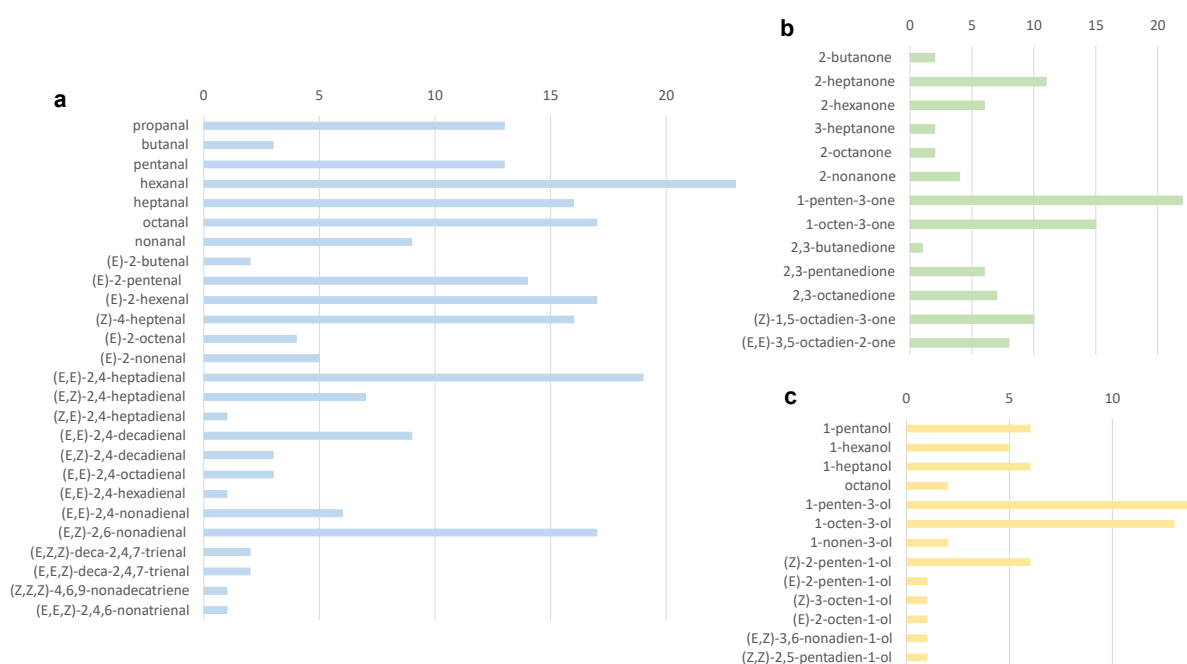


Figure 9: Main aldehydes (a), ketones (b) and alcohols (c) detected in oxidized food. Numbers indicates the number of occurrences of each of these compounds found in 40 papers on the subject.

Some volatile carbonyls were clearly identified as coming from lipid oxidation. This is for example the case of hexanal, which is considered as one the major indicator of lipid oxidation in muscle food⁴³. Hexanal comes from the oxidation of the omega-6 linoleic acid. As described in **Figure 10**, 2 different conjugated dienes can be obtained after the abstraction of a hydrogen atom from the 13th carbon atom, leading either to 13-hydroperoxide or 9-hydroperoxide. Both can lead to the formation of hexanal. On the contrary, some carbonyls, such as 2,3-pentanedione, could originate both from lipid oxidation and from microbial activity⁴⁹.

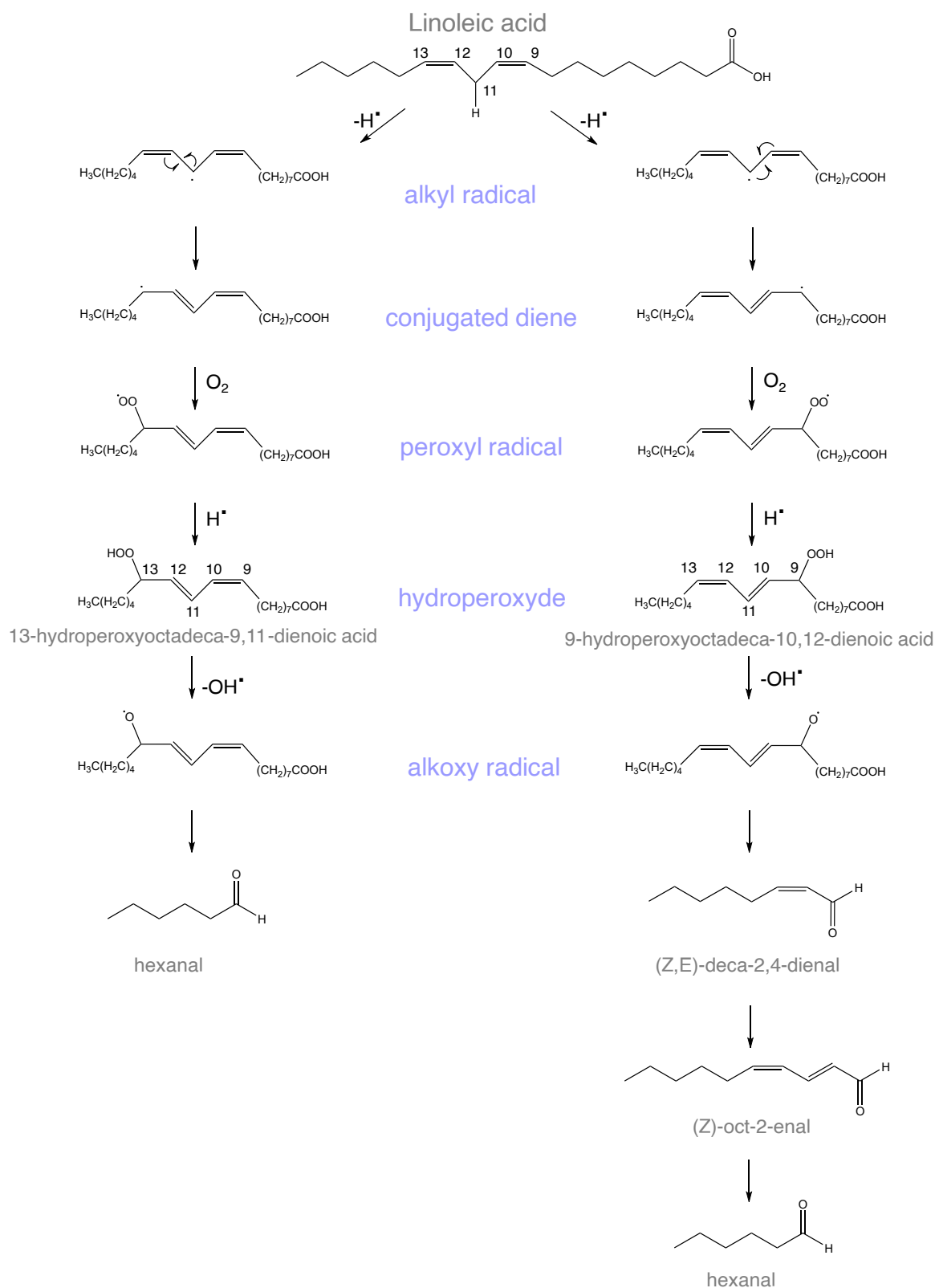


Figure 10: Transformation of linoleic acid into hexanal by the lipid oxidation mechanism [adapted from Shahidi and Oh⁵⁰].

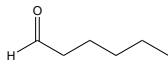
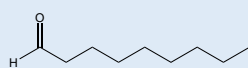
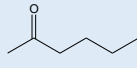
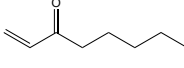
This study also highlighted that some volatile compounds were found to be good trackers of lipid oxidation regardless the original food matrix. This is for example the case of hexanal, which concentration increased during spoilage of meat^{19,51}, fish^{49,52–54}, fish oils⁵⁵ and vegetable oils^{43,56,57}. This is due to the fact that the omega-6 fatty acid from which originates hexanal is a common component of various food products, representing for example 66 weight percent (wt%) of sunflower oil⁵⁸; 12 wt% of pork and beef or 20 wt% of poultry⁵⁹; 2 wt% of menhaden and 5 wt% of tuna⁶⁰.

It was also noticed that in the case of thermal treatment, such as heating, cooking, or smoking; some additional volatile compounds were detected. Among them, furans, pyrazines, pyridines, pyrroles, thiophenes, thiazoles and thiazolines were identified^{61–63}. They come from another mechanism of degradation: the Maillard reaction. It involves amino groups from proteins and carbonyl groups from reducing sugars. This reaction brings changes in color (browning) and flavor (production of some new floral, nutty, caramel, toasty or malty flavors) to the food product, that are sometimes sought⁶⁴. As they do not represent lipid oxidation, these compounds were no longer considered.

According to this study, it was thus concluded that carbonyl compounds were the main representatives of lipid oxidation. They are also known to be major contributors to flavor deterioration of lipid-rich products^{45,65}. However, the odor is the first criterion used by consumer to reject a product⁶⁶, which generates a lot of food waste. It was thus of high interest to focus on the monitoring of carbonyls to track lipid oxidation.

The carbonyls presented in **Table 3** were thus chosen as trackers of lipid oxidation. They were selected because they were odorous with low thresholds, their concentration increased during spoilage, they were mostly found in several different food matrices and were identified as being released by lipid oxidation but originated from different fatty acids (see **Appendix A**). Moreover, they were commercially available at reasonable costs.

Table 3: Carbonyls chosen as lipid oxidation trackers in the context of this project.

NAME	STRUCTURE	ORIGIN	ODOR ⁶⁷	ODOR THRESHOLD IN WATER	EXPECTED QUANTITY IN SPOILED FOOD
Propanal		ω 3-linolenic acid	Earthy, whiskey, cocoa, nutty	9.5 $\mu\text{g/L}$ ^{68,69}	$\sim 0.5 \mu\text{g/g}$ (fish) ⁵²
Hexanal		ω 6-linoleic acid	Green, grass, woody	5 $\mu\text{g/L}$ ⁶⁸⁻⁷⁰	$\sim 1-150 \mu\text{g/g}$ (fish ^{52,71-73} , oil ⁷⁴) $\sim 1-5 \text{ mg/g}$ (chicken) ¹⁹
Nonanal		ω 9-oleic acid	Waxy, rose, orange, fatty, lemon	1 $\mu\text{g/L}$ ⁶⁸⁻⁷⁰	$\sim 1-90 \mu\text{g/g}$ (fish ^{52,71-73} , ham ¹⁹)
1-penten-3-one		ω 3-linolenic acid	Pungent, peppery, mustard, garlic	1.3 $\mu\text{g/L}$ ^{68,69}	$\sim 0.1-1.6 \mu\text{g/g}$ (fish ⁵² , oil ⁷⁴)
Hexan-2-one		ω 6-linoleic acid	Fruity, fungal, meaty, buttery	76 $\mu\text{g/L}$ ⁷⁵	$\sim 15 \mu\text{g/g}$ (oil ⁷⁴)
1-octen-3-one		ω 6-linoleic acid	Mushroom, herbal, earthy	0.003 $\mu\text{g/L}$ ^{70,76,77}	$\sim 1-73 \mu\text{g/g}$ (fish ⁷¹⁻⁷³)

2.3.4. Consequences of lipid oxidation

Lipid oxidation implies the degradation of both nutritional and organoleptic properties of food products^{19,45,78}. Besides the decrease in the quantity of the beneficial fatty acids, lipid oxidation implies the generation of off-flavors prohibitive for consumer acceptance⁶⁵. 2,4-decadienal is for example considered as responsible for rancid odors, and 1-octen-3-one is associated with fishy notes⁶⁷. They can be detected by human nose at trace levels (from ppb to ppm, depending on the olfactive molecule and the matrix)^{69,79,80}. In addition, off-flavors are responsible for most of food waste. According to a Swiss survey, the first criterion of rejection of a food product is in fact its odor, and more precisely its bad smell. This survey was conducted

by politbox on 2300 people in 2015 to have insights about the reason why so much food was wasted at the consumer level. At the question: “which criterion makes you throw away food in the first place?”, 35 % of the interrogated people answered: “the odor”, 27 % cited “the aspect” and only 21 % referred to the expiration date⁶⁶.

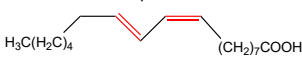
Furthermore, compounds released during lipid oxidation such as free radicals or reactive oxygen species (ROS) (like peroxides or singlet oxygen) may be harmful for consumer health, as they may damage DNA, disrupt proteins and enzymes functioning^{81–85}. Oxidative stress as well as the presence in the organism of some aldehydes generated by lipid oxidation, such as the 4-hydroxynonenal⁸⁶ were linked to many disorders such as Alzheimer’s disease^{87,88}. Finally, some products generated by lipid oxidation are dangerous, such as 1-penten-3-one, which is toxic if swallowed or inhaled (ingestion: LD50: 100 mg/kg, inhalation: 3 mg/L – 4 h).

2.4. CONVENTIONAL WAY TO DETERMINE THE OXIDATION LEVEL OF FOOD

Many methods exist for the determination of the lipid oxidation level of a food product. When focusing on the reactants, it is possible to monitor the oxygen amount in the surrounding environment of the product or measure the modifications in the number of unsaturations of the lipid. The most used techniques however measure the quantity of primary and secondary oxidation products produced. They are gathered in **Table 4**. The monitoring of primary products mainly relies on the measurement of the peroxide value (PV)³⁰ or the determination of the amount of conjugated diene (CD233)³¹. Secondary products are monitored by the estimation of the p-anisidine value³², by the thiobarbituric acid reactive substances (TBARS) assay³⁴, by chromatographic methods or sensory evaluation³⁵. PV and CD233 measurements can thus be used to track the early stages of lipid oxidation. However, as primary products are transitory, they cannot be used alone to assess the global lipid oxidation level of a product.

Table 4: Common techniques to determine the oxidation level of food.

METHOD	PRINCIPLE	TARGET	ADVANTAGES	DRAWBACKS	REF
PV	Iodometric titration of hydroperoxides $\text{ROOH} + 2 \text{I}^- + 2\text{H}^+ \rightarrow \text{ROH} + \text{I}_2 + \text{H}_2\text{O}$ $\text{I}_2 + 2 \text{S}_2\text{O}_3^{2-} \rightarrow 2 \text{I}^- + \text{S}_4\text{O}_6^{2-}$	Hydroperoxides	Standard method Rapid Simple	Partial oxidation level Anhydrous system Reference needed Solvent toxicity	30

CD233	UV-visible absorption (233 nm) of the conjugated diene/trienes 	Conjugated dienes and trienes	Standard method Rapid Simple	Oxidation of monounsaturated FA not detectable Interference of carbonyl absorption Solvent toxicity	31
p-anisidine value	UV-visible absorption (350 nm) of Schiff bases formed between carbonyls and p-anisidine (see Figure 11)	Carbonyls	Standard method	High toxicity Dependence of the intensity with the number of unsaturations Reference needed	32
TBARS	UV-visible absorption (530 nm) of a colored complex formed between malondialdehyde and thiobarbituric acid (see Figure 12)	Malondialdehyde (and more generally aldehydes)	Standard method	Long time for sample preparation (2 h min) High temperatures (95–100 °C) Strong acidic conditions Reference needed Solvent toxicity	34
Chromatographic methods	Separative technique coupled to different detectors based on the difference in the affinity of the analytes with the stationary and the mobile phase	All kind of products released by lipid oxidation	Low limit of detection Versatility (choice of columns and detectors)	Expensive device Reserved to trained personal Meticulous sample preparation Long analysis	/
Sensory evaluation	Detection and identification of the odors generated by volatile compounds	Volatile secondary products	Sensitivity	Need for a panel of trained experts Expensive Time-consuming	35

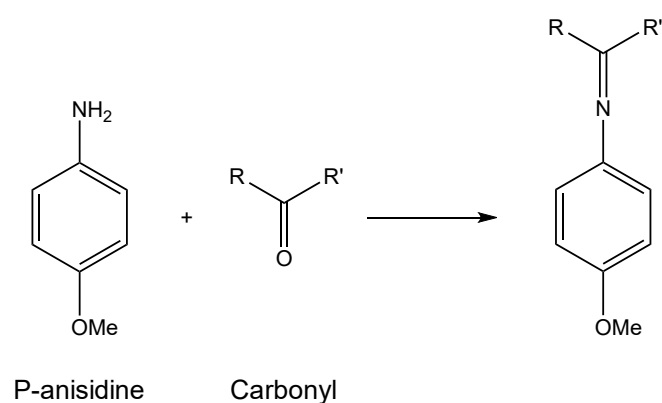


Figure 11: Reaction involved in the p-anisidine value test. R and R' are alkyl groups.

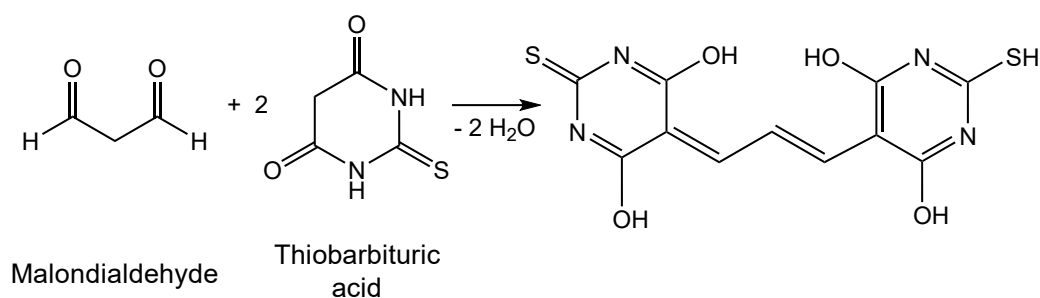


Figure 12: Reaction involved in the TBARS assay.

The majority of these methods have the great advantage to be standardized. However, they are usually expensive, dedicated to highly trained persons, require the use of some toxic chemicals and are time-consuming for sample preparation and analysis.

In addition, the study of the fatty moiety of a real food product generally requires a time-consuming extraction step. Moreover, due to the temperature applied, the lipids may be further oxidized during this procedure, implying biased oxidation level results. Finally, solvents used for the extraction are mostly hazardous and may induce health issues (*e.g.*, dichloromethane, methanol, chloroform)^{89,90}.

To cope with these issues, research is performed to find ways to monitor lipid oxidation in a fast, simple, and reliable way, with no use of toxic reactants and few sample preparation. In addition, a way to monitor lipid oxidation in real-time and during the entire shelf-life of the food product is attractive from both academic and industrial point of view.

2.5. OTHER METHODS TO MONITOR LIPID OXIDATION

In the recent years, several studies have emerged trying to find alternative methods for the determination of the lipid oxidation level of a sample using common analytical techniques⁹¹. This includes the use of differential scanning calorimetry (DSC)^{92,93} to evaluate the peak of exothermic reaction between lipids and oxygen or nuclear magnetic resonance (NMR)^{94,95} spectroscopy, Raman spectroscopy^{96,97} and near infrared spectroscopy (NIRS)^{98,99}, capable of identifying primary and secondary oxidation products.

Fluorescence was primarily used to characterize food products using the fluorescence of their components such as aromatic amino acids, tryptophan residues of proteins, riboflavin or vitamin A¹⁰⁰. This was particularly useful to fight against food fraud, as fluorescence provides

spectral signatures that can be used as fingerprints of the food products¹⁰⁰. Fluorescence was used to monitor lipid oxidation in 3 main ways.

First, the fluorescence spectrum of food products was measured using the front-face technique. As shown in **Figure 13**, the front-face fluorescence differs from the usual way to record fluorescence spectrum by positioning the sample on a rotating plate allowing to adjust angles with which the sample is excited and fluorescence detected. This allows to limit scattered and reflected light and reduce the risk of an overlap between the excitation and emission spectrum by reducing the width of the peaks. This is particularly interesting when recording the fluorescence of solid samples. This technique was used to analyze solid food samples such as fish¹⁰¹, chicken meat¹⁰², turkey meat¹⁰³, cakes¹⁰⁴ or butter¹⁰⁵ for example. They rely on the measurement of fluorescent compounds that are formed during the oxidation process, such as Schiff base products coming from the reaction of aldehydes generated by lipid oxidation with fluorescent amino acids such as tryptophan, tyrosine or phenylalanine contained in the food. The main drawback of this technique is that it is necessary to have an idea of which compounds are expected to adjust both excitation and emission wavelengths. Moreover, the measure is made on the surface of the food, which do not necessarily represent the oxidation level of the entire product in the case of the process is a non-homogeneous phenomenon.

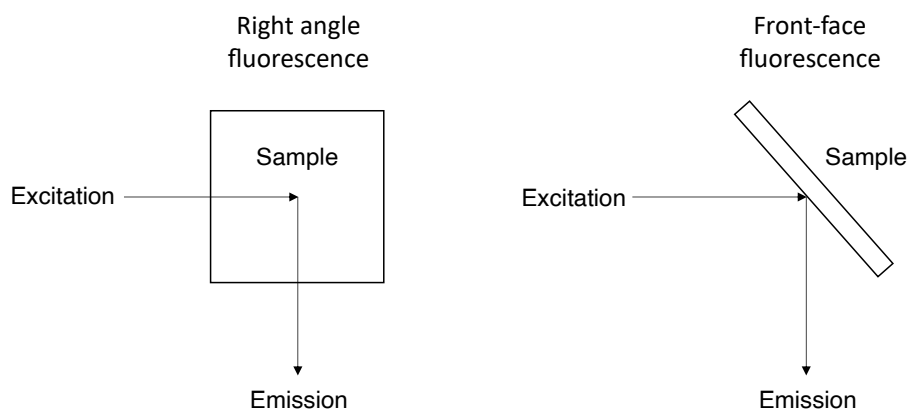


Figure 13: Illustration of the fluorescence front-face technique [adapted from Sádecká and Tóthová¹⁰⁶].

Second, the TBARS assay, usually performed with a UV-visible spectrophotometer, can also be performed using fluorescence. The TBARS assay in fact relies on the formation of a compound from the reaction of malonaldehyde with thiobarbituric acid, that is both colored and fluorescent. The color can thus be detected using a visible spectrophotometer (at 535 nm)

or the fluorescence property of the product can be exploited by a fluorescence spectrometer (with an excitation at 515 nm and emission recording at 553 nm)¹⁰⁷.

Third, chromatographic techniques can be coupled to fluorescent detectors, especially to monitor carbonyl compounds released during lipid oxidation. Such a method requires a derivatization step, usually with 2,4-dinitrophenylhydrazine, which forms fluorescent dinitrophenylhydrazone derivatives after reaction with carbonyl compounds^{83,108–110}.

3. FOOD PACKAGING

3.1. ROLE OF THE PACKAGING

The primary role of the packaging is to protect food products from contamination and spoilage, and to preserve their quality and freshness during transportation, storage, and sale. Therefore, their mechanical and barrier properties must be adapted to the specific requirements of the food product. They must also be designed to allow convenient use of the product. Finally, food packaging also provides consumers with regulatory information about the food product, such as the ingredients, nutritional information, and the expiration date.

As depicted in **Figure 14**, different types of materials are used by the food packaging industry. More than 40 % of the packaging are made of plastic, either flexible or rigid¹¹¹. Paper-derived packaging are usually used as secondary packaging but an increase in the demand of such materials was noticed in the last years as paper constitutes a sustainable alternative to non-biodegradable packing solutions¹¹². Metal packaging is commonly used for applications such as canned food. It provides extended shelf-life and high resistance against physical damages. Growing demand for glass packing is attributed to the chemical inertness and good recyclability of this material making it a good alternative to plastics¹¹².

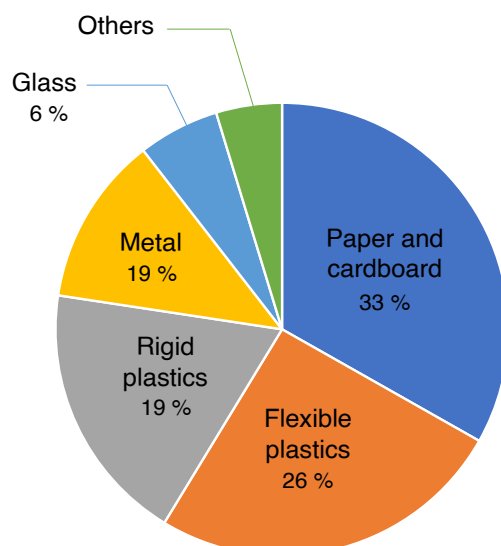


Figure 14: Global distribution of food packaging materials in 2019 (figures from statista¹¹¹).

Different types of polymers have been used as plastic packaging in the agri-food industry. Most common petrochemical examples are polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET). According to PlasticsEurope, these three polymers were the most used plastics in the packaging sector in 2019. In its last report of 2020, PlasticsEurope pointed out that 60 % of the plastic produced today could be used during 1 to 50 years whereas they are generally used for shorter period of time¹¹³. Such production trend cannot be part of a circular economy approach. Reduce packaging, collect more plastic wastes, recycle more and find sustainable alternatives materials constitutes the challenges facing the packaging industry today.

Biodegradable polymers constitute alternatives packaging solutions that were investigated in the last decades. However, their usual poor barrier properties limit their applications in the food sector¹¹⁴. According to Citeo, only 30 % of plastic packaging are recycled in France annually. The rest are either incinerated or buried. Main reasons are that some plastics are not recyclable (*e.g.*, multilayers). Other do not have dedicated collect channels (*e.g.*, polystyrene). Mechanical recycling is the most used today, but it mainly concerns PP and PET. Chemical recycling process is emerging and relies on the depolymerization of the material. This promising alternative is still expensive and was not applied on classical food packaging wastes yet¹¹⁵. For now, except for PET bottles, recycled plastics is little used by the food industry due to the high safety requirements and risks of cross-contamination. However, at the end of 2022, the European Commission revised the Packaging

and Packaging Waste Directive. The objective is that “all packaging on the EU market is reusable or recyclable in an economically viable way by 2030”. It is thus urgent to find solutions to achieve this goal. However, it will be important to ensure that in meeting these new rules, food products will be protected enough to avoid generating even more food waste.

3.2. TRADITIONAL WAY TO EXTEND FOOD SHELF-LIFE

According to the (EU) regulation n° 1169/2011, the shelf-life of a product is referred to as the “date of minimum durability” (replaced by the “use-by” date for the concerned food products). Therefore, shelf-life is the period during which a food maintains its characteristics under appropriate storage and handling conditions. These characteristics can be related to the safety or the quality of the product.

To extend food shelf-life by preventing microbial growth, technologies such as electric pulses, high pressures and irradiations have been employed. To take actions against lipid oxidation, it is important to understand which factors promote the oxidation reaction. Internal as well as external factors can be cited. Among internal factors, the type of fatty acids as well as the presence of pro-oxidant compounds such as metals (like iron) or enzymes (like lipoxygenase) will affect the oxidation rate. These parameters depend on the composition of the food product. Pro-oxidant iron can for example come from heme-proteins (myoglobin and hemoglobin) of animal tissues. Among external factors, temperature, light and oxygen are the major contributor to lipid oxidation⁴³. The agri-food industry relies on several techniques to prevent the action of these external factors and slow down the oxidation process. Most common techniques are vacuum packaging, modified atmosphere packaging (nitrogen-based for example), oxygen barrier packaging, opaque packaging and addition of antioxidants directly in the food product or in the packaging^{116–118}. Vacuum, modified atmosphere packaging and oxygen barrier packaging reduce the quantity of oxygen in contact with food. For certain type of products such as red meat, vacuum packaging is not always a solution of choice, as it will affect the desirable red color of the meat. Modified atmosphere packaging can slow down the oxidation process, but residual oxygen remains (around 1 %¹¹⁹) and can still degrade the food. The use of opaque packaging or at least packaging materials that absorb UV light is an efficient way to prevent photo-oxidation. Antioxidants can be antiradicals such as phenolic compounds, metal ion chelators like EDTA or oxygen reductors as ascorbic acid. They delay or slow down oxidation but cannot avoid it.

3.3. NEW WAY TO EXTEND FOOD SHELF-LIFE: ACTIVE PACKAGING

Active materials are defined by the EC regulation 450/2009 on active and intelligent materials and articles intended to come into contact with food. They can interact with food or its environment through a controlled sorption or release of compounds in order to extend the shelf life of the food product. Such active materials could be advantageously used when the precited packaging solutions are not sufficient. The first category of active packaging is based on sorption processes. They can for example rely on scavengers for gases such as oxygen, ethylene or carbon dioxide¹²⁰. The second category of active packaging rely on the release of compounds such as preservative agents or antioxidant in the surrounding environment of the food product in order to prevent spoilage and therefore extend its shelf-life. Preservatives can be for example allyl isothiocyanate¹²¹, an antibacterial agent, which is used to preserve food from the development of microorganisms. Antioxidants, such as ferulic acid¹²², aim to prevent lipid oxidation.

3.4. INTELLIGENT PACKAGING

Intelligent materials are defined in the EC regulation 450/2009 on active and intelligent materials and articles intended to come into contact with food. They are intended to “monitor the condition of packaged food or the environment surrounding the food”. Therefore, they gather information on the product and provide feedback on its condition during its shelf-life. Intelligent packaging and smart packaging are terms that are often used interchangeably. However, the EC 450/2009 regulation only defines active and intelligent materials. The terminology “smart packaging” is never mentioned. This leads to some confusion in the literature. “Smart packaging” could be defined as a system possessing features from both active and intelligent packaging. Therefore, this work is related to intelligent packaging.

According to Future Market Insights, the market of intelligent packaging is expected to account for US\$ 23.6 billion in 2023 and reach US\$ 56 billion in 10 years¹²³. Moreover, as shown in **Figure 15**, the number of publications related to intelligent packaging increased exponentially during the last 10 years.

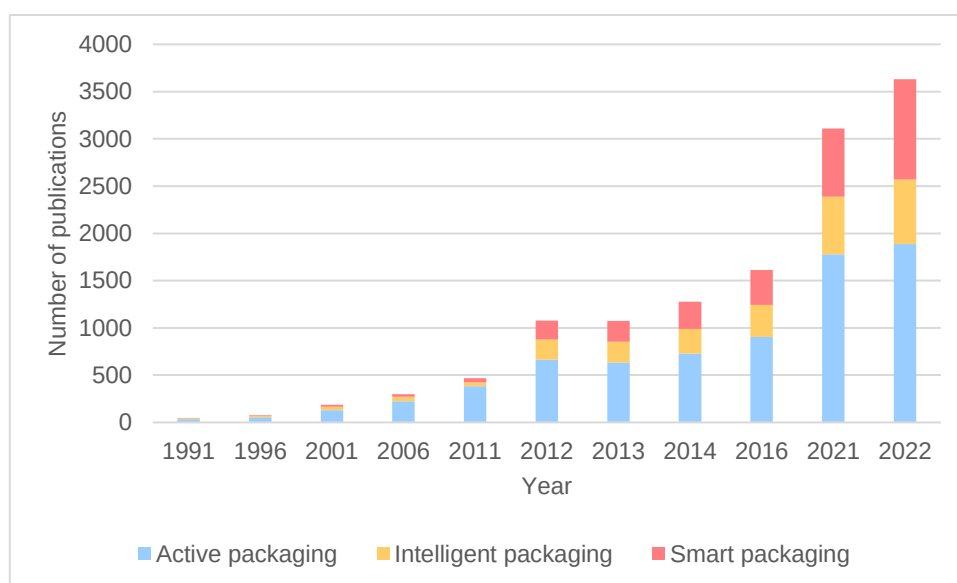


Figure 15: Number of publications related to “active packaging”, “intelligent packaging” and “smart packaging” as per year (figures from Web of Science).

Products undergo regular microbiological and chemical tests during production, often using the quality indicators listed previously. However, proper control after delivery to supermarkets and at the consumer level is often not possible. Intelligent packaging could fill this gap by tracking the quality of the product throughout the entire food supply chain by indicating the dynamic shelf life of the product to the retailer or consumer.

Intelligent packaging are usually categorized into three main categories: radiofrequency identification (RFID) tags, indicators and sensors. The classification of some innovations either in the category “indicator” or “sensor” is not always clear in the literature. Indicators can be defined as “substances that indicate the presence, absence or concentration of another substance” by means of a characteristic direct change such as a color modification¹²⁴. A sensor is a device containing two parts: a receptor and a transducer. Sensors could thus be differentiated from indicators by the need of a detection device to perceive the signal emitted by the sensor. Targeted users are different for each technology. RFID tags are meant for retailers and distributors, indicators can be used by consumers while sensors can be dedicated to retailers, distributors but also used for research and development in laboratories.

RFID tags are electronic systems able to transfer data from a tag attached to an item to a reader device using magnetic fields or electromagnetic waves. RFID tags also include barcodes, QR codes, holograms and NFC chips, data carriers which can be read with use of the

appropriate scanner. Examples of commercial RFID tags are given in **Table 5**. They can help ensuring product traceability and authenticity. As an example, the wine industry has already implemented the Prooftag's BubbleTag solution to fight against counterfeiting. It relies on the imprinting of a unique QR code, one part being on the glass bottle and the other on the capsule. This prevent the selling of an expensive bottle having been filled with a cheap wine, which does not correspond to the label.

Table 5: RFID tags defined as intelligent packaging.

RFID TECHNOLOGY	COMMERCIAL EXAMPLES
RFID tags	RFID (eProvenance, France) ThermAssureRF™ (Evidencia, USA) Turbotag™ (Sealed air, USA) BT foodnet (British Telecommunications, UK) * VarioSens® (KSW Microtech AG, Germany) *
QR codes	BubbleTag (Prooftag, UK)
Holograms	Hologram (De La Rue Holographics, UK)
NFC chips	NFC security tag (Selinko, Belgium)
Tagged inks	Traceless System (Kodak, USA)

* RFID tags no more commercialized.

As summarized in **Table 6**, there are 3 main types of indicators: the time-temperature indicators, the integrity/leakage indicators and the freshness indicators.

Table 6: Indicators defined as intelligent packaging.

INDICATOR	MEASURE	COMMERCIAL EXAMPLES
Time-temperature indicator	Temperature	Monitor Mark™ (3M, USA) Freeze Watch™ (3M, USA) Freshtag® (Vitsab®, Sweden) Fresh-Check® (Zebra, USA) SmartTag™ (Varcode, Israël) FreshTag (Insignia Technologies, Scotland) OnVu™ (Ciba Speciality Chemicals, Switzerland) * TopCryo (Cryolog, France) *

Integrity/leakage indicator	Gases (e.g., O ₂ , CO ₂)	Ageless Eye® (Mitsubishi Gas Chemical, Japan) IntellDot (Impak, USA) OxyEye™, OxySign™ (OxyFree, USA) Leak Detection Label (Insignia Technologies, Scotland) After Opening Label (Insignia Technologies, Scotland)
Freshness indicator	pH, volatile amines, specific metabolites	eO® (Cryolog, France) * TRACEO (Cryolog, France) * RipeSense® (Jenkins, New-Zealand) * SensorQ™ (Food Quality Sensor International Inc., USA) *

* Indicators no more commercialized.

Time-temperature indicators show whether the product have exceeded a temperature threshold and estimate the amount of time the product has spent in these inappropriate storage conditions¹²⁴. Integrity/leakage indicators detect the presence of gases such as O₂ or CO₂ and can inform the retailer if the integrity of the packaging has been compromised. Freshness indicators provide information resulting from microbial growth or chemical changes within a food product (e.g., pH changes, detection of volatile amine, ripeness state of food). As an example, the eO® freshness indicator, developed by Cryolog, was based on a dot in shape of flower containing a dye responsible for its initial color. Lactic acid bacteria were inoculated in the label and intended to grow at a rate mirroring the growth rate of the bacteria inside the packaging releasing lactic acid. The change in the pH affected the color of the label. Examples of indicators are displayed in **Figure 16**.

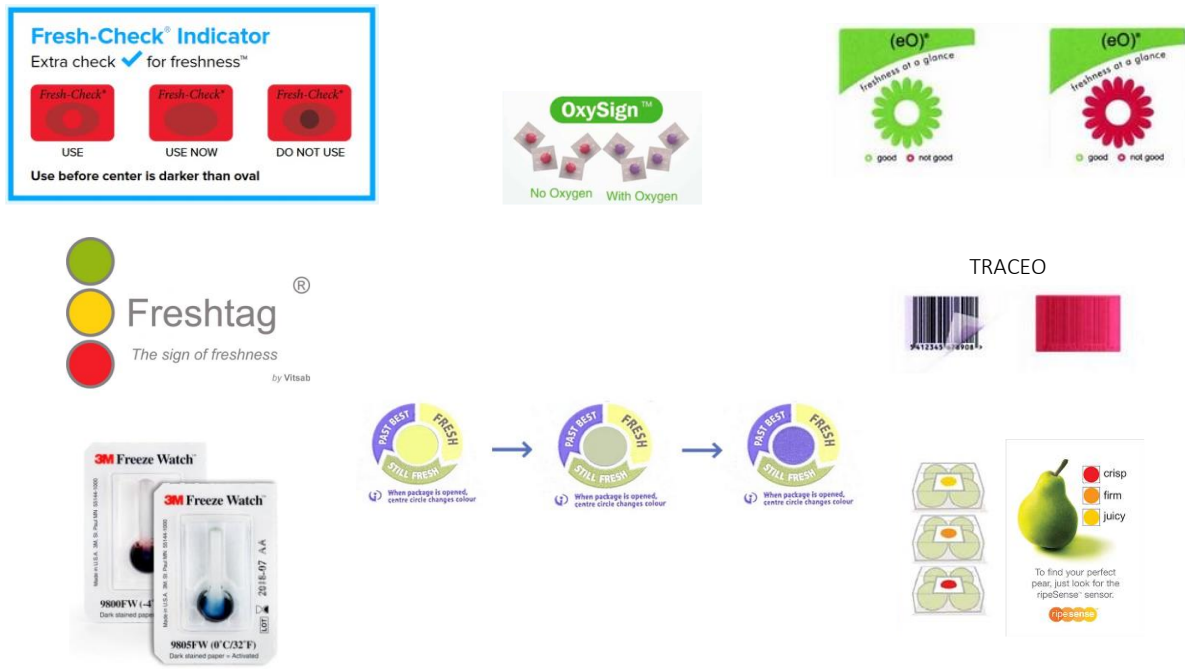


Figure 16: Examples of time-temperature indicators (left), integrity indicators (middle), freshness indicators (right)^a.

As depicted in **Table 7** intelligent packaging sensors include biosensors, gas sensors and chemical sensors.

Table 7: Sensors defined as intelligent packaging.

SENSOR	MEASURE	COMMERCIAL EXAMPLES
Biosensor	Food pathogens, toxins	Food Sentinel System® (SIRA Technologies) * ToxinGuard® (Toxin Alert, Canada) *
Gas sensor	O ₂ , CO ₂ , water vapor, ethylene	Breathe (Emco, UK) GasCheck (EMCO, UK) O2xyDot (OxySense) *
Temperature sensor	Temperature	SmartLabel (Vanprobe, India)
Chemical sensor	pH, hydrogen sulphide, volatile amines, biogenic amines	/

* Sensors no more commercialized.

^a Picture sources: <https://www.zebra.com>, <https://vitsab.com>, <https://www.3m.com>, <https://www.impakcorporation.com>, <https://www.insigniatechnologies.com>, <https://www.cryolog.com>, <https://www.packagingconnections.com> (15.03.2023)

Biosensors possess a bioreceptor made of organic or biological material such as antibodies as well as a transducer, either optical, acoustic or electrochemical¹²⁴. Gas sensors are sensitive to oxygen, carbon dioxide, water vapor or ethylene and are mainly used to control fruit ripeness, as exemplified in **Figure 17** (left). OxySense® used to develop O2xyDot®, a commercial fluorescence-based oxygen sensor (see **Figure 17**, right). It is based on a fluorescent ruthenium-based dye incorporated in a hydrophobic polymer, placed in the headspace of the packaging. Excitation and detection were performed using an external LED device. Oxygen is an excellent collisional quencher (more deeply discussed in the last chapter of this part). Its presence decreased the fluorescence of the dye entrapped within the dot. Chemical sensors, similarly to freshness indicators, provide information on the spoilage level of food. It is interesting to notice that no such sensors has been commercialized yet, even less to specifically monitor lipid oxidation. This could be attributed to the lack of miniaturized devices capable of reading the information given by the sensors. At the consumer level, the market may also not be ready for such innovations. Some brands such as Monoprix, Franprix and Metro implemented in France the use of time-temperature indicators by the past (Fresh-Check, TopCryo, OnVu respectively) but all these initiatives failed. The supplementary cost induced by the chips was not questioned. The main issue was the number of products that were returned to the distributor either due to misuse or lack of reliability of the indicator. If such was the case for time-temperature indicators, it can be understood that the same reasons hinder the implementation of chemical sensors monitoring food spoilage. Moreover, to date, consumers want less packaging and less confusing information on them. Such technology could be thus more profitable to the retailers and distributors. The chemical sensors could be applied to the fast and easy detection of food pathogens, adulterants and allergens for example.



Figure 17: Examples of gas sensors (left). O2xyDot® fluorescence-based oxygen sensor (right)^b.

Although the industrial applications of intelligent packaging sensors to monitor food spoilage are poor for the moment, the academic literature is full of work related to this field. These studies focus on the detection of volatile amine^{125–152} (mainly using pH-sensitive dyes), biogenic amines^{153–161}, sulfur compounds^{162–165}, xanthine¹⁶⁶, *E. coli*¹⁶⁷ or lytic enzymes¹⁶⁸. The majority of these sensors are colorimetric^{167–204}, some of them are fluorescent^{205–209} and the rest are electrochemical sensors^{166,210–217}. The literature on the detection of carbonyl compounds to track lipid oxidation in food sample is not so dense. Liu *et al.* developed a colorimetric sensor based on chitin films containing Congo red dye and hydroxylamine sulfate for the determination of aldehyde in oxidized edible oils²¹⁸. When focusing only on the sensing of carbonyls compounds Li *et al.* developed a colorimetric sensor based on aniline and hydrazine derivatives for the detection of aldehydes and ketones in gaseous phase of alcoholic beverages to ensure their authenticity and thus fight against frauds²¹⁹. Kim *et al.* proposed a colorimetric sensor for the detection of aldehyde using filter-paper soaked in methyl red dye for the monitoring of apple ripeness²²⁰. Fluorescent sensors did not seem to be applied to the field of food. The few existing examples of fluorescent sensors for the detection of carbonyls compounds are related to environmental^{221,222} or biological^{223,224} applications.

^b Picture sources: <https://www.emcotechnologies.co.uk>, <https://www.emcotechnologies.co.uk>, <https://industrialphysics.com>, <https://www.rycobel.com> (15.03.2023)

4. SENSING MATERIALS FOR CARBONYL MONITORING

4.1. OPTICAL SENSORS

A chemical sensor consists of 2 components: a sensitive material and a transducer. Different sensing techniques have been proposed in the last years, based on either optical (mainly colorimetric or fluorometric sensors), mass-sensitive or electrochemical properties. Optical sensors rely on color changes (*e.g.*, Tollen's reaction)²²⁵ or modulation of fluorescence emission (intensity or wavelength) (*e.g.*, aldimine or ketimine condensation; reaction with hydrazines)^{81,219,221,226}. They are usually coupled with the use of cameras, UV-visible spectrophotometers or spectrofluorometers. More recently, the detection by smartphone emerged and was applied to the monitoring of food quality, with the fluorescent detection of fusarium food contaminant in cereals²²⁷ or the measure of colorimetric changes of gelatin films containing anthocyanins for the detection of ammonia vapors in chicken breast²²⁸. Optical sensors, whether they are colorimetric or fluorometric are thus gaining increasing interest for the development of new user-friendly analytical methods. Sensors are solutions of choice for rapid, simple-to-use, reliable and portable solutions allowing on-site monitoring in real time. In this project, the developed fluorescent chemical sensors were made either of acrylate-based or silicate-based materials.

4.2. ACRYLATE-BASED MATERIALS

4.2.1. Definition and synthesis

Acrylate-based materials are organic polymers prepared from acrylate monomers, which are bifunctional molecules possessing both a carboxylate group and one or more vinyl groups, allowing the polymerization. Such polymers are typically produced through radical polymerization. The mechanism is the same as the one described in the chapter dedicated to lipid oxidation, with an initiation, propagation and termination step, which is summarized in **Figure 18**. This synthetic route requires the use of one or more functional monomers, a cross-linker, a polymerization initiator, and a solvent²²⁹. The selection of functional monomers should be based on the targeted application since they possess functional groups that provide specific reactivity to the polymer. The most common monomers include acrylamides and vinyl esters. The cross-linker provides mechanical stability to the final material, with divinylbenzene and ethylene glycol dimethacrylate (EGDMA) being the most commonly used ones. Radical

polymerization is typically performed either thermally or photoinitiated. 2,2'-azobis(2-methylpropionitrile) (AIBN) is classically used as a thermal initiator and 2,2-dimethoxy-1,2-diphenylethanone (DMPAP) is commonly used as a photoinitiator.

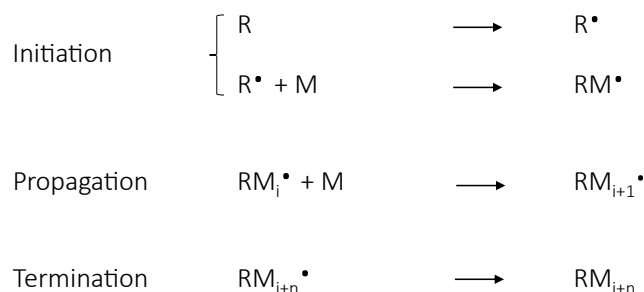


Figure 18: Mechanism of radical polymerization. R stands for radical and M for monomer.

4.2.2. Optical sensors based on acrylate-based materials

Optical sensors relying on acrylate-based materials were mostly developed in the frame of molecularly imprinted polymers (MIP), for the selective detection of analytes in food, environmental or biological samples. Some rely on the coating of MIP on rare earth nanoparticles²³⁰, on metal organic frameworks (metal ions coordinated to organic ligands to form porous structure)²³¹ or on the surface of carbon^{232–234} or quantum dots semiconductors^{235–237}. Here are a few examples of acrylate-based materials for colorimetric or fluorometric sensing of different analytes in food samples.

For the detection of drug residues in food samples, Zhang *et al.* proposed a coumarin-based fluorescent MIP selective for sulfadiazine²³⁸. For the detection of food spoilage trackers, Mattsson *et al.* developed a MIP for the detection of biogenic amines in fish samples. For the fluorescent detection of the analytes, the biogenic amines were labeled with the fluorescent dye BODIPY²³⁹. Sergeyeva *et al.* proposed a sensor for aflatoxin B1, a toxic mycotoxin. The fluorescence resulted of the irradiation of the analyte bound to the polymer²²⁷. Such sensor could also serve the fraud control with for example the fluorescent MIP based on carbon dots selective for tartrazine, a toxic additive used as fake saffron²⁴⁰.

For the detection of carbonyls as proposed in this work, it seems that no fluorescent acrylate-based material was recently developed. However, Rico-Yuste *et al.* studied the synthesis of an acrylate-based material for the colorimetric detection of furfural in beers²⁴¹. Furfural is a degradation product from the Maillard reaction. The material was obtained by the

co-polymerization of two functional monomers: 4-vinylaniline and 2-hydroxymethyl methacrylate, using EGDMA as a cross-linker.

4.3. SILICATE-BASED MATERIALS

4.3.1. Definitions

Silicon refers to the chemical element of symbol Si, which is rarely found as is on Earth due to its high chemical affinity for oxygen. Silica, also called silicon dioxide, is an oxide of silicon with formula SiO_2 , found in nature as quartz mineral. Silicates are polyatomic anions of silicon and oxygen, the most common family being the orthosilicates SiO_4^{4-} . Siloxanes are compounds of generic formula R_2SiO , where R is usually an alkane but can be organic. In this work, the name silicate-based material will be used to refer to materials based on the hydrolysis and condensation of tetraethyl orthosilicate ($\text{Si}(\text{OEt})_4$) according to the sol-gel process.

4.3.2. Synthesis of silicate-based materials

The most widely used strategy to synthesize silicon-derived materials is the sol-gel process. It relies on the generation of sol, a colloidal fluid containing the reactants, which eventually forms an insoluble three-dimensional network, called gel. The earliest work on this synthetic route was reported by Ebelmen in 1846²⁴², who observed hard monolithic products obtained after storage of tetraethylorthosilicate at room temperature for several months. The sol-gel process primarily relies on the hydrolysis and condensation of tetraalkylorthosilicates ($\text{Si}(\text{OR})_4$) where R is an alkyl group, such as tetramethyl orthosilicate (TMOS) or tetraethyl orthosilicate (TEOS). Due to the immiscibility of these precursors in water, a solvent, usually ethanol, is added to the mixture. Depending on whether water or alcohol is present in higher amount, the obtained gel is either called “hydrogel” or “alcogel”. The hydrolysis and condensations reactions occur under acidic or basic conditions²⁴³. In this project, basic conditions were systematically used. As displayed in **Figure 19**, hydrolysis generates silanol compounds that can condensate either with formation of water or alcohol.

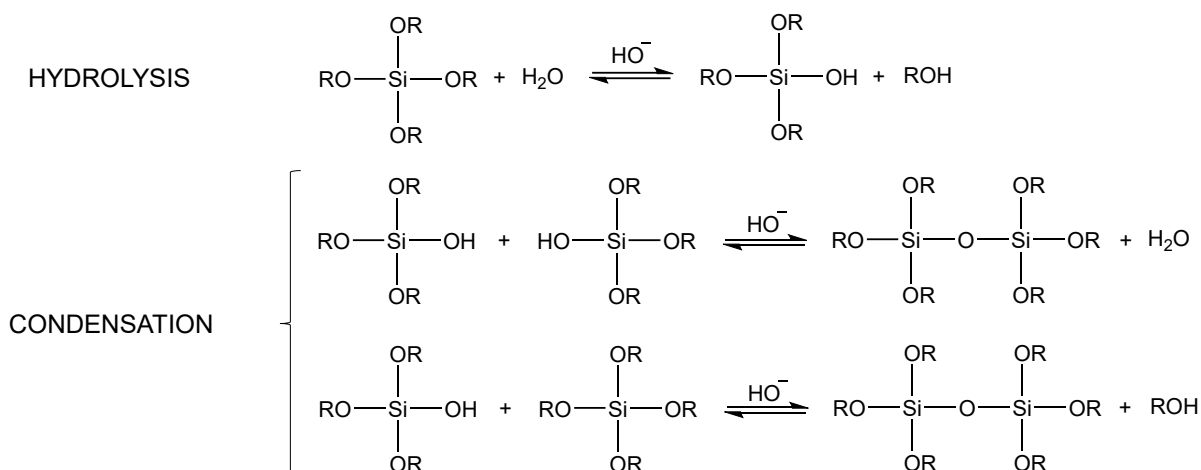


Figure 19: Hydrolysis and condensation occurring during the sol-gel process. R is an alkyl chain.

In the case of basic catalysis, hydrolysis relies on a nucleophilic substitution from $\text{S}_{\text{N}}2$ type, with nucleophilic attack of HO^- and formation of a pentacoordinate intermediate, as shown in **Figure 20**.

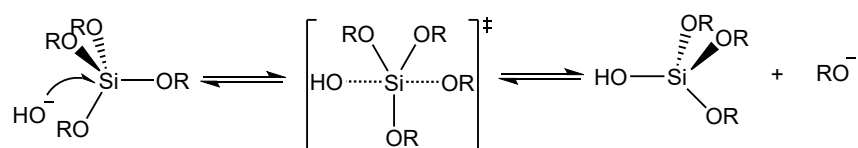


Figure 20: $\text{S}_{\text{N}}2$ mechanism of the hydrolysis step of the sol-gel process under basic catalysis. R is an alkyl chain.

As hydrolysis and condensation reactions occur simultaneously, these reactions are difficult to study. Therefore, the mechanism of condensation under basic conditions is not well understood. It was suggested that a $\text{S}_{\text{N}}2$ mechanism could govern this reaction²⁴⁴, with the deprotonation of a silanol by OH^- at first, followed by the attack of the resulting silanolate on another silanol molecule. The suggested corresponding mechanism is proposed in **Figure 21**.

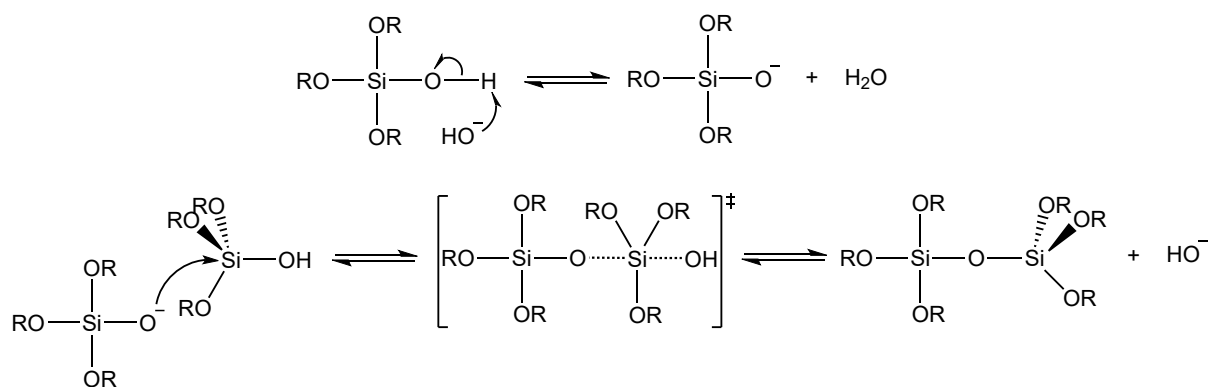


Figure 21: Proposed S_N2 mechanism for the condensation step of the sol-gel process under basic catalysis. R is an alkyl chain.

During the reaction, the viscosity of the medium increases. At the end of the process, the gel obtained can be let to dry. If drying is performed in ambient conditions, the resultant material is called a “xerogel”. If drying is done in supercritical conditions, an aerogel is produced. The drying conditions will affect the porosity of the resulting material. This porosity can be shaped by other procedures.

Prior to hydrolysis and condensation, the shape of the pores within the material can indeed be tuned by the so-called “Liquid Crystal Templating” (see **Figure 22**). Deeply investigated in the 90’s for the development of mesoporous silica-based materials^{245–248}, this route was made popular with the well-known MCM-41 material. This strategy relies on the addition of a surfactant in the reaction mixture, which will form specific arrangements of micelles according to the concentration. Hydrolysis and condensation of a tetraalkylorthosilicate is conducted around these micelles. The porosity of the resulting material will result from the removal of the micellar phase by calcination. The inner structure of these materials is thus well controlled, and their high porosity make them of high interest for many applications such as adsorption and catalysis.

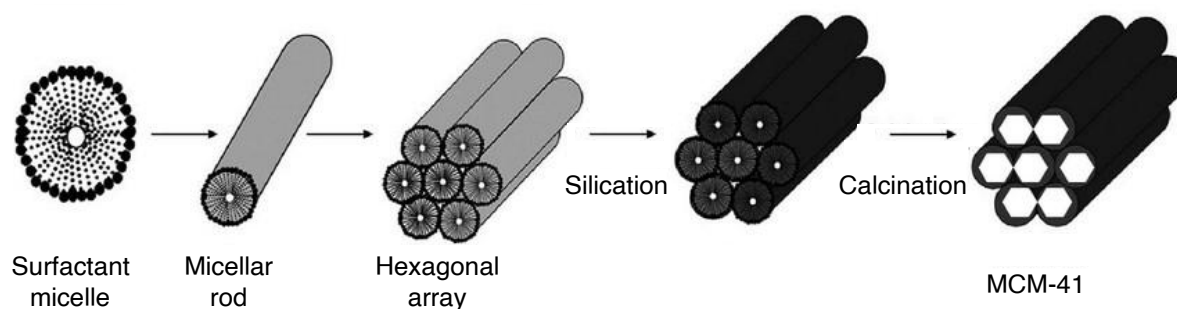


Figure 22: Principle of the "Liquid crystal Templating" in the example of the preparation of MCM-41 material [adapted from Mukhopadhyay *et al.*²⁴⁹].

4.3.3. Functionalization of silicate-based materials

To produce materials made of organic and inorganic moieties, several strategies can be investigated such as the co-condensation, the doping, the grafting and the impregnation. In this work, only the co-condensation and the doping were used. Such hybrid materials are named "ormosil" for "organically modified silicate".

The co-condensation relies on the use of one or more compounds during the sol-gel reaction, at least one of which being a tetraalkylorthosilicate for the generation of the inorganic backbone of the material, the others being alkoxy silanes (having Si-C bonds) implementing the organic moiety to the structure. This method allows to provide the material with tunable chemical properties, thanks to functional group covalently linked to the three-dimensional siloxane network. The co-condensation route generally leads to a more homogeneous distribution of organic functionalities into mesoporous matrices²⁵⁰.

For the doping, organic compounds are solubilized into the reaction mixture before hydrolysis and condensation of a tetraalkylorthosilicate. The organic molecule is then incorporated into the mineral network but is not covalently linked to it. In the case of the use of a surfactant, organic compounds can be solubilized within the micelles which are finally not calcinated. Such strategy was for example used for the extraction of metallic compounds from water²⁵¹.

4.3.4. Advantages of silicate-based materials

Silicate-based materials have gained increasing interest in the past decades because of their advantageous features, making them suitable for many applications. Silicate-based materials show better chemical, physical and thermal stability compared to their acrylate-based

counterparts. Moreover, only low toxicity reactants and no harmful solvents are used during the synthesis^{252,253}. Conducted at room temperature, this process is cost-effective and allows to build ormosil based on a wide choice of organosilane derivatives without risking any thermal degradation of the reactants. Moreover, as described previously, their porosity is easily tunable. Finally, the silicate-based materials can be shaped in different ways (powders, films, sol or gels) according to the targeted application.

4.3.5. Optical sensors based on silicate-based materials synthesized by the sol-gel process

Silicate-based materials synthesized by the sol-gel technique were only recently applied to the development of some optical chemical sensors.

The incorporation of a pH indicator or pH sensitive dyes into the sol-gel matrix allowed for the optical determination of the pH, either based on fluorescence or absorbance measurements. Lin J. and Liu D. for example developed a pH sensitive sensor using a mixture of several pH indicators entrapped in porous silica glass prepared by the sol-gel process²⁵⁴. Measurements were performed through the use of a UV-visible spectrophotometer. Such a sensor could be used in the food field for the determination of the microbial activity responsible for pH modification of the products.

Sol-gel based optical sensors have also been proposed for detection of different gases such as oxygen, carbon dioxide or ammonia. McDonagh *et al.* developed sol-gel films based on the fluorescence quenching of a ruthenium complex for oxygen sensing²⁵⁵. Von Bültzingslöwen C. *et al.* developed a sol-gel based fluorescent sensor for the monitoring of CO₂ in modified atmosphere food packaging²⁵⁶. Chen *et al.* proposed a fluorescent sol-gel material containing aminofluorescein for the detection of ammonia for the estimation of water pollution level²⁵⁷.

For the fluorescent detection of primary amines, Seok *et al.* recently proposed a sol-gel material functionalized with styrylbenzene derivatives and aldehyde groups²⁵⁸. They thus developed a material with the opposite objective of this project, which is based on the development of material containing amine groups to detect carbonyl compounds.

The detection of carbonyl compounds using sol-gel silica-based optical sensors is poorly represented in the literature. Only two papers related to the colorimetric determination of the formaldehyde air pollutant were found. Bunkoed *et al.* developed a colorimetric sensor for the selective determination of the formaldehyde air pollutant. The selective recognition was based

on the reaction of formaldehyde with either acetylacetone or methyl acetoacetate contained in a sol-gel film in the presence of ammonium acetate and acetic acid²⁵⁹. Hidayat *et al.* reported on a sol-gel material modified with alcohol oxidase and chlorophenol²⁶⁰.

5. FLUORESCENCE FOR SIGNAL TRANSDUCTION

As described in the previous chapter, optical sensors could either rely on a chromophore or a fluorophore, responsible either for color changes or modifications of fluorescence intensity/emission wavelength after the detection of the analytes. A chromophore is a molecule that absorbs certain wavelengths of the spectrum of the visible light. The color perceived is made of the wavelengths that are not absorbed but reflected by the molecule. The definition of a fluorophore is given below. This project relies on the development of fluorescent sensors.

5.1. FLUORESCENCE PRINCIPLE

Initially in the ground state, a fluorescent molecule undergoes excitation by the absorption of a photon. The molecule stays in the excited state for a very short period ($\sim 10^{-10}$ to 10^{-7} s) before returning to the ground state either via a radiative process (with emission of a photon) or a non-radiative process (without emission of a photon). A fluorophore can thus be defined as a compound that can emit a photon upon excitation.

Fluorescence thus implies modifications of the electronic states of the fluorophore. As schematized in **Figure 23**, a molecule in the excited state can either be in a singlet state or in a triplet state. In a singlet state, the excited electron is paired with the ground state electron. In the triplet state, the excited electron is no longer paired with the ground state electron and both electrons have the same spin. The direct transition from the ground state to an excited triplet state is very unlikely. A transition from the excited singlet state to the triplet state is however possible and is described in the following paragraph.

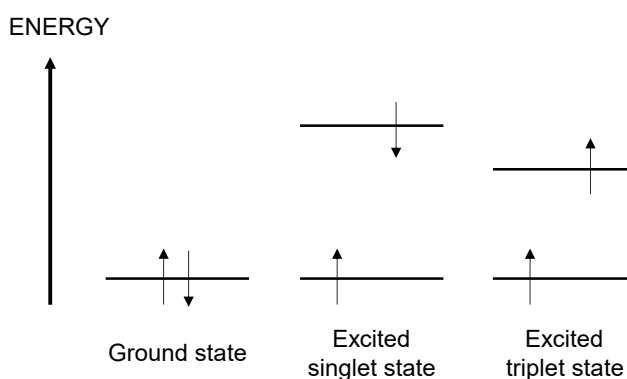


Figure 23: Description of the singlet and the triplet state [adapted from Valeur²⁶¹].

The Perrin-Jablonski diagram (see **Figure 24**) summarizes the electronic states of a molecule and the transitions between these states. The ground state is usually a singlet state (exception made for oxygen), noted S_0 . After absorption of a photon (—), the molecule is in an excited state from the same spin multiplicity as the ground state, noted S_n with $n > 0$ (example with S_1 and S_2 in the figure). Once excited and through a non-radiative mechanism called vibrational relaxation (), the molecule relaxes rapidly to the lowest vibrational energy state of each electronic state, which is called the fundamental vibrational state. If $n > 1$, excited molecules usually relax rapidly to the electronic state S_1 via a non-radiative process called internal conversion (). At this point, the excited molecule can take 3 relaxation paths to go from excited state S_1 to the ground state S_0 . The first one is the fluorescence (—) with the emission of a photon. The second one is the relaxation through quenching mechanisms () that are detailed later. The third one implies first a non-radiative process called intercrossing system () allowing the molecule to go from the singlet state S_1 to the triplet state T_1 , followed either by a radiative relaxation process called phosphorescence (—) or non-radiative processes (). Finally, there are only two radiative relaxation paths: fluorescence or phosphorescence. All other relaxation processes are non-radiative.

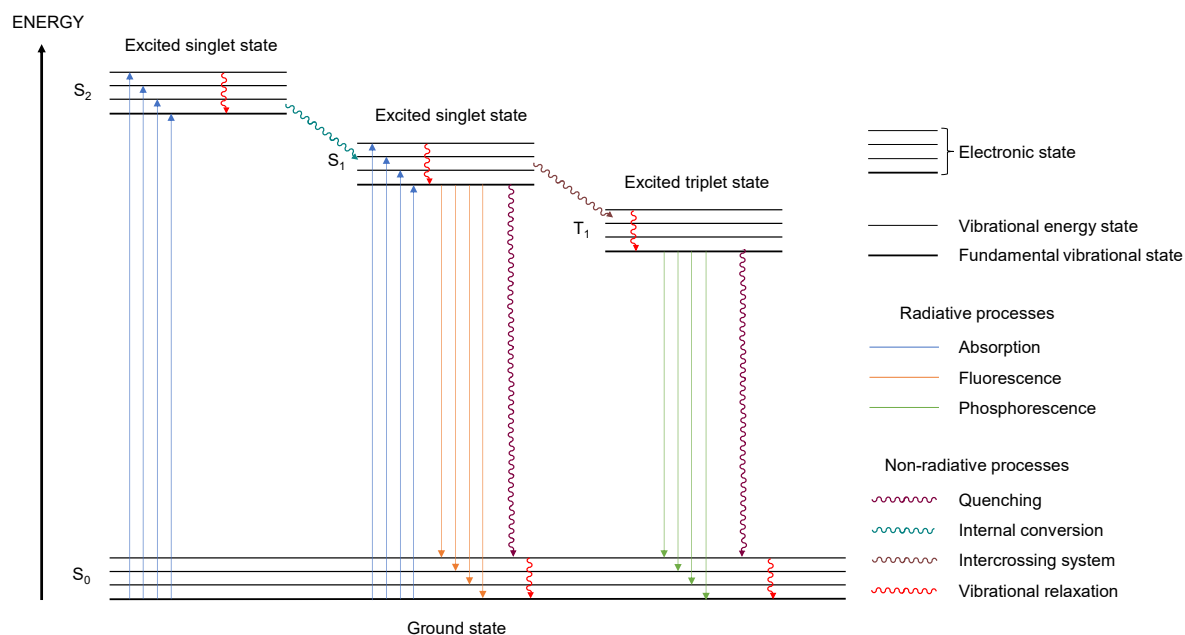
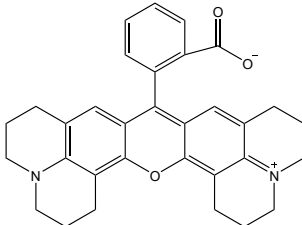
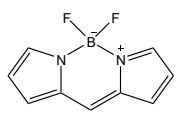
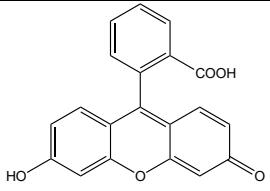
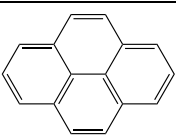
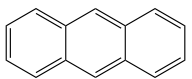
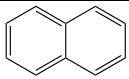
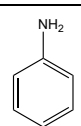


Figure 24: Perrin-Jablonski diagram representing the electronic states of a molecule and the transitions between them.

Common examples of fluorophores are rhodamine 101, BODIPY, fluorescein, pyrene, anthracene, or aniline to name a few. Some of their characteristics are given in **Table 8**. Their structural common point is the presence of aromatic rings and/or π -bonds. A fluorophore is often characterized by its excitation/emission wavelength, its Stokes shift, its fluorescence lifetime and its quantum yield. The maximum excitation wavelength corresponds to the maximum of adsorption of the molecule. Emission usually occurs at a longer wavelength and the difference between the excitation and the emission wavelength is called the “Stokes shift”. The lifetime of the fluorophore is the duration of the excited state before returning to the ground state. This parameter differentiate fluorescence from phosphorescence, the latter being characterized by longer period of time between excitation and emission (from a few microseconds to several hours²⁶²). The quantum yield is expressed as the number of emitted photons per absorbed photons and thus corresponds to the efficiency of transferring the energy from the exciting light to the emission of a photon. These parameters strongly depend on the structure of the fluorophore but also on its chemical environment. The fluorescence intensity of a sample depends on the concentration of the fluorescent molecule.

Table 8: Some examples of common fluorophores and their characteristics in ethanol.

NAME	FORMULA	EXCITATION	EMISSION	QUANTUM YIELD
Rhodamine 101 ²⁶³		560 nm	589 nm	1
BODIPY ²⁶⁴		507 nm	520 nm	0.81
Fluorescein ²⁶⁵		494 nm	521 nm	0.79
Pyrene ²⁶⁶		340 nm	452 nm	0.65
Anthracene ²⁶⁷		360 nm	377, 399, 423, 448 nm	0.27
Naphtalene ²⁶⁸		270 nm	400 nm	0.23
Aniline ²⁶⁹		290 nm	337 nm	0.08

Among optical sensors, fluorometric chemical sensors usually provide faster response and higher sensitivity compared to their colorimetric counterparts and do not require any reference^{270–273}. Fluorescence spectroscopy also allows non-destructive analysis¹⁰⁰ of complex samples without the need for derivatization or extraction²⁷⁴.

Some of them exhibit fluorescence-intensity enhancement, also called “turn-on” sensors whereas other are referred to as “turn-off” emission sensors when the fluorescence is

quenched by the interaction with the targeted analyte. In this work, only “turn-off” sensors were developed.

5.2. FLUORESCENCE QUENCHING MECHANISMS

Quenching refers to a process implying the decrease of the fluorescence intensity of a fluorescent compound. Quenching can result from the modification of the molecule at the ground state (static quenching) or from non-radiation relaxation processes from the excited state (dynamic quenching). In the second case, many mechanisms can be considered. These non-radiative processes compete with fluorescence emission and are eventually responsible for the fluorescence quenching.

5.2.1. Static quenching mechanisms

The static quenching process occurs in the ground state, before the excitation occurs. In this case, the fluorescent compound forms a non-fluorescent complex, usually by chemical reaction.

5.2.2. Dynamic quenching mechanisms

The dynamic quenching process occurs in the excited state. Phenomena responsible for the relaxation of the fluorescent probe to the ground state are performed through non-radiative processes.

Collisional quenching occurs when the excited fluorophore encounters a molecule called quencher, which induces non-radiative relaxation. Common quenchers are molecular oxygen, iodide ions, halogens, amines and more generally electron deficient molecules. The decrease in fluorescence intensity is described by the Stern-Volmer equation²⁷⁵:

$$\frac{I_0}{I} = 1 + K[Q] \quad (1)$$

with I_0 the initial fluorescence of the fluorophore and I its fluorescence in presence of the quencher at a concentration $[Q]$ and K the Stern-Volmer constant.

If only collisional quenching is at play, the Stern-Volmer equation follows a linear evolution. If its plot follows a non-linear behavior, it is acknowledged that both static and dynamic quenching processes are involved^{276–278}. When collisional quenching occurs, the quenching mechanism involved depends on the system fluorophore/quencher. It can rely on:

- Internal conversion, which is a non-radiative transition from a higher to a lower electronic state of the same spin multiplicity with emission of heat
- Intersystem crossing, which is a non-radiative transition from an electronic state to another of the same energy but from different spin multiplicity
- Electron, proton or charge transfer
- Conformational change
- Excimer or exciplex formation, which implies the formation of homonuclear (called excimer) or heteronuclear (called exciplex) species in the excited state that dissociate in the ground state
- Energy transfer, which Förster resonance energy transfer (FRET) is an example. This phenomenon implies 2 species: a donor and an acceptor with at least a partial recovery of the emission spectrum of the donor with the absorbance spectrum of the acceptor. The donor in its excited state will transfer energy to the acceptor in the ground state. This process is highly dependent from the distance between the 2 species.

5.2.3. Other quenching mechanisms

The fluorescence of the fluorophore can also be decreased by the absorption of the photons from the exciting source by other components in the solution. This phenomenon can occur when the excitation wavelength matches the maximum of absorption of some compounds of the system. In this case, the previous absorption of the exciting light by these compounds will not be used for the excitation of the fluorophore, resulting in the attenuation of its fluorescence.

5.2.4. Parameters affecting the quenching

Several parameters can influence the quenching process such as the solvent nature, the pH, the temperature or the viscosity of the medium. A change in the medium can impact the fluorescence emission spectrum of the fluorophore. As an example, a modification of the solvent from a non-polar to a polar one can lead to the formation of hydrogen bonds stabilizing the ground state, resulting in a decrease in fluorescence intensity. The temperature can influence the rate of static/dynamic quenching. An increase in the temperature will indeed favor dynamic quenching processes by increasing the mobility of the molecules. Non-radiative

relaxation and collisional quenching for example will therefore be more efficient at high temperatures. On the contrary to that, an increase in the temperature could impact the chemical stability of the non-fluorescent complex. An increase in the viscosity could reduce collisional quenching.

6. AMINE-CARBONYL REACTIVITY

This project aimed at developing materials for the chemical sensing of carbonyl compounds released by lipid oxidation. They were designed to contain amino groups (especially primary amines) providing the reactivity with the targeted compounds. Primary amines can react with aldehydes and ketones to form imines, also called “Schiff base” (see **Figure 25**).

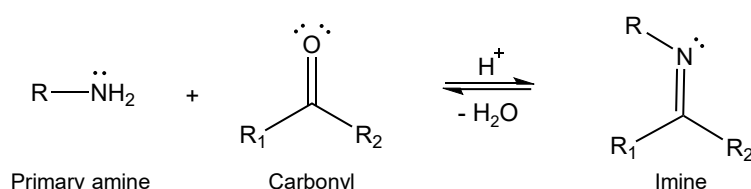


Figure 25: Formation of imines by reaction of primary amines with carbonyls. R, R₁, R₂ are alkyl chains.

As displayed in **Figure 26**, the reaction is governed by an addition/elimination mechanism²⁷⁹ under acidic catalysis. Some suggested that primary amines were weak bases, so the first step of the reaction was the protonation of the carbonyl group²⁸⁰. The second step is the nucleophilic attack of the nitrogen of the primary amine through its lone pair of electrons onto the electrophilic carbon atom from the carbonyl group. A tetrahedral intermediate is formed and leads to the formation of a carbinolamine. In the third step, an iminium ion is formed after the loss of a water molecule, which eventually lead to the imine once deprotonated.

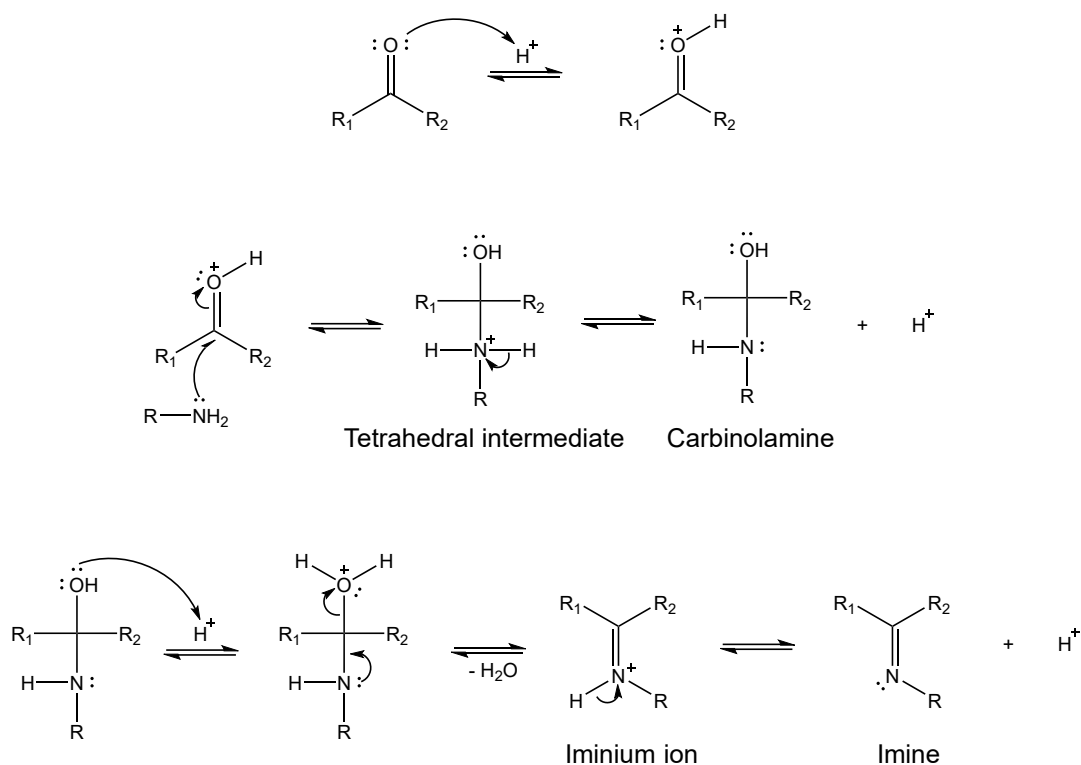


Figure 26: Mechanism of formation of imines by reaction of primary amines with carbonyls. R, R_1 , R_2 are alkyl chains.

Optimal conditions are mildly acidic conditions (around pH 5). Too acidic conditions would slow down the reaction as it would result in the protonation of the amine to form an ammonium ion. By losing its nucleophilic character, the ammonium ion cannot react with carbonyl compounds anymore. Imine can be hydrolyzed back to the corresponding primary amine under acidic conditions. Occasionally the term “aldimine” is used to refer to an imine made from an aldehyde and “ketimine” for an imine made from a ketone.

7. RESEARCH FOCUS OF THE THESIS AND EXPERIMENTAL APPROACH

As depicted in **Figure 27**, this thesis was conducted in several steps. The goal of this work was to develop intelligent packaging to monitor food spoilage in real-time in order to help reducing food waste while ensuring food safety. At the beginning of the project, a reflection was carried out in order to identify the categories of food product that would benefit from such an innovation. It was found that the food items that would benefit the most are packaged food items of high value, which are wasted a lot and have a short or a very long shelf-life. Fish and seafoods, meat, dairy products and edible oils meet these criteria and were therefore targeted. These products contain all significant amounts of lipids, which led to the decision to focus on

lipid oxidation among all possible food spoilage mechanisms. The impact of oxidation on these food matrices was investigated. The idea was to develop a non-destructive method to monitor lipid oxidation. During this degradation process, volatile compounds called “secondary oxidation products” are generated. A bibliographic review of these compounds highlighted that they are mostly composed of aldehydes, ketones and alcohols (see **Appendix A**). To be able to further apply the designed sensor on different food products, developing a “non-targeted” strategy thus seemed valuable. This means that the sensor would not be designed to detect a single analyte but rather a family of molecules that would be commonly found in different oxidized food matrices. In particular, it was decided to focus on the carbonyls’ family of molecules for several reasons. First, carbonyls were identified to be major contributors to the off-flavors generated during spoilage and the smell has been found to be the first criterion used by consumer to reject food as explained in 2.3.4. These compounds thus directly contribute to food waste. Second, carbonyls fulfill another important criterion: they are only found at low concentrations, or not at all in fresh products, and are mostly generated by the lipid oxidation process over time. New sensor for carbonyl compounds were thus designed. Providing the consumer with such an innovation could have proved counterproductive, leading to an increase in food waste, as argued in 3.4. It was thus decided to focus on the development of a tool dedicated to the retailers and distributors. This is why a fluorescent sensor was favored. Strategies were then investigated to synthesize new sensing materials for the fluorescent monitoring of carbonyl compounds. Two main categories of materials were considered: acrylate-based materials and silicate-based materials. In both cases, the criteria for the development were to be able to produce the materials at low cost, with commercially available, affordable reactants, using a simple to implement and rapid synthesis procedure. In order to make this project part of a green chemistry approach, the toxicity and the number of reactants were assessed. As a limited number of reactants was also required to make the procedure as simple as possible, it was decided at first to work with a single monomer bearing all the characteristic required to the development of the sensor. Fluorescent monomers able to interact with carbonyl compounds were thus sought. It was decided to develop a chemical sensor based on the reactivity of the amino groups for carbonyl compounds. Monomers bearing both amino groups and fluorescent core were purchased, and materials were synthesized either by radical polymerization or the sol-gel process. The performances of the sensors were then assessed. First, the interaction between the sensors and the carbonyls was

assessed by evaluating the adsorption capacity of the materials by GC-MS. Second, the modification of the fluorescence property of the sensor upon interaction with carbonyls was checked. In order to apply these new sensors on food products either with or without contact, the toxicity of all the newly designed sensors was assessed. One particular material, synthesized by the sol-gel process and based on aniline, was found promising for carbonyl sensing based on fluorescence and more deeply investigated. After systematic characterization, its performances were deeply studied after direct contact between the sensor and the carbonyls in liquid modelling media and in real food matrices: rapeseed oil and fish oil. Fish oil was chosen because its high content in EPA and DHA fatty acids makes it very sensitive to oxidation. Complete oxidation of the fish oil was monitored with the use of the sensor. To apply the sensor to the non-destructive analysis of the environment surrounding the food, the performance of the sol-gel material was also assessed by exposing it to a gas phase containing carbonyl vapors. The material was in this case integrated into a usual packaging polymer made of polylactic acid. Finally, to improve some characteristics of the sensor, new synthesis strategies were investigated for the design of sol-gel materials. The first new strategy relied on the use of 2 different monomers, one bearing an amino group, the other carrying the fluorescent core. The second was inspired of the “doped silica” strategy and relied on the solubilization of fluorescent probe inside micelles and the co-polymerization of a usual cross-linker with a silica-based monomer bearing an amino group.

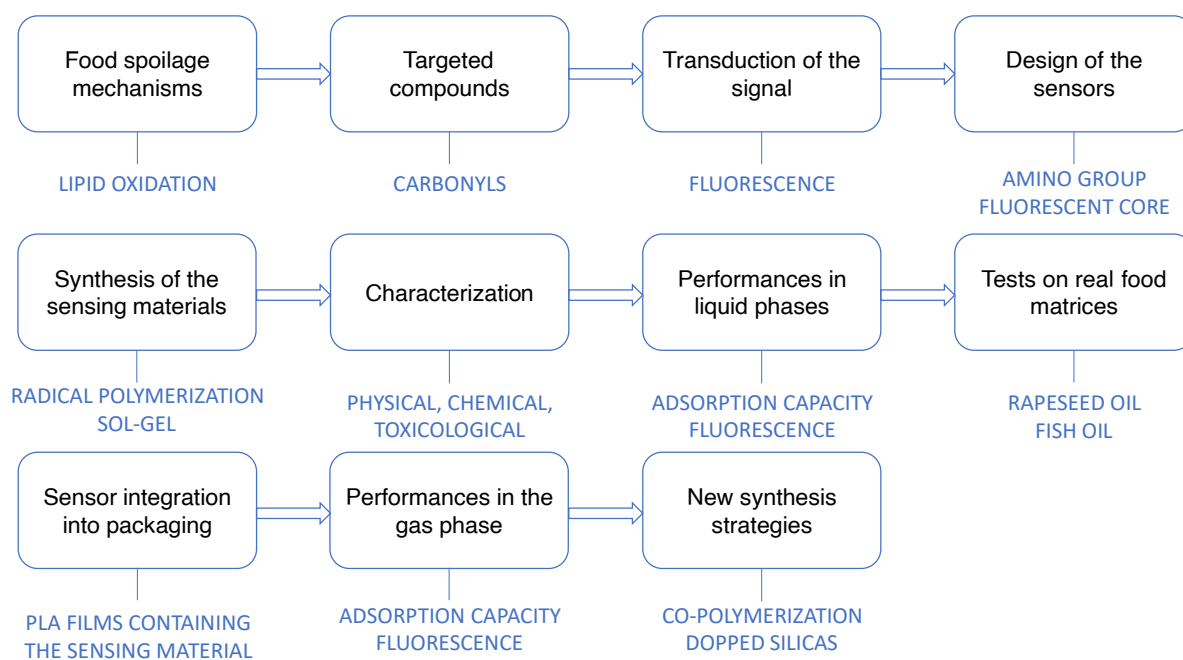


Figure 27: Steps of the thesis project.

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MATERIALS AND METHODS

MATERIALS AND METHODS

1. CHEMICALS

The chemical used for this thesis project are summarized in **Table 1**.

Table 1: Chemical used during the thesis.

Name	Abbreviation	CAS	Supplier
4-vinylaniline 97 %	4-VA	1520-21-4	Sigma-Aldrich
3-vinylaniline 97 %	3-VA	15411-43-5	Sigma-Aldrich
2-vinylaniline 95 %	2-VA	3867-18-3	abcr
4-trimethoxysilylaniline 97 %	4-TMSA	33976-43-1	abcr
1-naphtyltrimethoxysilane 95 %	/	18052-76-1	abcr
3-aminopropyltrimethoxysilane 97 %	/	13822-56-5	Sigma-Aldrich
hexadecyltrimethylazanium bromide 98 %	CTAB	57-09-0	Sigma-Aldrich
anthracene 97 %	/	120-12-7	Sigma-Aldrich
fluorescein (for fluorescence, free acid)	/	2321-07-5	Sigma-Aldrich
ethylene glycol dimethacrylate 98 %	EGDMA	97-90-5	Sigma-Aldrich
tetraethylorthosilicate 99 %	TEOS	78-10-4	Sigma-Aldrich
2,2-dimethoxy-1,2-diphenylethanone 99 %	DMPAP	24650-42-8	Sigma-Aldrich
2,2'-azobis(2-methylpropionitrile) 98 %	AIBN	78-67-1	Sigma-Aldrich
ammonium hydroxide 30 %	NH ₄ OH	1336-21-6	Sigma-Aldrich
hydrochloric acid	HCl	7647-01-0	Sigma-Aldrich
glacial acetic acid 99 %	CH ₃ CO ₂ H	64-19-7	Sigma-Aldrich
sodium hydroxide 0.1 M NaOH in water	NaOH	1310-73-2	Sigma-Aldrich
formic acid 98 %	/	64-18-6	Sigma-Aldrich
acetonitrile 99.9 %	CH ₃ CN	75-05-8	Honeywell
propanal 99 %	/	123-38-6	ACROS Organics™
hexanal 98 %	/	66-25-1	Alfa Aesar
nonanal 97 %	/	124-19-6	Thermo Scientific Chemicals
hexan-2-one 98 %	/	591-78-6	Acros Organics
1-penten-3-one 97 %	/	1629-58-9	Sigma-Aldrich
1-octen-3-one 96 %	/	4312-99-6	Sigma-Aldrich
<i>n</i> -pentane 99 %	/	109-66-0	Sigma-Aldrich
<i>n</i> -hexane 97 %	/	110-54-3	Sigma-Aldrich

<i>n</i> -octane 99 %	/	111-65-9	Merck
octan-1-ol 98 %	/	111-87-5	Sigma-Aldrich
polylactic acid	PLA	26100-51-6	NatureWorks®
ethanol 96 %	EtOH	64-17-5	Sigma-Aldrich
ethanol for UV-spectroscopy Uvasol®	EtOH	64-17-5	Sigma-Aldrich
ethyl acetate 99.8 %	EtAc	141-78-6	Sigma-Aldrich
cyclohexane 99.5 %	/	110-82-7	Chem-lab
propan-2-ol 95 %	/	67-63-0	Carlo Erba Reagents
1-butanol 99.9 %	/	71-36-3	Sigma-Aldrich
methanol (ACS reagent, 99.8 %)	MeOH	67-56-1	Sigma-Aldrich
sodium thiosulfate 98 %	Na ₂ S ₂ O ₃	7772-98-7	Sigma-Aldrich
potassium iodide 99 %	KI	7681-11-0	Sigma-Aldrich
2-sulfanylidene-1,3-diazinane-4,6-dione 98 %	2-thiobarbituric acid	98 504-17-6	Sigma-Aldrich
propyl gallate powder	/	121-79-9	Sigma-Aldrich
starch from potato powder	/	9005-25-8	Sigma-Aldrich
fish oil from menhaden	/	8002-50-04	Sigma-Aldrich

Deionized water was used, obtained from a MilliQ RG, Millipore apparatus.

2. MATERIAL SYNTHESSES

2.1. SYNTHESIS STRATEGIES

To design sensing materials to monitor carbonyl compounds using fluorescence, 3 strategies were investigated. All the materials possessed both an amino group providing the reactivity required with the carbonyl compounds and a fluorescent core for the signal transduction. In the first strategy, these 2 functional groups were born by one single monomer. Materials were synthesized either by radical polymerization or sol-gel process, using different functional monomers (4-vinylaniline, 3-vinylaniline, 2-vinylaniline for the radical polymerization and 4-trimethoxysilylaniline for the sol-gel synthesis). In the second strategy, sol-gel materials were synthesized based on 2 monomers, one with an amino group (3-aminopropyltrimethoxysilane) and the other with a fluorescent group (1-naphtyltrimethoxysilane in this case). In the third strategy, a fluorophore (either anthracene or fluorescein) was solubilized inside micelles prior to the polymerization of an aminosilane

derivative according to the sol-gel process. The reactants used for the 3 strategies are given in Figure 1.

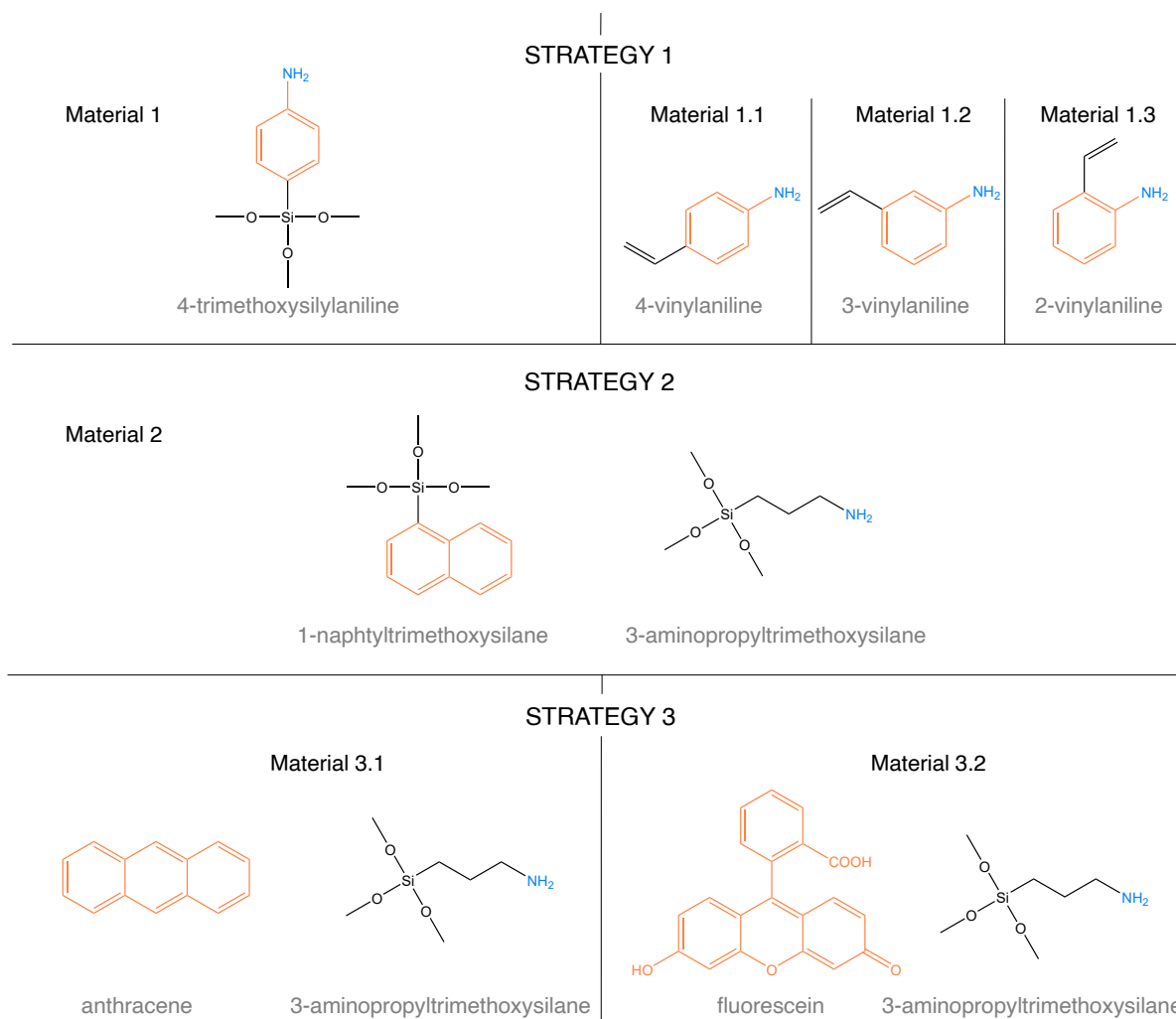


Figure 1: Main reactants used to synthesize the different materials studied in this thesis project according to the three strategies presented in 2.1. In orange are given the fluorescent cores and in blue the functional groups responsible for the interaction with carbonyls.

Control materials were systematically synthesized following the same procedure but without the functional monomers/fluorophores presented in **Figure 1**, to prove that the adsorption of carbonyl compounds and material fluorescence were strictly due to the presence of the required functional groups within the material structure.

2.2. EXPERIMENTAL PROCEDURES

2.2.1. Strategy 1

Synthesis of materials 1a, 1b, 1c: acrylate-based materials

The functional monomer (4-vinylaniline, 3-vinylaniline or 2-vinylaniline) was firstly dissolved in acetonitrile. The cross-linker ethylene glycol dimethacrylate (EGDMA) and the initiator (either DMPAP or AIBN) were then added. The following molar ratio was used: 4/20/1/50 for the functional monomer/cross-linker/initiator/solvent. Oxygen was removed from the reaction mixture by nitrogen bubbling for 10 minutes. For photoinitiated polymerization, the reactional medium was placed at 10 cm from an UV-lamp (366 nm) for 24 h. For thermally initiated polymerization, the reaction mixture was stirred at 250 rpm and heated at 60 °C for 24 h. The resulting powder was washed 3 times with acetonitrile by Büchner filtration using a 0.2 µm pore membrane. The material was dried 12 h at 60 °C, manually crushed and stored at 4 °C in the dark before use. A material with a higher ratio of functional monomer (20/20/1/50) was also synthesized following the same procedure. The reaction route is depicted in **Figure 2**.

The polymerization yield was calculated using the theoretical mass of material supposedly obtained.

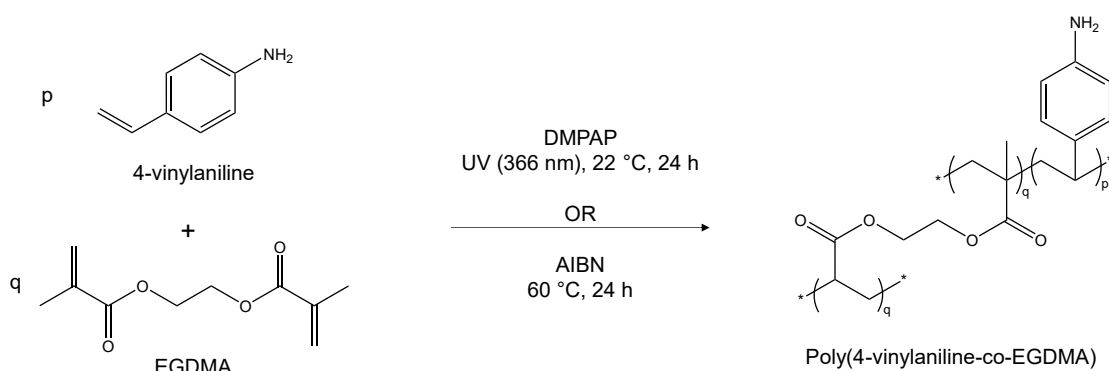


Figure 2: Reaction route followed for the synthesis of the acrylate-based material 1a, 1b, 1c according to the strategy 1. Example given using the 4-vinylaniline monomer.

Synthesis of material 1: silicate-based material

The material was synthesized using a sol-gel process. 4-trimethoxysilylaniline (4-TMSA) was firstly dissolved in an ethanol/water mixture (1/10 molar ratio) at 60 °C for 10 min.

Tetraethyl orthosilicate (TEOS) and ammonium hydroxide (30 % NH_4OH) were then added, using the following molar ratio: 1/5/31.25: 4-TMSA/TEOS/ NH_4OH . The mixture was stirred at 500 rpm for 24 h at 22 °C. Then, the solvent was evaporated, and the resulting powder was washed 3 times with an ethanol/water mixture (1/10 molar ratio) by Büchner filtration using a 0.2 μm pore membrane. The material was dried 12 h at 60 °C, manually crushed and stored at 4 °C in the dark before use. A material with a higher ratio of functional monomer (20/20/1/50) was also synthesized following the same procedure. The reaction route is depicted in **Figure 3**.

The polymerization yield was calculated from the assumed theoretical mass of material obtained, taking into account the loss of alcohol and water molecules during the hydrolysis and condensation processes.

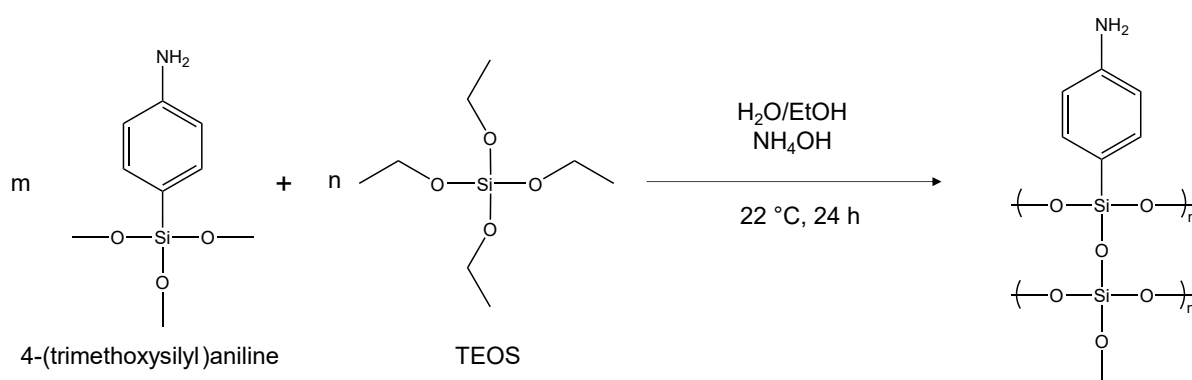


Figure 3: Reaction route followed for the synthesis of the silicate-based material, 1 according to the strategy 1.

2.2.2. Strategy 2

Synthesis of material 2

1-naphtyltrimethoxysilane (M1) was firstly dissolved in an ethanol in water mixture (1/10 molar ratio) at 60 °C for 15 min. 3-aminopropyltrimethoxysilane (M2), tetraethyl orthosilicate (TEOS) and ammonium hydroxide (30 % NH_4OH) were then added. The following molar ratio was used: 1/1/5/31.25/250/25 for M1/M2/TEOS/ NH_4OH / H_2O /EtOH. The mixture was stirred at 500 rpm for 24 h at 22 °C. Then, the solvent was evaporated under the hood, and the resulting powder was washed 3 times with an ethanol in water mixture (1/10 molar ratio) by Büchner filtration using a 0.2 μm pore membrane. The material was dried 12 h at 60 °C, manually crushed and stored at 4 °C in the dark before use. The reaction route is depicted in **Figure 4**.

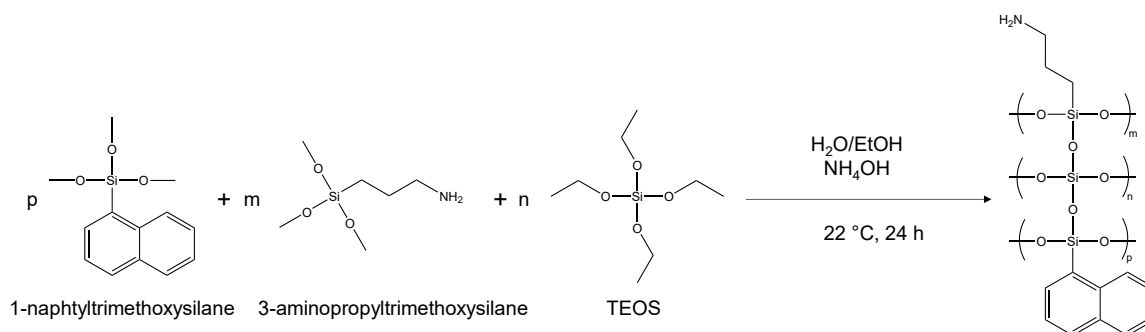


Figure 4: Reaction route followed for the synthesis of material 2.

2.2.3. Strategy 3

Synthesis of material 3.1: anthracene-based material

Hexadecyltrimethylazanium bromide (CTAB) was firstly dissolved in a solution of NaOH in water (0.1 M) at $60\text{ }^\circ\text{C}$ under stirring at 250 rpm for 10 min. Methanol, anthracene (F1), 3-aminopropyltrimethoxysilane (M) and tetraethyl orthosilicate (TEOS) were added. The following molar ratio was used: 0.18/0.6/0.6/1/13/140 for CTAB/F1/M/TEOS/MeOH/ H_2O (NaOH 0.1 M). The mixture was stirred for 1 h at 500 rpm at $60\text{ }^\circ\text{C}$ and further 24 h at $22\text{ }^\circ\text{C}$. Then, the solvent was evaporated under the hood and the powder was washed 3 times with water by Büchner filtration using a $0.2\text{ }\mu\text{m}$ pore membrane. The material was dried 12 h at $60\text{ }^\circ\text{C}$, manually crushed and stored at $4\text{ }^\circ\text{C}$ in the dark before use. The presumed structure of the material is depicted in **Figure 5**.

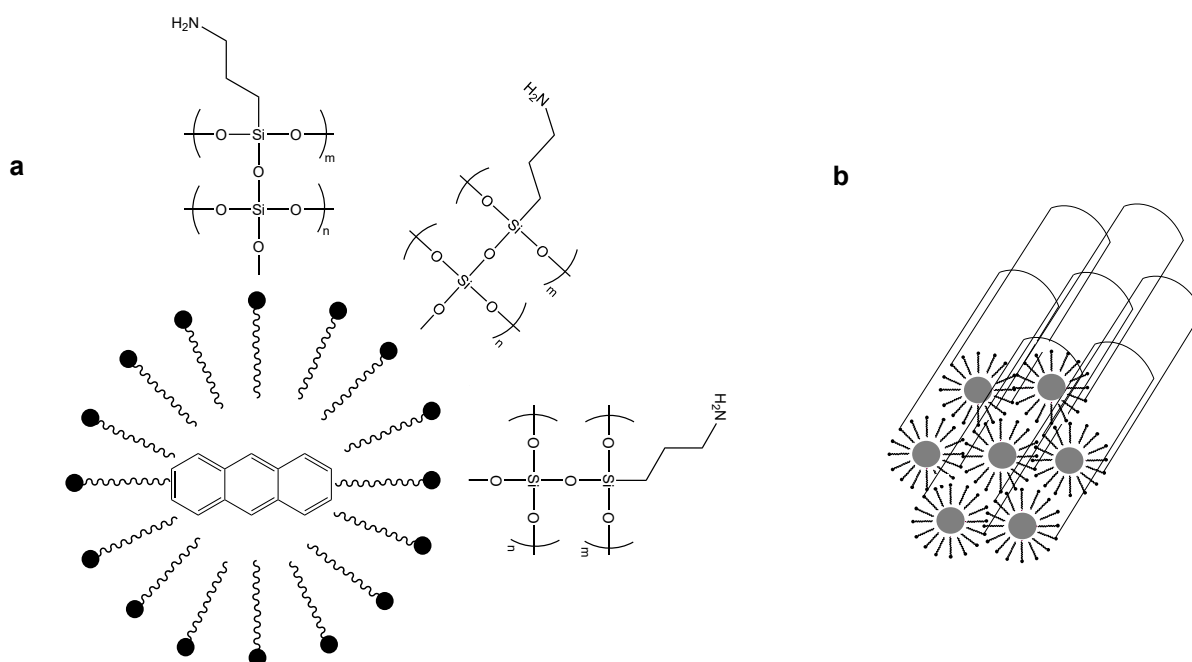


Figure 5: **a.** Schematic 2D representation of the material 3.1. **b.** In 3D, the CTAB micelles are in a hexagonal phase $\text{H}\alpha^1$.

Synthesis of material 3.2: fluorescein-based material

Material 3.2 was synthesized according to the same procedure as for material 3.1, replacing anthracene (F1) by fluorescein (F2).

2.3. FORM OF THE MATERIALS

For all the materials, the powder was used directly without any further preparation either for adsorption measurements in liquid and gas phases or for fluorescence experiments in the liquid phase. For fluorescence measurements in the gas phase with material 1, the material powder was incorporated inside polylactic acid (PLA) films. PLA was chosen because this polymer is non-fluorescent in the UV region, not water-soluble and has obtained the approbation of food contact material. 3 g of PLA were firstly dissolved in 50 mL of ethyl acetate for 3 h at 60 °C under agitation at 500 rpm. 10 mg of material powder were dispersed in 1.5 mL of PLA solution and placed in a glass petri dish. The solvent was then evaporated in oven at 35 °C for 2 h. Resulting films were cut into pieces of 1 cm x 2 cm. A reference film was prepared without material and its non-fluorescence was assessed.

3. MATERIAL CHARACTERIZATION

3.1. CHEMICAL CHARACTERIZATION

3.1.1. Solid-state nuclear magnetic resonance (NMR) spectroscopy

Solid-state NMR allows to determine the chemical structure of solids at the atomic level. This technique was used to determine the effect of carbonyl adsorption on the material 1. A 10 mg suspension of material in 2 mL of a solution of hexanal or hexan-2-one in ethanol (concentration: $4.99 \times 10^{-1} \text{ mol}\cdot\text{L}^{-1}$) was agitated at 50 rpm for 24 h at 22 °C. Samples were centrifugated at 10 000 rpm for 10 min. Supernatant was removed and the remaining powder was dried at 60 °C for 12 h. NMR spectra of the material after adsorption of hexanal or hexan-2-one were recorded and compared to the spectrum of the initial material before carbonyl adsorption. ^1H solid-state MAS NMR spectra were acquired at room temperature on an AVANCE 750 MHz wide bore spectrometer (BrukerTM) operating at a frequency of 750.12 MHz. Solid-state NMR spectra were deconvoluted using Dmfit software. The spectra were acquired at the “Institut de chimie de Strasbourg”, University of Strasbourg.

3.1.2. Thermal gravimetric analysis (TGA)

Thermal gravimetric analysis measures the mass changes of a sample when heated. It provides information on physical and chemical phenomena occurring during the sample heating. The decomposition of the organic part of the material 1 and 2 was analyzed using a Discovery TGA apparatus (TA Instruments). Around 15 mg of material was heated from room temperature to 800 °C with a heating rate of $20 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ under air flow ($25 \text{ mL}\cdot\text{min}^{-1}$). This measurement was performed at the “Ecole supérieure d'ingénieurs de recherche en matériaux et en infotronique” (ESIREM), University of Burgundy.

3.1.3. Elemental analysis (EA)

Elemental analysis consists of the measurement of the atomic emission of the elements present in a sample. Elemental analysis was performed on material 1 and 2 using a CHNS Thermo Scientific Electron Flash EA 1112 analyzer. The experiment was performed 4 times at the PACSMUB platform from the “Institut de chimie moléculaire” (ICMUB), University of Burgundy.

3.1.4. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis uses on infrared light to scan and identify the functional groups composing a sample based on their specific vibration modes. FTIR absorbance spectra of material 1 and its corresponding blank (obtained without functional monomer) were recorded at 25 °C using a Bruker Equinox 55 spectrometer equipped with a global source, DTGS detector and KBr splitter. Before analysis, around 1 mg of dried powder material was dispersed into KBr to obtain a 200 mg pellet. The absorbance spectra were recorded in transmission mode using a spectral range from 400 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1} and 100 scans per analysis, using a 200 mg KBr pellet as reference. These experiments were conducted at the “Laboratoire interdisciplinaire Carnot de Bourgogne” (ICB), University of Burgundy.

For the acrylate-based materials and material 3.1, FTIR absorbance spectra of the materials and their corresponding blank (obtained without functional monomer) were recorded at 25 °C using a Nicolet iS10 (Thermo Scientific™) spectrometer. Before analysis, around 1 mg of dried powder material was dispersed into KBr to obtain pellets of 200 mg. The transmission spectra were recorded in a spectral range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans per analysis, using a 200 mg KBr pellet as the reference.

3.2. PHYSICAL CHARACTERIZATION

3.2.1. Water vapor adsorption

The water vapor adsorption and desorption isotherms of the material were determined at 25 °C using a home-made McBain thermobalance equipped with a quartz spring under secondary vacuum. Around 10 mg of the material was first outgassed in situ at 80 °C under secondary vacuum (10^{-4} hPa) for 16 h before measurement. The variation of the sample mass was followed during water vapor adsorption or desorption by measuring the proportional elongation of the spring with a CCD camera. For each adsorption or desorption step, the mass variation was recorded up to equilibrium under controlled water vapor pressure, which varied between 10^{-4} to 30 hPa. These measurements were performed at ICB and more details about their home-made equipment can be found in previous work².

3.2.2. Scanning electron microscopy (SEM)

Scanning electron microscopy uses an electron beam to investigate the surface of a sample with nanoscale resolution. These measurements were performed on acrylate-based materials and material 1 at the MEB-CRO platform ("Institut de physique et de chimie des matériaux de Strasbourg" (IPCMS), University of Strasbourg) using a JEOL 6700F microscope equipped with a cold FEG electron source operating at 1 kV and both Everhart-Thornley and in-lense detectors. Prior to imaging, the powder was deposited on a carbon tape and the surface of the material was coated with carbon to prevent charging effects. Images were acquired at various magnifications between 100 and 20 000.

3.2.3. Laser diffraction

Laser diffraction is a particle size measurement technique for materials ranging from a few hundred nanometers to several millimeters. Laser diffraction equipment measures particle size distributions by measuring the angular variation in the intensity of light scattered as a laser beam passes through a sample of dispersed particles. The particle size distribution of acrylate-based materials and material 1 was determined using a Malvern MasterSizer 3000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK). A suspension of the material in ethanol was added to the circulating water until the obscuration reached 8 % to 10 %. Stirring speed was set to 1200 rpm. The refractive index of the particles (1.457) and their absorption index (0.01) were based on the silica model. Refractive index of the dispersing phase was set at 1.36. The Mie diffusion model was used to calculate the particle size. The temperature was maintained at 22 °C during all the experiment. Measurements were performed 3 times.

3.2.4. Nitrogen adsorption

The porosity of the material 1 was determined by nitrogen adsorption at -195.75 °C (77.4 K) with an ASAP 2020 Micromeritics apparatus. Prior to analysis, the sample was outgassed at 80 °C under secondary vacuum (10^{-4} hPa) for 16 h. The specific surface area of the material was calculated using the Brunauer-Emmett-Teller (BET) method for relative pressure between 0.06 and 0.25. The total pore volume was determined at a relative pressure of 0.99. The respective microporous and mesoporous volume and the pore size distribution were estimated by using the SAIEUS software³, which gave the cumulative pore volume and the pore

volume distribution as a function of the pore width by simulation using the “zeolithe H form 77 K NLDFT cylindrical model”. This experiment was performed at ICB.

3.3. TOXICITY EVALUATION

The toxicity of the material powder 1, 1.1, 2, 3.1 and 3.2 was firstly assessed. The potential migration of the material 1 incorporated inside PLA film was assessed afterwards. Firstly, the mutagenicity of the material powder was estimated by the Ames’ test performed on two bacterial strains: *Salmonella typhimurium TA98 and TA100* according to Maron and Ames⁴ and the OECD guideline no. 471 for the testing of chemicals⁵. More details about the experimental procedure can be found in previous work⁶. Each test was performed 3 times. Secondly, migration tests were performed on the PLA films incorporating the material (10 mg of material in films of 8.5 cm²) according to conditions MG2 from EU regulation no. 10/2011 on plastic materials and articles intended to come into contact with food⁷. 1.22 dm² of PLA films incorporating the material were placed in 200 mL ethanol for 10 days at 40 °C. Ames’ test was performed on the supernatant. A reference result was obtained following the same procedure using a PLA film without material. Each test was performed 3 times. These tests were realized by the DERRTECH PACKTOX platform (“Nutrition Toxicologie”, University of Burgundy).

3.4. MATERIAL AGING

Adsorption capacity, fluorescence properties and toxicity of the material powder 1 was assessed over time. Adsorption capacity and fluorescence emission spectrum of material 1 and material 1.1 were determined when the materials were freshly synthesized and after 3 months of storage at 4 °C in the dark as described in 4.2 and 5.2. Ames’ test was performed on material 1 either freshly synthesized or after 19 months of storage at 4 °C in the dark.

4. ADSORPTION CAPACITY OF CARBONYLS BY THE MATERIAL

Adsorption capacity is given as the mass of carbonyls adsorbed by 1 g of material (under air, at 22 °C and 30 % of relative humidity). It was determined by gas chromatography mass spectrometry.

4.1. PRINCIPLE OF GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Gas chromatography is a quantitative separative technique of volatile molecules thermally stable. Either liquid or gas samples can be studied. In the case of gaseous analytes, a pre-concentration step is recommended. This is usually achieved using the solid phase micro extraction (SPME) technique, which relies on the adsorption/absorption and desorption of the gaseous molecules onto a fiber. A chromatograph is composed of an injector allowing the vaporization of the sample and a column in which the analytes are carried (see **Figure 6**). Depending on the concentration of the sample, injection can either be performed in split or splitless mode. In split mode, the injection flow is divided, and partial amount of the sample is injected into the column to avoid the saturation of the detector. Depending on the affinity of the compounds for the stationary phase (polar or non-polar column) or for the mobile phase (an inert carrier gas, usually helium), the analyte will be retained to a greater or lesser extent by the column. The different compounds of a mixture are thus separated. The column is heated, and temperature gradients can be used to improve the separation between the analytes. The time between the injection and the detection of the compound is called "retention time". It is characteristic of a molecule and the chromatographic conditions used. This analytical method is quantitative since the area of the peak corresponding to the compound is proportional to the substance amount injected. The data are analyzed by means of a chromatogram, which represents the intensity of the detected signal as a function of the retention time. Different types of detectors can be coupled to the chromatograph such as flame ionization detector, thermal conductivity detector or mass spectrometer detector.

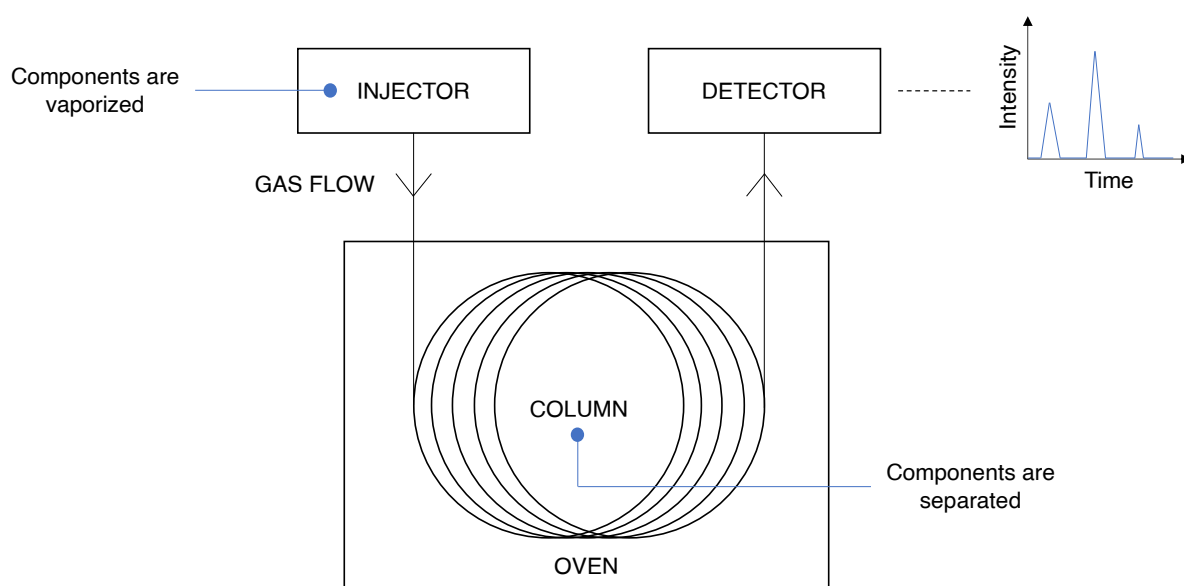


Figure 6: Principle of a gas chromatograph.

A mass spectrometer is composed of an ionization source, an analyzer and a detector (see **Figure 7**). Once injected, the sample is ionized, and the ionic fragments are ranked according to their mass over charge ratio (m/z). According to the molecular mass and the polarity of the molecules to analyze, different ionization sources can be considered such as electronic ionization (EI), chemical ionization (CI). Different analyzers exist such as quadrupole (Q) or time-of-flight (TOF).

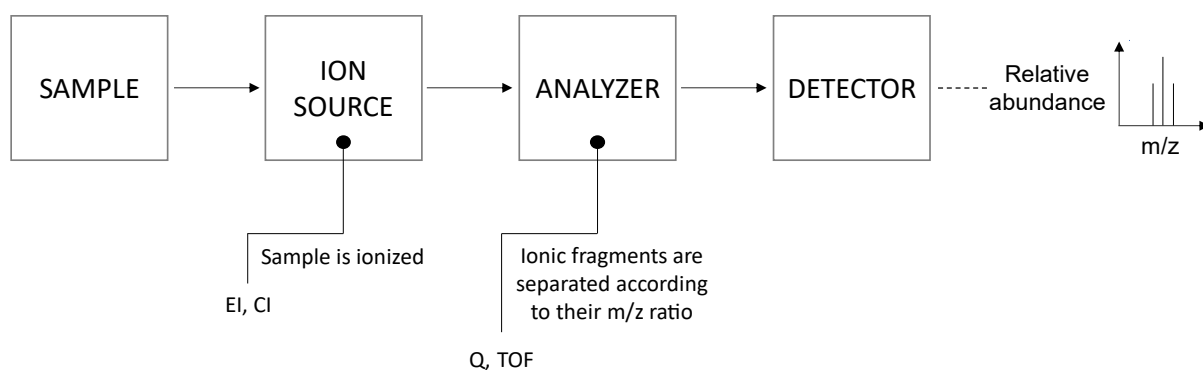


Figure 7: Principle of a mass spectrometer.

4.2. ADSORPTION CAPACITY OF CARBONYLS BY THE MATERIAL IN THE LIQUID PHASE

The adsorption capacity of carbonyls by the material in the liquid phase was calculated as follows:

$$\text{Adsorption capacity in the liquid phase} = \frac{(C_i - C_f) \times V_{liq.}}{m} \quad (1)$$

with C_i the initial concentration of hexanal or hexan-2-one in the liquid phase in contact with the material, C_f the concentration of hexanal or hexan-2-one remaining in the liquid phase at equilibrium, $V_{liq.}$ the volume of the hexanal or hexan-2-one solution and m the mass of the material. C_i and C_f were determined by gas chromatography coupled to mass spectroscopy (see Figure 8).

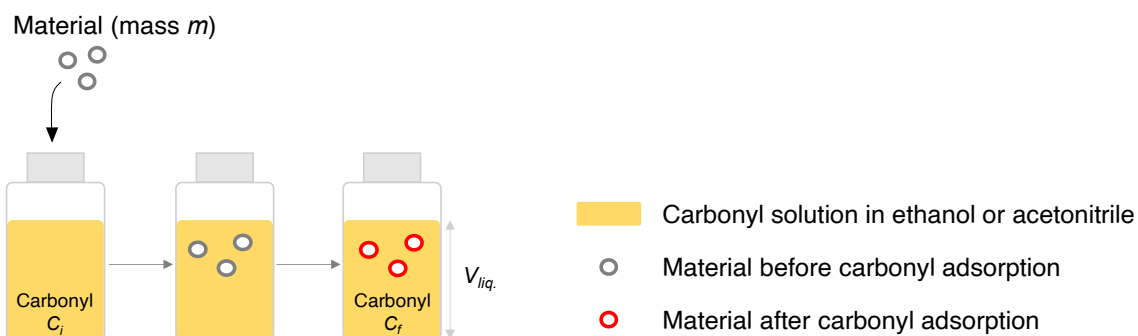


Figure 8: Schematic illustration of the experimental setup used for the determination of the adsorption capacity of carbonyls by the material in the liquid phase.

Adsorption capacity of material 1

A 10 mg suspension of the material in 2 mL of a solution of hexanal or hexan-2-one in ethanol was stirred at 50 rpm at 22 °C for 24 h to reach the equilibrium. Theoretical concentrations of hexanal or hexan-2-one in ethanol ranged from $2.95 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ (or $295 \text{ mg}\cdot\text{L}^{-1}$) to $25.4 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ (or $2551 \text{ mg}\cdot\text{L}^{-1}$). Samples were then centrifugated at 10 000 rpm for 10 min. 1 μL of the diluted liquid supernatant was injected thanks to an autosampler (Gerstel) on a 7890 A gas chromatograph (Agilent Technologies) equipped with a capillary column (30 m x 0.32 mm internal diameter, 0.5 μm thickness) coated with a DB-5MS stationary phase (Agilent J&W). Injections were performed in split mode (split ratio 50:1). The injection temperature was 250 °C. Helium was the carrier gas, at a flow rate of $1.4 \text{ mL}\cdot\text{min}^{-1}$. Mass spectrometer 5975C (Agilent) was used as detector (electronic impact 70 eV, ionization source at 230 °C, transmission line at 240 °C). The oven temperature was programmed from 50 °C to 65 °C at a first rate of $3 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$, and from 65 °C to 250 °C at a second rate of $30 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$. The oven temperature was finally maintained at 250 °C for 5 min. A solvent delay of 3 min was applied. Each adsorption experiment was carried out 4 times.

Adsorption capacity of material 2, 3.1 and 3.2

For materials 2, 3.1 and 3.2, only hexanal at one single concentration was tested (concentration: $2.49 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) following the exact same procedure as for material 1. Each adsorption experiment was carried out 3 times.

Adsorption capacity of material 1, 1.1, 1.2, 1.3

In the first article comparing the acrylate-based materials with material 1, the experimental procedure followed to determine their adsorption capacity slightly differ from the one described above. Only 5 mg of material were used, and an internal standard (octan-1-ol) was added. The chromatographic method was consequently adapted.

5 mg of material were suspended in 2 mL of an hexanal ($2.37 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) in acetonitrile solution. Octan-1-ol ($2.54 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) was added as an internal standard. Samples were agitated at 50 rpm for 24 h at 22 °C. They were centrifugated at 10 000 rpm for 10 minutes. The remaining concentration of hexanal in the liquid phase was determined by injecting 1 μL of the supernatant using an autosampler (Gerstel) on a 7890 A gas chromatograph (Agilent Technologies) equipped with a capillary column (30 m x 0.32 mm internal diameter, 0.5 μm thickness) coated with a DB-5MS stationary phase (Agilent J&W). Injections were performed in split mode (split ratio 50:1). The injection temperature was 250 °C. Helium was used as carrier gas at a flow rate of $1.4 \text{ mL}\cdot\text{min}^{-1}$. Mass spectrometer 5975C (Agilent) was used as the detector (electronic impact 70 eV, chemical ionization source at 230 °C, transmission line at 240 °C). The oven temperature was programmed from 50 °C to 65 °C at a rate of $3 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$, from 65 °C to 115 °C at $30 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$, then from 115 °C to 140 °C at $3 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ and from 140 °C to 250 °C at $30 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$. The oven temperature was finally maintained at 250 °C for 5 minutes. A solvent delay of 3 minutes was also applied. Each adsorption experiment was carried out 3 times.

4.3. ADSORPTION CAPACITY OF CARBONYLS BY THE MATERIAL IN THE GAS PHASE

This experiment was only conducted on material 1.

The adsorption capacity of carbonyls by the material in the gas phase was calculated as follows:

$$\text{Adsorption capacity in the gas phase} = \frac{(C_i - C_f) \times V_{\text{gas}}}{m} \quad (2)$$

with C_i the initial concentration of hexanal or hexan-2-one in the headspace of the vial before contact with the material, C_f the concentration of hexanal or hexan-2-one remaining in the headspace of the vial at equilibrium, V_{gas} the volume of the headspace of the vial and m the mass of the material. C_i and C_f were determined by solid phase micro extraction gas chromatography coupled to mass spectrometry (SPME-GC-MS) (see **Figure 9**).

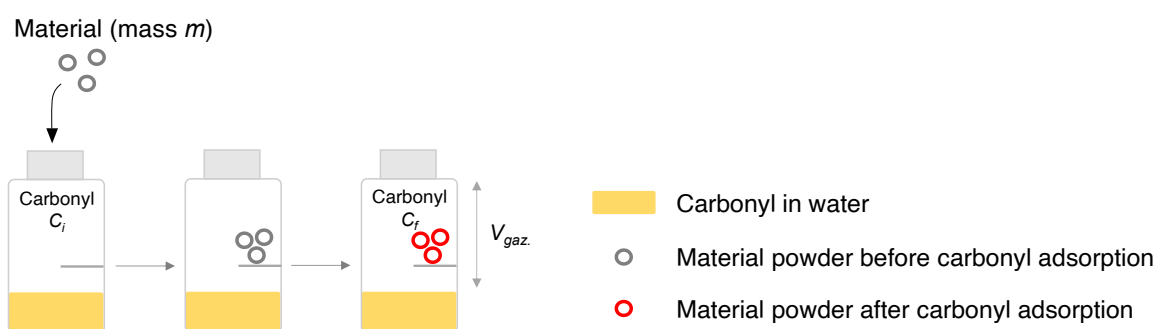


Figure 9: Schematic illustration of the experimental setup used for the determination of the adsorption capacity of carbonyls by the material in the gas phase.

SPME-GC-MS was used to analyze the headspace of 20 mL vials closed with a silicone/PTFE septum containing 50 μL of a solution of hexanal or hexan-2-one in water. Hexanal or hexan-2-one concentration in water ranged from $1.26 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ (12.6 $\text{mg}\cdot\text{L}^{-1}$) to $4.99 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ (50 $\text{mg}\cdot\text{L}^{-1}$). Vials were maintained at 60 $^{\circ}\text{C}$ under agitation at 250 rpm for 5 min before extraction, which lasted 2.5 min. Hexanal or hexan-2-one concentration in the headspace of the vials was determined in the presence or in the absence of 1 mg of material powder placed in the headspace of the vials (in caps at 1.5 cm from the bottom of the vial). Volatiles were adsorbed from the headspace onto a PDMS 100 μm SPME fiber (Supelco, USA) and were analyzed on a HP 6890 gas chromatograph (Hewlett Packard) equipped with a capillary column (30 m x 0.32 mm internal diameter, 0.5 μm thickness) coated with a DB-WAX stationary phase (J&W Scientific). Desorption was directly performed into the injector, using splitless mode at 250 $^{\circ}\text{C}$. Mass spectrometer HP 5973 (Agilent, USA) was used as detector (electronic impact 70 eV, transmission line at 240 $^{\circ}\text{C}$). Helium was the carrier gas at a flow rate of 1.4 $\text{mL}\cdot\text{min}^{-1}$. The oven temperature was programmed from 40 $^{\circ}\text{C}$ (maintained for 3 min) to

46 °C at a temperature rate of 1 °C·min⁻¹, and from 46 °C to 240 °C at a temperature rate of 100 °C·min⁻¹. This final temperature was maintained for 5 min. Each experiment was conducted 3 times.

5. FLUORESCENCE OF THE MATERIAL

5.1. PRINCIPLE OF FLUORESCENCE SPECTROSCOPY

Fluorescence spectroscopy is a quantitative investigative tool. The fluorescence properties of a compound can be measured using a spectrofluorometer. This device is conceived to measure various parameters of fluorescence, including the intensity and the distribution of emission wavelengths after excitation. Spectrofluorometers basically excite fluorophore molecules and measure its emitted fluorescence. As depicted in **Figure 10**, the spectrometer introduces ultra-violet or visible light using a photon source (from a laser, a xenon lamp, or LEDs). This light proceeds through a monochromator that selects a specific wavelength, which is then focused on the sample. Once excited, the fluorescent molecule emits photons that are conveyed to the detector. A computer software connected to the detector allows to analyze the data. To avoid any interference from the transmitted excitation light, the detector is typically set at a 90-degree angle to the light source. A fluorescence emission spectrum is obtained when the excitation wavelength is fixed, and the emission wavelength is scanned to get a plot of intensity vs. emission wavelength.

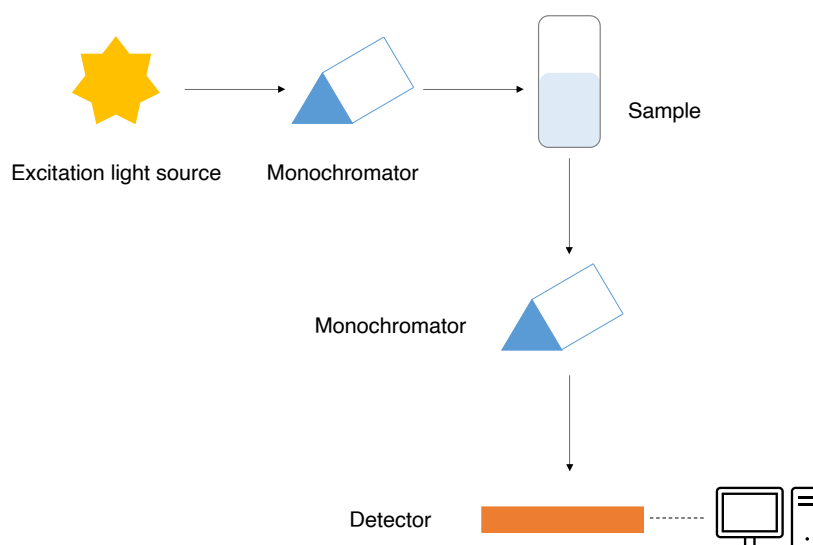


Figure 10: Principle of a spectrofluorometer device.

5.2. FLUORESCENCE OF THE MATERIAL AFTER EXPOSURE TO CARBONYLS IN THE LIQUID PHASE

5.2.1. General procedure

A spectrofluorometer Fluoromax4[®] (Horiba) was used with the software Fluorescence[®] and SUPRASIL[®] quartz cell (Hellma) of 10 mm light path. Excitation wavelength depended on the material studied (see **Table 2**). Preliminary experiments were conducted on each material to find the excitation wavelength providing the highest intensity of fluorescence emission. Fluorescence spectrum of the functional monomer/fluorophore were also recorded to identify the characteristic peaks of the fluorescent core of the material. Fluorescence emission spectrum was recorded using a 2 nm bandpass and a step interval of 1 nm.

Table 2: Excitation/emission wavelengths of the different materials studied.

	Excitation wavelength	Emission wavelength range
Material 1.1, 1.2, 1.3	280 ± 2 nm	300 nm – 500 nm
Material 1	290 ± 2 nm	300 nm – 500 nm
Material 2	270 ± 2 nm	300 nm – 600 nm
Material 3.1	360 ± 2 nm	370 nm – 500 nm
Material 3.2	494 ± 2 nm	500 nm – 700 nm

A 10 mg suspension of the material in a liquid phase was realized. Fluorescence emission spectrum of the material was recorded before and after exposure to carbonyls in the liquid phase. To avoid saturation of the detector, the quantity of materials 3.1 and 3.2 was 1000 times lower compared to the materials from strategy 1 and 2. Different parameters were investigated: contact time, carbonyl nature, carbonyl concentration and liquid phase (see **Figure 11**). The temperature was kept constant at 22 °C during the experiment. Each experiment was performed 3 times.

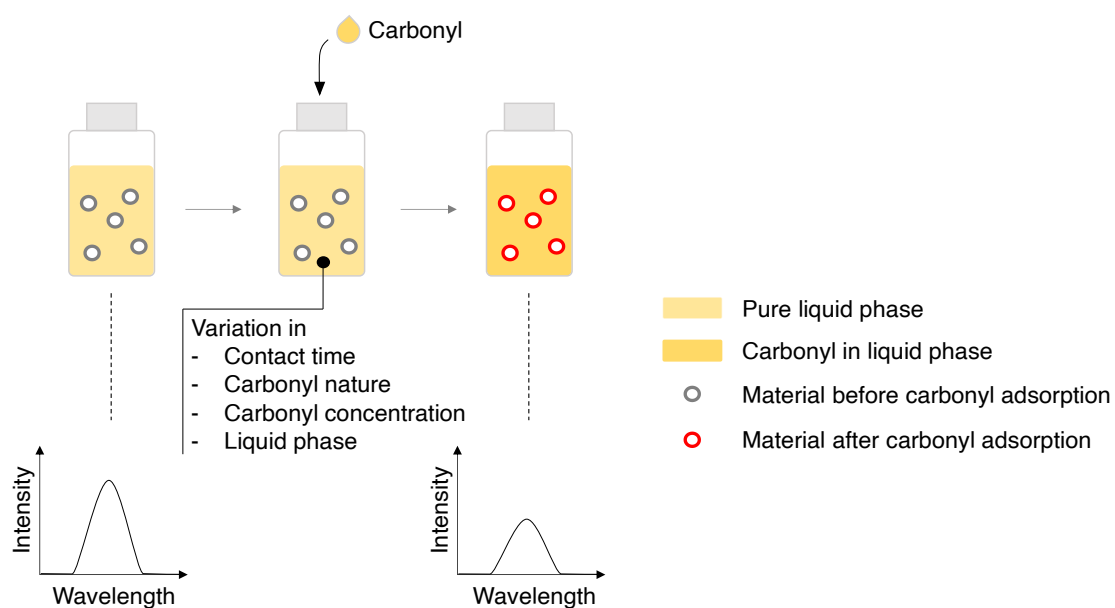


Figure 11: Experimental setup used for fluorescence measurements of the material in suspension in a liquid phase before and after interaction with carbonyls.

As presented in **Figure 12**, the percentage of fluorescence quenching, Q , was determined as follows:

$$Q = \frac{I_0 - I}{I_0} \times 100 \quad (3)$$

with I_0 the intensity of fluorescence of the material at its maximum of emission before addition of carbonyl and I its intensity after exposure to carbonyl at a given concentration for a given time. When the contact time was varied, I was named I_t . When the concentration in carbonyl was studied, I was named I_c .

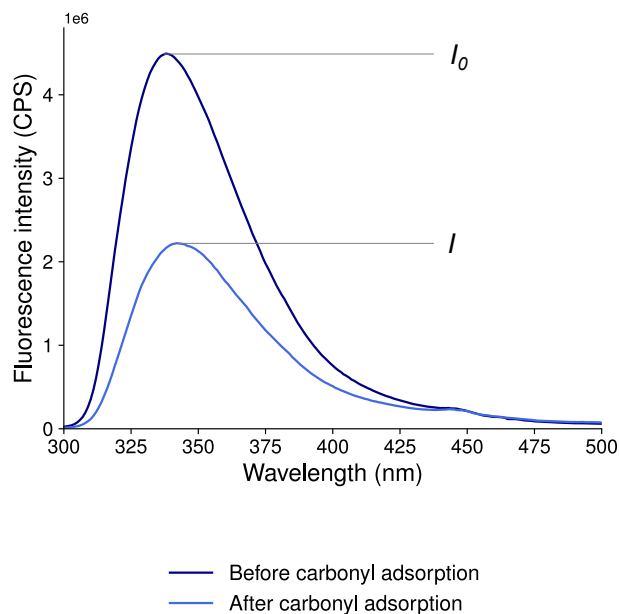


Figure 12: Values measured from fluorescence emission spectrum. I_0 is the fluorescence intensity of the material at its maximum of emission before addition of carbonyl in the liquid phase and I its intensity after exposure to carbonyl at a given concentration for a given time.

5.2.2. Percentage of fluorescence quenching at equilibrium

To study the impact of carbonyls on the materials fluorescence in different liquid phases, the percentage of fluorescence quenching was compared. In such case, a 10 mg suspension of the material in 2 mL of liquid phase was realized. After 10 seconds of stirring, the fluorescence emission spectrum of the suspended material was recorded. Then, 500 μL of a solution of carbonyl were added to obtain a concentration in the measurement cell of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. After 15 minutes of stirring at 80 rpm, the fluorescence emission spectrum of the material was recorded again. The percentage of fluorescence loss was determined according to **equation** $Q = \frac{I_0 - I}{I_0} \times 100$ (3), using the fluorescence intensity at the maximum emission wavelength after 15 min of interaction between the material and the carbonyl compound in the liquid phase as I . For material 3.1, the average loss of intensity measured at each maximum wavelength (377, 399, 423 and 448 nm) was considered.

5.2.3. Effect of the contact time

A 10 mg suspension of the material in 2 mL of liquid phase (either ethanol or ethanol/water (10/90, v/v) mixture) was realized. The suspension was stirred for 10 s and the

fluorescence emission spectrum of the material was recorded. Then, 500 μL of a solution of carbonyl (either propanal, hexanal, nonanal, 1-penten-3-one, 1-octen-3-one or hexan-2-one) in the liquid phase were added to obtain a concentration in carbonyl in the measurement cell of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. The cell was then continuously stirred at 80 rpm, and the fluorescence emission spectrum of the material was recorded after 30 s, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 2 h, 4 h and 7 h for material 1 in contact with hexanal and hexan-2-one in ethanol. For the other carbonyls in ethanol or in ethanol/water mixture, the experiment lasted 30 min.

The kinetics of fluorescence decrease of material 1 after interaction with hexanal and hexan-2-one was modelled by the pseudo-first order Lagergren model and the pseudo-second order model:

$$\ln\left(\frac{Q_e - Q_t}{Q_e}\right) = -kt \quad (4)$$

$$\frac{t}{Q_t} = \frac{1}{kQ_e^2} + \frac{t}{Q_e} \quad (5)$$

with Q_e the amount of carbonyl adsorbed at equilibrium ($\text{mg}\cdot\text{g}^{-1}$), Q_t the amount of carbonyl adsorbed at time t ($\text{mg}\cdot\text{g}^{-1}$), k the rate constant (min^{-1}) and t the time (min).

As the fluorescence loss of the material correlates with the quantity of adsorbed carbonyl, the value of Q_e and Q_t were calculated thanks to **equation** $Q = \frac{I_0 - I}{I_0} \times 100$ (3), with Q_e being obtained using the intensity of fluorescence after 7 h of contact and Q_t the intensity of fluorescence of the material after exposure to carbonyls during a period t .

5.2.4. Effect of the carbonyl concentration

A 10 mg suspension of the material in 1.8 mL of liquid phase (either ethanol or ethanol/water (10/90, v/v) mixture) was realized. After 10 s of stirring, the fluorescence spectrum of the material was recorded. A known amount of carbonyl (either propanal, hexanal, nonanal, 1-penten-3-one, 1-octen-3-one or hexan-2-one) in the liquid phase was periodically added to increase the carbonyl concentration in the solution from 0 to $0.54 \text{ mol}\cdot\text{L}^{-1}$. The solution was stirred at 80 rpm for 10 min to reach the equilibrium after each addition and the fluorescence emission spectrum of the material was recorded.

The limit of detection (LOD) and the sensitivity were determined by considering the first linear part of the curve, representing the percentage of fluorescence loss, Q , as a function of the carbonyl concentration. LOD for 10 mg of material was calculated according to the following equation⁸:

$$LOD = \frac{b+3\times\sigma_b}{a} \quad (6)$$

with a the slope and b the intercept of the linear regression of the percentage of fluorescence loss versus the carbonyl concentration and σ_b the standard deviation for b . The sensitivity was given by the slope a of this linear regression. Saturation point was determined when the fluorescence loss reached 100 %.

5.2.5. Effect of interfering compounds

To test the response of the sensor in the presence of interfering compounds, the influence of increasing concentration of n -pentane, n -hexane and n -octane on the fluorescence of the material was also assessed. The same experiment as previously described 5.2.3 and 5.2.4 was performed replacing the carbonyls by their alkane structural analogue in ethanol.

5.2.6. Effect of the nature of the liquid phase

Performances of the sensor were investigated in different liquid phases. First, ethanol modelling medium was used. Then, ethanol was replaced by the ethanol/water (90/10, v/v) mixture food simulant medium. In both of these liquid phases, the influence of contact time and concentration of different carbonyls on the fluorescence of the material were investigated. Finally, the percentage of fluorescence quenching of material 1 in rapeseed oil spiked with hexanal was assessed. In this case, the 10 mg suspension of material in 2 mL of rapeseed oil was homogenized for 30 s with vortex stirring before recording the fluorescence emission of the material. Then, 500 μ L of a solution of hexanal in rapeseed oil were added to obtain a concentration of hexanal in the measurement cell of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. The cell was stirred for 30 min before recording the fluorescence emission spectrum of the material between 300 nm and 800 nm. The emission spectrum of the rapeseed oil was also measured. The fluorescence intensity of the oil at the maximum emission wavelength of the material (551 nm) was weak and considered as negligible.

The influence of the polarity of the liquid phase was measured on material 1. To study the impact of the polarity of the liquid phase, the fluorescence emission spectrum of a 10 mg suspension of the material 1 in ethanol, ethanol/water (50/50, v/v) mixture, ethanol/water (10/90, v/v) mixture and water were recorded after 10 seconds of stirring.

The influence of the pH of the liquid phase was measured on material 1. The pH of ethanol/water (10/90, v/v) mixtures was adjusted using HCl or NaOH in order to obtain solutions at pH of 1.4, 3.6, 7.6, 9.3 and 12.8. The material was suspended in 2 mL of each of these solutions. After 24 h of stirring at 50 rpm, the fluorescence emission spectrum of the material was recorded. Then, 500 μL of a solution of 1-octen-3-one in ethanol were added to obtain a concentration in the measurement cell of $2.3 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. The solution was stirred at 80 rpm for 10 min in order to reach the equilibrium and then, the fluorescence emission spectrum of the material was recorded.

5.3. FLUORESCENCE OF THE MATERIAL AFTER EXPOSURE TO CARBONYLS IN THE GAS PHASE

As described in **Figure 13**, the fluorescence properties of the material after exposure to carbonyls in the gas phase was assessed after immobilizing the material in PLA films (see 2.3). The influence of contact time, carbonyl nature and carbonyl concentration on the fluorescence properties of the material were studied. Spectrofluorometer Fluoromax4[®] (Horiba) was used with the software Fluorescence[®]. Excitation wavelength was $290 \pm 2 \text{ nm}$. Fluorescence emission spectrum was recorded between 300 nm and 500 nm, using a 2 nm bandpass and a step interval of 1 nm. A 30 ° angle between the excitation beam and the detector was added to maximize the detection of the fluorescence of the film avoiding interfering photons coming directly from the excitation source. The temperature was kept constant at 22 °C during the experiment. Each experiment was performed 4 times.

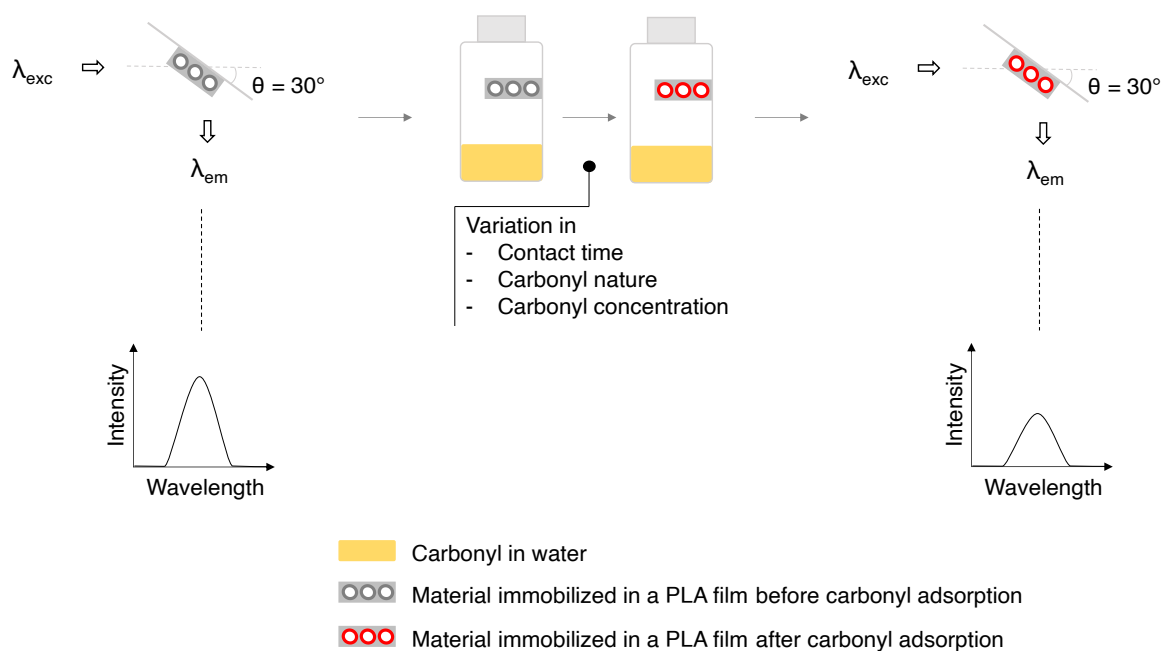


Figure 13: Experimental setup used for fluorescence measurements of the material immobilized in a PLA film before and after exposure to carbonyl vapors.

5.3.1. Effect of the contact time

1 mL of a solution of hexanal or hexan-2-one in water at $9.99 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ ($1 \text{ g}\cdot\text{L}^{-1}$) was placed in a 20 mL glass vial under agitation at 250 rpm at 60°C . A PLA film incorporating the material was fixed in the headspace of the vial (in caps glued at 1.5 cm from the bottom of the vial). The fluorescence emission spectrum of the film was recorded after 5 min, 15 min, 30 min, 1 h, 2 h, 4 h and 6 h of exposure to carbonyls vapors.

5.3.2. Effect of the carbonyl concentration

1 mL of a solution of hexanal in water with concentration ranging from $4.99 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ ($50 \text{ mg}\cdot\text{L}^{-1}$) to $2.49 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$ ($2.5 \text{ g}\cdot\text{L}^{-1}$) was placed in a 20 mL vial under agitation at 250 rpm at 60°C . A PLA film incorporating the material was hanged in the headspace of the vial (at 1.5 cm from the bottom of the vial) for 2 h to reach the equilibrium.

5.3.3. Influence of *n*-hexane vapors on the fluorescence of the material

n-hexane is a structural analogue of the carbonyl tested and considered as an interfering compound. 50 μL of *n*-hexane were placed in a 20 mL vial, stirred at 250 rpm and heated at 60°C . A PLA film incorporating the material was placed in the headspace of the vial (at 1.5 cm from the bottom of the vial) for 1 h.

6. MONITORING OF THE ACCELERATED AGEING OF A COMMERCIAL FISH OIL

Material 1 was applied to the real-time monitoring of the accelerated oxidation of a commercial fish oil. Fluorescence of the material was correlated to classical quality indices: peroxide value, thiobarbituric acid reactive substance assay and evolution of volatile compounds determined by SPME-GC-MS.

6.1. ACCELERATED OXIDATION OF MENHADEN OIL

19 g of menhaden oil were placed in opaque sealed jars of 200 mL (ratio oil/air: 0.1). Oil samples were submitted to an accelerated oxidation test, which was conducted in an oven at 60 °C for 21 days. Quality indices were determined in fresh oil, and after 1, 3, 5, 7, 14 and 21 days. Each day of measurement, 3 jars were taken from the oven to perform 3 repetitions of each quality index. The conditions of accelerated oxidation (ratio oil/air in the jars, presence or absence of light, amount of oxygen available) were optimized prior the experiment.

6.2. FLUORESCENCE OF THE MATERIAL

The same equipment as described in 5.2 was used. The excitation wavelength was 360 ± 2 nm. Fluorescence emission spectrum was recorded between 400 nm and 600 nm, with a 2 nm bandpass and a step interval of 1 nm. These acquisition parameters were optimized prior to this study using 3D fluorescence spectroscopy, allowing to visualize on a same graph excitation and emission spectra (see **Figure 14**). Both fluorescence spectra of the fish oil and the material in suspension in the oil were recorded. The excitation wavelength was finally chosen to minimize the fluorescence emission from the oil and to maximize the fluorescence emission from the material. The temperature was kept constant at 22 °C during this experiment.

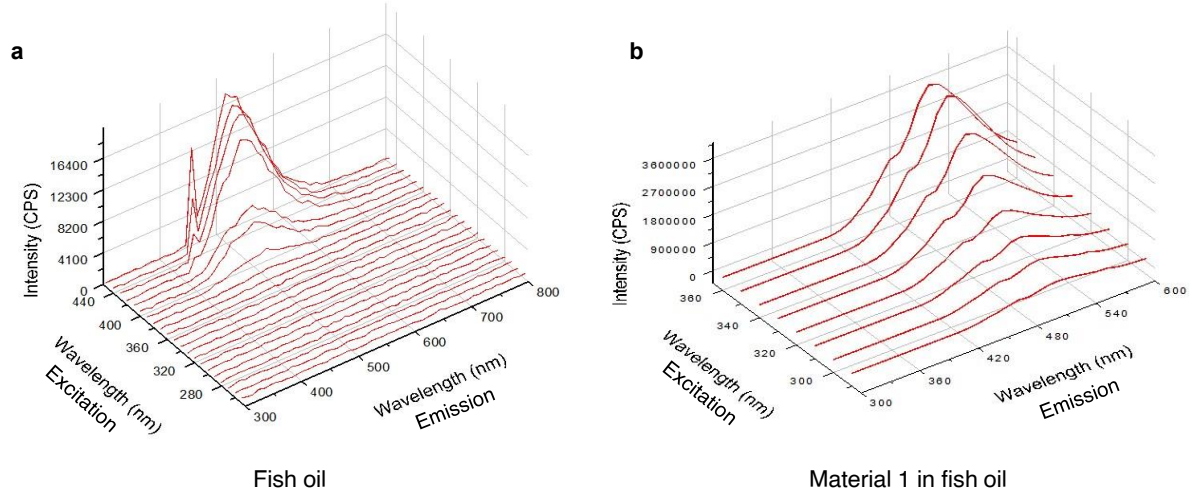
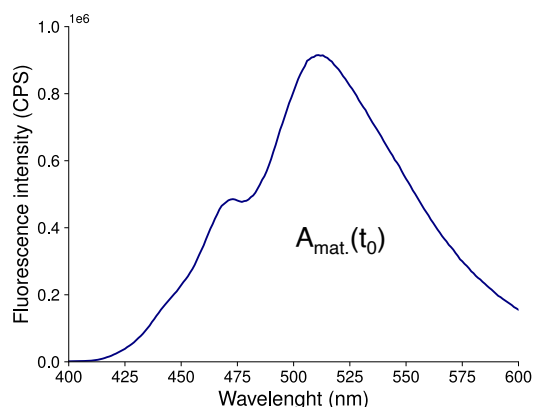


Figure 14: 3D fluorescence spectrum of the fish oil (a) and the material in suspension in the fish oil (b).

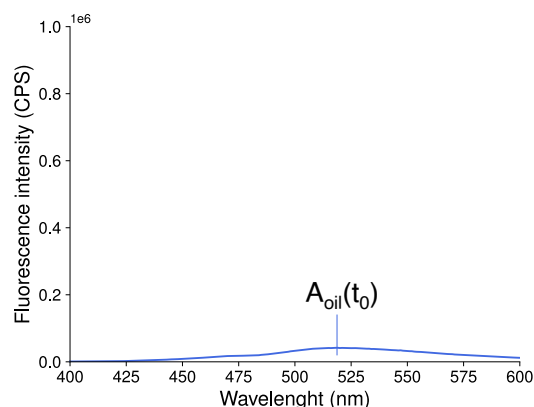
First, the fluorescence emission spectrum of 2.79 g of menhaden oil was recorded. Then, 10 mg of the material were added and the suspension was stirred for 1 min using a vortex and for 14 min at 80 rpm on a stirring plate. According to a previous kinetics measurement, the equilibrium was reached within 15 min. Then, the fluorescence emission spectrum of the material in suspension in the oil was recorded. The total surface area of the fluorescence emission curve from the oil alone and from the material in the oil were considered. The gradually oxidized oil displayed a weak fluorescence over the wavelength range studied. This was taken into account by systematically subtracting the surface area of the fluorescence emission curve of the oil at a given oxidation time to one of the material in suspension in the oil at the same time. The percentage of fluorescence loss of the material after interaction with carbonyl compounds released by lipid oxidation of the oil was finally determined as follows:

$$\text{Fluorescence loss} = \frac{[A_{mat.}(t_0) - A_{oil}(t_0)] - [A_{mat.}(t) - A_{oil}(t)]}{A_{mat.}(t_0) - A_{oil}(t_0)} \quad (7)$$

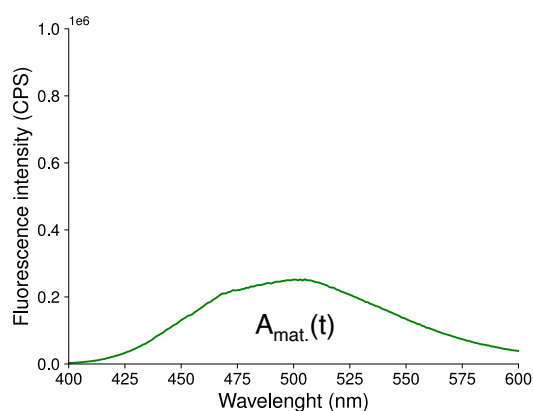
with $A_{mat.}$ being the total surface area of the fluorescence emission curve from the material in the oil and A_{oil} the total surface area of the fluorescence emission curve from the oil alone (see Figure 15).



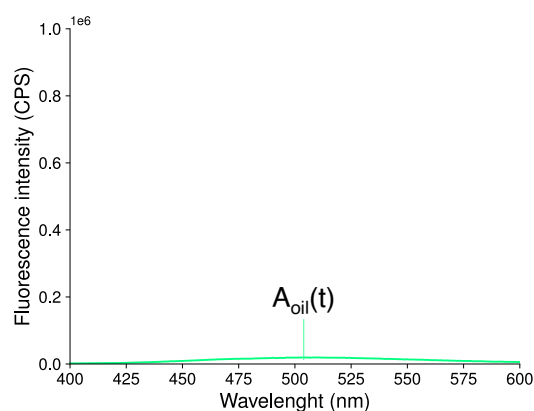
Fluorescence emission spectrum of the material in fresh oil (t_0)



Fluorescence emission spectrum of fresh oil (t_0)



Fluorescence emission spectrum of the material in oxidized oil (t days)



Fluorescence emission spectrum of oxidized oil (t days)

Figure 15: Area used for the calculation of the fluorescence loss over time during the oxidation monitoring of a commercial fish oil.

6.3. PEROXYDE VALUE DETERMINATION

The peroxide value evaluates the early stages of oxidation by measuring the quantity of hydroperoxydes formed by lipid oxidation. After 1, 3, 5, 7, 14 and 21 days of accelerated oxidation, the peroxide value (PV) of menhaden oil samples was determined according to the AOAC official method⁹. Between 0.5 and 2 g of oil were firstly dissolved in 10 mL of a mixture of cyclohexane/propan-2-ol/ethanol (5/7/2, v/v/v). 10 mL of anhydrous acetic acid and 5 mL of an aqueous solution saturated with potassium iodide were added. The reaction mixture was stirred for 1 min at 150 rpm and placed in the dark for 5 min. 75 mL of water were added and the mixture was titrated with a freshly prepared aqueous solution of sodium thiosulfate

($0.01 \text{ mol}\cdot\text{L}^{-1}$), using iodine-starch as a colorimetric indicator. A blank was used replacing oil with water. PV value was expressed in milliequivalents of oxygen per kg of oil.

6.4. THIOBARBITURIC ACID REACTIVE SUBSTANCE ASSAY

The thiobarbituric acid reactive substance assay allows to evaluate the quantity of aldehydes secondary products generated during lipid oxidation. After 1, 3, 5, 7, 14 and 21 days of accelerated oxidation, menhaden oil samples were also analyzed using the thiobarbituric acid reactive substance (TBARS) assay according to the AOAC official method¹⁰. Between 50 and 200 mg of oil were firstly dissolved in 1-butanol. 10 mg of propyl gallate were added. 5 mL of this mixture were mixed with 5 mL of 2-thiorbarbituric acid ($2 \text{ mg}\cdot\text{mL}^{-1}$). The reaction mixture was heated at 95°C for 2 h, before being cooled down at 22°C for 30 min. The absorbance of the sample was measured at 530 nm on a UV-visible spectrophotometer (mc1, SAFAS, Monaco) using 1-butanol as a reference. A blank was prepared following the same procedure without oil. The results were expressed as mg malondialdehyde (MDA)/kg of oil.

6.5. EVOLUTION OF VOLATILE COMPOUNDS

After 1, 3, 7, 14 and 21 days of accelerated oxidation, solid phase micro extraction was used to extract the volatiles from the headspace of 20 mL vials (closed with a silicone/PTFE septum) containing 0.93 g of menhaden oil. These vials were maintained at 60°C under agitation at 500 rpm for 5 min. Then an extraction was done during 2.5 min. Volatiles were adsorbed from the headspace onto a three-phase (DVB, CAR, PDMS) SPME fiber (Sigma Aldrich) and were analyzed on a 7890A gas chromatograph (Agilent Technologies) equipped with a capillary column (30 m x 0.32 mm internal diameter, $0.5 \mu\text{m}$ thickness) coated with a DB-5MS stationary phase (Agilent J&W). Desorption was directly performed into the injector, using splitless mode at 250°C . Mass spectrometer HP 5975 was used as a detector (electronic impact 70 eV , transmission line at 240°C). Helium was the carrier gas at a flow rate of $1.4 \text{ mL}\cdot\text{min}^{-1}$. The oven temperature was programmed from 40°C (maintained for 3 min) to 70°C at a temperature rate of $3^\circ\text{C}\cdot\text{min}^{-1}$, then from 70°C to 110°C at $5^\circ\text{C}\cdot\text{min}^{-1}$ and from 110°C to 250°C at $10^\circ\text{C}\cdot\text{min}^{-1}$. This final temperature was maintained for 5 min.

7. STRUCTURE DETERMINATION OF 1-PENTEN-3-ONE BY HPLC-MS

Results obtained with 1-penten-3-one were unexpected. Hypothesis was made that this molecule could eventually dimerize. The structure of the conjugated ketones studied in this work was thus investigated by HPLC-MS. Solutions of 1-penten-3-one and 1-octen-3-one in acetonitrile at 2 concentrations ($5 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ and $5 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) were filtrated and outgazed before injection in a 2695 liquid chromatograph (Waters) equipped with a C-18 column (4.6 x 50 mm internal diameter, 3.5 μm thickness, XBridge™) and an Acquity QDa mass spectrometer detector (Waters). The mass spectrometer was scanned in the 50-1200 m/z range. Analytical conditions were as follows: 0.8 kV capillary voltage, 30 V cone voltage, and 120 °C source block temperature. The compounds were analyzed using electrospray ionization (positive or negative modes). The separation was achieved using either acidic or basic conditions at a flow rate of $0.6 \text{ mL}\cdot\text{min}^{-1}$. The mobile phase was composed of a mixture of two solvents. For positive ion mode, solvent A was a formic acid/water mixture (0.1/99.9, v/v) and solvent B was a formic acid/acetonitrile mixture (0.1/99.9, v/v). For negative ion mode, formic acid was replaced by ammonium hydroxide. The following gradient of solvent A and B was used for the separation: 10 % of B was maintained for 2 min and the percentage of solvent B was increased up to 90 % in 5 min. 90 % of B was maintained for 2 min before being decreased to 10 % in 2 min and maintained at 10 % for 2 more min.

8. STATISTICS

Statistical analysis (ANOVA with Tukey's post hoc analysis) was performed using XLSTAT (Addinsoft) software. Significance was established at $p < 0.05$.

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RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Chap. 1. DESIGN AND SYNTHESIS OF FLUORESCENT MATERIALS FOR CARBONYL SENSING

At the beginning of the project, two main categories of materials were considered for the development of new fluorescent sensors for the monitoring of carbonyl compounds: acrylate-based materials, obtained by radical polymerization, and silicate-based materials, obtained by sol-gel synthesis. This chapter is dedicated to the design, synthesis optimization and comparison of the performances of different materials obtained by these two routes, in order to identify the best suited for the objective. More precisely, the impact of the synthesis route, the nature of the monomer, the initiation path in the case of radical polymerization, the molar ratio between the reactants on the adsorption capacity and the fluorescence properties of the materials upon interaction with hexanal in ethanol were studied.

Design and synthesis optimization of acrylate-based and silicate-based materials for carbonyl sensing based on fluorescence.

ABSTRACT

For their use as chemical sensors, the optimization of the performances of polymeric materials is a critical step in their development for the desired application. The main objective of this work was to identify the best suited materials to develop a sensor for carbonyl monitoring based on fluorescence. Two categories of materials were compared: acrylate-based materials, obtained by radical polymerization, and silicate-based materials, obtained by sol-gel synthesis. The performances of these materials in terms of yield of polymerization, carbonyl adsorption capacity and fluorescence property were compared. More precisely, the influence of various synthesis parameters such as polymerization type, radical polymerization initiation method, the nature of the functional monomer and the molar ratio of the different reactants were assessed. On the first hand, among acrylate-based materials, the one based on 4-vinylaniline showed better adsorption capacity compared to those based on 3-vinylaniline and 2-vinylaniline. Moreover, materials obtained by UV-polymerization showed a better adsorption capacity compared to those obtained by polymerization thermally initiated. On the other hand, the silicate-based material provided a better synthesis reproducibility, a higher adsorption capacity and a higher fluorescence intensity compared to its acrylate-based counterparts. Finally, contrarily to the acrylate-based materials tested, its adsorption capacity and its fluorescence properties were stable over time.

1. INTRODUCTION

The monitoring of carbonyl compounds is of high interest in many fields. They can provide information about the level of air pollution¹, be used as food quality trackers² or even as biomarkers in medical diagnoses³. Classical analytical methods for the determination of carbonyl compounds amounts in complex matrices often rely on chromatography⁴. Although this technique is very efficient, it relies on the use of expensive equipment dedicated to highly trained persons and requires time consuming sample preparation and analysis. This is why there is a demand for more simple but still sensitive and accurate analytical procedures. Optical sensors, whether they are colorimetric, fluorometric or sometimes chemiluminescent are gaining increasing interest for the development of new user-friendly analytical methods⁵.

Chemical sensors for carbonyl monitoring could rely on acrylate-based or silicate-based polymers. Acrylate-based materials are organic polymers, usually obtained by radical polymerization. This synthesis route requires the use of one or more functional monomers, a cross-linker, a polymerization initiator, and a solvent⁶. Functional monomers must be carefully chosen according to the application. They possess functional groups providing the targeted reactivity to the resulting polymer. The most widely used monomers are acrylamides and vinyl esters. The cross-linker confers mechanical stability to the final material. The most used cross-linkers are divinylbenzene and ethylene glycol dimethacrylate (EGDMA). Radical polymerization is usually performed either thermally or photoinitiated. 2,2'-azobis(2-methylpropionitrile) (AIBN) is classically used as a thermal initiator and 2,2-dimethoxy-1,2-diphenylethanone (DMPAP) is commonly used as a photoinitiator. As an example, Rico-Yuste *et al.* already studied the synthesis of an acrylate-based material for the colorimetric detection of furfural in beers⁷. The material was obtained by the co-polymerization of two functional monomers: 4-vinylaniline and 2-hydroxymethyl methacrylate, using EGDMA as a cross-linker.

Silicate-based materials are usually made of a mineral structure on which organic parts could be grafted. One way of preparation of such materials is the sol-gel process. It relies on the hydrolysis and condensation of alkoxy silane derivatives under acidic or basic conditions⁸. The co-condensation of an organosilicate monomer with a tetraalkoxy silane cross-linker, such as tetraethoxysilane (TEOS), provides tunable properties to the resulting material. Silicate-based materials show better chemical and physical stability compared to their acrylate-based counterparts. Moreover, no organic solvents and low toxicity reactants are used for the sol-gel synthesis⁹. Since the development of mesoporous silica for catalytical and adsorption applications, silicate-based materials have been extensively functionalized. However, to the best of our knowledge, no sol-gel material has been yet developed as fluorescent sensor for carbonyl monitoring. For the fluorescent detection of primary amines, Seok *et al.* recently proposed a sol-gel material functionalized with styrylbenzene derivatives and aldehyde groups¹⁰. The objective of this project is based on the opposite concept: to develop a fluorescent sensor containing amine groups to monitor carbonyl compounds.

This paper reports on the design and synthesis of acrylate-based and silicate-based materials intended to be used as chemical sensors for carbonyl monitoring. The first objective of the present work was thus to evaluate the best candidates for such a purpose, comparing

the more traditional materials based on acrylates to the less explored sol-gel materials. The synthesis parameters strongly influence the performance of the final materials. However, only a few studies detailed the synthesis optimization process and the impact of selected parameters on the properties of the materials. The second objective was therefore to study the influence of the most significant parameters on the materials performance: the polymerization route, the radical polymerization initiation method, the nature of the functional monomer, and the molar ratio of the different reactants. The resulting materials were compared in terms of adsorption capacity of hexanal and correlation with fluorescence properties.

2. MATERIALS AND METHODS

2.1. CHEMICALS

4-vinylaniline (4-VA, 97 %, CAS number 1520-21-4), 3-vinylaniline (3-VA, 97 %, CAS number 15411-43-5), ethylene glycol dimethacrylate (EGDMA, 98 %, CAS number 97-90-5), tetraethylorthosilicate (TEOS, 99 %, CAS number 78-10-4), 2,2-dimethoxy-1,2-diphenylethanone (DMPAP, 99 %, CAS number 24650-42-8), 2,2'-azobis(2-methylpropionitrile) (AIBN, 98 %, CAS number 78-67-1), octan-1-ol (98 %, CAS number 111-87-5) ammonium hydroxide (NH₄OH, 30 %, CAS number 1336-21-6) and ethanol for UV-spectroscopy Uvasol® (EtOH, 99,9 %, CAS number 64-17-5) were purchased from Sigma Aldrich, France. 2-vinylaniline (2-VA, 95 %, CAS number 3867-18-3) and 4-trimethoxysilylaniline (4-TMSA, 97 %, CAS number 33976-43-1) were purchased from abcr, Germany. Acetonitrile (CH₃CN, 99.9 %, CAS number 75-05-8) was obtained from Honeywell. Hexanal (98 %, CAS number 66-25-1) was purchased from Alfa Aesar, France. Deionized water was used, obtained from a MilliQ RG, Millipore apparatus.

2.2. MATERIALS SYNTHESIS

Only the final experimental procedures with optimized parameters are reported in the following. For the synthesis of acrylate-based materials, the initiator and solvent ratios were optimized. For silicate-based materials, the temperature of reaction and the volume of solvent were investigated.

2.2.1. Acrylate-based materials

The functional monomer (4-vinylaniline, 3-vinylaniline or 2-vinylaniline) was firstly dissolved in acetonitrile. The cross-linker ethylene glycol dimethacrylate (EGDMA) and the initiator (either DMPAP or AIBN) were then added. The following molar ratio was used: 4/20/1/50 for the functional monomer/cross-linker/initiator/solvent. Oxygen was removed from the reaction mixture by nitrogen bubbling for 10 minutes. For photoinitiated polymerization, the reactional medium was placed at 10 cm from an UV-lamp (366 nm) for 24 h. For thermally initiated polymerization, the reaction mixture was stirred at 250 rpm and heated at 60 °C for 24 h. The resulting powder was washed 3 times with acetonitrile by Büchner filtration using a 0.2 µm pore membrane. The material was dried 12 h at 60 °C, crushed and stored at 4 °C in the dark before use. A material with a higher ratio of functional monomer (20/20/1/50) was also synthesized following the same procedure. The polymerization yield was calculated using the theoretical mass of material supposedly obtained.

2.2.2. Silicate-based materials

4-trimethoxysilylaniline (4-TMSA) was firstly dissolved in an ethanol in water mixture (1/10 molar ratio) at 60 °C for 10 minutes. Tetraethyl orthosilicate (TEOS) and ammonium hydroxide (30 % NH₄OH) were then added. The following molar ratio was used: 1/5/31.25/250/25 for 4-TMSA/TEOS/NH₄OH/H₂O/EtOH. The mixture was stirred at 500 rpm for 24 h at 22 °C. Then, the solvent was evaporated under the hood, and the resulting powder was washed 3 times with an ethanol in water mixture (1/10 molar ratio) by Büchner filtration using a 0.2 µm pore membrane. The material was dried 12 h at 60 °C, crushed and stored at 4 °C in the dark before use. A material with a higher ratio of functional monomer (5/5/31.25/250/25) was synthesized following the same procedure. The polymerization yield was calculated from the assumed theoretical mass of material obtained, taking into account the loss of alcohol and water molecules during the hydrolysis and condensation processes.

In both cases (acrylate or silicate-based materials), control materials were systematically synthesized following the same procedure but without functional monomer. This aims at proving that the adsorption of carbonyl compounds and subsequent material fluorescence were strictly due to the presence of the monomer present in the structure of the material.

The materials were named according to the nature of their functional monomer, the polymerization route, the initiation of the polymerization, and the molar ratio of functional monomer. **Table 1** summarizes the name of the materials studied in this work.

Table 1: Names of the different materials synthesized, based on the main selected parameters for optimizing their synthesis.

4-VA UV stands for the material synthesized by photo-initiated radical polymerization using 4-vinylaniline monomer. 4-VA thermal refers to the material synthesized by radical polymerization thermally initiated using 4-vinylaniline monomer. 3-VA and 2-VA are used instead of 3-vinylaniline and 2-vinylaniline respectively. 4-TMSA sol-gel indicates that the material was synthesized by the sol-gel process using 4-trimethoxysilylaniline monomer. n.a. means not applicable.

Name of the material	Polymerization type	Initiation of the polymerization	Nature of functional monomer	Functional monomer/cross-linker ratio
4-VA UV	Radical polymerization	UV radiation	4-VA	4/20
3-VA UV	Radical polymerization	UV radiation	3-VA	4/20
2-VA UV	Radical polymerization	UV radiation	2-VA	4/20
4-VA thermal	Radical polymerization	Heat	4-VA	4/20
3-VA thermal	Radical polymerization	Heat	3-VA	4/20
2-VA thermal	Radical polymerization	Heat	2-VA	4/20
4-TMSA sol-gel	Sol-gel polymerization	n.a.	4-TMSA	1/5
4-VA x 5 UV	Radical polymerization	UV radiation	4-VA	20/20
4-TMSA x 5 sol-gel	Sol-gel polymerization	n.a.	4-TMSA	5/5

2.3. MATERIALS CHARACTERIZATION

2.3.1. Fourier Transform Infrared spectroscopy (FTIR)

FTIR absorbance spectra of the materials and their corresponding blank (obtained without functional monomer) were recorded at 25 °C using a Nicolet iS10 (Thermo Scientific™) spectrometer. Before analysis, around 1 mg of dried powder material was dispersed into KBr to obtain pellets of 200 mg. The transmission spectra were recorded in a spectral range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans per analysis, using a 200 mg KBr pellet as the reference.

2.3.2. Scanning electron microscopy (SEM)

The surface morphology of the materials was characterized by SEM using a JEOL 6700F microscope equipped with a cold FEG electron source operating at 1 kV and both Everhart-Thornley and in-lense detectors. Prior to imaging, the powder was deposited on a carbon tape and the surface of the material was coated with carbon. Images were acquired at various magnifications between 100 and 20 000.

2.3.3. Laser diffraction

The particle size distribution was determined by laser diffraction using a Malvern MasterSizer 3000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK). The material was added to the circulating water until the obscuration reached 8 % to 10 %. Stirring speed was set to 1200 rpm. The temperature was maintained at 22 °C during all the experiment. For the acrylate-based materials, the refractive index of the particles (1.488) was estimated using the polymethyl methacrylate model. For the silicate-based material, the refractive index of the particles (1.457) was based on the silica model. The refractive index of the dispersing phase was set at 1.36. The Mie diffusion model was used to calculate particle size. Each measurement was performed 3 times.

2.3.4. Material toxicity assessment

The mutagenicity of the material was estimated by the Ames' test, performed on two bacterial strains: *Salmonella typhimurium* TA98 and TA100, according to Maron and Ames¹¹ and the OECD guideline no. 471 for the testing of chemicals¹². More details about the experimental procedure can be found in previous work⁹.

2.4. ADSORPTION CAPACITY OF THE MATERIALS

The adsorption capacity of hexanal by the materials was determined by gas chromatography. 5 mg of material were suspended in 2 mL of a solution of hexanal ($2.37 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) in acetonitrile. Octan-1-ol ($2.54 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) was added as an internal standard. Samples were agitated at 50 rpm for 24 h at 22 °C. They were centrifugated at 10 000 rpm for 10 minutes. The remaining concentration of hexanal in the liquid phase was determined by injecting 1 μL of the supernatant using an autosampler (Gerstel) on a 7890 A gas chromatograph (Agilent Technologies) equipped with a capillary column (30 m x 0.32 mm internal diameter, 0.5 μm thickness) coated with a DB-5MS stationary phase (Agilent J&W). Injections were performed in split mode (split ratio 50:1). Injection temperature was 250 °C. Helium was used as carrier gas at a flow rate of $1.4 \text{ mL}\cdot\text{min}^{-1}$. Mass spectrometer 5975C (Agilent) was used as the detector (electronic impact 70 eV, chemical ionization source at 230 °C, transmission line at 240 °C). The oven temperature was programmed from 50 °C to 65 °C at a rate of $3 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$, from 65 °C to 115 °C at $30 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$, then from 115 °C to 140 °C at $3 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ and from 140 °C to 250 °C at $30 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$. The oven temperature was finally maintained at 250 °C for 5 minutes. A solvent delay of 3 minutes was also applied. Each adsorption experiment was carried out 3 times.

The adsorption capacity is defined as the mass of hexanal adsorbed by 1 g of material. It was calculated as follows:

$$\text{Adsorption capacity} = \frac{(C_i - C_f) \times V_{liq.}}{m} \quad (1)$$

with C_i the initial concentration of hexanal in the liquid phase in contact with the material, C_f the concentration of hexanal remaining in the liquid phase at equilibrium, $V_{liq.}$ the volume of the hexanal solution and m the mass of the material.

Statistical analysis (ANOVA with Tukey's post hoc analysis) was performed using XLSTAT (Addinsoft) software. Significance was established at $p < 0.05$.

2.5. FLUORESCENCE OF THE MATERIALS

The fluorescence emission spectrum of the materials was recorded before and after exposure to hexanal in ethanol. 10 mg of material were suspended in 2 mL of ethanol. After 10 seconds of stirring, the fluorescence emission spectrum of the suspended material was

recorded. Then, 500 μL of a solution of hexanal were added to obtain a concentration in the measurement cell of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. After 15 minutes of stirring at 80 rpm, the fluorescence emission spectrum of the material was recorded again. The fluorescence intensity of the material after exposure to hexanal was compared to its initial fluorescence. A Spectrofluorometer Fluoromax4[®] (Horiba) was used with the software Fluorescence[®]. A SUPRASIL[®] quartz cell (Hellma) of 10 x 10 mm dimension was employed. The excitation wavelengths were $280 \pm 2 \text{ nm}$ for acrylate-based materials and $290 \pm 2 \text{ nm}$ for silicate-based materials. Fluorescence emission spectra were recorded between 300 nm and 500 nm, with a 2 nm bandpass and a step interval of 1 nm. The temperature was kept at 22 °C during the experiment. Each experiment was performed 3 times.

The percentage of fluorescence quenching, Q , was determined as follow:

$$Q = \frac{I_0 - I_{eq}}{I_0} \times 100 \quad (2)$$

with I_0 the intensity of fluorescence at 337 nm of the material in ethanol before addition of carbonyl and I_{eq} its intensity after exposure to carbonyl at a concentration of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ for 20 min.

3. RESULTS AND DISCUSSION

3.1. DESIGN OF THE MATERIALS

The objective was to develop new materials for carbonyl sensing based on fluorescence. Tested synthesis routes and reactants were carefully selected to primarily meet the following requirements: a synthesis easy to implement and functional monomers commercially available at a reasonable cost. Moreover, to respect the principle of green chemistry related to the use of safe reactants, only chemicals with limited toxicity were chosen. This would enable the final use of the resulting sensor in fields requiring safe sensors such as food, cosmetics, or medicine.

Two main synthesis routes were considered. The first one was the radical polymerization of acrylate-based monomers. The second one was the sol-gel polymerization of silicate-based reactants.

In order to optimize the detection, a single functional monomer having the two characteristics required for the application was sought. It should be able to interact with

carbonyls and to be fluorescent. Aniline-based monomers perfectly suited that purpose, as the primary amine group can react with carbonyls and the aniline is a fluorescent core. Acrylate-based as well as silicate-based monomers with aniline cores were commercially purchased. The commercial offer in acrylate-based monomers is more abundant compared to silicate-based monomers. 3 monomers were selected for radical polymerization (4-vinylaniline, 3-vinylaniline, 2-vinylaniline) a single monomer for the sol-gel synthesis (4-trimethoxysilylaniline).

The adsorption capacities towards hexanal and the subsequent change in the fluorescence properties of the resulting materials were investigated to identify the best candidate to be used as a sensor for carbonyl monitoring.

3.2. SYNTHESIS OPTIMISATION

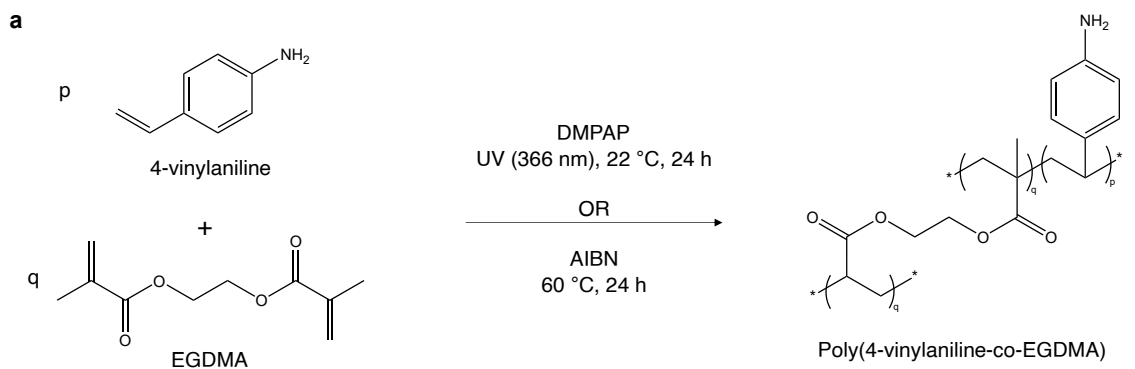
As a first step, synthesis parameters were optimized to maximize the yield of polymerization.

3.2.1. Acrylate-based materials

Acrylate-based materials were synthesized by radical polymerization by adapting a protocol from previous work¹³. The following parameters were studied for optimization:

- Initiation of polymerization: UV-light or heat (60 °C)
- Nature of the functional monomer: 4-vinylaniline, 3-vinylaniline, 2-vinylaniline
- Functional monomer/initiator molar ratio: 4/0.5 or 4/1
- Functional monomer/solvent molar ratio: 4/100 or 4/50.

Figure 1a summarizes the reaction route for the acrylate-based materials. It also gives the yields of polymerization obtained according to the different parameters tested.



UV-polymerization

	Molar ratio 1	Molar ratio 2	Molar ratio 3
Functional monomer (4-VA, 3-VA or 2-VA)	4	4	4
Cross-linker (EGDMA)	20	20	20
Initiator (DMPAP)	0.5	0.5	1
Solvent (CH ₃ CN)	100	50	50

Polymerization yield (%)			
4-VA	0.4	4	73 ± 16 **
3-VA	56 ± 28 *	not tested	not tested
2-VA	8 ± 12 *	not tested	not tested

* Synthesis repeated twice

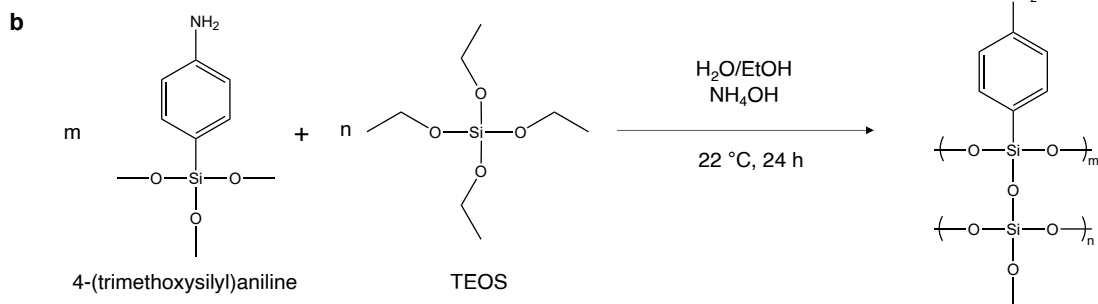
** Synthesis repeated 7 times

Thermal polymerization

	Molar ratio 1	Molar ratio 2
Functional monomer (4-VA, 3-VA or 2-VA)	4	4
Cross-linker (EGDMA)	20	20
Initiator (AIBN)	0.5	1
Solvent (CH ₃ CN)	100	50

Polymerization yield (%)		
4-VA	85	83 ± 12 ***
3-VA	51	not tested
2-VA	77	not tested

*** Synthesis repeated 3 times



	Molar ratio 1	Molar ratio 2	Molar ratio 3	Molar ratio 4	Molar ratio 5
Functional monomer (4-TMSA)	1	1	1	1	1
Cross-linker (TEOS)	5	5	5	5	5
Initiator (NH ₄ OH)	125	125	125	62.5	31.25
Solvent 1 (H ₂ O)	1000	1000	1000	500	250
Solvent 2 (EtOH)	100	100	100	50	25
Temperature	60 °C	40 °C	22 °C	60 °C	22 °C

Polymerization yields (%)	no material	no material	no material	no material	84 ± 6 ****

**** Synthesis repeated 13 times

Figure 1: Optimization of the synthesis with the studied parameters.

a. Reaction route and molar ratios used for the synthesis of acrylate-based materials using 4-vinylaniline (4-VA) as a functional monomer. Similar reactions were performed with 3-vinylaniline (3-VA) and 2-vinylaniline (2-VA) for the molar ratio 1. Radical polymerization was either photoinitiated or thermally initiated. An increased initiator molar ratio and a decreased volume of solvent allowed to maximize the polymerization yield. When no standard deviations for the yield of polymerization are given, the synthesis was performed only once. p and q are the molar ratio of functional monomer and cross-linker used for the synthesis of the acrylate-based materials, respectively. m and n are the molar ratio of functional monomer and cross-linker used for the synthesis of the functionalized sol-gel material, respectively.

b. Reaction route and molar ratios used for the synthesis of silicate-based material. The temperature as well as the solvent volume were reduced to favor material precipitation.

Firstly, considering UV-polymerization, a two-fold decrease in the volume of solvent led to improve the yield of polymerization from 0.4 % to 4 %, using 4-VA monomer. As shown in a previous work¹⁴, a reduced amount of solvent favors the precipitation of polymers from the reaction mixture. A change in the solvent nature was also tested, replacing acetonitrile by methanol. This impacted positively the yield of polymerization, which was 59 % using 4-VA monomer. But this material was no longer considered, as alternative strategies of polymerization gave similar or even better yields using less toxic solvents (acetonitrile and ethanol/water mixtures). A two-fold increase in the quantity of initiator also improved the yield of polymerization, from 4 % to 73 %, using 4-VA monomer. It could be explained by the residual amount of oxygen remaining in the reaction mixture, which can combine with free radicals generated during the first step of the radical polymerization and thus inhibits polymerization. Oxygen is supposedly removed before polymerization, but it is possible that nitrogen bubbling used for this purpose is not effective enough. A higher amount of initiator could thus compensate for the radicals inhibited by oxygen. The same hypothesis was considered to explain the high variability observed for the yield of reaction when the same synthesis was repeated many times (73 ± 16 % for 4-VA UV on 7 syntheses). Increasing even more the molar ratio of the initiator could probably solve this minor issue.

Secondly, for thermally initiated polymerization, neither the initiator ratio nor the solvent volume significantly impacted the yield of polymerization contrarily to the UV-polymerization. The best yields of polymerization were obtained when temperature (60 °C) was used to initiate the polymerization instead of UV-light (83 % and 73 %, with 4-VA, respectively). This was attributed to the better control of the temperature homogeneity in the reaction

mixture for thermally initiated polymerization. Actually, in the case of UV-polymerization, only a single side of the reaction tube was exposed to the UV-light, therefore limiting the opportunities for starting new polymeric chains in the whole reaction mixture.

3.2.2. Silicate-based materials

Silicate-based materials were synthesized using a sol-gel process. The temperature and the solvent molar ratio were optimized in order to favor the precipitation of the material.

Figure 1b displays the reaction route for the synthesis of the silicate-based materials. The yields of polymerization according to the different parameters investigated are also reported. It was impossible to synthesize any material when only the temperature of reaction was modified. It was only by reducing by 4 the solvents ratios that the material finally precipitated. Compared to acrylate-based materials, the synthesis repeatability is better controlled ($83 \pm 8 \%$ for 4-TMSA sol-gel and $73 \pm 16 \%$ for 4-VA UV respectively, based on 7 syntheses in each case). This is probably because oxygen does not interfere with the sol-gel polymerization as in the radical polymerization.

To conclude on that first optimization step, when dealing with radical polymerization, an important parameter to control in order to increase the yield of reaction appears to be related to the quantity of the residual oxygen amount dissolved in the reaction mixture. The molar ratio of initiator should therefore be chosen consequently. The solvent volume should also be limited in order to favor the material precipitation. This last consideration also applies for the sol-gel polymerization. Based on yield of polymerization and reproducibility of the synthesis, the classification between the different synthesis routes is as follows: sol-gel polymerization > thermal polymerization > UV-polymerization.

3.3. ADSORPTION CAPACITY OF THE MATERIALS

As the synthesized materials were intended to act as carbonyl sensors, their adsorption capacity for hexanal were systematically assessed. **Figure 2** depicts the adsorption capacity of the different materials exposed to a single concentration of hexanal in acetonitrile.

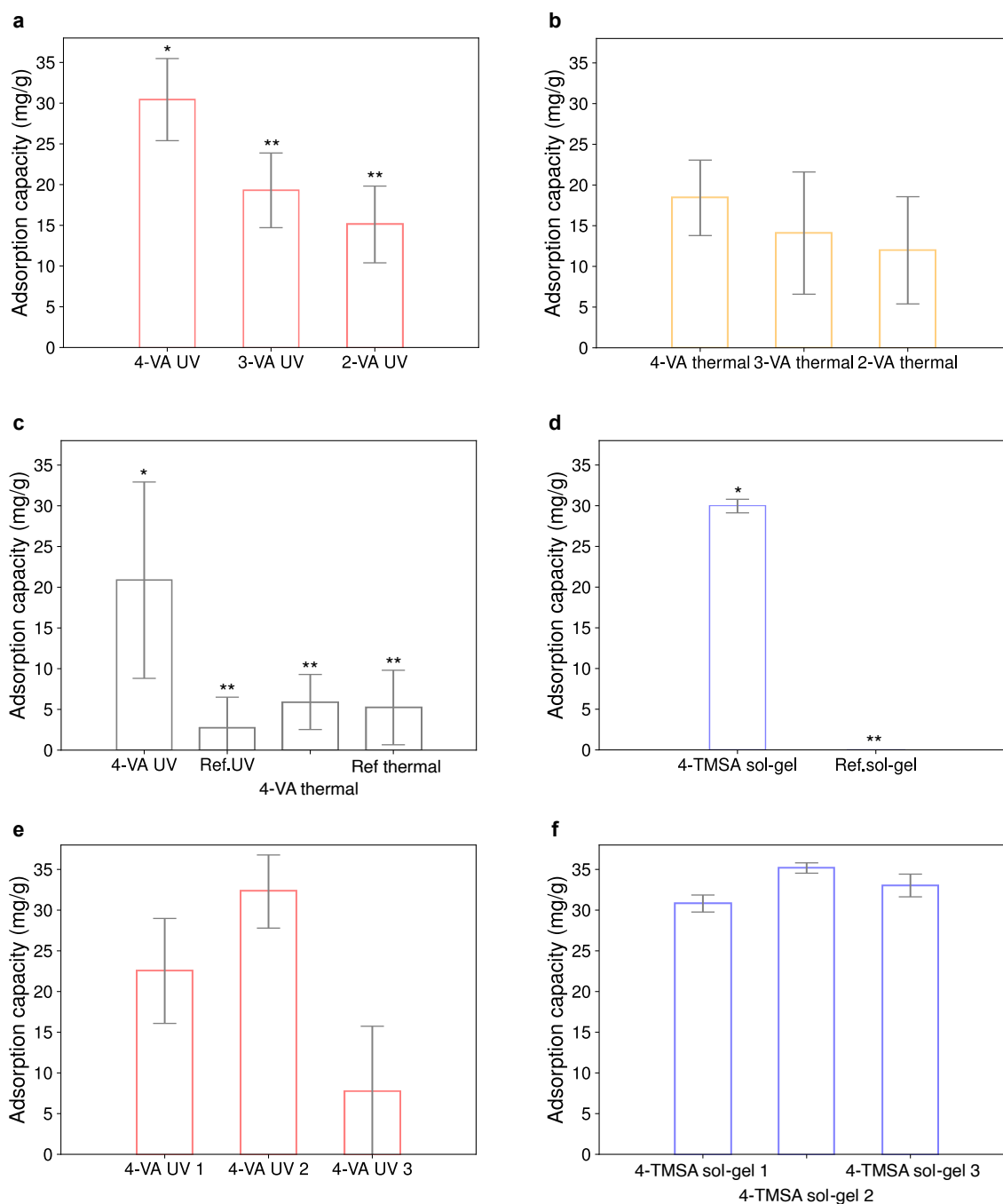


Figure 2: Adsorption capacity of the materials, reported in mg of hexanal adsorbed by g of material in acetonitrile (initial hexanal concentration: $2.37 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$).

a. Adsorption capacity of the materials obtained by UV-polymerization. The following molar ratio was used: 4/20/0.5/100 for the functional monomer/cross-linker/initiator/solvent.

b. Compared adsorption capacity of materials obtained by polymerization thermally initiated. The following molar ratio was used: 4/20/0.5/100 for the functional monomer/cross-linker/initiator/solvent.

c. Impact of the polymerization initiation on the adsorption capacity of the acrylate-based materials. The following molar ratio was used: 4/20/1/50 for the functional monomer/cross-linker/initiator/solvent.

d. Adsorption capacity of the sol-gel material, which significantly differ from that of its reference made without functional monomer.

e. Adsorption capacity of the 4-VA UV material obtained from 3 distinct synthesis batches. The following molar ratio was used: 4/20/1/50 for the functional monomer/cross-linker/initiator/solvent.

f. Adsorption capacity of the 4-TMSA sol-gel material obtained from 3 distinct synthesis batches.

In each case, the error bar represents the standard deviation obtained for three replicates. Statistical comparisons were done using one-way ANOVA test. Significantly different values ($p < 0.05$) are indicated by different number of stars.

Figure 2a and **Figure 2b** compares the influence of the nature of the functional monomer on the adsorption capacity of the resulting materials. **Figure 2a** focuses on the materials obtained by UV-polymerization. The material synthesized from the 4-vinylaniline monomer adsorbs a higher quantity of hexanal ($30 \pm 5 \text{ mg}\cdot\text{g}^{-1}$) compared to materials obtained with 3-vinylaniline and 2-vinylaniline ($19 \pm 5 \text{ mg}\cdot\text{g}^{-1}$ and $15 \pm 5 \text{ mg}\cdot\text{g}^{-1}$ respectively). The decrease in adsorption capacity can be attributed to the increasing steric effects depending on the position of the substituent groups on the aniline ring. The meta- and the ortho-substitutions lead to a reduced accessibility of hexanal to the primary amine function compared to the para-substitution. This is due to a closer proximity of the adsorption site to the polymeric chain. When radical polymerization was thermally initiated, a similar behavior was observed for the 3 materials (**Figure 2b**). Only the material obtained by UV-polymerization with the 4-vinylaniline adsorbed a significantly higher quantity of hexanal compared to the five other materials shown in **Figure 2a** and **Figure 2b**. **Figure 2c** compares the impact of the polymerization initiation route on the adsorption capacity of the materials synthesized using the 4-vinylaniline monomer. The material synthesized by UV-polymerization adsorbs about 3 times more compared to the material polymerized with temperature ($20 \pm 12 \text{ mg}\cdot\text{g}^{-1}$ and $6 \pm 3 \text{ mg}\cdot\text{g}^{-1}$, respectively). This could be explained by a difference in the distribution of the aniline groups at the surface of the material. With thermal initiation, adsorption sites could be entrapped within the bulk structure, thus being less accessible to hexanal. Piletska *et. al.* already suggested that photoinitiated polymerization produced materials with better performances¹⁴. Reference materials were systematically synthesized following the same experimental procedure but without using any monomer. The adsorption capacity of the 4-VA UV material significantly differs from the adsorption capacity of its reference. However, this is not the case for the material obtained by thermal initiation. It can also be noticed that both references adsorb a small quantity of hexanal

($3 \pm 3 \text{ mg}\cdot\text{g}^{-1}$ for the UV process reference material and $5 \pm 4 \text{ mg}\cdot\text{g}^{-1}$ for the thermal process reference material). This can be attributed to hydrogen bonding formed between hexanal and the ethylene glycol dimethacrylate cross-linker. **Figure 2a** and **Figure 2b** display the adsorption capacity for materials synthesized with a functional monomer/cross-linker/initiator/solvent molar ratio of 4/20/0.5/100 while materials reported in **Figure 2c** were obtained with a functional monomer/cross-linker/initiator/solvent molar ratio of 4/20/1/50. **Figure 2d** shows the adsorption capacity of the sol-gel material ($29 \pm 1 \text{ mg}\cdot\text{g}^{-1}$), which significantly differs from that of its reference. The standard deviation is drastically reduced using this synthesis route compared to the radical polymerization. This is in favor of a more homogeneous material, as already reported¹⁵. **Figure 2e** and **Figure 2f** compare the reproducibility of the performance of the materials when their synthesis was repeated several times. **Figure 2e** compares the adsorption capacity of the 4-VA material obtained by UV-polymerization for 3 distinct synthesis batches. The 3 materials show significant difference in their adsorption capacity (coefficient of variation of 58 %). On the contrary, the sol-gel material displayed only a very low variability (3 %) (**Figure 2f**). Therefore, although it seemed to be more difficult to achieve with radical polymerization, a very good control of the synthesis reproducibility was reached with the sol-gel process.

To conclude, 4-vinylaniline appeared to be the monomer providing the highest adsorption capacity to the material compared to 3-vinylaniline and 2-vinylaniline. A better adsorption capacity was obtained for materials synthesized by UV polymerization compared to those resulting from thermal polymerization. The sol-gel material showed both better adsorption capacity and higher reproducibility.

According to their adsorption capacity, the different materials could be classified in the following order: 4-TMSA sol-gel material > 4-VA UV > 4-VA thermal > 3-VA UV > 2-VA UV > 3-VA thermal > 2-VA thermal. For acrylate-based materials, those obtained using 3-vinylaniline and 2-vinylaniline as well as those resulting from thermal polymerization were no longer considered for further experiments since their adsorption capacities were lower than that of the 4-VA UV material and also more expensive to produce. Only the 4-VA UV and the 4-TMSA sol-gel materials were thus further investigated.

3.4. FLUORESCENCE PROPERTIES OF THE MATERIALS

In order to use the materials as fluorescent sensors for carbonyl monitoring, their fluorescence properties were investigated following the interaction with hexanal.

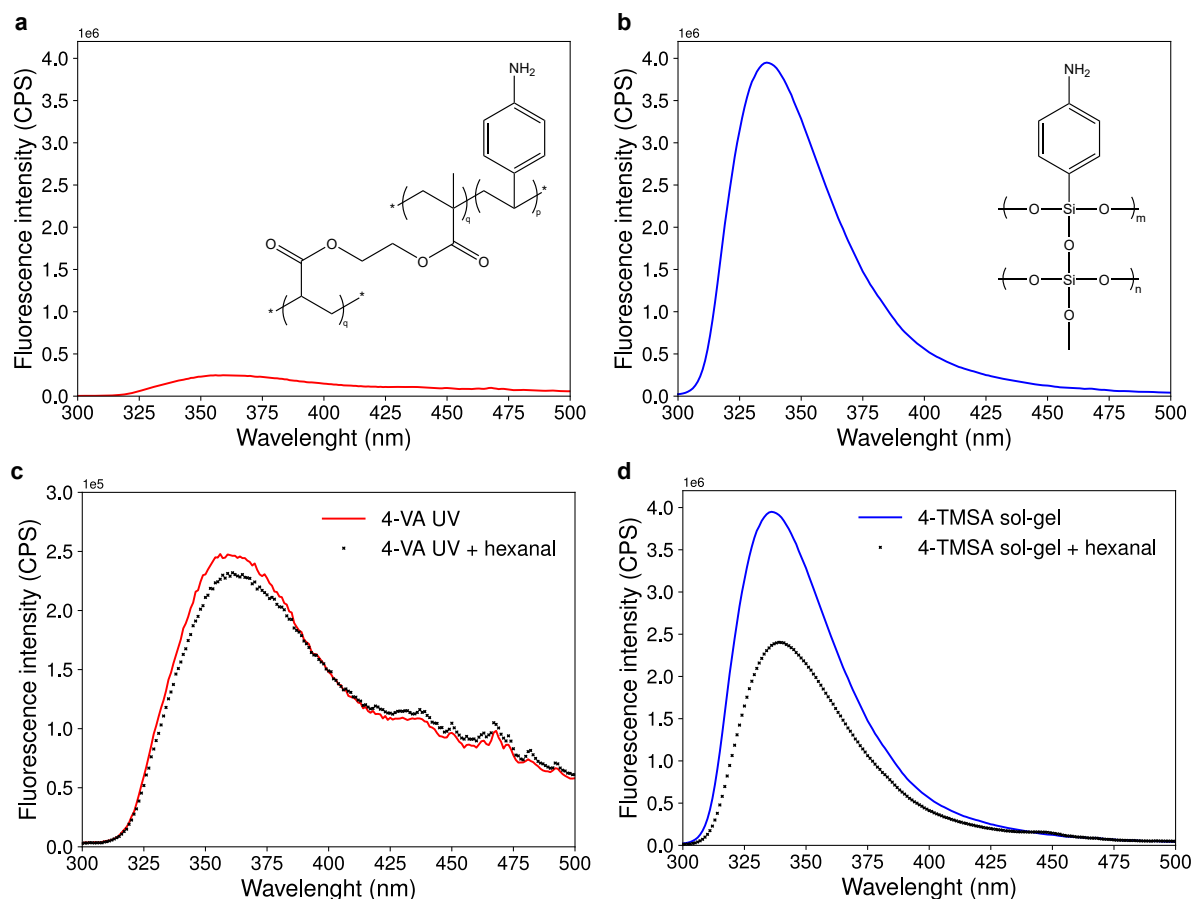


Figure 3: Fluorescence spectra of the materials and changes upon interaction with hexanal.

a. Fluorescence emission spectrum of a suspension of 10 mg of the acrylate-based material in ethanol. Excitation wavelength was set at 280 ± 2 nm. p and q are the molar ratio of functional monomer and cross-linker used for the synthesis of the acrylate-based materials, respectively.

b. Fluorescence emission spectrum of the silicate-based material (suspension of 10 mg of material in ethanol, excitation wavelength: 290 ± 2 nm). m and n are the molar ratio of functional monomer and cross-linker used for the synthesis of the functionalized sol-gel material, respectively.

c. Evolution of the fluorescence emission spectrum of the acrylate-based material suspended in ethanol before and after 15 minutes of contact with hexanal in the liquid phase at a concentration of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$.

d. Evolution of the fluorescence emission spectrum of the silicate-based material suspended in ethanol before and after 15 minutes of contact with hexanal in the liquid phase (concentration $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$).

As shown in **Figure 3a** and **Figure 3b**, displaying the fluorescence emission spectra of the acrylate-based and the silicate-based materials, both materials were fluorescent in the UV-region. The maximum of fluorescence intensity was at 360 nm and 337 nm for the acrylate-based and the silicate-based materials, respectively. The silicate-based material was 25 times more fluorescent compared to its acrylate counterpart. The corresponding reference materials, synthesized following the same experimental procedure but without any monomer, did not exhibit fluorescence. Therefore, only the functional monomers (4-vinylaniline for the acrylate-based material and 4-trimethoxysilylaniline for the silicate-based material) were responsible for the fluorescence of the materials.

Figure 3c and **Figure 3d** compare the fluorescence emission spectra of the acrylate-based and the silicate-based materials before and after interaction with hexanal in liquid phase. In both cases, a decrease in the fluorescence intensity of the material upon interaction with hexanal was observed. The percentage of fluorescence quenching by hexanal was 3.5 more important for the silicate-based material compared to the acrylate-based material (39.5 % and 11.5 % of fluorescence quenching respectively).

Both acrylate-based and silicate-based materials were thus fluorescent and showed significant changes in their fluorescence intensity after interaction with hexanal. These two materials could thus have been considered for carbonyl sensing. However, as the fluorescence of the material is quenched by carbonyls, the material with the highest fluorescence intensity will exhibit the highest limit of saturation and the lowest limit of detection. These are two important features for a sensor. The higher the initial fluorescence of the material is, the more carbonyls can be detected until fluorescence extinction. Moreover, the higher the percentage of fluorescence quenching is, the more likely it is that a small but significant decrease in fluorescence intensity would be detected. Therefore, the silicate-based material was unambiguously the best suited for the application targeted.

3.5. SPECIAL CASE OF MATERIALS WITH A HIGHER RATIO OF FUNCTIONAL MONOMER

To further improve the adsorption capacity and the fluorescence of the materials, the impact of the variation of a last parameter was studied. The two best candidates, the 4-VA UV and the 4-TMSA sol-gel materials, were synthesized in the previously optimized conditions but the amount of functional monomer was multiplied by 5 (see **Figure 4a**).

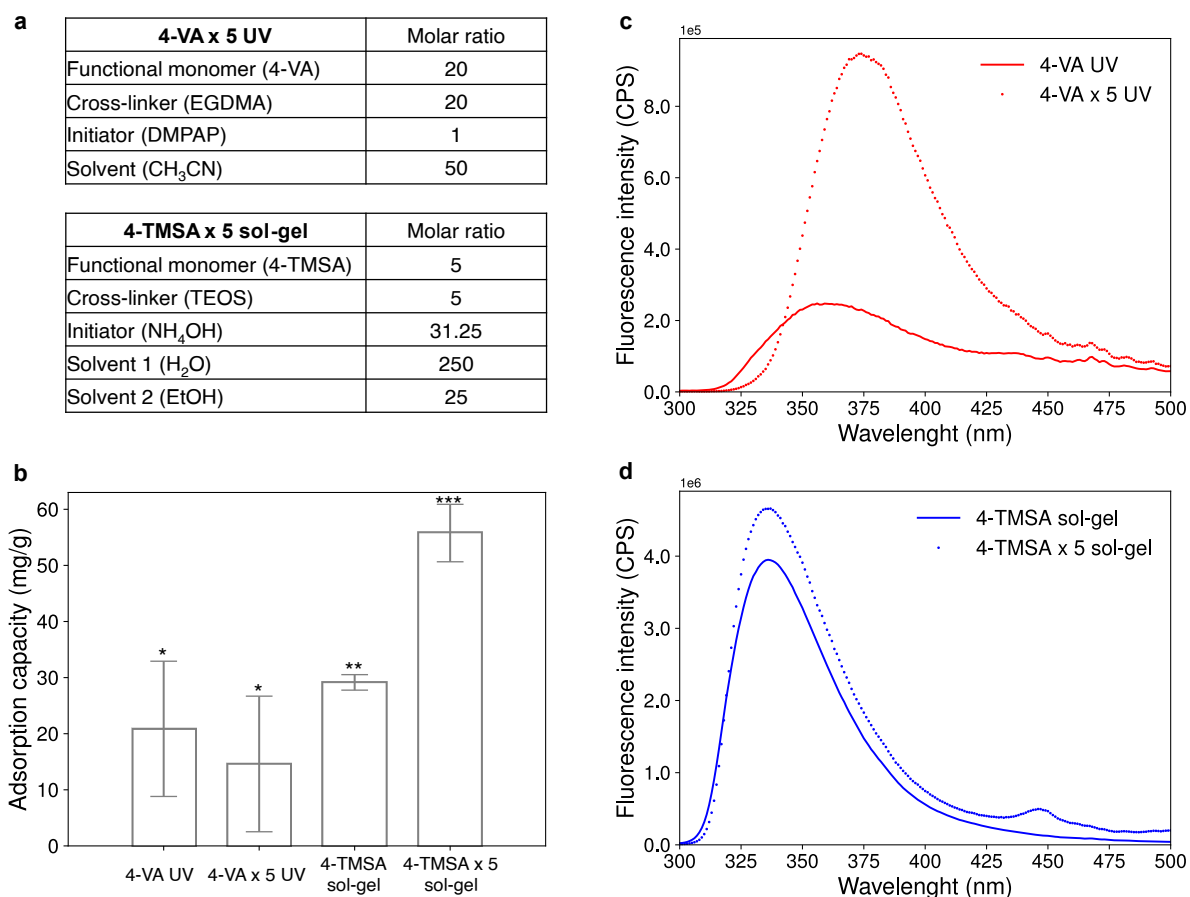


Figure 4: Adsorption capacity and fluorescence property of acrylate-based and silicate-based materials synthesized with a high ratio of functional monomer.

a. Molar ratios used to synthesize the acrylate-based and silicate-based material with higher amount of functional monomer.

b. Adsorption capacity of the acrylate-based and silicate-based materials synthesized with functional monomer/cross-linker molar ratio of 1/5 or 5/5. Significantly different values ($p < 0.05$) are indicated by different number of stars. Error bars represent the standard deviation obtained for three replicates. Statistical comparisons were done by ANOVA.

c. Fluorescence emission spectrum of the acrylate-based material initially synthesized (4-VA UV) and its analogue synthesized with 5 times more functional monomer (4-VA x 5 UV).

d. Fluorescence emission spectrum of the silicate-based material initially synthesized (4-TMSA sol-gel) and its analogue synthesized with 5 times more functional monomer (4-TMSA x 5 sol-gel).

Figure 4b compares the adsorption capacity of the acrylate-based and silicate-based materials synthesized with functional monomer/cross-linker molar ratio of 1/5 or 5/5. The adsorption capacity of the acrylate-based material did not significantly change with the

increase in the quantity of functional monomer. However, the adsorption capacity of the silicate-based material was almost doubled when the amount of functional monomer was multiplied by 5.

Figure 4c and **Figure 4d** display the influence of the increase in functional monomer on the fluorescence emission spectra of the acrylate-based and silicate-based materials. The fluorescence of the materials containing 5 times more functional monomer was enhanced in both cases. The fluorescence intensity was multiplied by 3 and 1.2 for the acrylate-based and the silicate-based material, respectively. Similarly to the initial materials 4-VA UV and 4-TMSA sol-gel, the fluorescence of the two materials synthesized with a higher amount of functional monomer was quenched upon interaction with hexanal (see **Figure S 1** in **Supporting Information**).

To sum up, the increase in the quantity of functional monomer during the synthesis had a strong impact on both the adsorption capacity and the fluorescence property of the silicate-based material and only on the fluorescence properties of the acrylate-based material. However, whatever the material (acrylate-based or silicate-based) neither the adsorption capacity nor the fluorescence intensity was increased by a factor 5, as it could have been expected by multiplying the amount of functional monomer by 5. Therefore, the increase in the functional monomer amount neither improve the adsorption capacity nor the fluorescence intensity to the expected level. In order to really increase the detection capacity of the material, both quantities of initiator and cross-linker should be optimized at the same time. However, the cost of the synthesis would consequently increase, leading to a compromise to be found between improved properties and cost of synthesis.

3.6. GLOBAL OVERALL COMPARISON OF THE ACRYLATE-BASED AND SILICATE-BASED MATERIALS

To put in a nutshell, the two types of materials fit the objective of developing a new material for carbonyl sensing using fluorescence. Both 4-VA UV and 4-TMSA sol-gel materials showed good adsorption capacity for hexanal as well as subsequent change in the fluorescence property upon interaction with hexanal. To sum up, these two materials were finally compared on specific points considering their synthesis, toxicity, and adsorption capacity as well as fluorescence property (see table in **Figure 5a**).

a

		Acrylate-based material	Silicate-based material
Synthesis	Functional monomer cost	39 €/g	33.5 €/g
	Synthesis route	Radical polymerization	Sol-gel synthesis
	Time of synthesis	24h	24h
	Energetic cost of the synthesis	Nitrogen bubbling UV lamp	Heating during 10 min at 60°C
	Yield of polymerization (%)	73 ± 16	85 ± 7
Characterisation	Toxicity of the monomers	H302, H312, H332 H315, H319, H335 H334 H351 H373	H302, H332 H312, H315, H319 H335
	Toxicity of the material	Not mutagenic (Ames' test)	Not mutagenic (Ames' test)
Properties	Adsorption capacity (mg/g) *	20.87 ± 12.05	29.95 ± 0.83
	Maximum fluorescence emission wavelength	366 nm (for $\lambda_{exc.} = 280$ nm)	337 nm (for $\lambda_{exc.} = 290$ nm)
	Fluorescence quenching by hexanal (%) **	11.5	39.5
	Fluorescence intensity at $\lambda_{em. max.}$ (CPS)	250 000	4 380 000
	Material ageing	Loss of adsorption capacity and fluorescence within 3 months	Adsorption capacity and fluorescence stable over 3 months

* For materials suspended in a solution of hexanal in ethanol at $2.37 \times 10^3 \text{ mol}\cdot\text{L}^{-1}$

** For materials suspended in a solution of hexanal in ethanol at $9.98 \times 10^3 \text{ mol}\cdot\text{L}^{-1}$ for 15 minutes

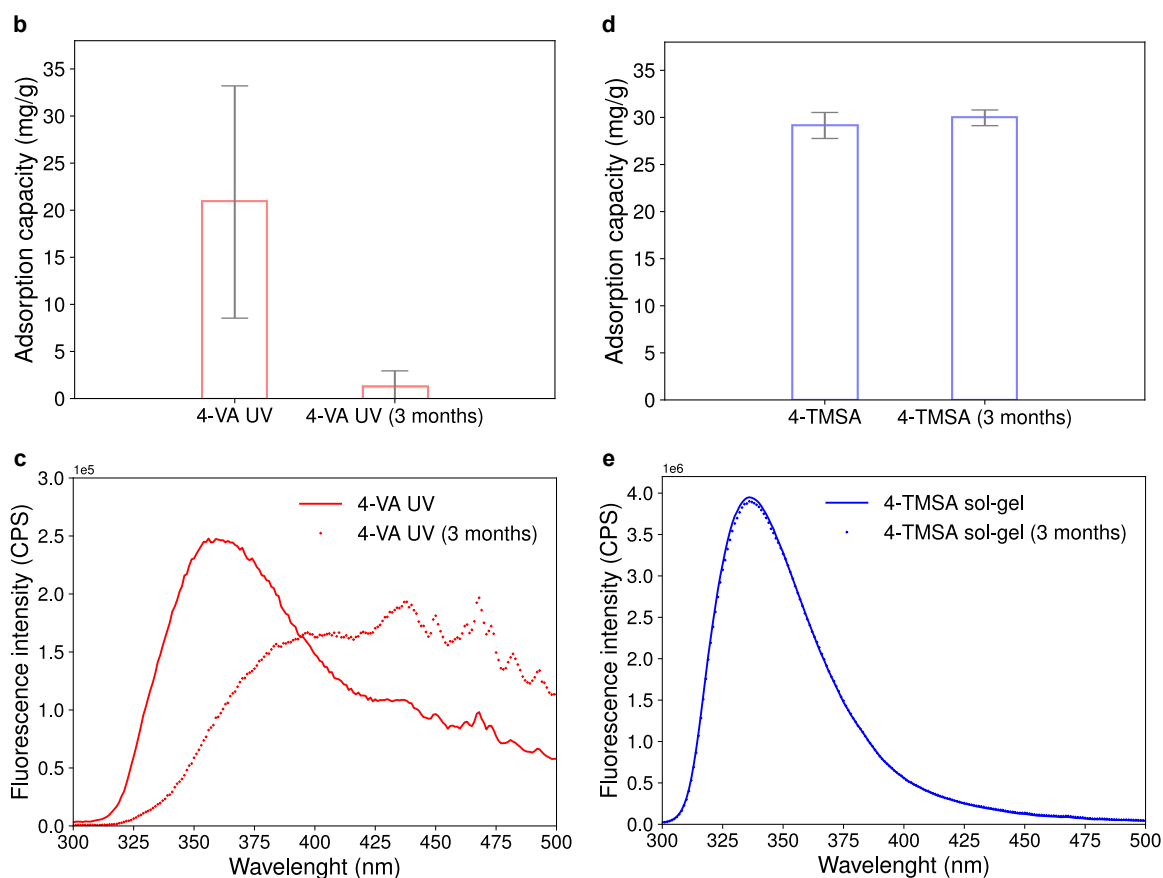


Figure 5: Global comparison of the acrylate-based and silicate-based materials.

a. Table comparing the acrylate-based and the silicate-based material regarding their synthesis, characterization and properties.

b. Evolution of the absorption capacity of the 4-VA UV material over 3 months. The material loss its adsorption capacity with time. Error bars represent the standard deviation obtained from 3 replicates.

c. Evolution of the fluorescence emission spectrum of the 4-VA UV material over 3 months. Modification of the fluorescence spectrum of the acrylate-based material and shift in its maximum emission wavelength suggest a modification of the material.

d. Evolution of the absorption capacity of the 4-TMSA sol-gel material over 3 months. The adsorption capacity of the material is maintained over time. Error bars represent the standard deviation obtained from 3 replicates.

e. Evolution of the fluorescence emission spectrum of the 4-TMSA sol-gel material over 3 months. The spectrum remains identical over time, suggesting a strong stability of the material over time.

Firstly, regarding the synthesis, both functional monomers (4-vinylaniline and 4-trimethoxysilylaniline) have a rather similar cost. However, acrylate-based materials are more widely used. Extensive choice in terms of monomers and synthesis conditions can be investigated. On the contrary, sol-gel polymerization is a less explored synthesis route. Commercial functional monomers are less numerous. The acrylate path thus gives more opportunities for potential further improvements of the structure of the material. However, the reproducibility of the synthesis considering yield of polymerization was better controlled with the sol-gel process compared to the radical polymerization.

Secondly, in terms of toxicity, solvents (ethanol and water) and reactants employed for the sol-gel synthesis (4-TMSA monomer and TEOS) have a much reduced toxicity level compared to those used for the synthesis of acrylate-based materials (especially 4-VA monomer, EGDMA and acetonitrile)⁹. In this example, the acrylate functional monomer may cause allergic reactions if inhaled, is suspected of causing cancer and may cause damage to organs depending on the exposure, while this is not the case for the silica functional monomer. However, both materials were not mutagenic for bacteria according to the Ames' test. The toxicity of the monomers was thus reduced once the covalent bonds were created with the crosslinker during polymerization.

Thirdly, in terms of functional properties for the desired application, the acrylate-based material seemed less performant compared to its silicate-based counterpart. On the first hand, the acrylate-based material showed lower adsorption capacity and with a higher variability. On the second hand, it exhibited by far a lower fluorescence intensity and thus a lower percentage of fluorescence quenching upon interaction with hexanal. Moreover, the acrylate-based material lost its adsorption capacity and fluorescence properties within 3 months (see **Figure**

5b and **Figure 5c**) while these characteristics remained stable over the same time for the silicate-based material (see **Figure 5d** and **Figure 5e**). The material internal structure of the acrylate-based material may have collapsed with time, rendering the adsorption sites inaccessible to hexanal molecules. However, the modification of the fluorescence spectrum of the acrylate-based material and the shift in its maximum emission wavelength, as presented in **Figure 5c**, also suggest changes in the near environment of fluorescent aniline groups or chemical transformations of the anilines. Such ageing of the material was therefore prohibitive for the use of the 4-VA UV material.

Finally, the sol-gel material seemed thus the most appropriate for the targeted application of carbonyl sensing using fluorescence.

3.7. CHARACTERISATION OF THE MATERIALS

In order to better understand the differences in the performance of the materials, especially the acrylate-based ones, their chemical composition as well as their morphology were compared.

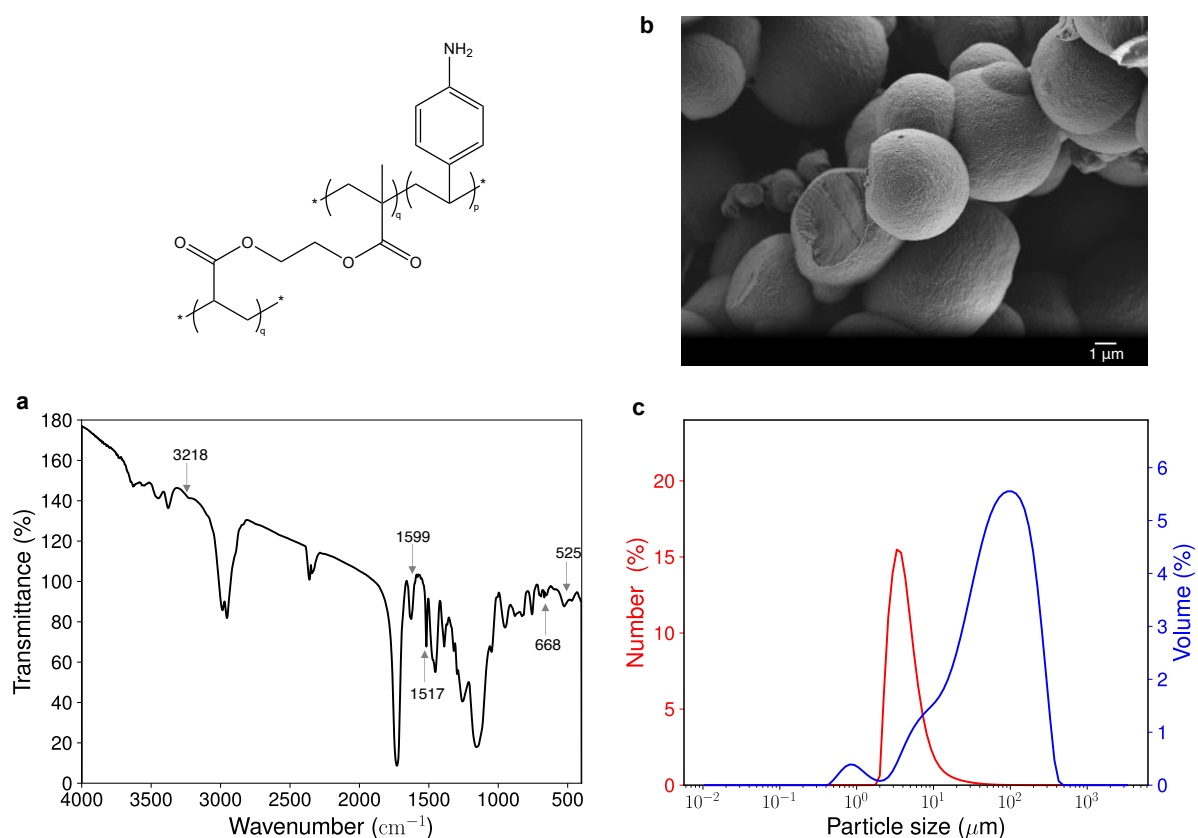


Figure 6: Characterization of the acrylate-based material.

p and q are the molar ratio of functional monomer and cross-linker used for the synthesis of the acrylate-based materials, respectively.

a. FTIR absorbance spectrum of the material. The arrows represent the absorbance peaks of characteristic functional groups composing the material (amine (3218 cm^{-1} , 1599 cm^{-1}) and aromatic ring (1517 cm^{-1} , 668 cm^{-1} , 525 cm^{-1}) proving the successful incorporation of the functional monomer into the material.

b. SEM image of the material showing aggregates of spherical particles (magnification 5000).

c. Particle size distribution of the material obtained by laser diffraction. The bimodal volumetric distribution reveals the presence of two populations having sizes of about $1\text{ }\mu\text{m}$ and $100\text{ }\mu\text{m}$. The number distribution indicates that most of the particles have a size around $4\text{ }\mu\text{m}$.

Figure 6a shows the Fourier-transform infrared (FTIR) spectrum of the acrylate-based material. $\nu_{\text{el}}(\text{C}=\text{C})$ at 1517 cm^{-1} corresponds to elongation vibrations of the aromatic ring of the aniline group. The peak at 668 cm^{-1} is attributed to an out of plane deformation of the ($\text{C}=\text{C}-\text{H}$) bounds of the aromatic ring and the one at 525 cm^{-1} is attributed to an out of plane aromatic ring deformation vibration of an 1,4-disubstituted benzene. These peaks clearly differentiate the spectrum of the material from that of its reference, obtained by polymerization of EGDMA only (see **Figure S 2a** in **Supporting Information**). This proves the successful incorporation of the aromatic ring of the 4-vinylaniline functional monomer into the polymer material. Less intense peaks $\nu_{\text{sym}}(\text{N}-\text{H})$ at 3218 cm^{-1} and $\delta_{\text{s}}(\text{N}-\text{H})$ at 1599 cm^{-1} suggest the presence of primary and aromatic amine. However, the resolution of the spectrum did not allow to determine if some secondary amines appeared during polymerization, which would have suggested the participation of the aniline group to the radical polymerization. The presence of water in the material can be noted by the presence of the following characteristic peaks: $\nu_{\text{sym}}(\text{O}-\text{H})$ at 3374 cm^{-1} and $\delta(\text{H}-\text{O}-\text{H})$ at 1625 cm^{-1} .

The morphology of the 4-VA UV material, as revealed by scanning electron microscopy (SEM), shows aggregates of spherical particles, having sizes in the micrometric range (see **Figure 6b**). The shape of the particle is consistent with the type of polymerization used, as spherical particles are often obtained with precipitation polymerization¹⁶. Particles are better defined and less agglomerated in the material compared to the reference material (see **Figure S 2b** in **Supporting Information**), suggesting a higher level of structuration of the material due to the addition of the functional monomer.

This is in good agreement with quantitative analysis performed by laser light diffraction, which also shows that the 4-VA UV material has a particle size in the micrometric range (**Figure 6c**). The bimodal volumetric distribution indeed revealed the presence of two populations having a near diameter of about 1 μm and 100 μm , respectively. However, the number distribution indicated that most of the particles have a size around 4 μm . The reference material exhibited a similar particle size distribution (see **Figure S 2c Supporting Information**). Most particles of the reference material have also a size around 4 μm . However, the volumetric distribution reveals the presence of bigger aggregates having sizes about 10 and 400 μm . This suggests a 10-fold shrinkage of the volume of the aggregates when the functional monomer is included within the material structure.

4-TMSA sol-gel material was deeply characterized in a previous work and showed a morphology comparable to that of its acrylate-based counterpart. FTIR analysis confirmed the successful incorporation of the 4-trimethoxysilylaniline monomer within the polymeric structure, resulting in a material made of irregular-shaped particles having sizes of about 1 μm and 100 μm , with most of the particles having a size around 1 μm . The important differences in terms of adsorption capacity and fluorescence property between the 4-VA UV material and the 4-TMSA sol-gel material thus cannot be explained by their morphological difference. Simon and Spivak suggested that the 4-vinylaniline functional monomer could inhibit the radical polymerization process¹⁷. This would result in a reduced amount of aniline available in the final material, which is responsible for both hexanal adsorption and fluorescence and could thus explain the lower performance of the acrylate-based material.

3.7.1. Difference between 4-VA UV and 4-VA thermal materials

No significant difference in the FTIR spectra of the acrylate-based materials obtained by UV-polymerization and by thermal initiation were noticed (see **Figure S 3a in Supporting Information**). Thus, the difference in adsorption capacity could not be attributed to any structural difference between the two materials. SEM images of the 4-VA thermal material showed similar morphology to the 4-VA UV material, with aggregates made of spherical particles. However, more spherical particles separated from each other were observed (see **Figure S 3b in Supporting Information**). Laser diffraction analysis revealed similar volume and number distributions for both photoinitiated and thermally initiated materials (see **Figure S 3c in Supporting Information**). Only the volume distribution of the particles obtained from 4-VA

thermal aggregates slightly differs from those of the 4-VA UV with more aggregates of 5 μm . These small morphological differences are in favor of the hypothesis that some adsorption sites are entrapped within the bulk structure of the 4-VA thermal material, leading to a less quantity of hexanal adsorbed by this material.

3.7.2. Difference between 4-VA UV et 4-VA x 5 UV

No significant morphological difference was noted when the molar ratio of the functional monomer was increased, except that the volume distribution obtained by laser diffraction revealed the presence of bigger aggregates of 200 μm for the-VA x 5 UV material instead of 100 μm for the-VA UV material.

3.7.3. Difference between 4-VA UV freshly synthesized and 4-VA UV after 3 months of ageing

No significant difference in the FTIR spectra of the acrylate-based materials freshly synthesized and after 3 months of ageing were noticed (see **Figure S 4a in Supporting Information**). It is possible that a chemical or physical degradation of the material occurred. However, the characteristic peaks attributed to the primary amine were already low in intensity compared to those from the α,β -unsaturated ester from the EGDMA crosslinker ($\nu(\text{C}=\text{O})$ at 1723 cm^{-1} , and $\nu(\text{C}-\text{O})$ at 1282 cm^{-1} and at 1144 cm^{-1}) in the freshly synthesized material, due to the ratio used for the synthesis of the material (five times more cross-linker than functional monomer). A degradation of this functional group with time was thus hardly noticeable on FTIR spectra. A collapse of the internal structure of the material could have explained the loss of absorption and fluorescence properties over time. This hypothesis was supported by laser diffraction analysis that revealed a shrinkage of the particle size over time, from 4 μm for freshly synthesized material to 0.6 μm for aged material (after 3 months) (see **Figure S 4b in Supporting Information**). Aniline groups entrapped within the material structure are thus probably less accessible to hexanal for adsorption at the material surface, leading to a decrease in the adsorption capacity of the material with time. Similarly, remaining aniline groups could not be excited if entrapped within the polymeric structure, resulting in a decrease in the fluorescence of the material.

4. CONCLUSION

In the aim of designing a carbonyl sensor based on fluorescence, many material synthesis parameters were studied, including polymerization type, radical polymerization initiation method, the nature of the functional monomer and the molar ratio of the different reactants. They significantly impacted the performance of the materials in terms of yield of polymerization, adsorption capacity and fluorescence property. It was shown that a reduced solvent volume favored material precipitation. Materials based on 4-vinylaniline monomer showed a better adsorption capacity compared to those based on 3-vinylaniline and 2-vinylaniline. Materials obtained by UV-polymerization displayed a better adsorption capacity compared to those obtained by thermally initiated polymerization. However, compared to all acrylate-based materials tested, the silicate-based material was considered the best choice for the desired application. The good reproducibility of the synthesis, its higher adsorption capacity and its higher fluorescence intensity were important parameters that supported such selection. The stability of its adsorption capacity and fluorescence over time was also decisive.

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SUPPORTING INFORMATION

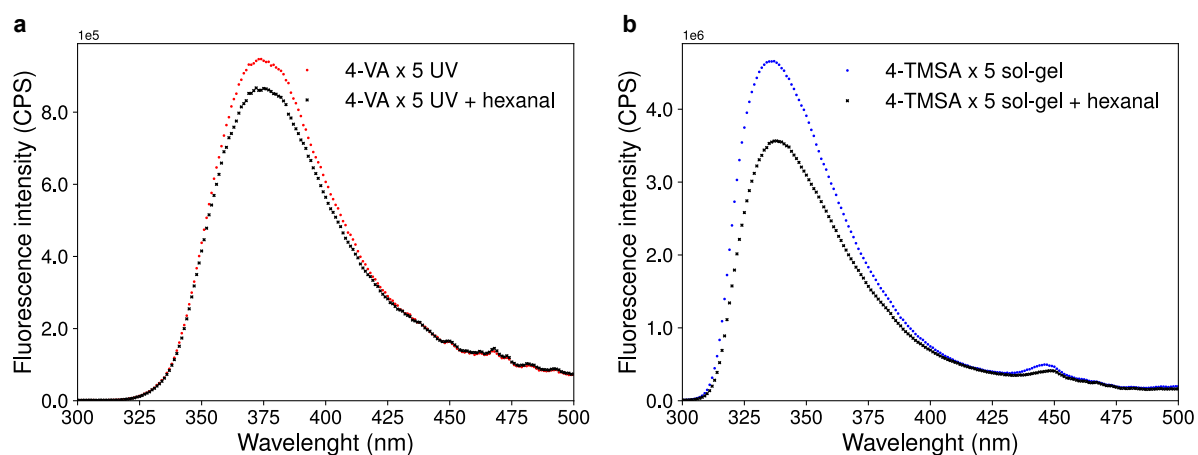


Figure S 1: Evolution of the fluorescence emission spectrum of the acrylate-based and silicate-based material synthesized with higher ratio of functional monomer in presence of hexanal.

a. Evolution of the fluorescence emission spectrum of the 4-VA x 5 UV material suspended in ethanol before and after 15 minutes of contact with hexanal in the liquid phase (concentration $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$).

b. Evolution of the fluorescence emission spectrum of the 4-TMSA x 5 sol-gel material suspended in ethanol before and after 15 minutes of contact with hexanal in the liquid phase (concentration $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$).

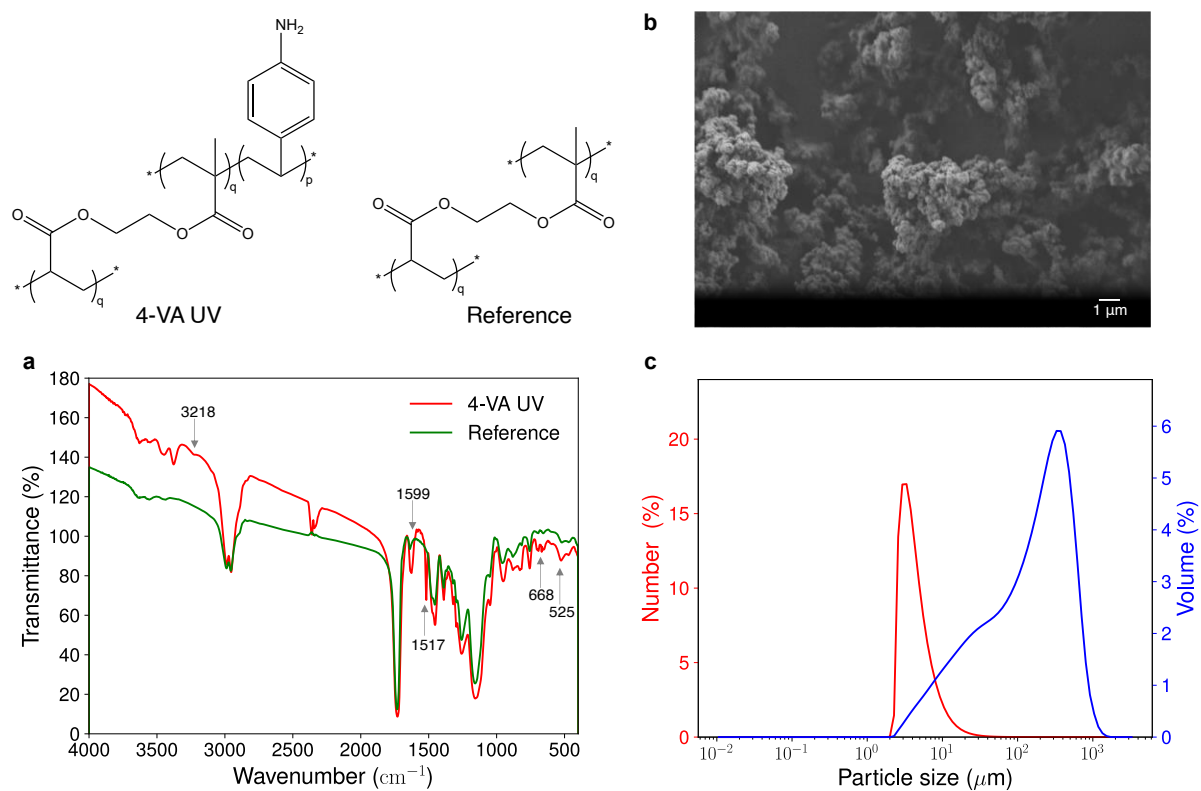


Figure S 2: Chemical and physical characterization of the reference material.

p and q are the molar ratio of functional monomer and cross-linker used for the synthesis of the acrylate-based materials, respectively.

a. Compared FTIR absorbance spectra of the 4-VA UV material and its reference. Characteristic peaks (amine (3218 cm⁻¹, 1599 cm⁻¹) and aromatic ring (1517 cm⁻¹, 668 cm⁻¹, 525 cm⁻¹) in the 4-VA UV material differentiate the two spectra, suggesting the successful incorporation of the functional monomer into the material.

b. SEM image (magnification 5000) of the reference material showing aggregates of spherical particles less defined and more agglomerated compared to the 4-VA UV material.

c. Particle size distribution of the reference material obtained by laser diffraction. The bimodal volumetric distribution reveals the presence of two populations having sizes of about 1 μm and 400 μm. The number distribution indicates that most of the particles have a size around 4 μm.

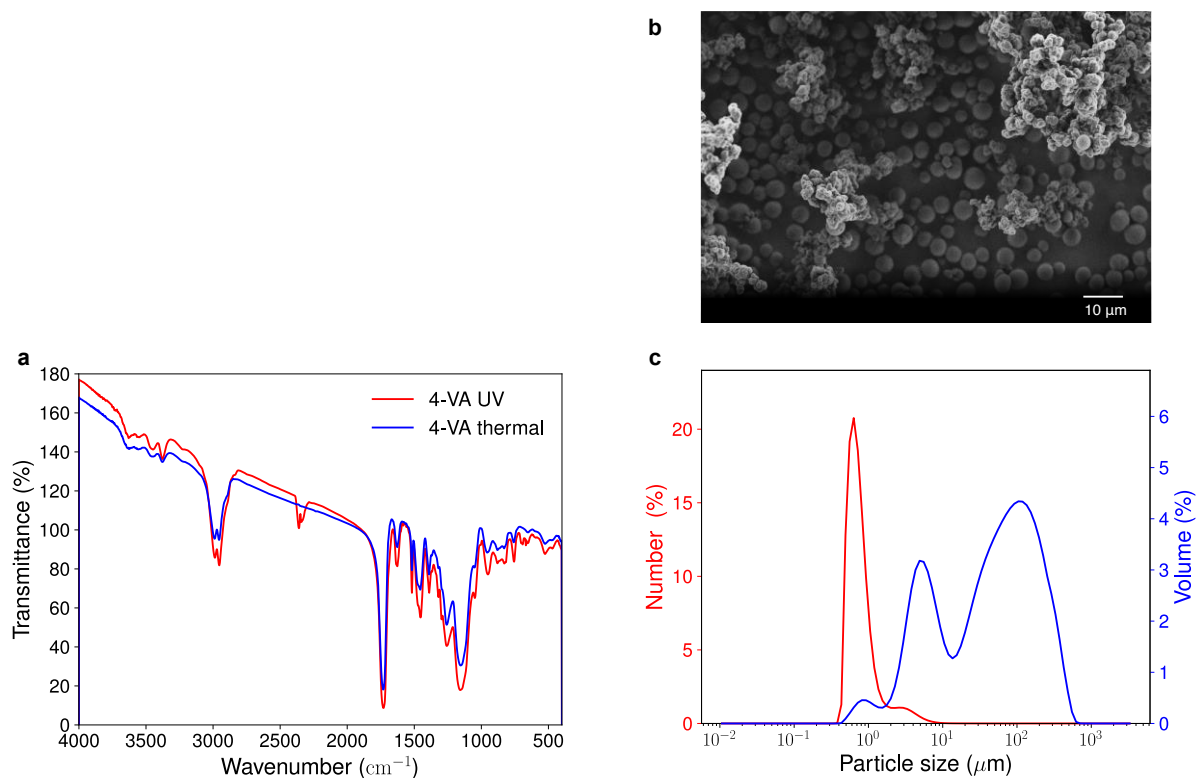


Figure S 3: Chemical and physical characterization of the acrylate-based material synthesized by radical polymerization thermally initiated.

a. Compared FTIR absorbance spectra of the 4-VA UV and 4-VA thermal material, showing no significant differences.

b. SEM image (magnification 1000) of the 4-VA thermal material showing aggregates of spherical particles with more stand-alone particles compared to the 4-VA UV material.

c. Particle size distribution of the 4-VA thermal material obtained by laser diffraction. The volumetric distribution reveals the presence of three populations having sizes of about 1 μm , 5 μm and 100 μm . The number distribution indicates that most of the particles have a size around 4 μm .

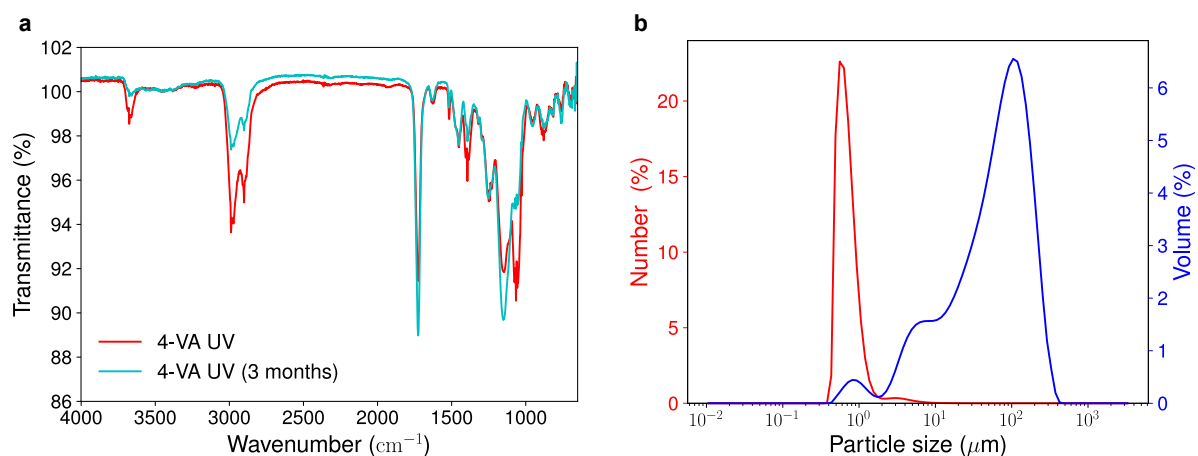


Figure S 4: Chemical and physical characterization of the acrylate-based material 3 months old.

a. Compared FTIR absorbance spectra of the 4-VA UV freshly synthesized and 4-VA UV 3 months old material, showing no significant differences. The resolution of the spectra may not be good enough to distinguish small changes.

b. Particle size distribution of the 3 months old material obtained by laser diffraction. The volumetric distribution reveals the presence of three populations having sizes of about 1 μm , 5 μm and 100 μm . The number distribution indicates that most of the particles have a size around 0.6 μm . A 6-fold shrinkage of the particle size is observed, suggesting a collapse of the structure of the material with time.

Chap. 2. CHARACTERIZATION AND PERFORMANCE OF THE SOL-GEL MATERIAL FOR THE DETECTION OF CARBONYLS IN LIQUID MEDIA

At the end of the last chapter, one of the materials synthesized by the sol-gel process was particularly promising. Compared to its acrylate-based counterparts, its synthesis was more reproducible, its hexanal adsorption capacity was higher, it exhibited higher fluorescence intensity and these performances were stable over time. This sol-gel material was thus more deeply investigated. In this chapter, it was thoroughly chemically and physically characterized. Its adsorption capacity towards both aldehydes (hexanal) and ketones (hexan-2-one) was assessed, when exposed to carbonyls in a liquid modelling medium (ethanol). Its fluorescence properties upon interaction with these carbonyls in ethanol was also explored. Finally, preliminary results of fluorescence experiments in a food simulating medium (ethanol/water mixture) and in a real medium (vegetable oil) are presented.

Intelligent material to track carbonyls.

ABSTRACT

Carbonyl compounds are ubiquitous quality trackers. They provide information about air and water pollution level. They traduce degradation of food products. They are biomarkers for medical diagnosis. Their fast detection and easy quantification, with more user-friendly sensors, are thus highly relevant. The synthesis, characterization and performance assessment of a new intelligent material, based on aniline fluorescence, to monitor carbonyls in real-time is reported. A cost-effective synthesis, using a straightforward sol-gel process, led to a non-toxic silica-based material with high porosity, which can be used with almost no sample preparation. It exhibits a rapid (< 1 min) fluorescence decrease upon interaction with carbonyls. The limit of detection is as low as $\sim 5 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ for hexanal, while fluorescence extinction occurs at much higher concentrations ($5 \times 10^{-1} \text{ mol}\cdot\text{L}^{-1}$), providing the sensor a very broad range of detection. Real-time monitoring is possible since the fluorescence loss correlates with the concentration of carbonyl. The performance was validated in modelling as well as in real media, making this sensor promising for a wide range of applications.

1. INTRODUCTION

The monitoring of carbonyl compounds in liquid or gas phase is valuable in many fields. In fact, carbonyls are good quality trackers of environmental, food as well as biological samples. Firstly, acetaldehyde, benzaldehyde and formaldehyde are common air pollutants. Their quantification, especially in indoor places, is particularly required. These substances can be responsible for allergic reactions. They can be toxic and even carcinogenic in some cases at very low levels¹⁻⁵. As an example, the Office of Environmental Health Hazard Assessment has set the maximum exposure to acetaldehyde level at 140 ppb in air for chronic exposure⁶. Moreover, carbonyls can be involved in chemical reactions that produce harmful pollutants (*e.g.*, nitrogen oxides NO_x)⁷. Secondly, the release of carbonyl compounds by food products and beverages can indicate lipid and protein oxidation. It is thus linked to a deterioration of both nutritional and organoleptic properties⁸⁻¹⁰. 2,4-decadienal, considered as responsible for rancid odors, and 1-octen-3-one, associated with fishy notes¹¹, are generated by lipid oxidation. They can be detected by human nose at trace levels (from ppb to ppm, depending on the olfactive molecule and the matrix)¹²⁻¹⁴. Some specific carbonyl compounds can thus be used as quality trackers

for food and beverages. Acetaldehyde is a marker of fruit ripeness¹⁵, and a furfural concentration increase in beers is responsible for their flavor depreciation¹⁶. Moreover, carbonyls tracking has already been proposed to assess the authenticity of liquors and thus struggle with frauds¹⁷. Thirdly, oxidative stress in cells and tissues can generate carbonyls byproducts that can damage DNA and disturb proteins and enzymes functions^{18–22}. Biomarkers such as acrolein can be used to detect some diseases such as diabetes, neurodegenerative conditions (*e.g.*, Parkinson's disease and Alzheimer's disease), and even cancers in biological samples (*e.g.*, like in urine)^{22–24}. As an example, ppb levels of hexanal, heptanal, malonaldehyde as well as 2-butanone and 3-hydroxy-2-butanone were identified as biomarkers of lung cancer in exhaled breath of sick patients^{25–27}.

Classical methods for carbonyl detection include sensory evaluation and separation techniques, such as gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled to different types of detectors (*e.g.*, ultraviolet, fluorescence, mass spectrometry (MS))^{5,20,22,28,29}. A standard method used by the food industry to titrate aldehydes produced by lipid oxidation in foods is the thiobarbituric acid reactive substances (TBARS) test³⁰. It relies on UV-visible spectrophotometry. Although these common methods provide accurate results with rather low limits of detection (*e.g.*, down to $\mu\text{g}\cdot\text{L}^{-1}$ for HPLC-MS³¹), they are usually expensive, dedicated to highly trained persons and time-consuming for sample preparation and analysis. Therefore, there is a need for rapid, simple-to-use, reliable and portable solutions allowing on-site carbonyl monitoring in real time with low level of detection. Intelligent materials could offer a solution of choice. In the field of food, they are referred to as systems that can monitor the quality of a product and provide information to its user (EC 450/2009).

Different sensing techniques have been proposed in the last years, based on either optical (colorimetric, fluorometric and chemiluminescent sensors), mass-sensitive or electrochemical properties. Optical sensors rely on color changes (*e.g.*, Tollen's reaction)³² or modulation of fluorescence emission (intensity or wavelength) (*e.g.*, aldimine or ketimine condensation; reaction with hydrazines)^{1,17,18,24}. They can be coupled with the use of cameras, UV-visible spectrophotometers or spectrofluorometers. Among optical sensors, fluorometric chemical sensors usually provide faster response and higher sensitivity compared to their colorimetric counterparts and do not require any reference or blank^{23,33,34}. Some of them

exhibit fluorescence-intensity enhancement, also called “turn-on” sensors whereas other are referred to as “turn-off” emission sensors when the fluorescence is quenched by the interaction with the targeted analyte. More recently, there was an increasing research interest for sensors based on metal-organic frameworks (MOFs) and quantum dots (QDs) in order to achieve better selectivity and sensitivity³⁵. However, most of these sensors are not cheap and easy-to-use solutions for monitoring carbonyls in real time. Moreover, many do not propose safe alternatives for food or medical applications with toxicological issues. Materials synthesized by the sol-gel process have thus gained increasing interest in the past decades because of their low toxicity, high mechanical resistance, high porosity, green synthesis at room temperature, thus making them attractive for many applications including food and medical purposes^{36,37}.

In this paper, we report on the synthesis, characterization, and application of a new intelligent material for the monitoring of carbonyl compounds by fluorescence spectroscopy. 4-trimethoxysilylaniline was chosen as the functional monomer, in which the aniline group was intended to both interact with the carbonyl compounds and serve as the fluorescent core. The material was synthesized by the sol-gel process. Its adsorption capacity as well as the modification of its fluorescence property were determined after exposure to hexanal and hexan-2-one in ethanol. Fluorescence of the material after interaction with hexanal was also recorded in an ethanol/water mixture and in rapeseed oil to test the sensor in a food and cosmetic simulating medium as well as in real conditions of use. The successful design of such a sol-gel material constitutes a novel breakthrough for the fast sensing of carbonyl compounds.

2. MATERIALS AND METHODS

2.1. CHEMICALS

Tetraethylorthosilicate (TEOS, 99 %, CAS number 78-10-4), ammonium hydroxide (NH₄OH, 30 %, CAS number 1336-21-6), ethyl acetate (99.8 %, CAS number 141-78-6), ethanol (EtOH, 96 %, CAS number 64-17-5, used for synthesis) and ethanol for UV-spectroscopy Uvasol® (EtOH, 99,9 %, CAS number 64-17-5, used for chromatographic and fluorescence experiments) were purchased from Sigma Aldrich (France), 4-trimethoxysilylaniline (4-TMSA, 97 %, CAS number 33976-43-1) from abcr (Germany), hexanal (98 %, CAS number 66-25-1)

from Alfa Aesar (France), hexan-2-one (98 %, CAS number 591-78-6) from Acros Organics. Water used was deionized from a MilliQ RG, Millipore apparatus.

2.2. SYNTHESIS OF THE MATERIAL

The material was synthesized using a sol-gel process. 4-trimethoxysilylaniline (4-TMSA) was firstly dissolved in an ethanol/water mixture (1/10 molar ratio) at 60 °C for 10 min. Tetraethyl orthosilicate (TEOS) and ammonium hydroxide (30 % NH₄OH) were then added, using the following molar ratio: 1/5/31.25: 4-TMSA/TEOS/NH₄OH. The mixture was stirred at 500 rpm for 24 h at 22 °C. Then, the solvent was evaporated, and the resulting powder was washed 3 times with an ethanol/water mixture (1/10 molar ratio) by Büchner filtration using a 0.2 µm pore membrane. The material was dried 12 h at 60 °C, crushed and stored at 4 °C in the dark before use. A control material was synthesized following the same procedure but without 4-trimethoxysilylaniline, to prove that the adsorption of carbonyl compounds and material fluorescence were strictly due to the presence of the monomer within the material structure.

2.3. MATERIAL CHARACTERIZATION

2.3.1. Evaluation of the toxicity of the material

The mutagenicity of the material was evaluated by the Ames' test performed on two bacterial strains: *Salmonella typhimurium* TA98 and TA100 according to Maron and Ames³⁸ and the OECD guideline no. 471 for the testing of chemicals³⁹. More details about the experimental procedure can be found in previous work³⁷. Each test was performed 3 times.

2.3.2. Solid-state nuclear magnetic resonance (NMR) spectroscopy

Solid-state NMR was used to determine the effect of carbonyl adsorption on the material at the molecular scale. A suspension of 10 mg of material in 2 mL of a solution of hexanal or hexan-2-one in ethanol (concentration: $4.99 \times 10^{-1} \text{ mol} \cdot \text{L}^{-1}$) was agitated at 50 rpm for 24 h at 22 °C. Samples were centrifugated at 10 000 rpm for 10 min. Supernatant was removed and the remaining powder was dried at 60 °C for 12 h. NMR spectra of the material after adsorption of hexanal or hexan-2-one were recorded and compared to the initial spectrum of the material before carbonyl adsorption. ¹H solid-state MAS NMR spectra were acquired at room temperature on an AVANCE 750 MHz wide bore spectrometer (Bruker™) operating at a frequency of 750.12 MHz. Solid-state NMR spectra were deconvoluted using Dmfit software.

2.3.3. Thermal gravimetric analysis (TGA)

The decomposition of the organic part of the material was analyzed by TGA using a Discovery TGA apparatus (TA Instruments). Around 15 mg of material was heated from room temperature to 800 °C with a heating rate of 20 °C·min⁻¹ under air flow (25 mL·min⁻¹).

2.3.4. Elemental analysis (EA)

Elemental analysis was performed using a CHNS Thermo Scientific Electron Flash EA 1112 analyzer. The experiment was performed 4 times.

2.3.5. Fourier transform infrared spectroscopy (FTIR)

FTIR absorbance spectra of the material and its corresponding blank (obtained without functional monomer) were recorded at 25 °C using a Bruker Equinox 55 spectrometer equipped with a global source, DTGS detector and KBr splitter. Before analysis, around 1 mg of dried powder material was dispersed into KBr to obtain a 200 mg pellet. The absorbance spectra were recorded in transmission mode using a spectral range from 400 cm⁻¹ to 4000 cm⁻¹ with a resolution of 4 cm⁻¹ and 100 scans per analysis, using a 200 mg KBr pellet as reference.

2.3.6. Water vapor adsorption

The water vapor adsorption and desorption isotherms of the material were determined at 25 °C using a home-made McBain thermobalance equipped with a quartz spring under secondary vacuum following the procedure detailed in the previous work⁴⁰. Around 10 mg of the material was first outgassed in situ at 80 °C under secondary vacuum (10⁻⁴ hPa) for 16 h before measurement. The variation of the sample mass was followed during water vapor adsorption or desorption by measuring the proportional elongation of the spring with a CCD camera. For each adsorption or desorption step, the mass variation was recorded up to equilibrium under controlled water vapor pressure, which varied between 10⁻⁴ to 30 hPa.

2.3.7. Scanning electron microscopy (SEM)

The surface morphology of the material was characterized by SEM using a JEOL 6700F microscope equipped with a cold FEG electron source operating at 1 kV and both Everhart-Thornley and in-lense detectors. Prior to imaging, the powder was deposited on a carbon tape and the surface of the material was coated with carbon to prevent charging effects. Images were acquired at various magnifications between 100 and 20 000.

2.3.8. Laser diffraction

The particle size distribution was determined by laser diffraction using a Malvern MasterSizer 3000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK). A suspension of the material in ethanol was added to the circulating water until the obscuration reached 8 % to 10 %. Stirring speed was set to 1200 rpm. The refractive index of the particles (1.457) and their absorption index (0.01) were based on the silica model. Refractive index of the dispersing phase was set at 1.36. The Mie diffusion model was used to calculate the particle size. The temperature was maintained at 22 °C during all the experiment. Measurements were performed 3 times.

2.3.9. Nitrogen adsorption

The porosity of the material was determined by nitrogen adsorption at -195.75 °C (77.4 K) with an ASAP 2020 Micromeritics apparatus. Prior to analysis, the sample was outgassed at 80 °C under secondary vacuum (10^{-4} hPa) for 16 h. The specific surface area of the material was calculated using the Brunauer-Emmett-Teller (BET) method for relative pressure between 0.06 and 0.25. The total pore volume was determined at a relative pressure of 0.99. The respective microporous and mesoporous volume and the pore size distribution were estimated by using the SAIEUS software⁴¹, which gave the cumulative pore volume and the pore volume distribution as a function of the pore width by simulation using the “zeolithe H form 77 K NLDFT cylindrical model”.

2.4. ADSORPTION CAPACITY OF CARBONYLS BY THE MATERIAL

Adsorption capacity of the material after exposure to carbonyls was determined by gas chromatography coupled to mass spectroscopy. Adsorption capacity is given as the mass of carbonyls adsorbed by 1 g of material (under air, at 22 °C and 30 % of relative humidity).

A suspension of 10 mg of the material in 2 mL of a solution of hexanal or hexan-2-one in ethanol was stirred at 50 rpm at 22 °C for 24 h to reach the equilibrium. Theoretical concentrations of hexanal or hexan-2-one in ethanol ranged from $2.95 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ (or 295 $\text{mg}\cdot\text{L}^{-1}$) to $25.4 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ (or 2551 $\text{mg}\cdot\text{L}^{-1}$). Samples were then centrifugated at 10 000 rpm for 10 min. 1 μL of the diluted liquid supernatant was injected thanks to an autosampler (Gerstel) on a 7890 A gas chromatograph (Agilent Technologies) equipped with a capillary column (30 m x 0.32 mm internal diameter, 0.5 μm thickness) coated with a DB-5MS

stationary phase (Agilent J&W). Injections were performed in split mode (split ratio 50:1). The injection temperature was 250 °C. Helium was the carrier gas, at a flow rate of 1.4 mL·min⁻¹. Mass spectrometer 5975C (Agilent) was used as detector (electronic impact 70 eV, ionization source at 230 °C, transmission line at 240 °C). The oven temperature was programmed from 50 °C to 65 °C at a first rate of 3 °C·min⁻¹, and from 65 °C to 250 °C at a second rate of 30 °C·min⁻¹. The oven temperature was finally maintained at 250 °C for 5 min. A solvent delay of 3 min was applied. Each adsorption experiment was carried out 4 times.

The adsorption capacity of the material for carbonyls was calculated as follows:

$$\text{Adsorption capacity} = \frac{(C_i - C_f) \times V_{liq.}}{m} \quad (1)$$

with C_i the initial concentration of hexanal or hexan-2-one in the liquid phase in contact with the material, C_f the concentration of hexanal or hexan-2-one remaining in the liquid phase at equilibrium, $V_{liq.}$ the volume of the hexanal or hexan-2-one solution and m the mass of the material.

2.5. FLUORESCENCE OF THE MATERIAL

Fluorescence emission spectrum of the synthesized material was recorded before and after its exposure to carbonyls. Both contact time and concentration of carbonyl compounds were studied. A spectrofluorometer Fluoromax4® (Horiba) was used with the software Fluorescence® and SUPRASIL® quartz cell (Hellma) of 10 mm light path. Excitation wavelength was 290 ± 2 nm. Fluorescence emission spectrum was recorded between 300 nm and 500 nm, using a 2 nm bandpass and a step interval of 1 nm. The temperature was kept at 22 °C during the experiment. Each experiment was performed 3 times.

2.5.1. Effect of the contact time

A suspension of 10 mg of the material in 2 mL of ethanol was stirred for 10 s and the fluorescence emission spectrum of the material was recorded. Then, 500 µL of a solution of hexanal or hexan-2-one in ethanol were added to obtain a concentration in carbonyl in the measurement cell of 9.98×10^{-3} mol·L⁻¹. The cell was then continuously stirred at 80 rpm, and the fluorescence emission spectrum of the material was recorded after 30 s, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 2 h, 4 h and 7 h. Each experiment was performed 3 times.

The kinetics of fluorescence decrease was modelled by the pseudo-first order Lagergren model and the pseudo-second order model:

$$\ln\left(\frac{Q_e - Q_t}{Q_e}\right) = -kt \quad (2)$$

$$\frac{t}{Q_t} = \frac{1}{kQ_e^2} + \frac{t}{Q_e} \quad (3)$$

with Q_e the amount of carbonyl adsorbed at equilibrium ($\text{mg}\cdot\text{g}^{-1}$), Q_t the amount of carbonyl adsorbed at time t ($\text{mg}\cdot\text{g}^{-1}$), k the rate constant (min^{-1}) and t the time (min).

As the fluorescence loss of the material correlates with the quantity of adsorbed carbonyl, the value of Q was given by:

$$Q = \frac{I_0 - I_t}{I_0} \quad (4)$$

with I_0 the intensity of fluorescence at 337 nm of the material in ethanol before addition of carbonyl and I_t its intensity after exposure to hexanal or hexan-2-one during a period of time t .

2.5.2. Effect of the carbonyl concentration

A suspension of 10 mg of the material in 1.8 mL of ethanol was placed in a quartz cell. After 10 s of stirring, the fluorescence spectrum of the material was recorded. A known amount of hexanal or hexan-2-one in ethanol was periodically added to increase the carbonyl concentration in the solution from $0 \text{ mol}\cdot\text{L}^{-1}$ to $0.54 \text{ mol}\cdot\text{L}^{-1}$. The solution was stirred at 80 rpm for 10 min to reach the equilibrium after each addition and the fluorescence emission spectrum of the material was recorded. Each experiment was performed 3 times.

The percentage of fluorescence quenching, Q_c , was determined as follows:

$$Q_c = \frac{I_0 - I_c}{I_0} \times 100 \quad (5)$$

with I_0 the intensity of fluorescence at 337 nm of the material in ethanol before carbonyl addition and I_c its intensity after 10 min of contact with hexanal or hexan-2-one at a concentration c .

The limit of detection (LOD) and the sensitivity were determined by considering the first linear part of the curve, representing the percentage of fluorescence loss as a function of the

carbonyl concentration. *LOD* for 10 mg of material was calculated according to the following equation⁴²:

$$LOD = \frac{b+3\times\sigma_b}{a} \quad (6)$$

with *a* the slope and *b* the intercept of the linear regression of the percentage of fluorescence loss versus the carbonyl concentration and σ_b the standard deviation for *b*. The sensitivity was given by the slope *a* of this linear regression. Saturation limit was determined when the fluorescence loss reached 100 %.

2.5.3. Effect of the liquid phase

In addition to the evaluation of the performance of the material in ethanol, other dispersing phases were tested in order to test the possibility to use the material for carbonyl sensing in real samples. The fluorescence emission spectrum of the material in suspension in 2 other liquid phases was recorded. Firstly, ethanol was replaced by an ethanol/water (90/10, v/v) mixture to simulate hydrophilic food or cosmetic samples. Secondly, the material was introduced in rapeseed oil. In both cases, 10 mg of material were placed in 2 mL of the liquid phase. After 30 s of vortex stirring, the fluorescence emission spectrum of the material was recorded. Then, 500 μ L of a solution of hexanal in the dispersing phase (either ethanol/water mixture or rapeseed oil) were added to obtain a concentration of hexanal in the measurement cell of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. The cell was stirred for 30 min before recording the fluorescence emission spectrum of the material. For the experiment in rapeseed oil, the emission was recorded between 300 nm and 800 nm. The emission spectrum of the rapeseed oil was also measured. The fluorescence intensity of the oil at the maximum emission wavelength of the material (551 nm) was weak and considered as negligible. Each experiment was performed 3 times.

2.6. STATISTICS

Statistical analysis (ANOVA with Tukey's post hoc analysis) was performed using XLSTAT (Addinsoft) software. Significance was established at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. SYNTHESIS AND CHARACTERISATION OF THE MATERIAL

3.1.1. Development of the intelligent material

The design of an intelligent material capable of detecting aldehydes and ketones must meet several requirements. First, the material should be able to interact with carbonyls. Second, upon interaction, its spectroscopic properties should change. Such a material was successfully synthesized by the sol-gel process. Primary amine function reactivity, fluorescence quantum yield of the fluorescent core, cost, toxicity, and commercial availability were first considered to select the appropriate monomer. The chosen functional monomer (4-trimethoxysilylaniline) possessed both 1) an amino group for chemical reaction with carbonyl compounds and 2) an aniline core playing the role of the fluorescent probe and acting as a transmitter upon reaction with carbonyls (see **Figure 1**). This functional monomer was copolymerized with tetraethyl orthosilicate (TEOS) crosslinker, providing mechanical stability to the material. Yield of reaction was 85 % in average.

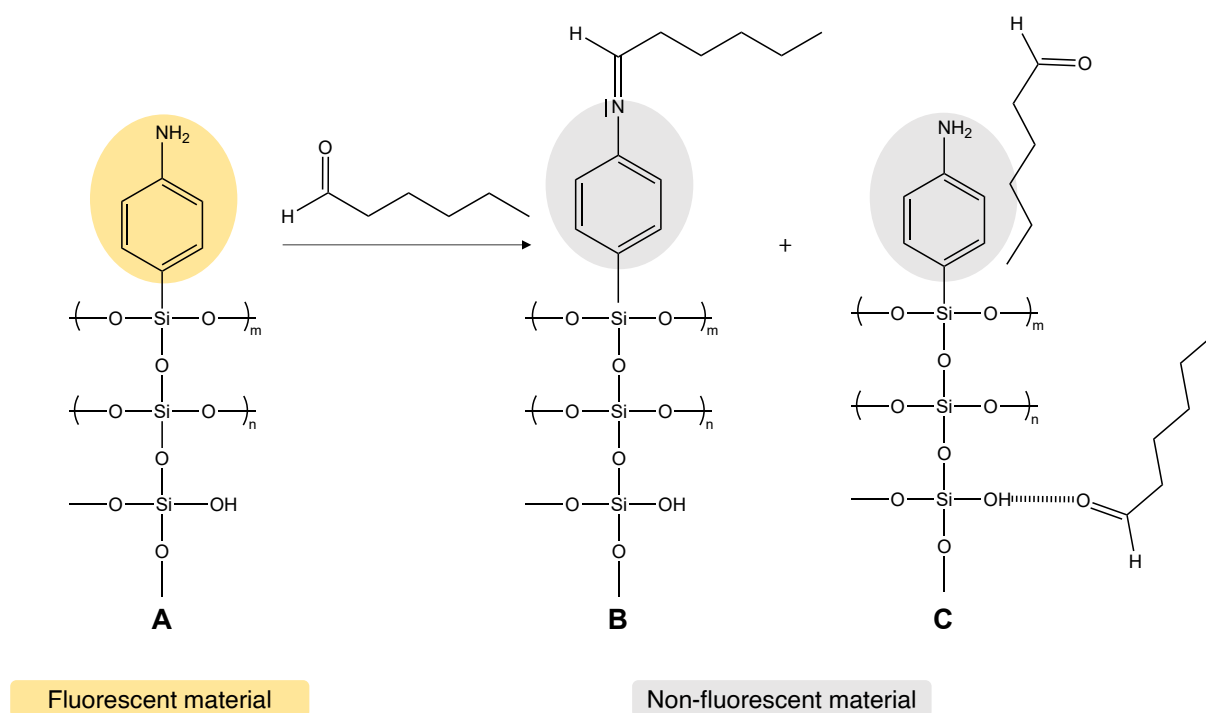


Figure 1: Structure, principle, and interaction mechanisms of the synthesized intelligent material.

A depicts the structure of the intelligent material synthesized by polymerization of 4-trimethoxysilylaniline functional monomer and tetraethyl orthosilicate crosslinker. Once polymerization is achieved, the amino group is available for reaction with carbonyls and the aniline is responsible for material

fluorescence. **(B)** The material can chemically react with carbonyls (example given with hexanal) through the formation of an imine bond after nucleophilic addition of carbonyls. **(C)** The material can also physically interact with carbonyls (example given with hexanal) either by hydrogen bonds between carbonyl groups and uncondensed silanols or via hydrophobic interactions between the hydrocarbon chain and the aromatic ring of aniline. **(B and C)** The fluorescence intensity of the initial material is quenched by interaction with carbonyls. m and n are the molar ratio of functional monomer and cross-linker used for the synthesis of the functionalized sol-gel material, respectively.

It is very likely that carbonyls could have been both chemically and physically adsorbed by the material. As displayed in **Figure 1**, chemical reaction occurs through the primary amine functions located at the surface of the material, which can form imine functions after nucleophilic addition of carbonyls. Physical interactions could be either hydrogen bonds between carbonyl groups and uncondensed silanols or hydrophobic interactions between the hydrocarbon chain and the aromatic ring of anilines (logP (hexanal): 1.8, logP (hexan-2-one): 1.4). From solid state nuclear magnetic resonance (NMR) spectroscopy analysis, the ^1H spectrum of the material (**Figure S 1 in Supporting Information**) showed a broad and intense peak at 4.3 ppm, attributed to an overlap between the amine of the material and water⁴³. ^1H DP/MAS spectra being quantitative, the comparison of the deconvoluted ^1H NMR spectra of the material before and after hexanal adsorption showed a decrease in the amine peak area of 26 %. In the case of hexan-2-one, it was only 5 %. The presence of peaks in the region between 0 and 2 ppm, corresponding to the aliphatic part of the carbonyls, highlighted interactions of the material with hexanal and hexan-2-one. The comparison of spectra obtained after adsorption of hexanal and hexan-2-one revealed broader peaks in the case of hexanal compared to hexan-2-one. This suggests that multiples interactions occurred with hexanal, compared to hexan-2-one. It can thus be assumed that hexanal both chemically reacts with the amino group of the material and is also physically adsorbed, while hexan-2-one is preferentially physically adsorbed on the surface of the material.

The safety of the resulting material was also assessed based on the EU regulation on food contact material ((EU) 10/2011), which is particularly restrictive. The resulting material powder was not mutagenic for bacteria according to the Ames' test performed on two bacterial strains: *Salmonella typhimurium* TA98 and TA100 neither when it was freshly synthesized nor after 19 months of storage at 4 °C in the dark.

3.1.2. Chemical characterization

The amount of the organic part of the material was determined by thermogravimetric (TGA) and elemental analysis (EA). TGA showed two distinct mass losses (see **Figure S 2** in **Supporting Information**). The first one (5.9 % mass loss) occurred between 25 °C and 150 °C and was attributed to the desorption of physisorbed water. The second one, between 150 °C and 800 °C, corresponded to the degradation of the aniline functional group, from the 4-trimethoxysilylaniline monomer. This last organic moiety loss accounted for 19.8 % of the material mass. On a dry basis, the ratio of mineral moiety was therefore 74.3 %. This result was in good agreement with initial stoichiometric coefficients of the reactants used for the synthesis. The material was obtained from the copolymerization of 1 organic functional monomer carrying the aniline group (4-trimethoxysilylaniline) for 5 mineral crosslinkers (tetraethyl orthosilicate).

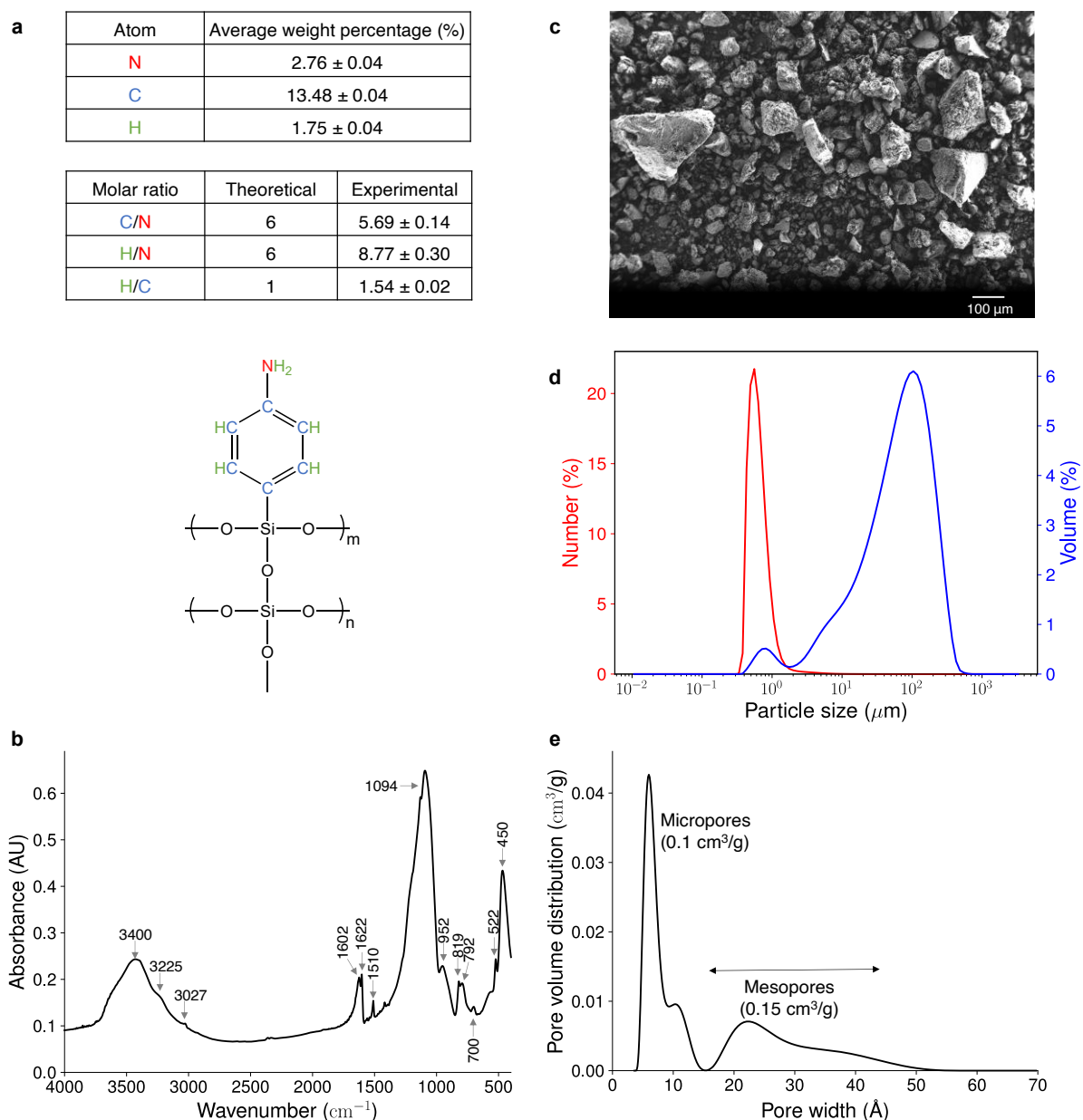


Figure 2: Chemical and physical characterization of the material.

a. Elemental analysis of the material. The experimental C/N molar ratio is in line with the theoretical one, while the H/N and the H/C ratios imply the presence of 2 extra hydrogens, due to physisorbed water in the material. m and n are the molar ratio of functional monomer and cross-linker used for the synthesis of the functionalized sol-gel material, respectively.

b. FTIR absorbance spectrum of the material, proving the successful incorporation of the functional monomer into the material. The arrows represent the absorbance peaks of characteristic chemical groups of the material (amine, aromatic ring, siloxane and water).

c. SEM image of the material showing aggregates of irregular-shaped particles (magnification 102).

d. Particle size distribution of the material obtained by laser diffraction. The bimodal volume distribution reveals the presence of two populations having size of about 1 μm and 100 μm , while the monomodal number distribution indicates that most of the particles have a size around 1 μm .

e. Pore volume distribution of the material obtained by the software SAIEUS, showing a bimodal pore size distribution. The total volume of the pores is estimated to be 0.25 $\text{cm}^3\cdot\text{g}^{-1}$, resulting from the presence of micropores (volume: 0.15 $\text{cm}^3\cdot\text{g}^{-1}$, size: 0.5 – 1 nm) and mesopores (volume: 0.10 $\text{cm}^3\cdot\text{g}^{-1}$, size: 2 – 5 nm).

The mass percentage of nitrogen, carbon and hydrogen in the material, as provided by elemental analysis, is displayed in **Figure 2a**. Nitrogen represented 2.76 ± 0.04 % of the mass of the material, carbon 13.48 ± 0.18 % and hydrogen 1.75 ± 0.04 %. This gave a C/N molar ratio of 5.69 ± 0.14 . It was in line with the theoretical ratio, as it was expected that one aniline functional group was introduced in one repeating unit of the polymer, resulting in 1 nitrogen for 6 carbon atoms. The H/N ratio of 8.77 ± 0.30 revealed an excess of 2 hydrogens in a repeating unit. This was attributed to the presence of physically adsorbed water in the material, as previously suggested by TGA.

The successful incorporation of the 4-trimethoxysilylaniline functional monomer into the polymer material after its hydrolysis and condensation with the tetraethyl orthosilicate (TEOS) crosslinker was further supported by Fourier-transform infrared (FTIR) spectroscopy analysis (**Figure 2b**). The peaks at 3225 and 1602 cm^{-1} were attributed to the N-H bond stretching and bending of the primary amine, respectively. The peaks at 3027 and 1510 cm^{-1} corresponded to the stretching vibrations of the C-H and C=C bonds of the aromatic ring of the aniline group, respectively. The peak at 700 cm^{-1} could be attributed to an out of plane deformation of the C-H bond of the aromatic ring and the one at 522 cm^{-1} was attributed to an out of plane aromatic ring deformation vibration of the 1,4-disubstituted benzene. As expected, all these peaks did not appear in the spectrum of the blank material obtained by polymerization of TEOS only. The vibration peaks $\nu_{\text{as}}(\text{Si-O-Si})$ at 1094 cm^{-1} , $\nu_{\text{sym}}(\text{Si-O-Si})$ at 819 cm^{-1} , $\delta(\text{Si-O-Si})$ at 792 cm^{-1} and $\nu_{\text{sym}}(\text{Si-O-Si})$ at 450 cm^{-1} were characteristic of the mineral silica structure of the material coming from the condensation of silanol groups. The band $\nu_{\text{as}}(\text{Si-OH})$ at 952 cm^{-1} referred to remaining silanol groups that did not condense during the polymerization process. The presence of water in the material was also noted by the following characteristic peaks: $\nu(\text{O-H})$ at 3400 cm^{-1} and $\delta(\text{H-O-H})$ at 1622 cm^{-1} .

The water vapor adsorption isotherm (see **Figure S 3** in **Supporting Information**) gave a more accurate understanding of the water vapor uptake capacity of the material. At 10 hPa of water vapor pressure, which corresponds to 30 % relative humidity (usual conditions of use), the mass uptake of the material was about 8 %. The maximum amount of water adsorbed by the material corresponded to 25 % of its dry mass. This is in favor of a hydrophilic character of the material. Such behavior can be explained by the hygroscopic nature of silica materials due to the presence of uncondensed silanol groups capable of forming hydrogen bonds with water, as displayed in **Figure 1**. Moreover, the adsorption of water vapor showed a strong hysteresis, which is due to the presence of mesopores in the material.

3.1.3. Physical characterization

The morphology of the material, as revealed by SEM, showed aggregates of irregular-shaped particles having sizes in the micrometric range, with only a few particles having length about 50 μm (see **Figure 2c**). This was in good agreement with quantitative analysis by laser diffraction, which also displayed particle size in the micrometric range (**Figure 2d**). The bimodal volume distribution indeed revealed the presence of two populations having sizes of about 1 μm and 100 μm . However, the number distribution unambiguously indicated that most of the particles have a size around 1 μm .

The material porosity was assessed by nitrogen physisorption. A type Ib isotherm was observed (see **Figure S 4** in **Supporting Information**). This revealed the presence of both micropores (< 2 nm) and mesopores (2-50 nm)⁴⁴. Such isotherm is characterized by a steep increase of the adsorbed amount of N_2 in narrow micropores at $p/p^\circ < 0.15$ followed by a more gradual N_2 uptake in narrow mesopores (in this case 2-5 nm) for p/p° between 0.15 and 0.25. Adsorption-desorption phenomenon was reversible, with only a slight hysteresis loop from 0.15 to 0.99 p/p° . The specific surface area of the material, calculated using the Brunauer-Emmett-Teller (BET) method for relative pressure between 0.06 and 0.25, was estimated to be 452 $\text{m}^2\cdot\text{g}^{-1}$. Although the internal porosity was not shaped by the use of a surfactant prior to polymerization, as it is the case with traditional silica-based MCM-41 materials, the material studied nevertheless had half of the porosity expected from such a MCM-41 material⁴⁵. Therefore, this could be considered as a rather high porosity according to the simple synthesis route implemented. The total volume of the pores determined from the amount of N_2 adsorbed at $p/p^\circ = 0.99$ is equal to 0.25 $\text{cm}^3\cdot\text{g}^{-1}$ and the microporous volume is 0.15 $\text{cm}^3\cdot\text{g}^{-1}$, as estimated

by the t-plot method. The difference between these values allowed to estimate the mesoporous volume of the material ($0.1 \text{ cm}^3 \cdot \text{g}^{-1}$). Because of the presence of both micropores and mesopores, the classical Barrett-Joyner-Halenda (BJH) method could not be used to determine the size and distribution of the pores in the whole pore size range. The software SAIEUS⁴¹, was thus used instead to plot the pore size distribution (see **Figure 2e**). It described a bimodal distribution of pores sizes, with micropores of 0.5 nm (with a shoulder at 1 nm) and a broader peak for mesopores with pore size between 2 and 5 nm. The important porosity of the material is an asset because only a small amount of powder is thus further required to obtain satisfying adsorption results.

3.2. DETECTION AND QUANTIFICATION OF CARBONYLS

3.2.1. Adsorption capacity of the material

The adsorption capacity referred to as the mass of carbonyl adsorbed by 1 g of material (under air, at 22 °C, 30 % of relative humidity) was measured as shown in **Figure 3a**. **Figure 3b** displays the adsorption capacity of hexanal by the material after 24 h of contact in liquid phase (ethanol). The quantity of hexanal adsorbed by the material increased with the hexanal concentration. For an initial concentration of hexanal in ethanol of $25 \text{ mmol} \cdot \text{L}^{-1}$, the adsorption capacity of the material was $83 \pm 8 \text{ mg} \cdot \text{g}^{-1}$. This represents a filling of 41 % of the porosity of the material with hexanal. The saturation of the material did not seem to be reached and yet its adsorption capacity was already 1.5 times higher than the maximum amount of hexanal adsorbed by a reference silica-gel (which adsorbed 546 mg of hexanal with 10 g of silica material)⁴⁶. This result is promising for the use of the material as a portable sensor because only a small amount of the material is required for the adsorption of high quantities of carbonyls. A similar behavior was observed with hexan-2-one, but the variability was more important compared to hexanal. Nevertheless, these results allow to conclude that the material adsorbs both aldehydes and ketones.

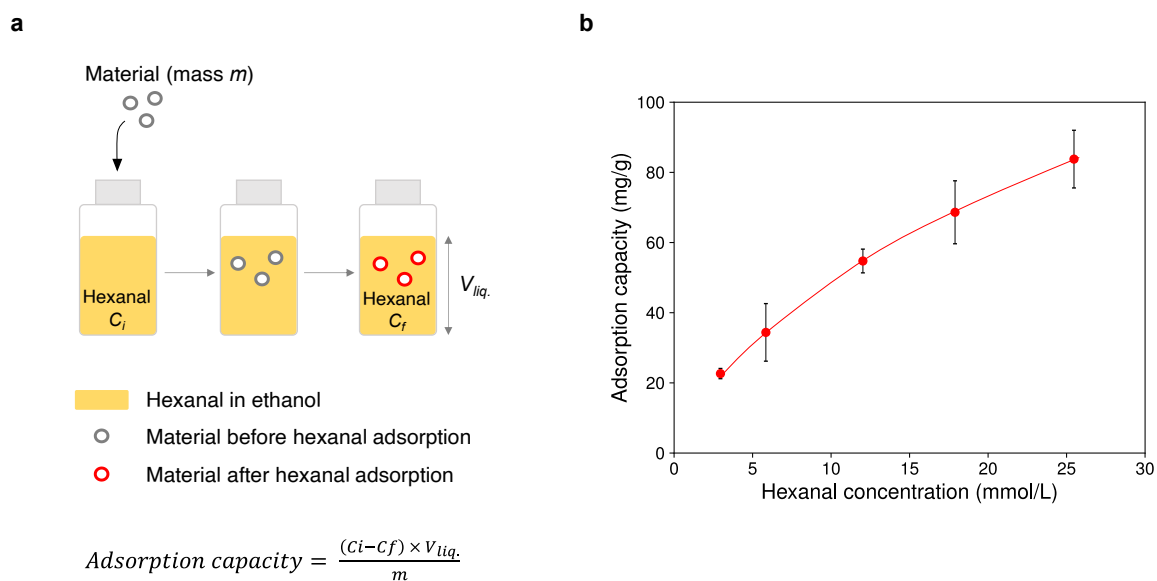


Figure 3: Adsorption capacity of the material.

a. Schematic representation of the experimental setup used for the determination of the adsorption capacity of hexanal by the material at 22 °C after 24 h of contact in liquid phase (ethanol). C_i and C_f were determined by GC-MS.

b. Adsorption capacity of hexanal by the material. Error bars represent the standard deviation obtained from 4 replicates. Red points are experimental data, solid line is a guide for the eyes. The normalized mass of hexanal adsorbed by the material increases with the initial hexanal concentration in the liquid phase.

3.2.2. Change in fluorescence of the material upon exposure to carbonyls

The fluorescence emission spectra of the material were recorded as shown in **Figure 4**.

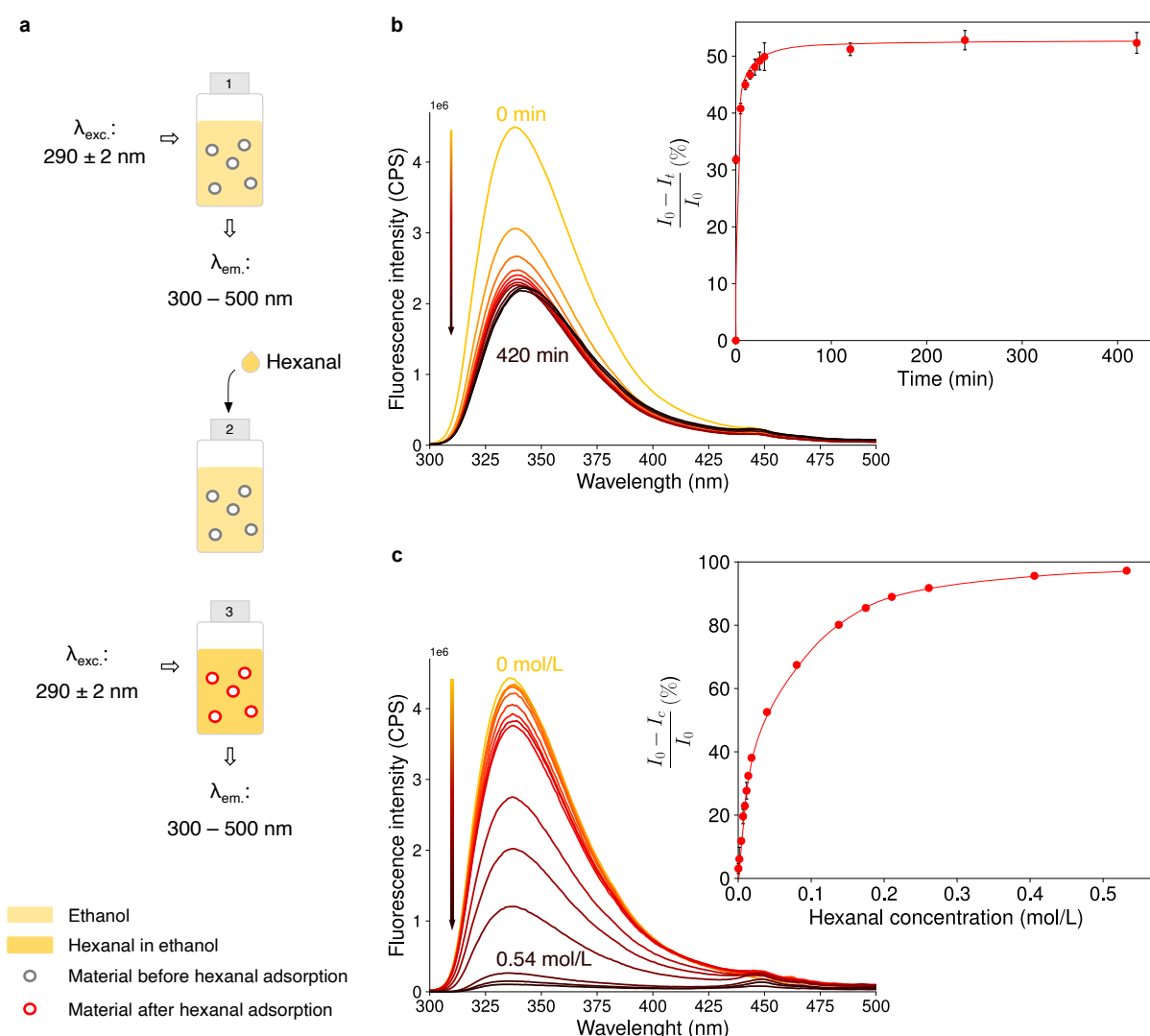


Figure 4: Fluorescence of the material upon exposure to carbonyls.

a. The schematic illustrates the experimental setup used for the determination of the influence of contact time and carbonyl concentration on the fluorescence of the material (example given with hexanal). A suspension of 10 mg of material in ethanol was analyzed at 22 °C. Excitation was set at 290 nm and fluorescence emission spectrum of the material was recorded between 300 nm and 500 nm. Hexanal was added and fluorescence emission spectrum of the material was recorded once again.

b. Evolution over time of the fluorescence emission spectrum of the material in a solution of hexanal in ethanol (concentration: $9.98 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$) and corresponding kinetics of fluorescence quenching of the material upon interaction with hexanal. The yellow spectrum corresponds to the initial fluorescence of the material in ethanol before contact with hexanal. The other spectra depict the fluorescence emission of the material over time after addition of hexanal in the liquid phase. A significant decrease in the fluorescence intensity at 337 nm is observed after 30 s of contact between the material and hexanal. For the plot of the kinetics curve, the intensity of fluorescence of the material is measured at its maximum of emission (337 nm). Normalized fluorescence loss is calculated as $\frac{I_0 - I_t}{I_0}$ with I_0 the intensity of fluorescence of the material in ethanol before addition of hexanal and I_t

its intensity after exposure to hexanal during a period of time t . Red points are experimental data, solid line is a guide for the eyes. Error bars represent the standard deviation obtained from 3 replicates.

c. Evolution of the fluorescence emission spectrum of the material with hexanal concentration ranging from 0 (yellow curve) to $0.54 \text{ mol}\cdot\text{L}^{-1}$ (dark curve). The yellow curve corresponds to the fluorescence of the material in ethanol before addition of hexanal in the liquid phase. A decrease in the fluorescence intensity at 337 nm is observed when hexanal concentration increases up to fluorescence extinction for an hexanal concentration of $0.54 \text{ mol}\cdot\text{L}^{-1}$. For the plot of the percentage of fluorescence quenching as a function of the carbonyl concentration, the intensity of fluorescence of the material is measured at its maximum of emission (337 nm). Normalized fluorescence loss is calculated as $\frac{I_0 - I_c}{I_0}$ with I_0 the intensity of fluorescence of the material in ethanol before addition of hexanal and I_c the intensity of fluorescence emission of the material in contact with hexanal in ethanol for 10 min at a concentration c . Red points are experimental data, solid line is a guide for the eyes. Error bars represent the standard deviation obtained from 3 replicates.

As presented in **Figure 4b** and **Figure 4c**, the material showed a maximum of fluorescence intensity at 337 nm once excited at 290 nm. This characteristic signal was attributed to the aniline group in ethanol⁴⁷. After exposure to carbonyls, the fluorescence intensity of the material decreased. The plot of the Stern-Volmer equation resulted in non-linear curves, both for hexanal and hexan-2-one (see **Figure S 5** in **Supporting Information**). This means that both static and dynamic fluorescence quenching mechanisms were involved^{48–50}. Static quenching occurs in the ground state and rely on the transformation of the fluorescent probe into a non-fluorescent compound. Dynamic quenching occurs at the excited state, implying non-radiative relaxation processes. In each case, several mechanisms can be considered. Firstly, static quenching may happen in the case of chemical reaction between the material and the carbonyl compound. Imine molecules are formed by nucleophilic addition of carbonyl groups onto the primary amine functions of the material. Such imines can be non-fluorescent or at least not excited at 290 nm. The lone pair of electrons of the nitrogen atom in the imine molecule can be responsible for intramolecular quenching by electron transfer or charge transfer at excited state^{51,52}. Progressive reduction in the number of fluorescent aniline groups in the material could thus explain the decrease in the fluorescence of the material. Moreover, non-radiative energy can be dissipated by intramolecular motion of the hydrocarbon chain added to the material through the formation of the imine^{53–55}. Si *et al.*^{56,57} also suggested that the photoisomerization of the imine bond was responsible for fluorescence quenching because of non-radiative relaxation of the excited state of the imine molecules. The energy required for the *cis-trans* isomerization is between 0.2 and 0.3 eV, which corresponds

to wavelengths between 248 nm and 372 nm⁵⁸. This phenomenon could therefore happen with an excitation wavelength at 290 nm. Secondly, dynamic quenching mechanisms can result from physical interactions between carbonyls and the material. Intermolecular hydrogen bonds between the carbonyl groups and uncondensed silanols may stabilize the ground state, decreasing the fluorescence of the material. In the case of hydrophobic interactions, electron transfer can additionally happen. Collisional quenching can occur between amines from the material and carbonyl compounds in the liquid phase. Carbonyls being electron deficient, can then be responsible for intermolecular quenching. Finally, a competition for absorption of photons from the excitation source between carbonyl compounds in solution and the material in suspension can be considered. Hexanal (which absorbs at 221 nm and 290 nm) and hexan-2-one (which absorbs at 240 nm and 276 nm) (see **Figure S 6 in Supporting Information**) can theoretically absorb photons from the excitation source before it excites the aniline group of the material, resulting in no excitation of the material and consequently fluorescence quenching. However, this mechanism was considered negligible because the extinction coefficient of the functional monomer at the excitation wavelength (290 nm) is between 100 and 200 times higher than these of hexanal and hexan-2-one. Photons from the excitation source would thus be preferentially absorbed by the material and not by the carbonyl compounds.

Figure 4b represents the kinetics of fluorescence quenching of the material after exposure to hexanal in the liquid phase. A significant decrease in the fluorescence intensity at 337 nm was observed after 30 s of contact between the material and hexanal. Equilibrium was reached very fast, after 10 min. Similar results were obtained with hexan-2-one (see **Figure S 7a in Supporting Information**). Such fast adsorption kinetics of carbonyls gave rise to promising applications of the material as a sensor, for which the time of analysis should be as short as possible. Kinetics were modelled by the pseudo-first order Lagergren model and pseudo-second order model. For the pseudo-first order model, linear regression between $\ln\left(\frac{Q_e - Q_t}{Q_e}\right)$ (with Q_e the amount of carbonyl adsorbed at equilibrium and Q_t the amount of carbonyl adsorbed at time t) and time did not fit the experimental data (correlation coefficients r of -0.82 and -0.61 for hexanal and hexan-2-one, respectively). On the contrary, for the pseudo-second order model, linear regression between $\frac{t}{Q_t}$ and time perfectly fitted the experimental data, with correlation coefficients r of 0.99 for both hexanal and hexan-2-one (see **Figure S 8 in**

Supporting Information). The “pseudo” rate constant k was 5 times higher for hexan-2-one compared to hexanal, revealing a faster fluorescence quenching of the material by ketones compared to aldehydes.

Figure 4c displays the influence of the hexanal concentration on the fluorescence of the material. A decrease in the fluorescence intensity of the material at 337 nm was observed when the hexanal concentration increased. For concentrations above $0.5 \text{ mol}\cdot\text{L}^{-1}$, an extinction of the fluorescence was noticed. Quenchable sensors have sometimes been criticized for the difficulty to detect high concentrations of analytes⁵⁹. However, we report here on a “turn-off” sensor with a wide range of detection (from $0.5 \text{ mmol}\cdot\text{L}^{-1}$ to $0.5 \text{ mol}\cdot\text{L}^{-1}$) with only 10 mg of material. A shift in the maximum fluorescence emission wavelength from 337 nm to 345 nm was also noticed when the hexanal concentration increased from $0 \text{ mol}\cdot\text{L}^{-1}$ to $0.5 \text{ mol}\cdot\text{L}^{-1}$. As the fluorescence emission of a fluorescent core is strongly influenced by its chemical environment, this shift could be explained by the modification of the environment of the fluorescent aniline groups due to the adsorption of carbonyl compounds by the material. This phenomenon was already observed by Si *et al.*⁵⁶. Similar results were obtained with hexan-2-one (see **Figure S 7b** in **Supporting Information**). In both cases, the influence of hexanal and hexan-2-one concentration on the fluorescence intensity of the material presented two distinct evolutions, with a first step reached for carbonyl concentration up to $0.01 \text{ mol}\cdot\text{L}^{-1}$ and a second step up to $0.54 \text{ mol}\cdot\text{L}^{-1}$. This could be attributed to the filling of the 2 types of pores, with the larger porosity (mesopores) being first filled with carbonyls followed by micropores for higher concentrations. Such fluorescence intensity decrease in 2 steps could also be explained by the 2 types of adsorption mechanisms: chemical reaction and physical interaction.

Based on the evolution of the fluorescence loss with the carbonyl concentration, the sensitivity and the limit of detection (LOD) of the sensor for hexanal and hexan-2-one were determined. The sensitivity, given by the slope of this curve at low concentrations was 87 for hexanal and 47 for hexan-2-one, traducing a higher affinity of the material for the aldehydes compared to the ketones. The LOD is defined as the smallest quantity of carbonyls that can be brought in contact with the material resulting in a significant decrease in its fluorescence intensity. The $LODs$ were $5.7 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ ($57 \text{ mg}\cdot\text{L}^{-1}$) and $8.0 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ ($80 \text{ mg}\cdot\text{L}^{-1}$) for hexanal and hexan-2-one, respectively. This could be considered a rather high limit of detection for applications requiring the detection of a single compound. However, this sensor is not

specific to a single molecule but it is intended to be used to determine the global carbonyl content of a system. Chen *et al.* already reported the need for new methods for multi-aldehydes determination to improve the signal detectability²⁰. In the case of lipid oxidation for example, this would allow the food spoilage to be globally followed by detecting the sum of carbonyls released.

3.3. APPLICATION OF THE SENSOR IN SIMULATING AND REAL CONDITIONS

In addition to the evaluation of the performance of the sensor in ethanol as the liquid phase, it was further investigated in an ethanol/water (10/90, v/v) mixture, simulating hydrophilic food or cosmetic samples according to the (EU) 10/2011 regulation (**Figure 5a**).

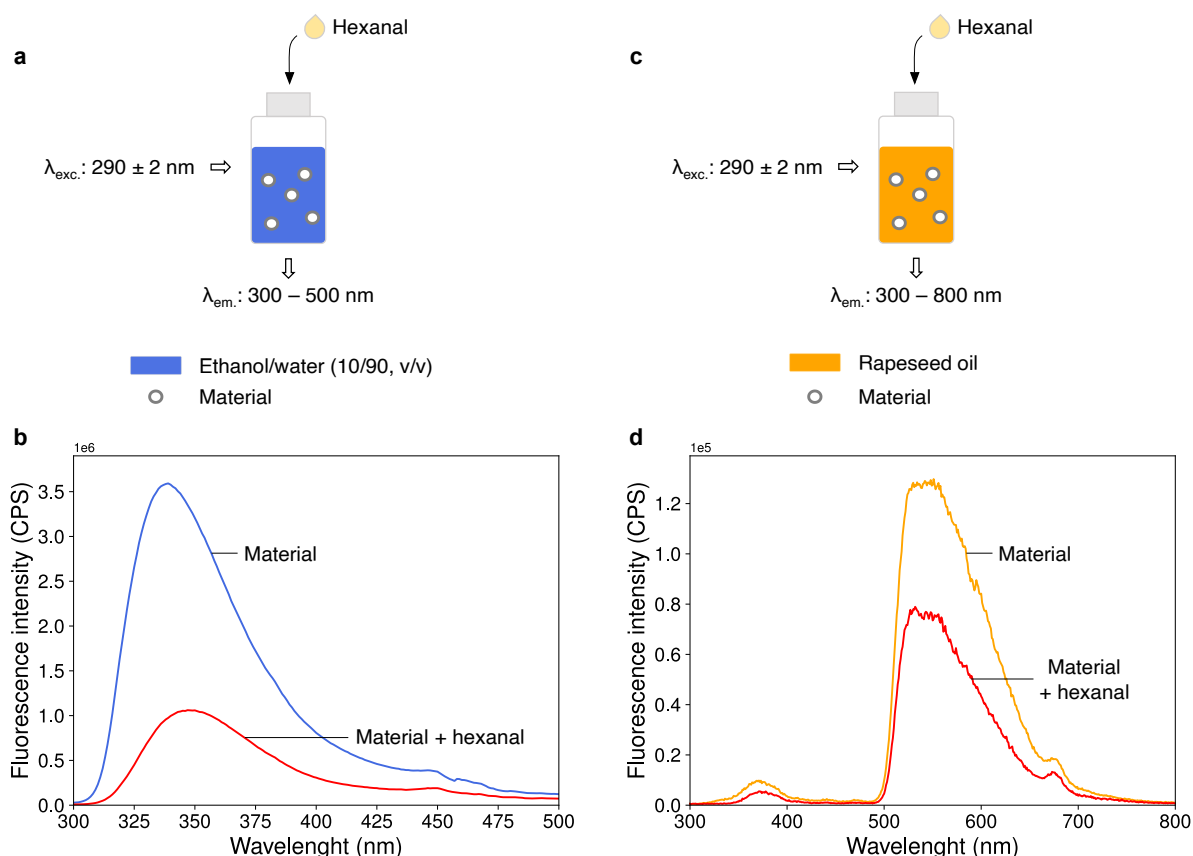


Figure 5: Impact of hexanal on the fluorescence of the material in simulating and real media.

a. The schematic illustrates the experimental setup used for the measurement of the fluorescence emission spectrum of 10 mg of the material in suspension in an ethanol/water (10/90, v/v) mixture, used as simulating medium of hydrophilic food or cosmetic samples. Excitation was set at 290 nm and fluorescence emission spectrum of the material was recorded between 300 nm and 500 nm. Temperature was kept constant at 22 °C during the experiment. Spectra were recorded before and 30 min after addition of hexanal (concentration: $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) in the dispersing phase.

b. Fluorescence emission spectrum of the material before and after interaction with hexanal in an ethanol/water (10/90, v/v) mixture. The maximum of fluorescence intensity was at 339 nm. Fluorescence was significantly quenched by hexanal.

c. The schematic illustrates the experimental setup used for the measurement of the fluorescence emission spectrum of 10 mg of the material in suspension in rapeseed oil. Excitation was set at 290 nm and fluorescence emission spectrum of the material was recorded between 300 nm and 800 nm. Temperature was kept constant at 22 °C during the experiment. Spectra were recorded before and 30 min after addition of hexanal (concentration: $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) in the dispersing phase.

d. Fluorescence emission spectrum of the material before and after interaction with hexanal in rapeseed oil. The maximum of fluorescence intensity was at 551 nm. Fluorescence was significantly quenched by hexanal.

Figure 5b represents the fluorescence emission spectrum of the material before and after interaction with hexanal in the ethanol/water mixture. Similarly to the results obtained in ethanol, the material exhibited a maximum of fluorescence intensity at 339 nm, which was quenched upon interaction with hexanal. The applicability of the sensor to the determination of carbonyl compounds in real conditions was also performed using rapeseed oil spiked with hexanal, as shown in **Figure 5c**. **Figure 5d** depicts the fluorescence emission spectrum of the material in rapeseed oil before and after addition of hexanal. In this last case, the material emitted fluorescence in the visible region at 551 nm. Such as in ethanol and in ethanol/water mixture, the fluorescence intensity of the material was quenched after addition of hexanal in the rapeseed oil dispersing phase. In all cases, a shift in the maximum emission wavelength of the material toward higher wavelengths (bathochromic effect) was observed. This could be attributed to a charge, proton or electron, transfer in the singlet excited state^{60–62}. Fluorescence intensity at the maximum emission wavelength was lower in these dispersing phases compared to ethanol. This can be attributed to interactions of the material with components of dispersing phase, especially through hydrogen bonds (with ethanol and water or with oleic acid, linoleic acid, α -linolenic acid, vitamins E and K in the case of rapeseed oil). Such interactions would stabilize the ground state^{63,64} and reduce the fluorescence intensity of the material. These results demonstrated the applicability of the sensor to monitor the global carbonyl content in real matrices.

4. CONCLUSION

This paper reports on the successful design, synthesis and performance assessment of a novel intelligent material for the monitoring of carbonyl compounds based on fluorescence. A single sensor for multi-analytes detection constitutes a very original approach in the field of sensing and allows the global content of carbonyls to be determined. A cost-effective and straightforward sol-gel process with silica-based reactants was employed for the synthesis of a non-toxic fluorescent material. Although sol-gel materials are rarely used for sensing applications, their reduced toxicity makes them suitable for critical applications such as in the food or medical fields. The successful design of such a fluorescent sol-gel material thus constitutes a breakthrough for the detection and quantification of carbonyl compounds. The material displayed particles having size in the micrometrical range, with a high porosity, resulting from the presence of both micropores and mesopores. Such material was able to

successfully interact with both aldehydes and ketones. Chemical reaction as well as physical interactions occurred. The fluorescence of the material was quenched rapidly (less than 1 min) by carbonyls. Both static and dynamic quenching mechanisms were involved. Only limited sample preparation was required. The sensor operated over a wide range of concentration, of 3 orders of magnitude. All these interesting and advantageous features make this sensor suitable for a wide range of applications. The applicability of the sensor to monitor lipid oxidation in food or cosmetics samples was successfully demonstrated in an ethanol/water simulating medium. The sensor even provided significant response in a real medium consisting of rapeseed oil. Next steps will be to extend the use of the developed material for monitoring carbonyls in even more complex matrices or environments according to the desired field of application (*e.g.*, food, cosmetics, environment or biology).

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SUPPORTING INFORMATION

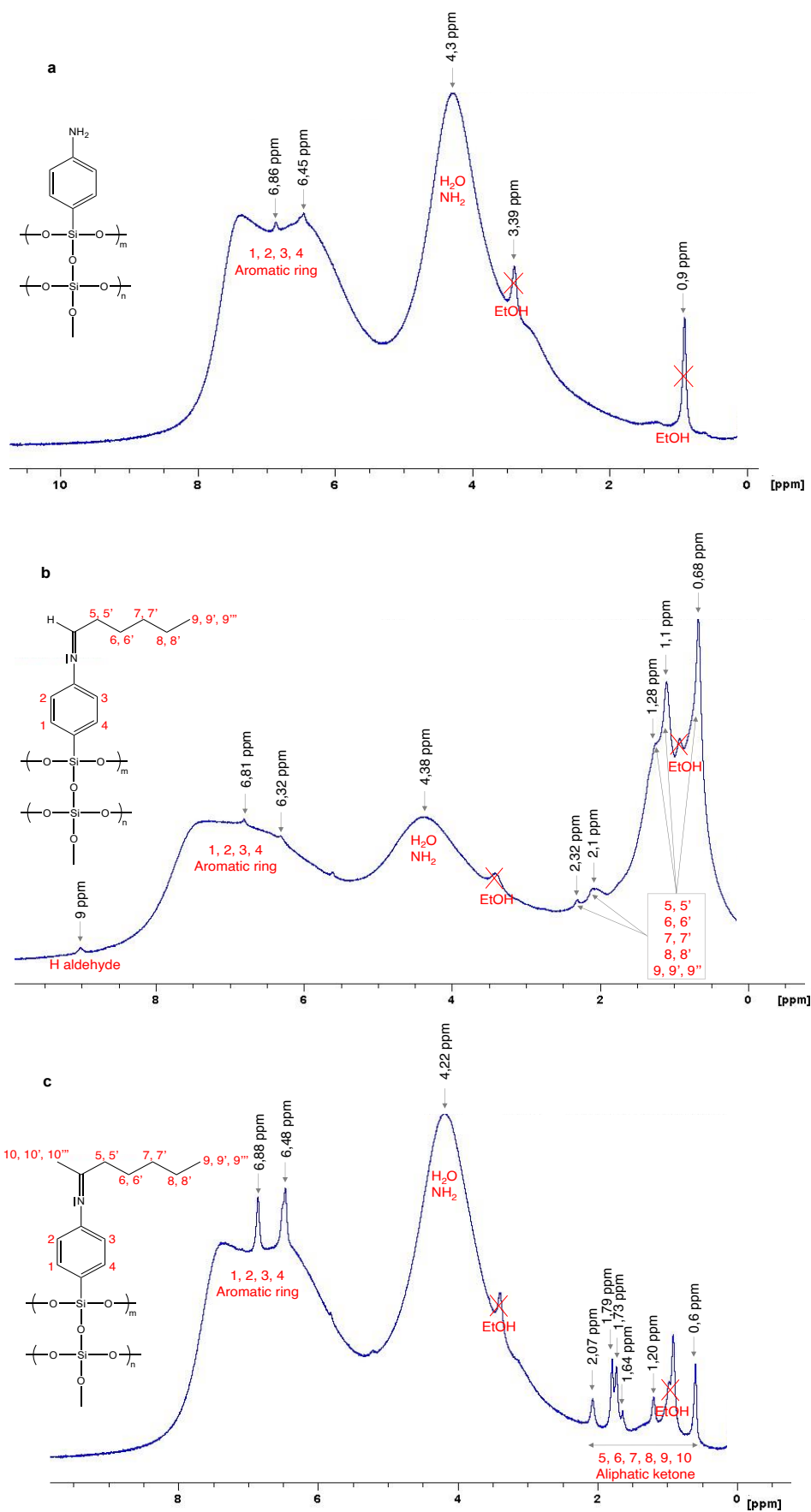


Figure S 1: ^1H NMR spectrum of the (a) starting material; (b) after adsorption of hexanal and (c) after adsorption of hexan-2-one.

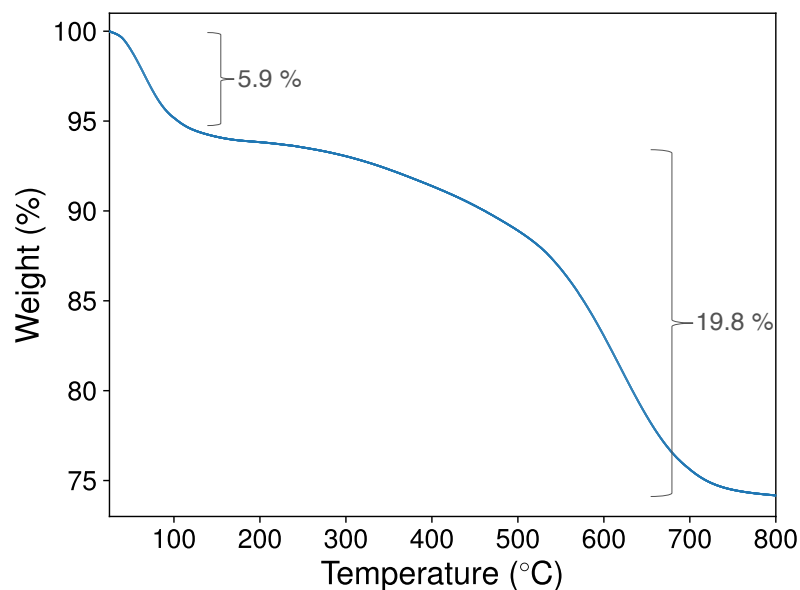


Figure S 2: TGA of the material obtained by heating the material under air flow.

A first mass loss, relative to 5.9 %, occurred between 25 °C and 150 °C and is attributed to the desorption of physisorbed water. The second one occurred between 150 °C and 800 °C and corresponds to the degradation of the functional aniline group, representing an organic fraction loss of 19.8 %. On a dry basis, the ratio of mineral fraction is therefore 74.3 %.

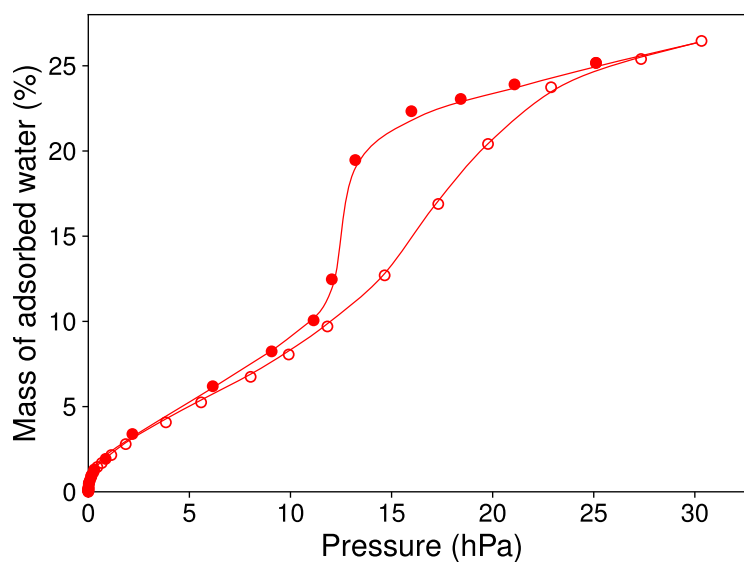


Figure S 3: Adsorption isotherm of water vapor on the material, performed at 25 °C, displaying adsorption (○) and desorption (●).

The amount of water vapor adsorbed or desorbed at equilibrium is given in gram of water per 100 gram of dry material. At 10 hPa of water vapor pressure, which corresponds to 30 % relative humidity (usual conditions of use), the mass uptake of the material is about 8 %. The hysteresis is related to the presence of mesopores in the material. The solid lines are guide for the eyes.

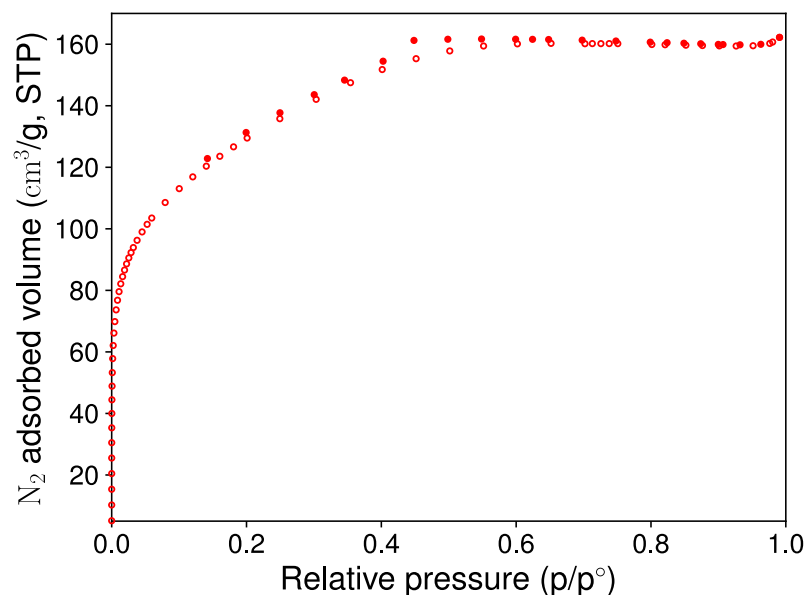


Figure S 4: Porosity of the material as determined by nitrogen adsorption.

Nitrogen adsorption isotherm of the material, displaying adsorption ($\circ$) and desorption ($\bullet$). p is the N_2 pressure at equilibrium and p° is the saturated vapor pressure at -195.8°C (1013.25 hPa). The isotherm is of type Ib, which reveals the presence of both micropores ($< 2\text{ nm}$) and mesopores (2-50 nm) in the material. A slight hysteresis loop from 0.15 to 0.99 relative pressure was observed.

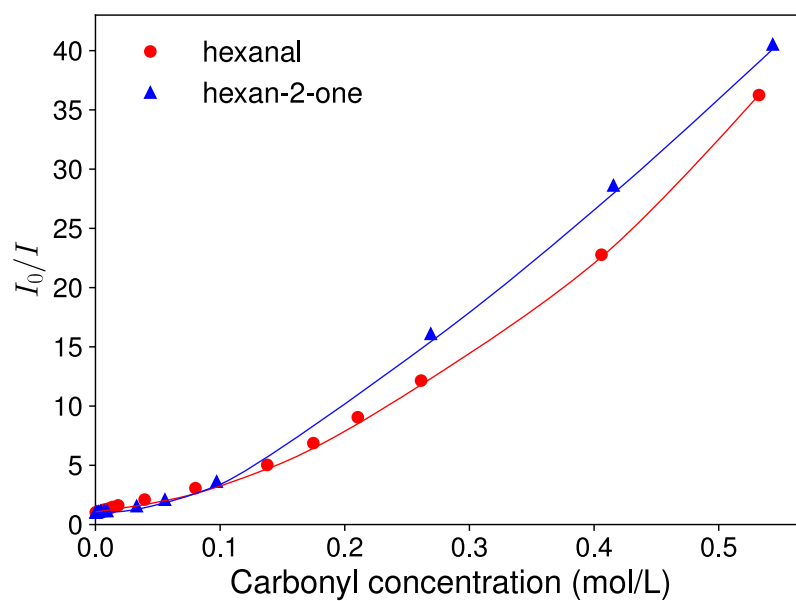


Figure S 5: Stern-Volmer plot of the tested carbonyls.

I_0 is the intensity of fluorescence emission of the material at 337 nm and I its fluorescence after 10 min of interaction with carbonyls at a given concentration. The non-linear relationship suggests that both static and dynamic fluorescence quenching mechanisms are involved. Solid lines are guides for the eyes.

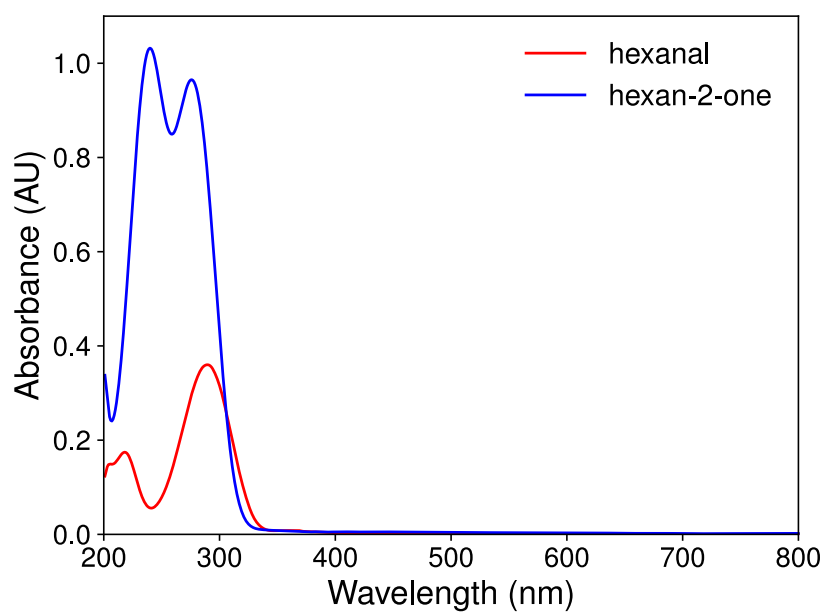


Figure S 6: Absorbance spectra of hexanal and hexan-2-one in ethanol. Hexanal absorbs at 221 nm and 290 nm and hexan-2-one absorbs at 240 nm and 276 nm.

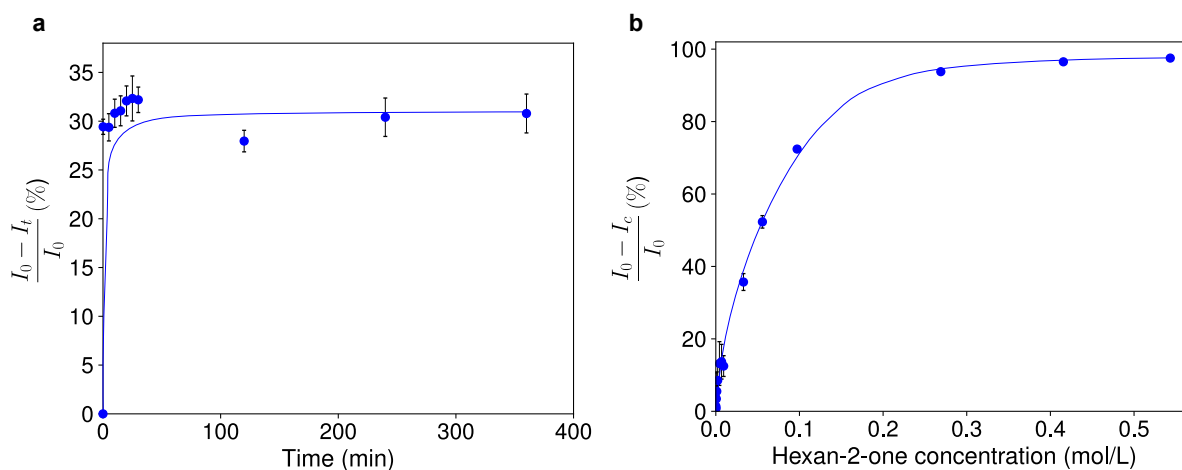


Figure S 7: Influence of contact time and concentration of hexan-2-one on the fluorescence of the material.

a. Kinetics of fluorescence decrease of the material upon interaction with hexan-2-one. The intensity of fluorescence of the material is measured at its maximum of emission (337 nm). Normalized fluorescence loss is calculated as $\frac{I_0 - I_t}{I_0}$ with I_0 the intensity of fluorescence of the material in ethanol before addition of hexan-2-one and I_t its intensity after exposure to hexan-2-one during a period of time t . Blue points are experimental data, solid line is a guide for the eyes. Error bars represent the standard deviation obtained from 3 replicates.

b. Influence of hexan-2-one concentration on the percentage of fluorescence quenching of the material at 22 °C. The intensity of fluorescence of the material is measured at its maximum of emission (337 nm). Normalized fluorescence loss is calculated as $\frac{I_0 - I_c}{I_0}$ with I_0 the intensity of fluorescence of the material in ethanol before addition of hexan-2-one and I_c the intensity of fluorescence emission of the material in contact with hexan-2-one in ethanol for 10 minutes at a concentration c . Blue points are experimental data, solid line is a guide for the eyes. Error bars represent the standard deviation obtained from 3 replicates.

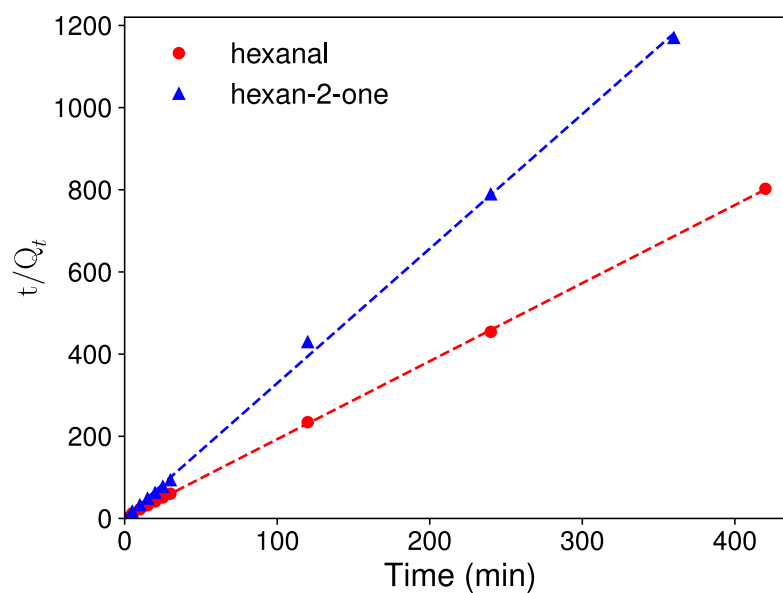


Figure S 8: Pseudo-second order modelling of the kinetics of fluorescence quenching of the material induced by hexanal (circles) and hexan-2-one (triangle). Qt is calculated as $\frac{I_0 - I_t}{I_0}$ with I_0 the intensity of fluorescence of the material in ethanol before addition of carbonyl and I_t its intensity after exposure to carbonyl during a period of time t .

Linear regression fitted the experimental data with correlation coefficients r of 0.99 both for hexanal and hexan-2-one. The “pseudo” rate constant k was 5 times higher for hexan-2-one compared to hexanal, revealing a faster fluorescence quenching of the material by ketones compared to aldehydes. Dash lines are linear regressions.

Chap. 3. INFLUENCE OF THE ANALYTE AND THE LIQUID MEDIA ON THE PERFORMANCE OF THE SENSOR

In the previous chapter, only two carbonyls were tested (hexanal and hexan-2-one) and the fluorescence of the material was deeply explored in ethanol and less investigated in other liquid media. However, this sensor was developed for multi-analytes detection in various food matrices. Therefore, in this chapter, the influence of the carbonyl nature and structure (propanal, hexanal, nonanal, 1-penten-3-one and 1-octen-3-one) as well as the polarity and pH of the liquid medium on the fluorescence properties of the sol-gel material was studied.

Monitoring of lipid oxidation using a fluorescent sol-gel functionalized material.

ABSTRACT

A sol-gel material based on the fluorescence of aniline was developed for the monitoring of secondary products of lipid oxidation in foods. More precisely, it has been designed for the sensing of the global carbonyl content. The material exhibited high fluorescence intensity, which was quenched upon interaction with carbonyls. Various conditions were tested by changing the nature of the carbonyl compound, the food simulant medium and its pH. First, the impact of the nature and the structure of the analyte, namely propanal, hexanal, nonanal, 1-penten-3-one and 1-octen-3-one, on the fluorescence property of the material was studied. These molecules were identified as good trackers of the degradation of different fatty acids. The size and hydrophobicity of the analyte impacted the kinetics and the percentage of fluorescence quenching as well as the limit of detection and the saturation limit of the sensor. Moreover, the nature of the solvent also had a strong impact on the fluorescence of the material. The fluorescence in ethanol medium was higher compared to the more polar ethanol/water (10/90, v/v) mixture, used as food simulant. Finally, the optimal pH range to operate the sensor was determined to be from 7 to 9. The material was thus tested in various conditions to determine its range of applicability as a sensor for carbonyl monitoring to assess lipid oxidation of food products.

1. INTRODUCTION

Lipids are essential nutrients in human diet. They are made of polyunsaturated fatty acids such as $\omega 3$ for example, which are known to play a crucial role in reducing the risk of cardiovascular disease or in regulating inflammatory reactions^{1,2}. However, lipids are very sensitive to oxidation, which is a natural chemical degradation process. Initiated either by heat, light, sensitizer or metals, the radical process involved is unstoppable³. During lipid oxidation, many different reaction products are generated. The first ones, so-called “primary products”, are mainly hydroperoxides. They are further transformed into “secondary products”. Among them, aldehydes, ketones, alcohols and carboxylic acids are the most released molecules⁴. Many of them are volatile and responsible for the unpleasant rancid odor, characteristic of lipid oxidation⁵.

Failing to avoid lipid oxidation, its monitoring is of high importance. Many common and standard methods for the determination of the lipid oxidation state of a food product already exist. The monitoring of primary products relies on the measurement of the peroxide value (PV)⁶ or on the determination of the amount of conjugated dienes (CD233)⁷. Secondary products are usually monitored by the estimation of the p-anisidine value⁸, by the thiobarbituric acid reactive substances (TBARS) assay⁹, by chromatographic methods or even by sensory evaluation. Even if all these methods have been used for decades, they require highly trained persons, lab skills, a lot of time and expensive equipment.

In the present work, the performance of a user-friendly sensor intended to monitor lipid oxidation was thoroughly evaluated. Based on the fluorescence of an aniline derivative and synthesized by the sol-gel process, a material was developed to track secondary products of lipid oxidation, especially carbonyl compounds. Aniline was chosen to act as fluorescent probe. Its primary amine function was intended to chemically react with carbonyl compounds. The synthesized material exhibited high fluorescence intensity, which was quenched upon interaction with carbonyls. Details about the synthesis and characterization of this material can be found in previous work. As this sensor was designed to indicate the global content in carbonyls, the impact of the nature and the structure of the analyte as well as the influence of the solvent polarity and its pH on the fluorescence property of the material were assessed. More precisely, the kinetics of the fluorescence decrease and the percentage of fluorescence quenching of the material by different carbonyls (namely propanal, hexanal, nonanal, 1-penten-3-one and 1-octen-3-one) as well as the limit of detection and saturation limit for each carbonyl were compared in ethanol and in an ethanol/water (10/90, v/v) mixture, used as food simulant medium. Moreover, the impact of interfering compounds (*n*-pentane, *n*-hexane, *n*-octane) on the fluorescence of the material was assessed. The pH range in which the sensor was operational was finally determined.

2. MATERIALS AND METHODS

2.1. CHEMICALS

4-trimethoxysilylaniline (4-TMSA, 97 %, CAS number 33976-43-1) was obtained from abcr, Germany. Tetraethylorthosilicate (TEOS, 99 %, CAS number 78-10-4), ammonium hydroxide (NH₄OH, 30 %, CAS number 1336-21-6), hydrochloric acid (HCl, CAS number 7647-

01-0), sodium hydroxide (NaOH, CAS number 1310-73-2), 1-octen-3-one (96 %, CAS number 4312-99-6), *n*-pentane (99 %, CAS number 109-66-0), *n*-hexane (97 %, CAS number 110-54-3), 1-penten-3-one (97 % CAS number 1629-58-9), formic acid (98 %, CAS number 64-18-6), ammonium hydroxide solution (28-30 %, CAS number 1336-21-6) and ethanol for UV-spectroscopy Uvasol® (EtOH, 99,9 %, CAS number 64-17-5) were purchased from Sigma Aldrich, France. *n*-octane (99 %, CAS number 111-65-9) was supplied by Merck, Germany. Propanal (99 %, CAS number 123-38-6) was obtained from ACROS Organics™. Hexanal (98 %, CAS number 66-25-1) was purchased from Alfa Aesar, France. Nonanal (97 %, CAS number 124-19-6) was bought from Thermo Scientific Chemicals. Deionized water was used, obtained from a MilliQ RG, Millipore apparatus.

2.2. FLUORESCENCE EXPERIMENTS

2.2.1. General procedure

A suspension of the sol-gel material in ethanol or ethanol/water mixture (50/50, v/v or 10/90, v/v) was realized. After 10 s of stirring, the fluorescence emission spectrum of the material in suspension was recorded. A carbonyl solution in ethanol at a given concentration (either propanal, hexanal, nonanal, 1-penten-3-one or 1-octen-3-one) was added to the dispersing phase and left in contact with the material in suspension for a given time before the fluorescence emission spectrum of the material was recorded once again. Both influence of contact time and concentration of carbonyl were studied. A spectrofluorometer Fluoromax4® (Horiba) was used with the software Fluorescence® and a SUPRASIL® quartz cell (Hellma) of 10 mm light path. Excitation wavelength was 290 ± 2 nm. The fluorescence emission spectrum was recorded between 300 nm and 500 nm, using a 2 nm bandpass and a step interval of 1 nm. The temperature was kept constant at 22 °C during the experiment. Each experiment was performed 3 times.

2.2.2. Kinetics of decrease of the fluorescence intensity of the material after interaction with different types of carbonyls

A suspension of 10 mg of material in 2 mL of ethanol was stirred for 10 s and the fluorescence emission spectrum of the material was recorded. Then, 500 µL of a solution of carbonyl (either propanal, hexanal, nonanal, 1-penten-3-one or 1-octen-3-one) in ethanol were added to obtain a concentration in the measurement cell of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. The cell was

then continuously agitated at 80 rpm, and the fluorescence emission spectrum of the material was recorded after 30 s, 5 min, 10 min, 15 min, 20 min, 25 min and 30 min. The fluorescence intensity of the material at 337 nm was compared at each time.

2.2.3. Percentage of fluorescence quenching

The percentage of fluorescence quenching, Q , was determined as follows:

$$Q = \frac{I_0 - I_{eq}}{I_0} \times 100 \quad (1)$$

with I_0 the intensity of fluorescence at 337 nm of the material in ethanol before addition of carbonyl and I_{eq} its intensity after exposure to carbonyl for 20 min.

2.2.4. Influence of the concentration of the analyte on the fluorescence of the material

A suspension of 10 mg of the material in 1.8 mL of ethanol was placed in a quartz cell. After 10 s of stirring, the fluorescence spectrum of the material was recorded. A known amount of carbonyl in ethanol was periodically added to increase the carbonyl concentration in the dispersing phase from 0 mol·L⁻¹ to 0.54 mol·L⁻¹. The solution was agitated at 80 rpm for 10 min to reach the equilibrium after each addition and the fluorescence emission spectrum of the material was recorded.

To test the response of the sensor in the presence of interfering compounds, the influence of increasing concentration of *n*-pentane, *n*-hexane and *n*-octane on the fluorescence of the material was also assessed. The same experiment as previously described was performed replacing the carbonyls by their alkane structural analogue.

2.2.5. Impact of the polarity of the solvent on the fluorescence of the material

After 10 s of stirring, the fluorescence emission spectrum of a 10 mg suspension of the material in ethanol, ethanol/water (50/50, v/v) mixture, ethanol/water (10/90, v/v) mixture and water were recorded. Each spectrum was recorded 3 times. The kinetics of decrease of fluorescence intensity, the percentage of fluorescence quenching and the influence of the concentration of the analyte on the fluorescence of the material were determined, as described in 2.2.2, 2.2.3 and 2.2.4.

2.2.6. Impact of the pH of the dispersing phase on the fluorescence of the material

The pH of ethanol/water (10/90, v/v) mixtures was adjusted using HCl or NaOH in order to obtain solutions at pH of 1.4, 3.6, 7.6, 9.3 and 12.8. A 5 mg suspension of material was realized in 2 mL of each of these solutions. After 24 h of stirring at 50 rpm, the fluorescence emission spectrum of the material was recorded. Then, 500 μL of a solution of 1-octen-3-one in ethanol were added to obtain a concentration in the measurement cell of $2.3 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. The solution was agitated at 80 rpm for 10 min in order to reach the equilibrium and then, the fluorescence emission spectrum of the material was recorded. The measurements were performed 3 times for each pH.

2.3. STRUCTURE DETERMINATION OF 1-PENTEN-3-ONE BY HPLC-MS

Results obtained with 1-penten-3-one were unexpected. Hypothesis was made that this molecule could eventually dimerize. The structure of the conjugated ketones studied in this work was thus investigated by HPLC-MS. Solutions of 1-penten-3-one and 1-octen-3-one in acetonitrile at 2 concentrations ($5 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ and $5 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) were filtrated and outgazed before injection in a 2695 liquid chromatograph (Waters) equipped with a C-18 column (4.6 x 50 mm internal diameter, 3.5 μm thickness, XBridge™) and an Acquity QDa mass spectrometer detector (Waters). The mass spectrometer was scanned in the 50-1200 m/z range. Analytical conditions were as follows: 0.8 kV capillary voltage, 30 V cone voltage, and 120 °C source block temperature. The compounds were analyzed using electrospray ionization (positive or negative modes). The separation was achieved using either acidic or basic conditions at a flow rate of $0.6 \text{ mL}\cdot\text{min}^{-1}$. The mobile phase was composed of a mixture of two solvents. For positive ion mode, solvent A was a formic acid/water mixture (0.1/99.9, v/v) and solvent B was a formic acid/acetonitrile mixture (0.1/99.9, v/v). For negative ion mode, formic acid was replaced by ammonium hydroxide. The following gradient of solvent A and B was used for the separation: 10 % of B was maintained for 2 min and the percentage of solvent B was increased up to 90 % in 5 min. 90 % of B was maintained for 2 min before being decreased to 10 % in 2 min and maintained at 10 % for 2 more min.

2.4. STATISTICS

Statistical analysis (ANOVA with Tukey's post hoc analysis) was performed using XLSTAT (Addinsoft) software. Significance was established at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. INFLUENCE OF THE ANALYTE CHAIN LENGTH ON THE FLUORESCENCE PROPERTY OF THE MATERIAL

Propanal, hexanal and nonanal were selected in order to study the influence of the chain length of the aldehyde on the fluorescence property of the material. These three aldehydes are produced by the oxidation of ω 3, ω 6 and ω 9 fatty acids, respectively^{10,11}. Besides, only a few mono-ketones released during lipid oxidation are aliphatic. In order to have an example of ketones coming from the oxidation of different types of fatty acids and thus to be able to compare them, 2 conjugated ketones were also chosen. 1-penten-3-one and 1-octen-3-one are also good trackers of ω 3 and ω 6 fatty acids degradation, respectively¹². The experiments were first conducted in ethanol to assess the effect of the nature of the analyte, and particularly the effect of the chain length on the fluorescence property of the material.

3.1.1. Influence of the nature of the analyte on the kinetics of fluorescence decrease of the material

Figure 1a and **Figure 1b** depict the kinetics of the decrease of the fluorescence intensity of the material after interaction with different analytes. For all of the tested carbonyls, the equilibrium was reached in less than 5 min. From the chosen representation of I_t/I_{eq} in **Figure 1a** and **Figure 1b**, it is easy to determine for which analyte the equilibrium was reached the fastest. The fluorescence of the material was quenched quicker with the ketones compared to the aldehydes. Moreover, the longer the hydrocarbon chain was, the faster was the quenching. Molecules which quenched the fluorescence faster ranked as follows: for aldehydes, nonanal (logP: 3.3) > hexanal (logP: 1.8) > propanal (logP: 0.6) and for ketones, 1-octen-3-one (logP: 2.4) > 1-penten-3-one (logP: 1.0). Therefore, it seemed that the more hydrophobic the molecules were, the faster they interacted with hydrophobic anilines groups of the material, resulting in rapid fluorescence quenching.

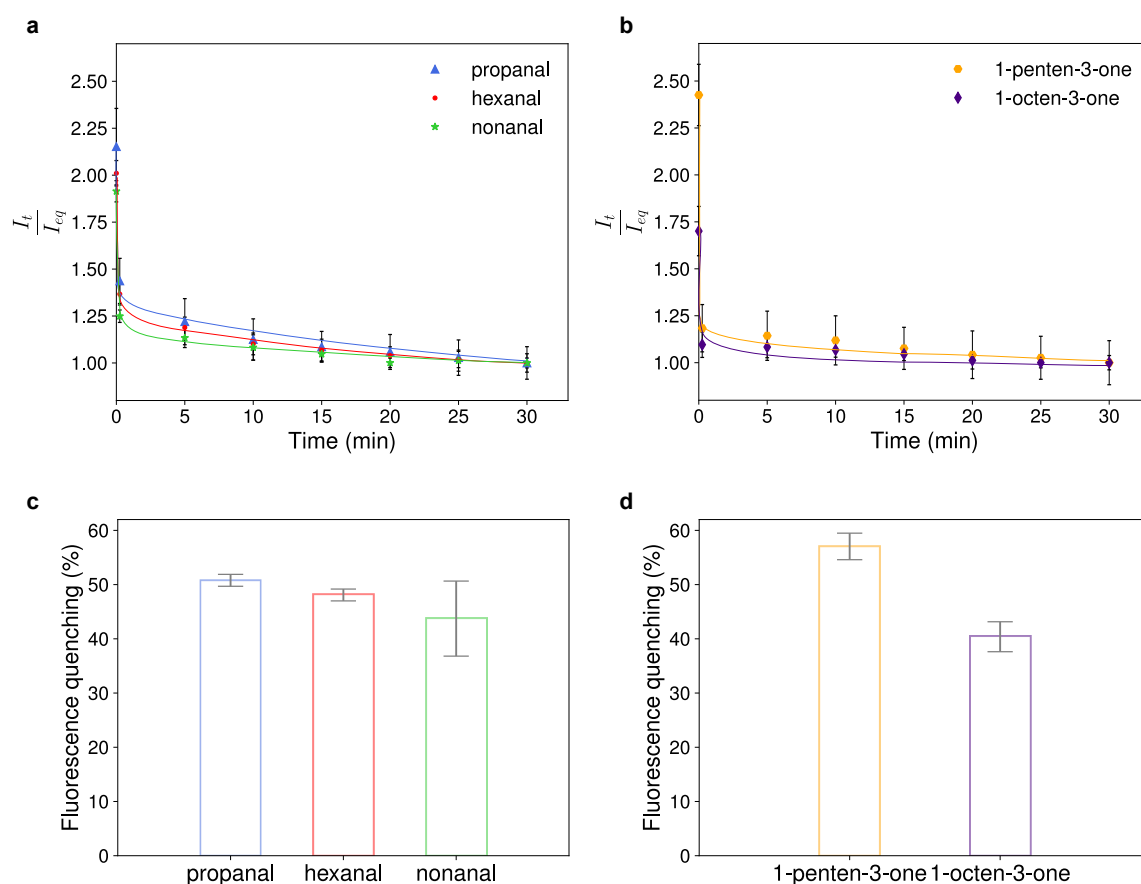


Figure 1: Kinetics of the fluorescence decrease of the material after interaction with the selected carbonyls and corresponding percentage of fluorescence quenching at equilibrium.

a. b. Kinetics of the fluorescence decrease of the material after interaction with aldehydes (**a**) and ketones (**b**). The intensity of fluorescence of the material in suspension in a solution of each carbonyl in ethanol (concentration: $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) was followed over time. I_t corresponds to the fluorescence intensity of 10 mg of the material at 337 nm at a given time and I_{eq} represents the fluorescence intensity of the material at 337 nm at equilibrium (considered at 30 min). Error bars represent the standard deviations obtained from 3 replicates. Solid lines are guides for the eyes.

c. d. Percentage of fluorescence quenching of the material by aldehydes (**c**) and ketones (**d**) for the same carbonyl concentration ($9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) after 20 min of stirring of the material in suspension in each carbonyl solution in ethanol. Error bars represent the standard deviations obtained from 3 replicates.

3.1.2. Influence of the nature of the analyte on the fluorescence quenching of the material

Figure 1c and **Figure 1d** display the percentage of fluorescence quenching upon interaction of the material with each target. It was observed that the shortest the molecule was, the more important the fluorescence decrease was. For example, propanal was responsible for 50 % of fluorescence quenching while nonanal was only responsible for 43 % of

fluorescence extinction at the same concentration ($9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$). Among ketones, the molecular size impacted the percentage of fluorescence quenching in a similar manner to aldehydes. 1-penten-3-one was responsible for 57 % of fluorescence loss while 1-octen-3-one quenched the fluorescence only to the 40 % extent. Such a ranking of the molecules responsible for the most important quenching was obtained: for aldehydes, propanal > hexanal > nonanal > and for ketones, 1-penten-3-one > 1-octen-3-one. This could be explained by the fact that smaller molecules are more likely to easily access to more aniline sites and especially those that are entrapped within the bulk polymeric structure of the material. Smaller molecules could thus quench more aniline cores than molecules with a longer aliphatic chain for a given analyte concentration.

3.1.3. Influence of the nature of the analyte on the limit of detection and saturation limit of the sensor

The limit of detection (LOD) and the saturation limit of the material were determined thanks to the plot of the percentage of fluorescence quenching of the material according to the analyte concentration (**Figure 2a** and **Figure 2b**). In its application as a sensor for carbonyl monitoring, the LOD has to be as low as possible. The saturation limit is also an important parameter to consider, as it determines the concentration range in which the sensor can operate. Actually, in order to be able to monitor the dynamic shelf-life of a product, the fluorescence extinction must occur above a given carbonyl concentration, corresponding to the spoilage threshold of the food. Saturation limit should thus be as high as possible. **Figure 2c** and **Figure 2d** display the limit of detection and saturation limit of the material for the different carbonyls tested. It was noteworthy that for aldehydes, the LOD increased, and the saturation limit decreased with the chain length. Among the three aldehydes tested, propanal thus exhibited the lowest LOD and the highest saturation limit. The molecular size and the hydrophobicity of the molecule could explain such a ranking in LOD. As previously suggested, smaller molecules could probably more easily enter inside the porosity of the material. Thus, they have more opportunities to interact with aniline groups entrapped within the bulk structure of the material and to give rise to fluorescence quenching. As the material displayed both micropores (from 0.5 to 1 nm) and mesopores (from 2 to 5 nm), low concentrations of short-chain molecules are thus more likely to be detected by fluorescence quenching. The accessibility to quenching sites seemed also linked to the hydrophobicity of the molecule. The

material was composed of 80 % hydrophilic mineral fraction and 20 % hydrophobic organic aniline. As a consequence, longer molecules, but also more hydrophobic due to their longer aliphatic chain may have restricted accessibility to the inner structure of the material. In order to better explain the differences observed for the saturation limit, the fluorescence quenching mechanisms need to be referred to. Upon the reaction of an aniline group with a carbonyl, an imine molecule is formed. The fluorescence quenching of the material can first rely on intramolecular motions of the aliphatic part of the aldimine or the ketimine responsible for non-radiative relaxation^{13–15}. It can be assumed that the longer the aliphatic chain is, the more energy will be dissipated by the motion of the hydrocarbon chain upon formation of the imine and thus the more the fluorescence will be quenched. Therefore, carbonyls with longer hydrocarbon chain will quench the fluorescence to a larger extent at a given concentration. This could explain why the saturation limit was reached at lower concentrations with longer molecules.

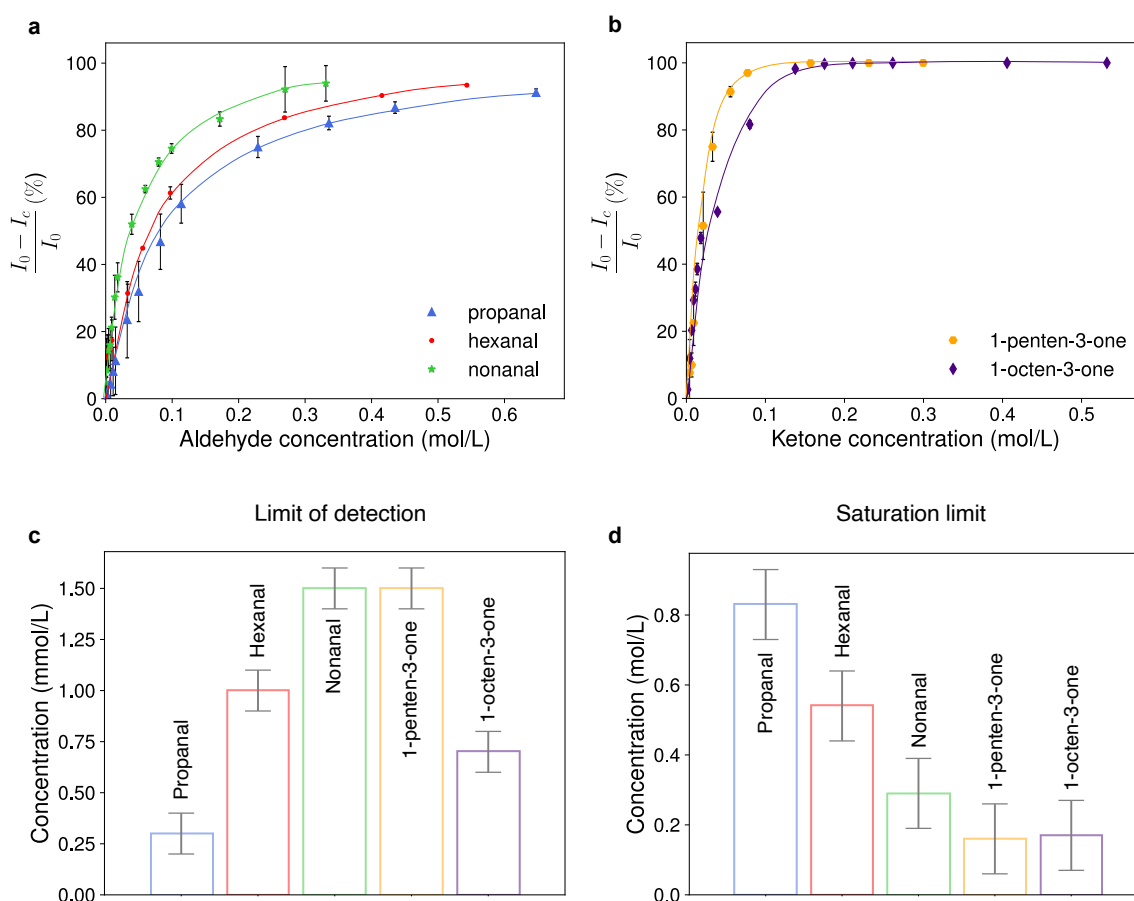


Figure 2: Influence of the concentration of the analyte on the percentage of fluorescence loss of the material, limit of detection and saturation limit.

a. b. Influence of the concentration of aldehydes (**a**) and ketones (**b**) on the percentage of fluorescence loss of the material. I_0 represents the initial fluorescence intensity at 337 nm of 10 mg material in suspension in ethanol. I_c represents the fluorescence intensity of the material at 337 nm after 10 min of stirring of the suspension of the material in the carbonyl solution in ethanol at concentration c . Error bars represent the standard deviations obtained from 3 replicates. Solid lines are guides for the eyes.

c. d. Limit of detection and saturation limit of each carbonyl. Error bars represent the standard deviations obtained from 3 replicates.

Dealing with ketones, the 1-octen-3-one exhibited a smaller LOD compared to 1-penten-3-one, although they both displayed similar saturation limits. Contrarily to aldehydes, the shortest ketone had the highest LOD. Such difference could be explained by the potential degradation of 1-penten-3-one. HPLC-MS analysis of the 1-penten-3-one solution revealed the presence of 4-methyl-1-nonene-3,7-dione, resulting from the dimerization of 1-penten-3-one¹⁶ as well as 1-(6-ethyl-3,4-dihydro-2H-pyran-2-yl)-1-propanone and 4-hydroxy-3,8-decanedione, being both products of the Diels-Alder reaction¹⁶. The molecular size and hydrophobicity of these molecules strongly differ from the properties of 1-penten-3-one, which could explain the inconsistency of the results obtained with the ketones.

3.1.4. Influence of interfering compounds on the fluorescence property of the material

The impact of the presence of interfering compounds on the fluorescence property of the material was also investigated. *n*-pentane, *n*-hexane and *n*-octane were chosen as alkane structural analogues of the different carbonyls tested. It was noteworthy that *n*-pentane did not affect the fluorescence emission spectrum of the material at all. However, *n*-hexane and *n*-octane were both responsible for 30 % of fluorescence quenching. **Figure 3a** compares the influence of the concentration of hexanal and *n*-hexane on the fluorescence of the material. Hexanal was able to quench 100 % of the fluorescence of the material, while a plateau at 30 % was reached with *n*-hexane. Only physical interactions such as hydrophobic interactions could explain the quenching of the fluorescence of the material by the alkane. It could be deduced that several quenching mechanisms are involved when the material interacts with hexanal, and that some of them are related to physical interactions. **Figure 3b** compares the kinetics of fluorescence quenching either induced by hexanal or by *n*-hexane. It was observed that the equilibrium was reached faster for *n*-hexane compared to hexanal. This is in line with previous results revealing that the more hydrophobic the molecules were, the faster the equilibrium was reached. For the application as a sensor, the potential quenching by interfering compounds will

thus have to be taken into account by previous calibration of the sensor according to the matrix analyzed if such structural analogues exist in the product (which is not the case for foods).

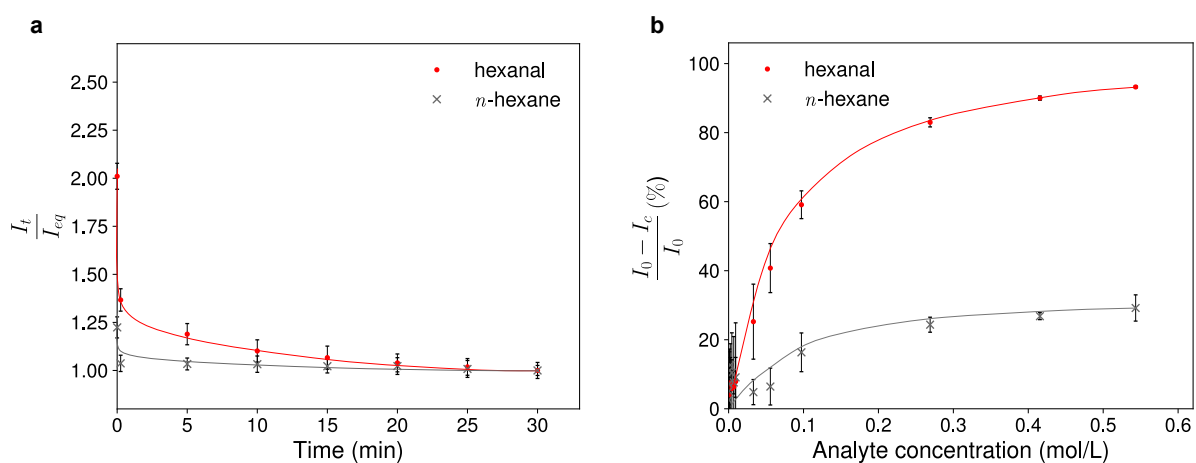


Figure 3: Influence of the presence of an interfering compound (*n*-hexane) on the fluorescence property of the material.

a. Kinetics of the fluorescence decrease of the material after interaction with hexanal and an alkane structural analogue, *n*-hexane, playing the role of interfering compound. Error bars represent the standard deviations obtained from 3 replicates. Solid lines are guides for the eyes.

b. Influence of the concentration of hexanal and *n*-hexane on the percentage of fluorescence loss of the material. Error bars represent the standard deviations obtained from 3 replicates. Solid lines are guides for the eyes.

3.2. INFLUENCE OF THE SOLVENT ON THE FLUORESCENCE PROPERTY OF THE MATERIAL

3.2.1. Influence of the polarity of the solvent on the fluorescence of the material

The impact of the nature of the solvent on the fluorescence property of the material was assessed by analyzing the fluorescence emission spectrum of the material in suspension in four different solvents of increasing polarity: ethanol, ethanol/water mixture (50/50, v/v), ethanol/water mixture (10/90, v/v) and water (see **Figure 4a**). In ethanol, the material was 1.7 times more fluorescent as in water, and in mixtures of ethanol and water the fluorescence intensity decreased when the water content increased. Thus, the fluorescence intensity of the material decreased when the polarity of the solvent increased. Moreover, a slight bathochromic shift of the maximum emission wavelength of the material was noticeable when the solvent polarity increased: $\lambda_{\max}(\text{ethanol}) = 338 \text{ nm} > \lambda_{\max}(\text{ethanol/water, 50/50, v/v}) = 339 \text{ nm} > \lambda_{\max}(\text{water}) = 340 \text{ nm}$. It is known that changing

the solvent may impact the fluorescence maximum wavelength and the fluorescence intensity of fluorescent probes^{17,18}. In protic solvents such as ethanol and water, intermolecular hydrogen bonds between the nitrogen atom of the aniline group and the -OH groups of the solvents may occur and stabilize the ground state^{19,20}. When the polarity of the solvent increases, the fluorescence quenching is generally attributed to charge/proton transfer or electron transfer^{18,19,21}. Therefore, in the next experiments, an ethanol/water mixture (10/90, v/v) was used as food simulant for lipid-rich products such as fish, meat or cheese, according to the EU regulation n° 10/2011. The same parameters as previously evaluated in ethanol were analyzed in order to determine the impact of the nature of the solvent on the fluorescence property of the material.

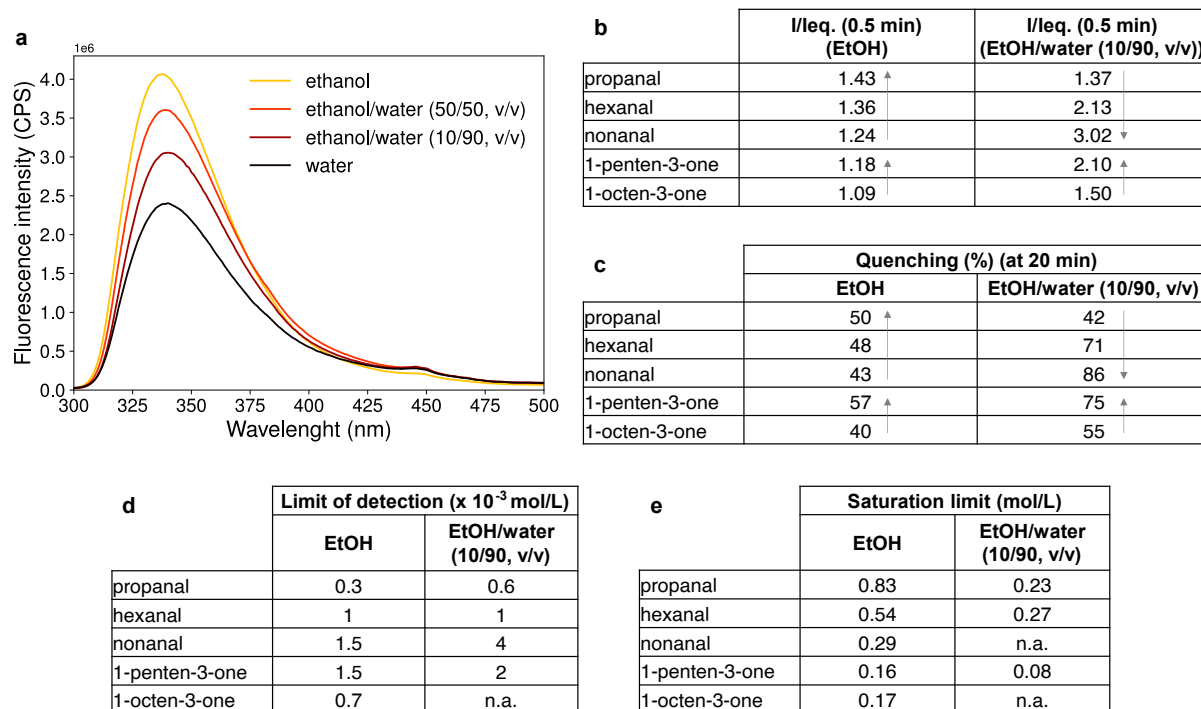


Figure 4: Influence of the solvent polarity on the fluorescence of the material (kinetics of the fluorescence decrease, percentage of fluorescence quenching, limit of detection and saturation limit of each carbonyl).

a. Evolution of the fluorescence spectrum of a 10 mg suspension of material in four dispersing phases of increasing polarity (ethanol; ethanol/water, (50/50, v/v); ethanol/water, (10/90, v/v) and water).

b. Kinetics of the fluorescence decrease of the material after interaction with carbonyls in ethanol (EtOH) and ethanol/water (10/90, v/v) mixture.

c. Percentage of fluorescence quenching of 10 mg of material by carbonyls, in ethanol and ethanol/water (10/90, v/v) mixture at the same carbonyl concentration ($9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$).

d. e. Limit of detection (**d**) and saturation limit (**e**) of the different carbonyls in ethanol and ethanol/water (10/90, v/v) mixture. (n.a.: not applicable due to solubility limitations).

3.2.2. Influence of the polarity of the solvent on the kinetics of fluorescence quenching of the material after interaction with the analytes

As depicted in **Figure 4** (Table b), the rate of adsorption of the analyte by the material was assessed in an ethanol/water mixture (10/90, v/v) and compared to that obtained in ethanol. For aldehydes, it was observed that the fastest fluorescence quenching occurred in the following order: propanal > hexanal > nonanal. Thus, the shortest the molecule was, the fastest the equilibrium state was reached. In ethanol, the opposite behavior was observed. This could be explained by the fact that polar molecules are more soluble in polar medium and are thus more likely to be conveyed up to the material compared to more hydrophobic molecules. Moreover, concerning ketones, 1-octen-3-one quenched more rapidly the fluorescence of the material compared to 1-penten-3-one. These molecules did not follow the trend of their aldehydes' counterparts. This is probably due to the mixture of molecules derived from 1-penten-3-one which did not permit any comparison between the 2 ketones.

3.2.3. Influence of the polarity of the solvent on the fluorescence quenching of the material by the analytes

As displayed in **Figure 4** (Table c), the fluorescence of the material was more efficiently quenched by aldehydes with longer aliphatic chains at a given concentration. Nonanal accounted for 86 % of quenching while propanal only quenched 42 % of the fluorescence at the same concentration ($9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$). The ranking of the aldehydes quenching the more the fluorescence was as follows: nonanal > hexanal > propanal. Except for propanal, the percentage of fluorescence quenching was higher with each analyte in the ethanol/water mixture (10/90, v/v) compared to ethanol. This is probably due to interactions between the material and the more polar solvent, resulting in non-radiative relaxation processes of the excited material.

3.2.4. Influence of the polarity of the solvent on the limit of detection and saturation limit of the sensor

The influence of the concentration of the different analytes on the fluorescence of the material was also assessed in the ethanol/water mixture (10/90, v/v) and compared to the

results obtained in ethanol. As 1-octen-3-one was not soluble in the ethanol/water mixture, it was no longer considered for this experiment. As shown in **Figure 4** (Table d), the limit of detection of all of the carbonyls tested was higher in the ethanol/water mixture compared to ethanol. The saturation limit was rather difficult to estimate in the ethanol/water mixture due to the limitation brought by the solubility of some carbonyls (especially nonanal) at high concentrations. As depicted in **Figure 4** (Table e), the saturation limit was lower in the ethanol/water mixture. The fluorescence extinction was thus reached at lower concentrations of carbonyls (propanal, hexanal and 1-penten-3-one) in the ethanol/water solvent. Given the facts that the fluorescence intensity of the material decreases after interaction with the analyte and that the initial fluorescence of the material is lower in more polar solvents, the detectable range of carbonyl concentration is therefore more limited in ethanol/water mixtures. However, even if the range of detection of the sensor in water-rich solvents is reduced, it is still largely enough for the monitoring of lipid oxidation among food products. Actually, food products may release from a few $\mu\text{g}\cdot\text{g}^{-1}$ to a few $\text{mg}\cdot\text{g}^{-1}$ of each carbonyl during the oxidative spoilage^{22–24}. Moreover, it can be considered that around 40 carbonyls molecules are commonly released by lipid-rich products, among which the five tested in this study. Considering an average food product in its packaging, it could be estimated that in the worst-case scenario, no more than $0.25\text{ mol}\cdot\text{L}^{-1}$ of carbonyls should be released. This corresponds to the saturation limit of the sensor presented. Saturation limit is thus high enough to monitor the global content of carbonyls released during the lipid oxidation of a food product. This brings an additional proof that the material could also be successfully used for carbonyl monitoring in water-rich products.

3.3. INFLUENCE OF THE pH ON THE FLUORESCENCE PROPERTY OF THE MATERIAL

When 10 mg of material were placed in suspension in 2 mL of ethanol, the pH was 7.82 ± 0.02 . The impact of the pH of the dispersing phase on the fluorescence property of the material was also studied. **Figure 5** depicts the fluorescence intensity of the material at its maximum emission wavelength (337 nm) before and after interaction with 1-octen-3-one according to the pH of the ethanol/water (10/90, v/v) mixture in which the material was placed in suspension. At pH 1.4, the fluorescence intensity of the material was close to zero. Under acidic conditions, the aniline is protonated ($\text{pK}_{\text{a}1} = 4.6$) and the anilinium ion is non-fluorescent²⁵. This is probably due to the loss of the lone pair of electrons from the nitrogen atom resulting in reduced delocalization of the π -electrons over the benzene ring, which is

responsible for the initial fluorescence of aniline. As the fluorescence of the material is extremely weak at this pH, it cannot be further quenched by interaction with 1-octen-3-one. Moreover, protonated amines are less likely to chemically react with carbonyl compounds by nucleophilic addition. Thus, 1-octen-3-one did not have any impact on the fluorescence of the material at this pH. Similar behavior was observed at pH 12.8: aniline was deprotonated ($pK_{a2} = 9.3$) and the material became non-fluorescent. In such a case, the aniline anion is probably not fluorescent because of charge/proton transfer non-radiative relaxation process. Moreover, a dissolution of the silicate-based material started to occur under basic conditions. This phenomenon was already noticed at $pH > 9$ by Krauskopf²⁶. It could be related to the polarization of Si-O bonds in the bulk by the negatively charged Si-O groups on the surface of the material, leading to a weakening the Si-O bonds and thus promoting the dissolution of the silica-based material²⁷. At pH 7.6 and 9.3, the fluorescence intensity of the material was at its maximum. This is in good agreement with the observations previously made by Williams, who highlighted that the maximum fluorescence of anilines was obtained at a pH between 7 and 10²⁵. Therefore, the aniline grafted onto a silica structure seemed to respond similarly to the variation in pH as the aniline in solution. Over this pH range, the fluorescence of the material was significantly quenched by 1-octen-3-one. At intermediate pH, the amino group of the aniline is not charged. Molecular aniline is thus both highly fluorescent and highly reactive compared to its ionic forms. According to these results, the optimal conditions for using the material are at a pH between 7 and 9. Such material could thus be used for the monitoring of lipid oxidation for a lot of different food products, as usual pH of common lipid-rich products (milk²⁸, butter²⁸, fish²⁹, meat³⁰) is around 6 – 7.

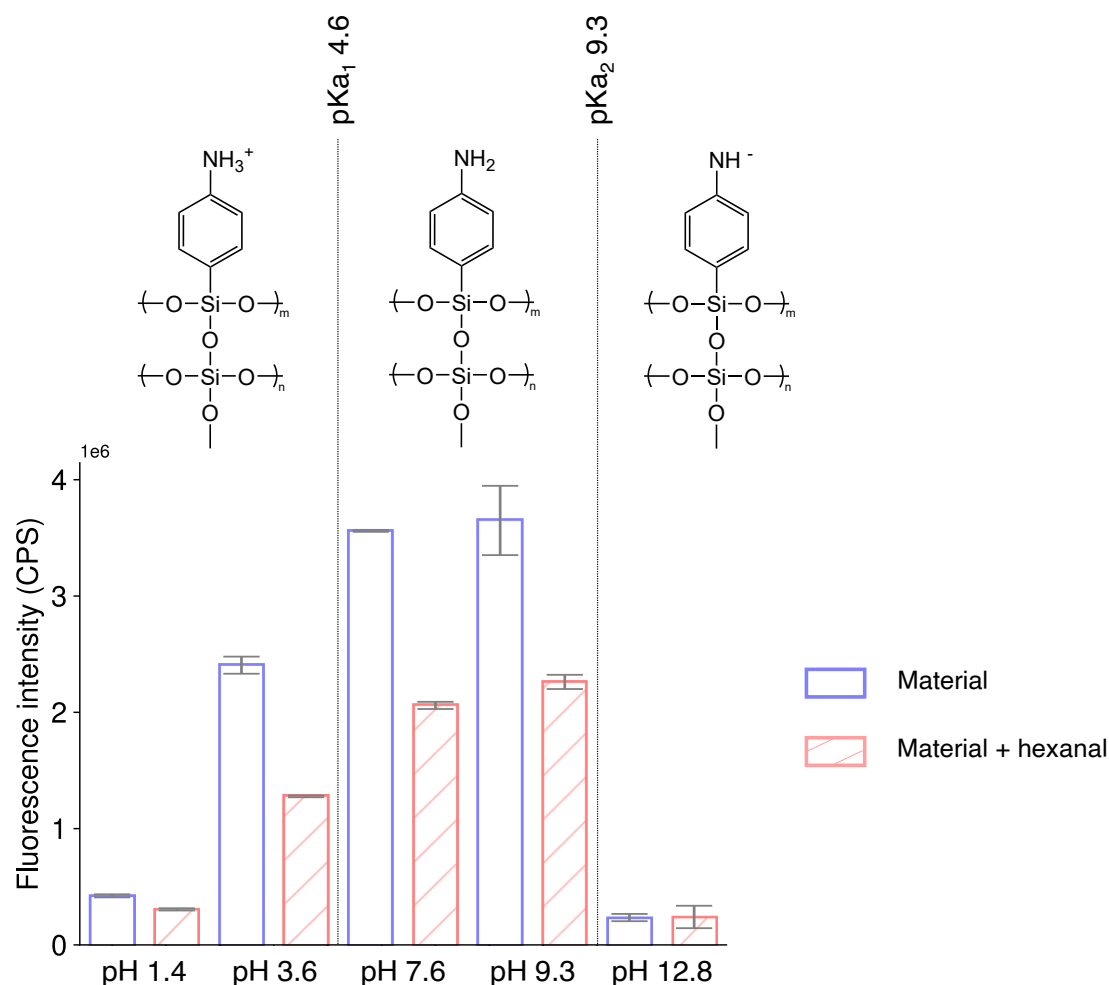


Figure 5: Influence of the pH of the dispersing phase on the fluorescence property of the material.

Evolution of the fluorescence intensity at 337 nm of 5 mg of material, before (blue empty bar) and after (red hatched bar) interaction with 1-octen-3-one for 10 min, according to the pH of the ethanol/water (10/90, v/v) dispersing phase. Error bars represent the standard deviations obtained from 3 replicates. Corresponding theoretical evolution of the structure of the aniline group of the sol-gel material according to the pH. m and n are the molar ratio of functional monomer and cross-linker used for the synthesis of the functionalized sol-gel material, respectively.

4. CONCLUSION

To conclude, the performances of a new fluorescent material intended to be used as a sensor for the monitoring of carbonyls secondary products of lipid oxidation was assessed. The impact of the nature of the carbonyl, including the aliphatic chain length, as well as the solvent polarity and its pH on the fluorescence property of the material was investigated. The designed sol-gel material exhibited high fluorescence that was quenched upon interaction with

carbonyls. In ethanol, the kinetics of fluorescence decrease induced by the tested carbonyls (propanal, hexanal, nonanal, 1-penten-3-one and 1-octen-3-one) was fast (less than 5 min). The molecular size and the hydrophobicity of the analyte seemed to be the major parameters that affected the adsorption rate, the percentage of fluorescence quenching as well as the limit of detection and saturation limit of the sensor. The presence of interfering compounds, *n*-hexane and *n*-octane selected as structural analogues of the carbonyls tested, had a limited contribution to the quenching of the fluorescence of the material. This will have to be taken into account in the analysis of complex matrices if such molecules are obviously present in the matrix studied. In more polar solvents, the fluorescence intensity of the material was lower because of charge/proton transfer or electron transfer mechanisms. However, the kinetics of fluorescence decrease remained fast (less than 5 min), and the fluorescence quenching by carbonyls was significant enough to detect carbonyls over a wide range of concentrations (from 0.6 mmol·L⁻¹ to 0.27 mol·L⁻¹). The optimal pH range for the use of the sensor was 7 – 9, corresponding to molecular aniline form, conferring to the material both high fluorescence and high reactivity towards carbonyls. The sensor was thus proven to be efficient in different food simulants, at pH corresponding to most of the lipid-rich food products, and with a range of detection allowing the lipid oxidation to be monitored all along the shelf-life of the food product.

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Chap. 4. APPLICATION OF THE SOL-GEL MATERIAL TO THE MONITORING OF THE OXIDATION OF A COMMERCIAL OIL

In this chapter, the sol-gel material was applied to the monitoring of the oxidation of a real food matrix: a commercial fish oil. The indications of fluorescence provided by the sensor were correlated with usual classical methods used to determine the oxidation level of food: the peroxide value, the thiobarbituric acid reactive substances assay and the solid phase microextraction coupled with gas chromatography-mass spectrometry.

A new, fast and simple method for lipid oxidation monitoring based on a fluorescent sol-gel sensing material: application to edible oil.

ABSTRACT

A new sensor for the fast and simple detection of the lipid oxidation level of food products was developed. Based on aniline and synthesized by a cost-effective and straightforward sol-gel process, a highly fluorescent material was newly designed. Its fluorescence was quenched upon interaction with carbonyls secondary products of lipid oxidation. Its successful application to the monitoring of the accelerated aging of a fish oil from menhaden is reported. It was possible to positively correlate the percentage of fluorescence intensity loss of the developed sensor to the peroxide value and the result of the thiobarbituric acid reactive substance assay. This new analytical method is 225 times more sensitive than the TBARS assay. The evolution of volatile carbonyls, in particular hexanal, heptanal, 2,4-heptadienal and 3,5-octadien-2-one, was monitored by SPME-GC-MS during oxidation. The sum of the surface area of the corresponding chromatogram peaks of each of these compounds was also correlated to the fluorescence loss of the sensor. This study demonstrates the possibility of using this new sensor instead of classical methods for the fast, simple and sensitive determination of the lipid oxidation level of food products, with little sample preparation and no manipulation of any toxic reactant required.

1. INTRODUCTION

Lipids are major macronutrients in human consumption contributing to the daily energy intake. They are mainly composed of triglycerides, which are glycerol molecules esterified with 3 carboxylic acids, also called “fatty acids”. α -linolenic acid (C18:3, ω 3), linoleic acid (C18:2, ω 6) and oleic acid (C18:1, ω 9) are among the most abundant fatty acids. Eicosapentaenoic acid (EPA) (C20:5, ω 3) as well as docosahexaenoic acid (DHA) (C22:6, ω 3) are omega 3 fatty acids found in fish-derived products. Due to their high level of unsaturation, these unsaturated lipids are very sensitive to oxidation. Lipid oxidation is an unstoppable and inevitable degradation process occurring in any product containing unsaturated lipids exposed to oxygen. Lipid oxidation follows a radical mechanism, either initiated by heat, light or catalysts such as metals, pigments or enzymes¹. Hydroperoxides are usually generated at first. Then, they are mainly

converted into aldehydes, ketones, alcohols and carboxylic acids². Carbonyls compounds are major contributors to flavor deterioration of lipid-rich products^{3,4}.

To slow down the lipid oxidation process, the agri-food industry relies on different strategies. Antioxidants can be added to the food to extend the shelf-life. Opaque food packaging, high oxygen barrier materials and the use of vacuum or modified atmosphere packaging confer additional protection against food oxidation. However, residual oxygen always remains in the food product and in the packaging headspace, leading to the oxidation of lipids. Failing to completely avoid lipid oxidation, it is thus of high importance to be able to determine the oxidation level of food products. Indeed, lipid oxidation implies the degradation of both nutritional and organoleptic properties, generating off-flavors that are prohibitive for consumer acceptance⁴. Moreover, the reaction products from lipid oxidation, such as peroxides, free radicals and aldehydes, may be harmful for the health, since oxidative stress has been linked to many disorders such as Parkinson's and Alzheimer's disease⁵ or cardiac inflammatory diseases⁶.

Among the most classical methods used to determine the oxidation level of food are the peroxide value (PV), the quantity of conjugated dienes, the p-anisidine value, the thiobarbituric acid reactive substance (TBARS) assay, chromatographic methods as well as sensory evaluation. Although many of them are standard methods⁷⁻¹⁰ used for decades, they are time-consuming, require high amounts of solvents and toxic reactants and may rely on the use of expensive equipment by highly trained personnel. Therefore, there is a real need for new, fast and simple analytical method for the determination of the lipid oxidation level in food products.

Some previous works already intended to correlate some analytical techniques with classical indices of lipid oxidation. As an example, PV was successfully correlated with near-infrared spectroscopy (NIRS) measurements for the determination of the oxidation level of crude palm oil¹¹. NIRS was also recently correlated to the quantity of conjugated dienes in oil in water (O/W) emulsions¹². Supported by HPLC-MS analysis, Lin M. and Liu S. synthesized a fluorescent probe based on naphthalimide hydrazine. They successfully detected hexanal and malondialdehyde in model linoleic acid O/W emulsions¹³.

In the present work, we report the successful application of a new fluorescent sensing material for the monitoring of the oxidation of a commercial lipid-rich fish oil during accelerated

aging. Based on aniline and synthesized using a straightforward and cost-effective sol-gel process, the sensing material exhibits high fluorescence that is quenched upon interaction with carbonyl compounds. Details about the synthesis and the characterization of this material can be found in previous work. The fluorescence of the sensor, PV and TBARS value were followed. The evolution of the volatile compounds released during accelerated oxidation of the oil was also determined by SPME-GC-MS over time. Correlations between the loss of fluorescence intensity of the sensor with the results obtained by classical methods (PV, TBARS and volatile carbonyls) could thus be assessed.

2. MATERIALS AND METHODS

2.1. CHEMICALS

4-trimethoxysilylaniline (4-TMSA, 97 %, CAS number 33976-43-1) was obtained from abcr, Germany. Cyclohexane (99.5 %, CAS number 110-82-7) was bought from Chem-lab. Propan-2-ol (95 %, CAS number 67-63-0) was provided by Carlo Erba Reagents. Tetraethylorthosilicate (TEOS, 99 %, CAS number 78-10-4), ammonium hydroxide (NH₄OH, 30 %, CAS number 1336-21-6), glacial acetic acid (CH₃CO₂H, 99 %, CAS number 64-19-7), sodium thiosulfate (Na₂S₂O₃, 98 %, CAS number 7772-98-7), potassium iodide (KI, 99 %, CAS number 7681-11-0), 2-sulfanylidene-1,3-diazinane-4,6-dione (2-thiobarbituric acid, 98 %, CAS number 98 504-17-6), propyl gallate powder (98 %, CAS number 121-79-9), starch from potato powder (CAS number 9005-25-8), fish oil from menhaden (CAS number 8002-50-04), 1-butanol (99.9 %, CAS number 71-36-3), ethanol (EtOH, 96 %, CAS number 64-17-5, used for the thiobarbituric acid reactive substance assay) and ethanol for UV-spectroscopy Uvasol® (EtOH, 99,9 %, CAS number 64-17-5, used for fluorescence experiments) were purchased from Sigma Aldrich, France. Deionized water was used, obtained from a MilliQ RG, Millipore apparatus.

2.2. ACCELERATED OXIDATION OF MENHADEN OIL

19 g of menhaden oil were placed in opaque sealed jars of 200 mL (ratio oil/air: 0.1). Oil samples were submitted to an accelerated oxidation test, which was conducted in an oven at 60 °C for 21 days. Quality indices were determined in fresh oil, and after 1, 3, 5, 7, 14 and 21 days. At each measurement day, three jars were taken from the oven to perform three repetitions of each quality indices measurements.

2.3. FLUORESCENCE OF THE SENSING MATERIAL

A Spectrofluorometer Fluoromax4® (Horiba) was used with the software Fluorescence®. A SUPRASIL® quartz cell (Hellma) of 10 x 10 mm dimension was employed. The excitation wavelength was 360 ± 2 nm to minimize the fluorescence emission from the oil and to maximize the fluorescence emission from the material. Fluorescence emission spectrum was recorded between 400 nm and 600 nm, with a 2 nm bandpass and a step interval of 1 nm. The temperature was kept constant at 22 °C during this experiment. Each experiment was performed 3 times.

First, the fluorescence emission spectrum of 2.79 g of menhaden oil was recorded. Then, 10 mg of the sensing material were added and the suspension was stirred for 1 min using a vortex and for 14 min at 80 rpm on a stirring plate. Then, the fluorescence emission spectrum of the material in suspension in the oil was recorded. The total surface area of the fluorescence emission curve from the oil alone and from the material in the oil were considered. The gradually oxidized oil displayed a weak fluorescence over the wavelength range studied, which was taken into account by systematically subtracting the corresponding surface area of the fluorescence emission curve of the oil at a given oxidation time to one of the material in suspension in the oil at the same time. The percentage of fluorescence loss of the material after interaction with carbonyl compounds released by lipid oxidation of the oil was finally determined as follows:

$$\text{Fluorescence loss} = \frac{[A_{mat.}(t_0) - A_{oil}(t_0)] - [A_{mat.}(t) - A_{oil}(t)]}{A_{mat.}(t_0) - A_{oil}(t_0)} \quad (1)$$

with $A_{mat.}$ being the total surface area of the fluorescence emission curve from the material in the oil A_{oil} the total surface area of the fluorescence emission curve from the oil alone.

2.4. PEROXYDE VALUE DETERMINATION

After 1, 3, 5, 7, 14 and 21 days of accelerated oxidation, the peroxide value (PV) of menhaden oil samples was determined according to the AOAC official method⁷. Between 0.5 and 2 g of oil were firstly dissolved in 10 mL of a mixture of cyclohexane/propan-2-ol/ethanol (5/7/2, v/v/v). 10 mL of anhydrous acetic acid and 5 mL of an aqueous solution saturated with potassium iodide were added. The reaction mixture was stirred for 1 min at 150 rpm and placed in the dark for 5 min. 75 mL of water were added and the mixture was titrated with a freshly

prepared aqueous solution of sodium thiosulfate ($0.01 \text{ mol}\cdot\text{L}^{-1}$), using iodine-starch as a colorimetric indicator. A blank was used replacing oil with water. PV was expressed in milliequivalents of oxygen per kg of oil.

2.5. THIOBARBITURIC ACID REACTIVE SUBSTANCE ASSAY

After 1, 3, 5, 7, 14 and 21 days of accelerated oxidation, menhaden oil samples were also analyzed using the thiobarbituric acid reactive substance (TBARS) assay according to the AOAC official method¹⁰. Between 50 and 200 mg of oil were firstly dissolved in 1-butanol. 10 mg of propyl gallate were added. 5 mL of this solution were mixed with 5 mL of 2-thiorbarbituric acid ($2 \text{ mg}\cdot\text{mL}^{-1}$). The final solution was heated at 95°C for 2 h, before being cooled down at 22°C for 30 min. The absorbance of the sample was measured at 530 nm on a UV-visible spectrophotometer (mc1, SAFAS, Monaco) using 1-butanol as a reference. A blank was prepared following the same procedure without oil. The results were expressed as mg malondialdehyde (MDA)/kg of oil.

2.6. EVOLUTION OF VOLATILE COMPOUNDS

After 1, 3, 7, 14 and 21 days of accelerated oxidation, Solid Phase Micro Extraction was used to analyze the headspace of 20 mL vials (closed with a silicone/PTFE septum) containing 0.93 g of menhaden oil. These vials were maintained at 60°C under agitation at 500 rpm for 5 min. Then an extraction was done during 2.5 min. Volatiles were adsorbed from the headspace onto a three-phase (DVB, CAR, PDMS) SPME fiber (Sigma Aldrich) and were analyzed on a 7890A gas chromatograph (Agilent Technologies) equipped with a capillary column (30 m x 0.32 mm internal diameter, $0.5 \mu\text{m}$ thickness) coated with a DB-5MS stationary phase (Agilent J&W). Desorption was directly performed into the injector, using splitless mode at 250°C . Mass spectrometer HP 5975 was used as a detector (electronic impact 70 eV, transmission line at 240°C). Helium was the carrier gas at a flow rate of $1.4 \text{ mL}\cdot\text{min}^{-1}$. The oven temperature was programmed from 40°C (maintained for 3 min) to 70°C at a temperature rate of $3^\circ\text{C}\cdot\text{min}^{-1}$, then from 70°C to 110°C at $5^\circ\text{C}\cdot\text{min}^{-1}$ and from 110°C to 250°C at $10^\circ\text{C}\cdot\text{min}^{-1}$. This final temperature was maintained for 5 min.

2.7. STATISTICS

Statistical analysis (ANOVA with Tukey's post hoc analysis) was performed using XLSTAT (Addinsoft) software. Significance was established at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. MONITORING OF OXIDATION WITH A FLUORESCENT SENSING MATERIAL

Figure 1a displays the fluorescence emission spectrum of the sensing material in suspension in menhaden oil. In fresh oil (day 0), the material exhibited high fluorescence with a maximum of emission at 512 nm. As the oil oxidized during the accelerated aging, the fluorescence intensity of the material decreased. The fluorescence of the material was quenched by interaction with carbonyl compounds released by lipid oxidation. **Figure 1b** represents the percentage of fluorescence loss over time. Saturation of the sensor is reached at 100 % of fluorescence loss, meaning total extinction of the fluorescence of the material. This happened from the 7th day of oxidation. Between day 0 and day 7, the percentage of fluorescence loss can be correlated to the increasing oxidation level of the oil.

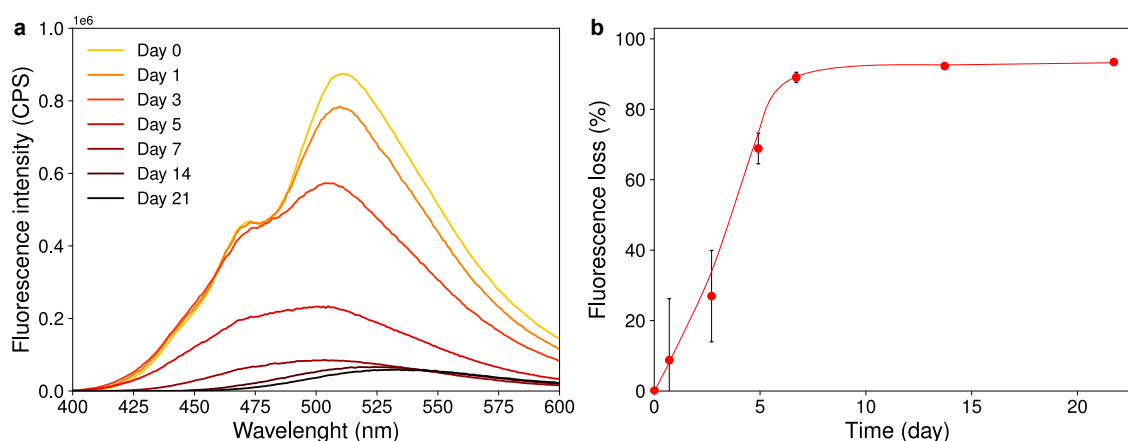


Figure 1: Evolution of the fluorescence of the sensor during the oxidation of menhaden oil.

a. Evolution of the fluorescence emission spectrum of 10 mg of material in suspension in 2.79 g of fresh menhaden oil (yellow curve) and oxidized oil (other curves). Excitation was set at 360 ± 2 nm.

b. Evolution of the percentage of fluorescence loss of 10 mg of material during oxidation. Error bars represent the standard deviation from 3 replicates. Solid line is a guide for the eyes.

3.2. EVOLUTION OF THE PEROXYDE VALUE DURING OXIDATION

Figure 2a depicts the evolution of the PV with oxidation time. The PV first increased from day 0 to day 7, stabilized between day 7 and 14 and finally decreased after day 14. The shape of the curve representing the PV as a function of time is typical of lipid oxidation. This test monitors the evolution of the concentration of primary products of lipid oxidation (mainly

hydroperoxides), which are transitory and further transformed into secondary products^{3,14}. **Figure 2b** simultaneously represents the percentage of fluorescence loss of the developed sensing material with the evolution of the PV during oxidation. The EU regulation (EC 796/2002) established an upper limit for the PV for the commercialization of virgin olive oil at 20 meq/kg. Above this PV, it is considered that oxidation started to occur in the oil. This limit was already reached between the day 0 and the day 1, confirming that the temperature initiated the oxidation process and that menhaden oil is very sensitive to oxidation. **Figure 2c** correlates the percentage of fluorescence loss of the sensing material with the PV. Linear regression between the two indices displayed positive correlation with a Pearson's coefficient, which measures the linear correlation between two sets of normally distributed observed variables of 0.99. The developed sensor can thus be used to determine early stages of lipid oxidation.

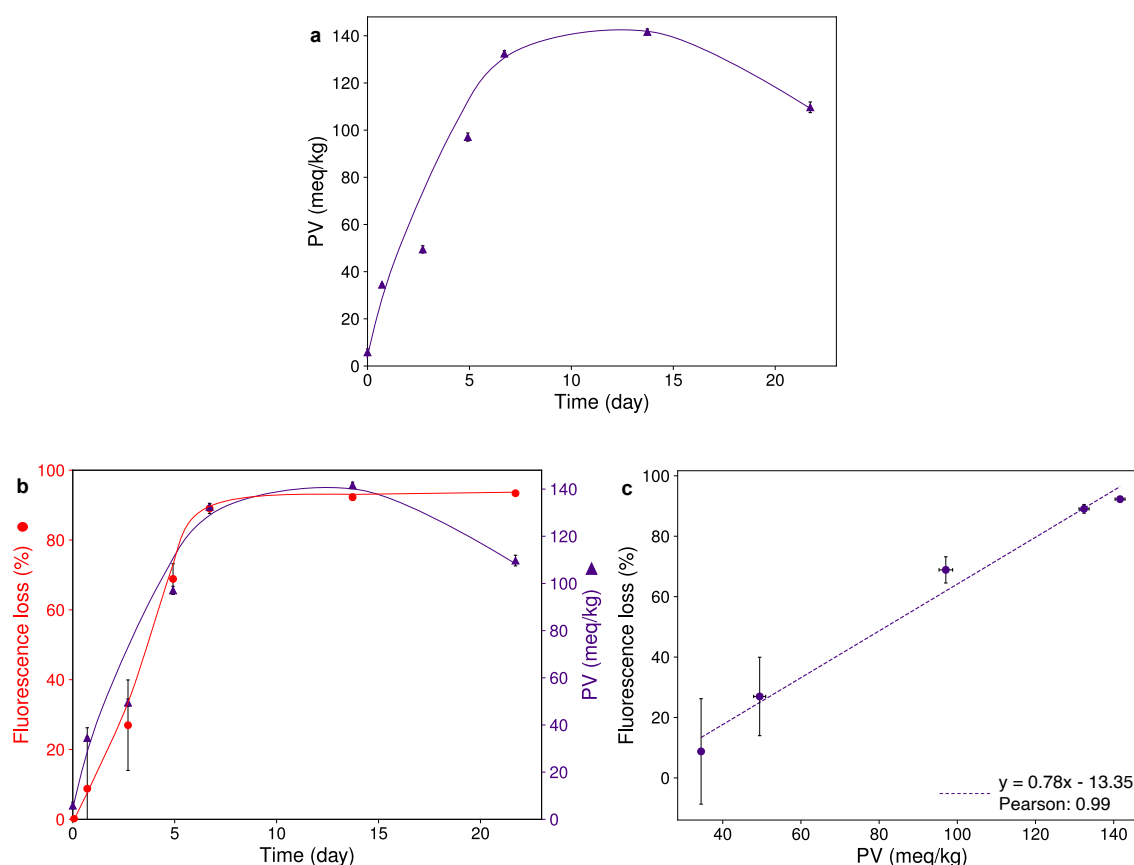


Figure 2: Evolution of the peroxide value (PV) during the oxidation of menhaden oil and correlation with the fluorescence loss of the sensing material.

a. Evolution of the PV during the oxidation of the oil. Error bars represent standard deviation from 3 replicates. Solid line is a guide for the eyes.

b. Evolution of the percentage of fluorescence loss of the sensing material and of the PV over time. Error bars represent standard deviation from 3 replicates. Solid lines are guides for the eyes.

c. Correlation between the fluorescence loss of the sensor and the PV. Error bars represent standard deviation from 3 replicates. The 2 last points where the saturation of the fluorescent sensor was reached and PV started to decrease are not included.

3.3. EVOLUTION OF THE THIOBARBARBITURIC ACID REACTIVE SUBSTANCE VALUE DURING OXIDATION

The TBARS assay follows the evolution of the secondary products of lipid oxidation (mainly aldehydes). Usually, the TBARS value is constant and close to zero at the beginning of the oxidation process and then gradually increases. This is exactly what was observed, as shown in **Figure 3a**, which depicts the evolution of the TBARS value over time. The TBARS value did not change between day 0 and day 1 and then linearly increased from day 3 up to the end of the experiment. This is in good agreement with the evolution of the PV previously described. Oxidation started between day 0 and day 1 with the generation of primary products, which were transformed into secondary products from day 1. Secondary products were thus detectable from day 3 by the TBARS assay. **Figure 3b** simultaneously represents the percentage of fluorescence loss of the sensor and the TBARS value during oxidation. There is no regulatory threshold for the value of TBARS. In the literature, a limit of TBARS value in correlation with sensory analysis has already been reported at 2 mg MDA/kg for highly oxidized beef^{15,16}. Even if the food matrix is not the same, this value can be used as a limit not to exceed in the case of menhaden oil. This limit was reached between day 14 and day 21. In addition, it is noteworthy that the slope of the fluorescence loss (18.02) was steeper than that of the TBARS value (0.08). The new analytical method proposed was thus 225 times more sensitive than the TBARS assay. **Figure 3c** correlates the percentage of fluorescence loss of the sensing material with the TBARS value. Linear regression showed positive correlation between the two indices with a Pearson's coefficient of 0.96. These results further confirm the possibility to use this new analytical method for the monitoring of lipid oxidation in a more sensitive manner compared to the TBARS assay.

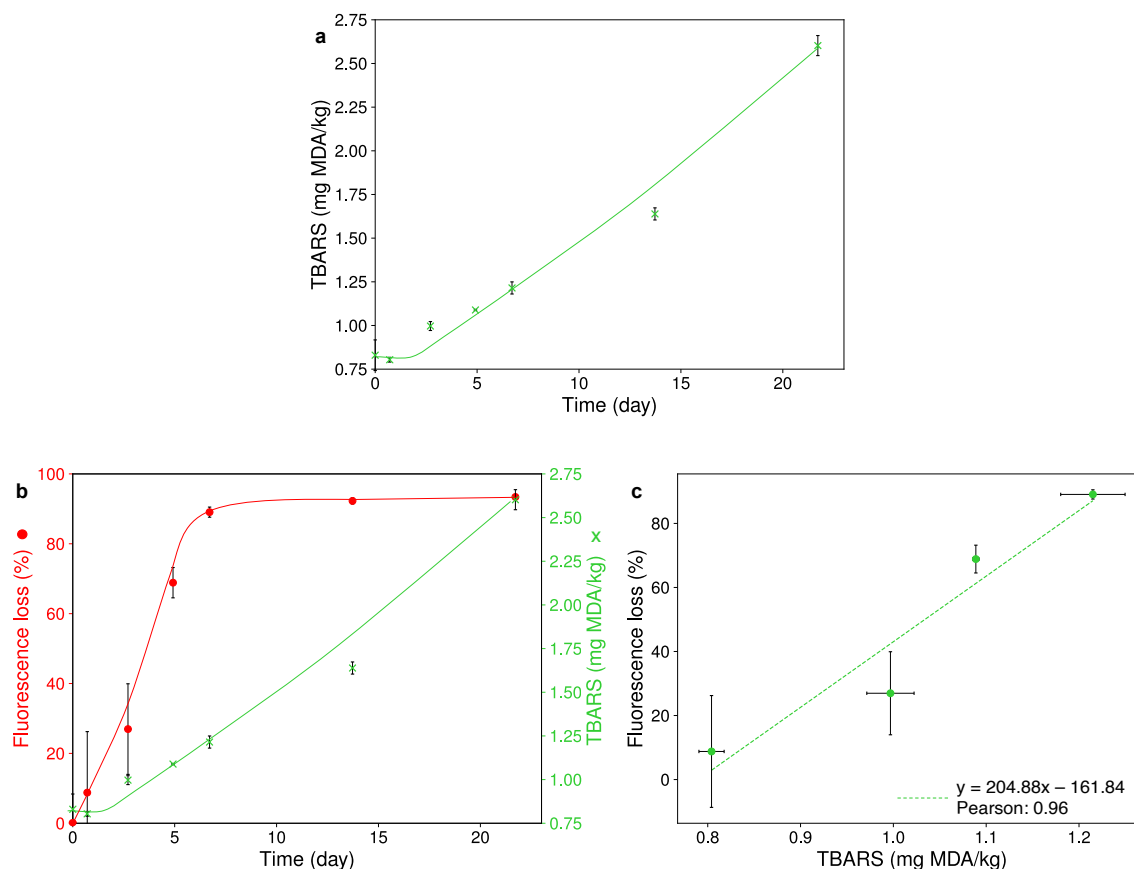


Figure 3: Evolution of the TBARS value during the oxidation of menhaden oil and correlation with the fluorescence loss of the sensing material.

a. Evolution of the TBARS value during the oxidation of the oil. Error bars represent standard deviation from 3 replicates. Solid line is a guide for the eyes.

b. Evolution of the percentage of fluorescence loss of the sensing material and of the TBARS value over time. Error bars represent standard deviation from 3 replicates. Solid lines are guides for the eyes.

c. Correlation between the fluorescence loss of the sensor and the TBARS value. Error bars represent standard deviation from 3 replicates. The 2 last points where the saturation of the fluorescent sensor was reached are not included.

3.4. EVOLUTION OF VOLATILE COMPOUNDS DURING OXIDATION

The monitoring of volatile compounds during oxidation was carried out by SPME-GC-MS. The evolution of four carbonyl compounds, considered as classical secondary products of lipid oxidation^{17,18}, were followed over time: hexanal, heptanal, 2,4-heptadienal and 3,5-octadien-2-one. **Figure 4a** represents the evolution of the surface area of the corresponding chromatogram peak of each of these compounds during oxidation. 2,4-heptadienal and 3,5-

octadien-2-one displayed an increase from day 3 to day 14 followed by a decrease between day 14 and day 21. This suggests that these unsaturated carbonyls were further transformed into lower molecular weight carbonyls^{19,20}. Short and aliphatic aldehydes such as hexanal and heptanal showed an increase from day 3 up to the end of the experiment. The evolution of their concentration with time in the headspace is reported in **Figure 4b**. The quantity of carbonyls in the oil therefore started to increase from day 3. This is line with the results of the TBARS assay, which also differ significantly from the initial value only from day 3. **Figure 4c** displays the simultaneous evolution of the percentage of fluorescence loss of the sensor and the sum of the surface areas of the chromatogram peaks corresponding to the four targeted carbonyls (hexanal, heptanal, 2,4-heptadienal and 3,5-octadien-2-one) over time. From day 14, the production of the specific carbonyls monitored reached a plateau because the decrease in quantity of unsaturated carbonyls compensated the increase in hexanal and heptanal concentrations. **Figure 4d** depicts the correlation between the percentage of fluorescence loss of the sensor with the production of the targeted carbonyls. Linear regression showed positive correlation between these two indices with a Pearson's coefficient of 0.99. Therefore, the sensor can unambiguously be used for the monitoring of the evolution of carbonyl compounds.

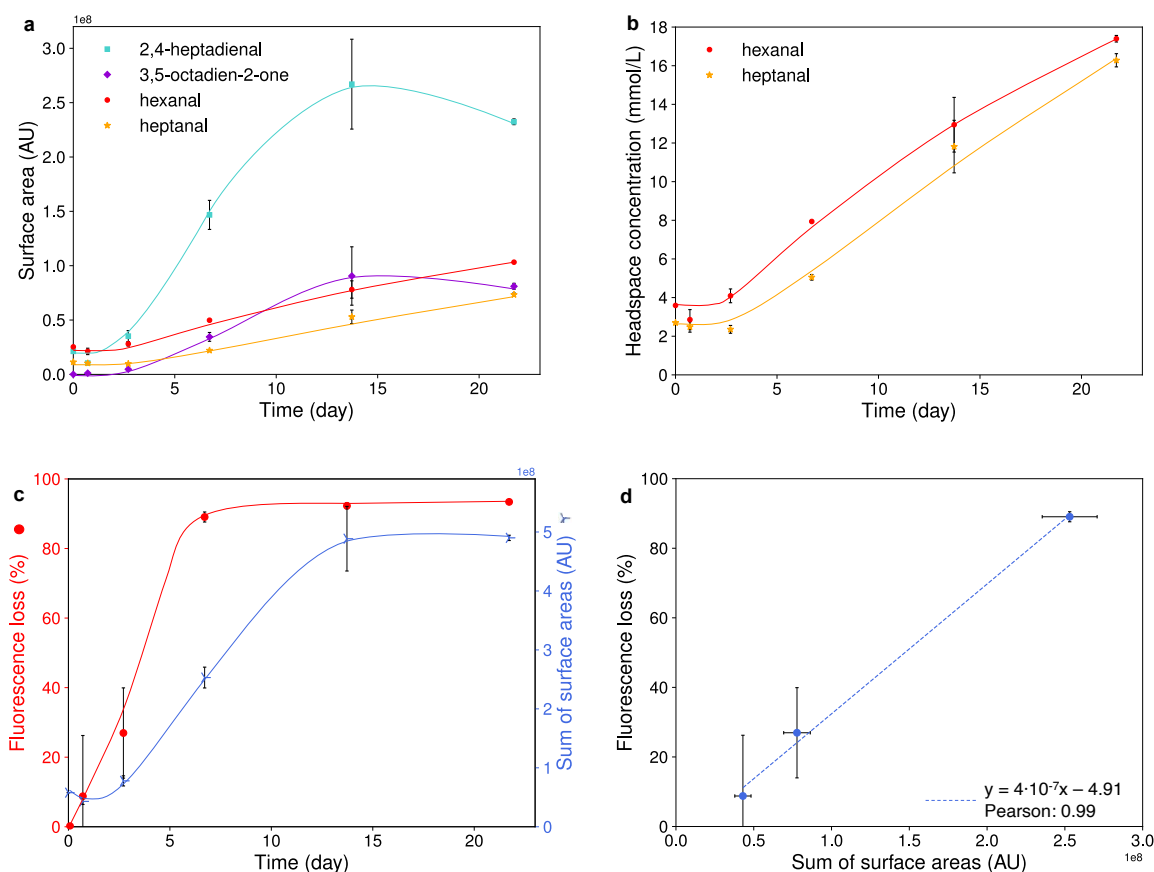


Figure 4: Evolution of volatile compounds during the oxidation of menhaden oil and correlation with the fluorescence loss of the sensing material.

a. Evolution of the surface area of the chromatogram peaks from SPME-GC-MS analysis of four volatile compounds considered as characteristics products of lipid oxidation (hexanal, heptanal, 2,4-heptadienal, 3,5-octadiene-2-one) during oxidation. Errors bars represent the standard deviation obtained from 3 replicates. Solid lines are guides for the eyes.

b. Evolution of the concentration of hexanal and heptanal in the headspace of closed vials containing oxidized oil. Errors bars represent the standard deviation obtained from 3 replicates. Solid lines are guides for the eyes.

c. Evolution of the percentage of fluorescence loss of the sensing material and of the surface area of the chromatogram peaks corresponding to hexanal, heptanal, 2,4-heptadienal and 3,5-octadiene-2-one obtained by SPME-GC-MS during oxidation of the oil. Error bars represent standard deviation from 3 replicates. Solid lines are guides for the eyes.

d. Correlation between the fluorescence loss of the sensor and the surface area of the chromatogram peaks corresponding to hexanal, heptanal, 2,4-heptadienal and 3,5-octadiene-2-one. Error bars represent standard deviation from 3 replicates. The experiment was not performed at day 5. The 2 last points where the saturation of the fluorescent sensor was reached are not included.

4. CONCLUSION

To conclude, this work reported the successful use of a new fluorescent sensor for monitoring lipid oxidation in food products. Based on aniline and synthesized by a cost-effective and straightforward sol-gel process, the fluorescence of this sensing material was quenched upon interaction with carbonyl compounds. Tested on a real food matrix, fish oil from menhaden, the developed sensor exhibited high fluorescence in fresh oil, which decreased when the oil oxidized. The percentage of fluorescence quenching was correlated with three classical methods used for the determination of the oxidation level of food products: the peroxide value, the thiobarbituric acid reactive substances assay and the determination of the composition in volatile compounds of the sample by SPME-GC-MS. Positive correlations were obtained between the loss of fluorescence intensity of the sensor and the values of PV and TBARS with Pearson's coefficients of 0.99 and 0.96, respectively. Moreover, the new analytical method was 255 times more sensitive than the TBARS assay. The evolution of four representative volatile carbonyls compounds (hexanal, heptanal, 2,4-heptadienal and 3,5-octadien-2-one) was also followed over time. The concentration in hexanal and heptanal increased linearly during oxidation while the quantity of unsaturated carbonyls increased up to day 14 and then decreased. A positive linear correlation was obtained between the percentage of fluorescence loss of the sensor and the production of these four targeted carbonyls. To go further, the amount of sensing material could be adjusted in order to detect a more advanced oxidation stage before fluorescence extinction. Nevertheless, all of these results demonstrated the possibility of using this new fluorescent sensor for the monitoring of lipid oxidation in food products in a more sensitive way compared to conventional methods, with a simple and fast method, requiring almost no sample preparation and no use of solvents or toxic reactants. This method could even be adapted to the non-destructive in-situ monitoring of the oxidation level of samples in real-time.

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Chap. 5. DETECTION OF CARBONYL VAPORS USING THE FLUORESCENT SOL-GEL MATERIAL

In the last chapters, the performance of the designed sensor based on the sol-gel material was assessed in liquid media. For further application of the sensor to the non-destructive analysis of the environment surrounding food, the sensor was integrated inside a selected packaging matrix (polylactic acid). In this chapter, the adsorption capacity and fluorescence properties of the material exposed to carbonyl vapors was investigated.

1. ADSORPTION CAPACITY OF CARBONYLS BY THE MATERIAL IN THE GAS PHASE

Adsorption capacity of carbonyls by the material in the gas phase was determined as shown in **Figure 1a**. **Figure 1b** depicts the adsorption capacity of hexanal and hexan-2-one by the material in the gas phase. Similarly to the results obtained in the liquid phase, the mass of hexanal adsorbed by the material increased with the carbonyl concentration in the gas phase. For an initial concentration of hexanal in the gas phase of $0.11 \text{ mmol}\cdot\text{L}^{-1}$, the adsorption capacity of the material was $133 \pm 25 \text{ mg}\cdot\text{g}^{-1}$. The saturation of the material did not seem to be reached and yet its adsorption capacity was already more than 2 times higher than the maximum amount of hexanal adsorbed by a reference silica-gel (which adsorbed 546 mg of hexanal with 10 g of silica material)¹. The adsorption capacity curves display a similar behavior for both hexanal and hexan-2-one but the sensitivity of the material for hexanal vapors seemed to be slightly higher than for hexan-2-one vapors (1.2 times). As a conclusion, results showed that the material can also adsorb aldehydes and ketones in the gas phase.

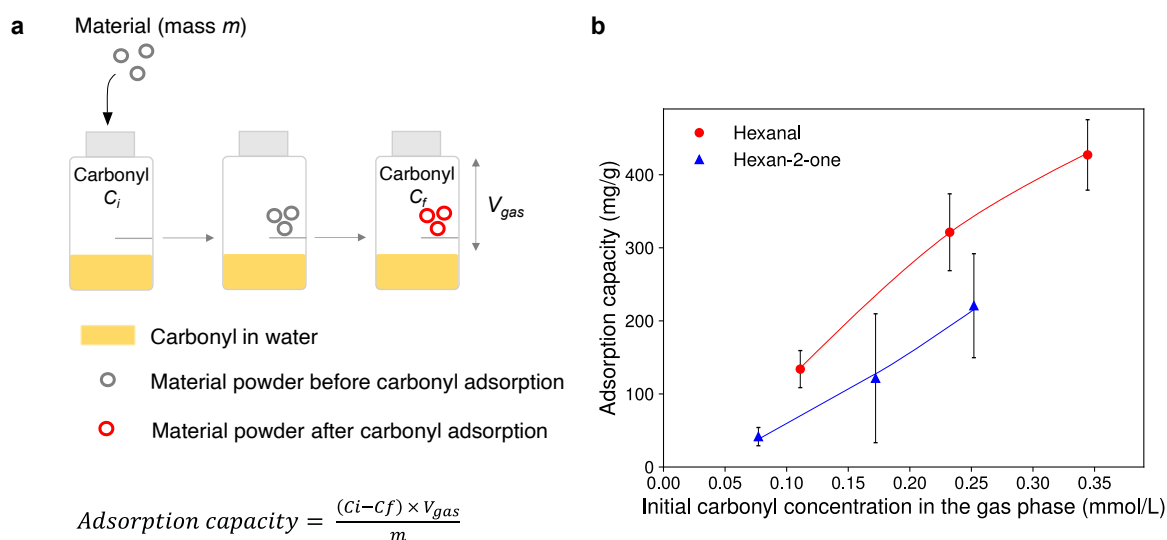


Figure 1: Adsorption capacity of the material when exposed to carbonyls in the gas phase.

a. Schematic representation of the experimental setup used for the determination of the adsorption capacity of carbonyls in the gas phase by the material. C_i and C_f were determined by SPME-GC-MS.

b. Adsorption capacity of hexanal and hexan-2-one by the material in the gas phase. Error bars represent the standard deviation obtained from 3 replicates. Red points are experimental data, solid line is a guide for the eyes.

2. CHANGE IN FLUORESCENCE OF THE MATERIAL UPON EXPOSURE TO CARBONYLS IN THE GAS PHASE

The material was immobilized as a powder dispersed into a polylactic acid (PLA) matrix in order to obtain a film. Films thickness was $0.187 \pm 0.012 \mu\text{m}$. Reproducibility of the film thickness was good, providing similar diffusion of carbonyls inside the film. Moreover, the polylactic acid films incorporating the material were not mutagenic for bacteria according to the Ames' test performed on two bacterial strains: *Salmonella typhimurium* TA98 and TA100.

The influence of the contact time and carbonyl concentration on the fluorescence of the material incorporated in PLA films was studied, as shown in **Figure 2a**. The influence of the presence of an interfering compound (*n*-hexane) in the gas phase on the fluorescence of the material was also assessed.

Figure 2b depicts the kinetics of fluorescence intensity decrease of PLA films incorporating the intelligent material after exposure to hexanal vapors. The fluorescence intensity of the film significantly decreased after 5 min. As observed in the liquid phase, the quenching kinetics in the gas phase was thus fast. After 4 h, condensation of hexanal vapors started to occur on the surface of the films. Standard deviations were quite important and could be attributed to some difficulties encountered to manage perfect repartition of the intelligent material powder inside the PLA films. The good reproducibility of the fluorescence of each piece of film was thus somehow difficult to control perfectly. Similar results were observed with hexan-2-one, but the percentage of fluorescence quenching was lower compared to hexanal for a similar initial concentration of carbonyl in water. This could either be attributed to the lower volatility of hexan-2-one or to the lower affinity of the intelligent material for the ketone.

The impact of hexanal concentration in the gas phase on the fluorescence decrease of the material incorporated in PLA films was performed, with hexanal concentration in the aqueous phase ranging from $4.99 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ ($50 \text{ mg}\cdot\text{L}^{-1}$) to $2.49 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$ ($2.5 \text{ g}\cdot\text{L}^{-1}$) (see **Figure 2c**). First results allowed to estimate the limit of detection to $5 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ ($50 \text{ mg}\cdot\text{L}^{-1}$), which is similar to the limit of detection estimated in the liquid phase. Saturation seemed to occur at $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ ($1 \text{ g}\cdot\text{L}^{-1}$), which is a huge concentration compared to the concentrations of carbonyls released in the gas phase during lipid oxidation for example (a few $\text{mg}\cdot\text{L}^{-1}$ for each compound in average)^{2,3}. In the future, this limit could be easily modulated

by adjusting the amount of intelligent material in the film. As observed in the liquid phase, the material operated over a wide range of detection.

n-hexane vapors, structural analogue of hexanal and hexan-2-one without carbonyl group, did not seem to significantly quench the fluorescence of the films ($I_{337\text{ nm}}$ without *n*-hexane: $1.05 \pm 0.07 \times 10^7$, $I_{337\text{ nm}}$ with *n*-hexane: $1.01 \pm 0.04 \times 10^7$). Interfering compounds in the gas phase did not impact the proper operating process of the sensor. The response of the intelligent material only in the case of interaction with carbonyl compounds in the gas phase is an asset for its application on the monitoring of food complex samples.

To conclude, the same intelligent material could easily be used in both liquid and gas phases, providing the possibility of conducting sample analysis either in direct contact with the product or non-destructively with the monitoring of its surrounding environment.

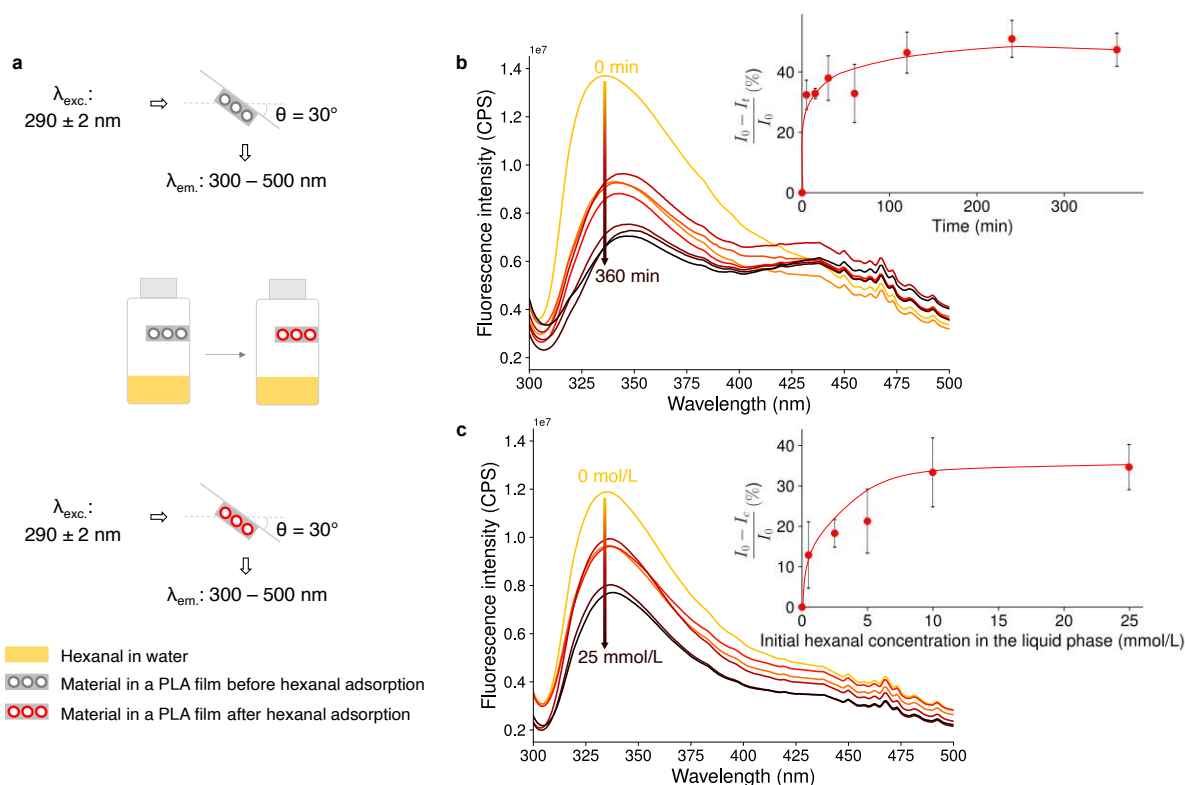


Figure 2: Fluorescence of the material immobilized in PLA films exposed to carbonyls in the gas phase.

a. Schematic representation of the experimental setup used for the determination of the influence of contact time and carbonyl concentration on the fluorescence of the material when exposed to carbonyls in the gas phase (example given with hexanal). The material was immobilized into PLA films, which were placed in the headspace of closed vials saturated with hexanal vapors.

b. Evolution over time of the fluorescence emission spectrum of PLA films incorporating the material and corresponding kinetics of fluorescence quenching of the material upon interaction with hexanal (hexanal concentration in water: $9.99 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$). The yellow spectrum corresponds to the fluorescence of the PLA film incorporating the material before contact with hexanal in the gas phase. Other spectra depict the fluorescence emission of the PLA films incorporating the material after having been placed in contact with hexanal vapors for a determined period of time. For the plot of the kinetics curve, the intensity of fluorescence of the material is measured at its maximum of emission (337 nm). Normalized fluorescence loss is calculated by using the formula $\frac{I_0 - I_t}{I_0}$ with I_0 the intensity of fluorescence of the material immobilized in the PLA film before exposure to hexanal vapors and I_t its intensity after exposure to hexanal during a period of time t . Red points are experimental data, solid line is a guide for the eyes. Error bars represent the standard deviation obtained from 4 replicates. Similar results were obtained with hexan-2-one.

c. Evolution of the fluorescence emission spectrum of the material immobilized in PLA films after exposure to hexanal concentration ranging from 0 (yellow curve) to $0.25 \text{ mmol} \cdot \text{L}^{-1}$ in the gas phase. For the plot of the percentage of fluorescence quenching as a function of the carbonyl concentration, the intensity of fluorescence of the material is measured at its maximum of emission (337 nm). Normalized fluorescence loss is

calculated by using the formula $\frac{I_0 - I_c}{I_0}$ with I_0 the intensity of fluorescence of the material immobilized in PLA films before exposure to hexanal and I_c the intensity of fluorescence emission of the material after contact with hexanal at a concentration c for 2 h. Red points are experimental data, solid line is a guide for the eyes. Error bars represent the standard deviation obtained from 4 replicates.

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Chap. 6. ALTERNATIVE STRATEGIES INVESTIGATED FOR THE DESIGN OF FLUORESCENT MATERIALS FOR CARBONYL SENSING

In this chapter, two new strategies for the synthesis of materials by the sol-gel process were investigated in order to improve the performances of the sensor. In particular, the emission of fluorescence in the visible region was sought. Two main reasons for this: 1) get rid of any detection device to perceive the spectroscopic modifications of the sensors 2) most of the plastics used in the packaging industry are fluorescent in the UV region, which will hinder the reading of the response of the sensor once integrated inside the packaging. The first strategy relied on the use of two distinct monomers bearing the characteristic groups required for the fluorescent sensing of carbonyls (one with an amino group: the 3-aminopropyltrimethoxysilane, the other with a fluorescent core: the 1-naphthyltrimethoxysilane). The second strategy gave rise to two materials and was inspired by the synthesis of the “doped silica” materials. Common fluorescent probes (anthracene or fluorescein) were solubilized in micelles around which a usual cross-linker (tetraethylorthosilicate) was co-polymerized with a silicate-based monomer bearing an amino group. The toxicity, adsorption capacity and fluorescence properties upon interaction with hexanal of these newly synthesized materials were compared to the sol-gel material previously investigated.

Synthesis strategies of silica-based functionalized materials for the sensing of carbonyl compounds.

ABSTRACT

Silica-based materials for sensing applications were underestimated for a long time. In this work, several strategies were investigated in order to synthesize sol-gel materials that could serve as sensors for carbonyl monitoring using fluorescence. Materials were designed to possess both a free amino group for the reaction with carbonyls and a fluorescent core. The first strategy was the simplest, using a single functional monomer, the 4-trimethoxysilylaniline, meeting the two previously cited criteria required. In order to have more choices in terms of reactants, and especially to change the fluorescent core, a second strategy was considered, using two functional monomers: the first one possessing an amino group (3-aminopropyltrimethoxysilane) and the other one being fluorescent (1-naphthyltrimethoxysilylaniline). Lastly, to have even more choice in terms of fluorescent probes, a third strategy, based on the solubilization of common fluorescent compounds (anthracene or fluorescein) into oil-in-water micelles before the polymerization of a silica structure around them. Four different materials were thus synthesized according to these 3 strategies. Their ability to be used for carbonyl sensing based on their fluorescence properties was investigated. More precisely, their adsorption capacity and fluorescence property after interaction with hexanal as well as their toxicity were assessed. Although the materials obtained following the last strategy were 1000 times more fluorescent than the 2 other materials, they suffered from leakage of the surfactant and the fluorescent probe, rendering the materials not safe enough for many applications, contrarily to the first and second strategies.

1. INTRODUCTION

Among the synthesis routes of silica-based materials, the sol-gel process, relies on the hydrolysis and condensation of alkoxysilane derivatives in acid or basic medium¹. This synthetic route has been used for example for the development of silica-based molecularly imprinted silicas (MIS)^{2,3}. Firstly proposed in the 1930s⁴ and more investigated in the 1950s^{5–8}, this route has attracted increasing interest in the last decades since these materials show better physical stability and less toxicity compared to the acrylate-based molecularly imprinted polymers (MIPs)^{3,9}.

In the 1990's, a new synthetic route for mesoporous silica-based materials, the so-called "Liquid Crystal Templating", have been made popular¹⁰⁻¹³, in particular with the development of the well-known MCM-41 material. Prior to polymerization, the use of a surfactant at a given concentration determines the shape of the pores of material, obtained after calcination for the removal of the micellar phase. The inner structure of these materials is thus well controlled, and their high porosity make them of high interest for applications such as adsorption and catalysis.

More recently, this synthetic route was adapted to generate "doped silica materials", following the same principle, but without the removal of the organic micellar phase by calcination. The micellar phase was used to solubilize organic compounds conferring specific properties to the material with the incorporation of functional groups within the material. It has been proposed for example for the extraction of metallic compounds (copper and europium) from water using a mesostructured silica doped with two 4-acyl-5-hydroxy-pyrazole¹⁴.

In this work, four materials synthesized according to the sol-gel process in basic conditions were evaluated as potential candidates for the use as carbonyl sensors. More precisely, their adsorption capacity and fluorescence property after interaction with hexanal were assessed. The toxicity of the materials was also evaluated.

2. MATERIALS AND METHODS

2.1. CHEMICALS

4-trimethoxysilylaniline (4-TMSA, 97 %, CAS number 33976-43-1) and 1-naphthyltrimethoxysilane (95 %, CAS number 18052-76-1) were bought from abcr, Germany. Tetraethylorthosilicate (TEOS, 99 %, CAS number 78-10-4), 3-aminopropyltrimethoxysilane (97 %, CAS number 13822-56-5), hexadecyltrimethylazanium bromide (CTAB, 98 %, CAS number 57-09-0), anthracene (97 %, CAS number 120-12-7), fluorescein (for fluorescence, free acid, CAS number 2321-07-5), ammonium hydroxide (NH₄OH, 30 %, CAS number 1336-21-6), sodium hydroxide (0.1 M NaOH in water, CAS number 1310-73-2), methanol (ACS reagent, 99.8 %, CAS number 67-56-1), ethanol (EtOH, 96 %, CAS number 64-17-5, used for synthesis) and ethanol for UV-spectroscopy Uvasol® (EtOH, 99.9 %, CAS number 64-17-5, used for chromatographic and fluorescence experiments) were purchased from Sigma Aldrich, France.

Hexanal (98 %, CAS number 66-25-1) was provided by Alfa Aesar, France. Deionized water was used, obtained from a MilliQ RG, Millipore apparatus.

2.2. MATERIALS SYNTHESIS

2.2.1. Material 1

4-trimethoxysilylaniline (4-TMSA) was firstly dissolved in an ethanol/water mixture (1/10 molar ratio) at 60 °C for 10 min. Tetraethyl orthosilicate (TEOS) and ammonium hydroxide (30 % NH₄OH) were then added. The following molar ratio was used: 1/5/31.25/250/25 for 4-TMSA/TEOS/NH₄OH/H₂O/EtOH. The mixture was stirred at 500 rpm for 24 h at 22 °C. Then, the solvent was evaporated under the hood, and the resulting powder was washed 3 times with an ethanol/water mixture (1/10 molar ratio) by Büchner filtration using a 0.2 µm pore membrane. The material was dried 12 h at 60 °C, crushed and stored at 4 °C in the dark before use.

2.2.2. Material 2

1-naphtyltrimethoxysilane (M1) was firstly dissolved in an ethanol in water mixture (1/10 molar ratio) at 60 °C for 15 min. 3-aminopropyltrimethoxysilane (M2), tetraethyl orthosilicate (TEOS) and ammonium hydroxide (30 % NH₄OH) were then added. The following molar ratio was used: 1/1/5/31.25/250/25 for M1/M2/TEOS/NH₄OH/H₂O/EtOH. The mixture was stirred at 500 rpm for 24 h at 22 °C. Then, the solvent was evaporated under the hood, and the resulting powder was washed 3 times with an ethanol in water mixture (1/10 molar ratio) by Büchner filtration using a 0.2 µm pore membrane. The material was dried 12 h at 60 °C, crushed and stored at 4 °C in the dark before use.

2.2.3. Material 3.1

Hexadecyltrimethylazanium bromide (CTAB) was firstly dissolved in a solution of NaOH in water (0.1 M) at 60 °C under stirring at 250 rpm for 10 min. Methanol, anthracene (F1), 3-aminopropyltrimethoxysilane (M) and tetraethyl orthosilicate (TEOS) were added. The following molar ratio was used: 0.18/0.6/0.6/1/13/140 for CTAB/F1/M/TEOS/MeOH/H₂O (NaOH 0.1 M). The mixture was stirred for 1 h at 500 rpm at 60 °C and further 24 h at 22 °C. Then, the solvent was evaporated under the hood and the powder was washed 3 times with water by Büchner filtration using a 0.2 µm pore membrane. The material was dried 12 h at 60 °C, crushed and stored at 4 °C in the dark before use.

2.2.4. Material 3.2

Material 3.2 was synthesized according to the same procedure as for material 3.1, replacing anthracene (F1) by fluorescein (F2).

2.3. ADSORPTION CAPACITY OF THE MATERIALS

The adsorption capacity of hexanal by the materials was determined by gas chromatography. 10 mg of the material were suspended in 2 mL of an hexanal in ethanol solution (concentration: $2.49 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$). Samples were agitated at 50 rpm for 24 h at 22 °C. They were centrifugated at 10 000 rpm for 10 min. The supernatant was diluted 2.5 times before analysis. The remaining concentration of hexanal in the liquid phase was determined by injecting 1 μL of the diluted supernatant using an autosampler (Gerstel) on a 7890 A gas chromatograph (Agilent Technologies) equipped with a capillary column (30 m x 0.32 mm internal diameter, 0.5 μm thickness) coated with a DB-5MS stationary phase (Agilent J&W). Injections were performed in split mode (split ratio 50:1). The injection temperature was 250 °C. Helium was used as carrier gas at a flow rate of $1.4 \text{ mL}\cdot\text{min}^{-1}$. Mass spectrometer 5975C (Agilent) was used as the detector (electronic impact 70 eV, ionization source at 230 °C, transmission line at 240 °C). The oven temperature was programmed from 50 °C to 65 °C at a first rate of $3 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$, and from 65 °C to 250 °C at a second rate of $30 \text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$. The oven temperature was finally maintained at 250 °C for 5 min. A solvent delay of 3 min was also applied. Each adsorption experiment was carried out 3 times.

The adsorption capacity is defined as the mass of hexanal adsorbed by 1 g of material. It was calculated as follows:

$$\text{Adsorption capacity} = \frac{(C_i - C_f) \times V_{\text{liq.}}}{m} \quad (1)$$

with C_i the initial concentration of hexanal in the liquid phase in contact with the material, C_f the concentration of hexanal remaining in the liquid phase after 24 h contact, $V_{\text{liq.}}$ the volume of the hexanal solution and m the mass of the material.

2.4. FLUORESCENCE OF THE MATERIALS

The fluorescence emission spectrum of the material was recorded before and after exposure to hexanal in ethanol. For materials 1 and 2, 10 mg of material were suspended in 2 mL of ethanol. After 10 seconds of stirring, the fluorescence emission spectrum of the

suspended material was recorded. Then, 500 μL of a solution of hexanal were added to obtain a concentration in the measurement cell of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$. After 15 min of stirring at 80 rpm, the fluorescence emission spectrum of the material was recorded again. The fluorescence intensity of the material after exposure to hexanal was compared to its initial fluorescence. To avoid saturation of the detector, the quantity of materials 3.1 and 3.2 used for the fluorescence measurements was 1000 times lower compared to materials 1 and 2. A Spectrofluorometer Fluoromax4[®] (Horiba) was used with the software Fluorescence[®]. A SUPRASIL[®] quartz cell (Hellma) of 10 x 10 mm dimension was employed. The excitation wavelengths were $290 \pm 2 \text{ nm}$ for material 1, $270 \pm 2 \text{ nm}$ for material 2, $360 \pm 2 \text{ nm}$ for material 3.1 and $494 \pm 2 \text{ nm}$ for material 3.2. Fluorescence emission spectra were recorded with a 2 nm bandpass and a step interval of 1 nm. The temperature was kept constant at 22 °C during the experiment. Each experiment was performed 3 times.

The percentage of fluorescence quenching, Q , was determined as follows:

$$Q = \frac{I_0 - I_{eq}}{I_0} \times 100 \quad (2)$$

with I_0 the intensity of fluorescence of the material in ethanol before addition of hexanal and I_{eq} its intensity after exposure to hexanal for 15 min. Intensities were measured at 337 nm for material 1, 400 nm for material 2 and 521 nm for material 3.2. For material 3.1, the average loss of intensity measured at each maximum wavelength (377, 399, 423 and 448 nm) was considered.

2.5. EVALUATION OF THE TOXICITY OF THE MATERIALS

The mutagenicity of the materials was estimated by the Ames' test performed on two bacterial strains: *Salmonella typhimurium* TA98 and TA100 according to Maron and Ames¹⁵ and the OECD guideline no. 471 for the testing of chemicals¹⁶. More details about the experimental procedure can be found in previous work⁹. Each test was performed 3 times.

2.6. STATISTICS

Statistical analysis (ANOVA with Tukey's post hoc analysis) was performed using XLSTAT (Addinsoft) software. Significance was established at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. DESIGN OF THE MATERIALS

Several strategies can be considered for the design of silica-based materials for the sensing of carbonyls based on fluorescence. Considering the classical reaction between amine and carbonyl groups, the materials should possess an amino group capable of reacting with the carbonyl compounds and a fluorescent core. The fluorescence property of the materials should then be modified by the interaction with carbonyls. **Figure 1** depicts the main reactants used for the synthesis of each material studied in this work. All of the materials were synthesized according to a sol-gel process in basic conditions, using tetraethyl orthosilicate (TEOS) as the cross-linker. The first strategy relied on a single monomer possessing both an amino group and a fluorescent core. Material 1 was obtained using 4-trimethoxysilylaniline as the functional monomer (**Figure 1a**). Therefore, the resulting material was based on aniline fluorescence properties, which fluoresces in the UV region (at 337 nm in ethanol)¹⁷. As the commercial offer of silica-based functional monomers was limited, the choice of reactants for this first strategy was very restricted. In order to shift the fluorescence emission of the material in the visible region, other fluorophores should have been considered. To have more choices of reactants, and especially in the use of fluorophores, it was thus proposed to co-polymerize 2 functional monomers: one possessing an amino group and the other one being fluorescent. Material 2 was synthesized according to the same experimental procedure as for material 1 but using both 3-aminopropyltrimethoxysilane and 1-naphthyltrimethoxysilaniline as functional monomers (**Figure 1b**). In these 2 first strategies, the functional monomers were covalently linked to the mineral silica structure. This required to find reactants possessing siloxane groups for the polymerization. However, the commercial availability of such monomers was very poor and very expensive when they existed. Therefore, in order to have even more choices in terms of fluorescent probes and be able to use classical ones, a last strategy was considered. Hexadecyltrimethylazanium bromide (CTAB) surfactant was used in basic medium to create oil in water (O/W) micelles into which a common fluorophore (either anthracene for material 3.1 or fluorescein for material 3.2) was solubilized. 3-aminopropyltrimethoxysilane was also added to provide an amino group to the material in order to ensure reactions with carbonyls. TEOS was polymerized around the pre-formed micelles (**Figure 1c** and **Figure 1d**). Contrarily to materials 1 and 2, in this last strategy the fluorophore was thus not covalently linked to the

silica structure. All four materials were used as is, without calcination of the organic moiety, as it would have been the case for traditional mesoporous silica-based materials. The impact of the strategy employed for the synthesis of the different materials on their ability to be used for carbonyl sensing was further assessed. In particular, the adsorption capacity and the fluorescence property of each material after interaction with hexanal was determined.

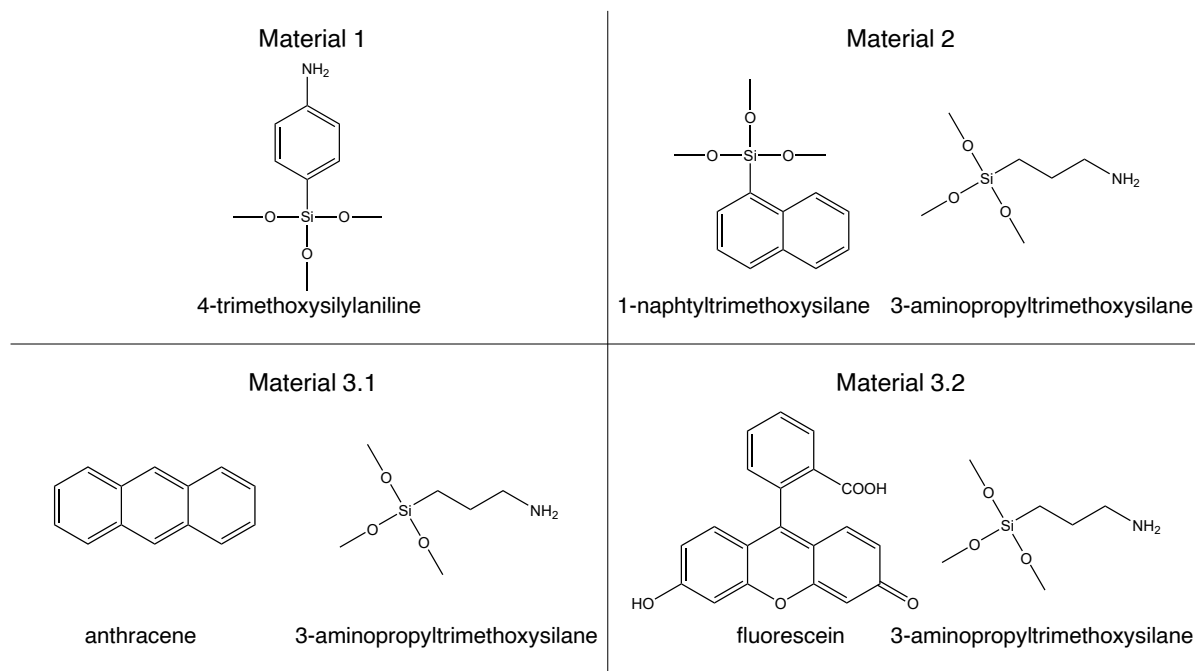


Figure 1: Reactants used for the synthesis of the different fluorescent materials for carbonyl sensing applications. Tetraethylorthosilicate (TEOS) was used as the cross-linker for the polymerization of all four materials according to a sol-gel process in basic conditions.

3.2. ADSORPTION CAPACITY OF THE MATERIALS

To be used as sensor for the determination of the carbonyl content of a given sample, the adsorption capacity of hexanal by each material was assessed. **Figure 2** displays the adsorption capacity of each material after interaction with hexanal at a selected concentration in ethanol ($2.49 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$). 1 g of material 1 was able to adsorb $22.7 \pm 1.5 \text{ mg}$ of hexanal. Although the molar ratio between the functional monomer possessing the amino group and the TEOS cross-linker was the same for material 1 and material 2 (1 to 5 ratio), material 1 adsorbed almost 2.5 times more hexanal than material 2. The latter only adsorbed $9.1 \pm 1.4 \text{ mg} \cdot \text{g}^{-1}$ of hexanal. Due to the addition of a functional monomer in the synthesis of the material 2, the repartition between the amino groups on the surface of the silica structure is probably more heterogeneous than the one achieved for material 1. Amino groups in material 2 could

potentially be less accessible to hexanal molecules in comparison to material 1. This can also be attributed to the fact that the amino group of material 1 is spatially closer to the hydrophobic part of the material, provided by the aniline group. In material 2, the amino group is surrounded by the hydrophilic siloxane part of the material. However, it has already been observed that the analyte preferentially adsorbed by a material containing functional monomers having comparable hydrophobicity¹⁸. As interactions with hexanal may be favored by a hydrophobic environment, material 1 can adsorb more hexanal than material 2. It is also interesting to notice that materials 3.1 and 3.2 did not adsorb the same amount of hexanal although the molar ratio of 3-aminopropyltrimethoxysilane/TEOS was the same for both materials (0.6/1). Material 3.1 adsorbed $29.9 \pm 7.3 \text{ mg}\cdot\text{g}^{-1}$ of hexanal, which is 4.5 times more than material 3.2. This could be attributed to the more hydrophobic nature of material 3.1, based on anthracene ($\log P$: 4.4) compared to material 3.2 ($\log P$: 3.4), as previously described. GC-MS analysis of the supernatant of the suspensions of the materials 3.1 and 3.2 in ethanol unfortunately revealed the leakage of anthracene and fluorescein in the dispersing phase. This is unambiguously linked to the synthesis method, as anthracene and fluorescein were just solubilized into the O/W micelles formed with CTAB and were not covalently linked to the bulk structure. According to the adsorption capacity of each material, the best suited ones for hexanal adsorption were thus ranked as follows: material 3.1 > material 1 > material 2 > material 3.2.

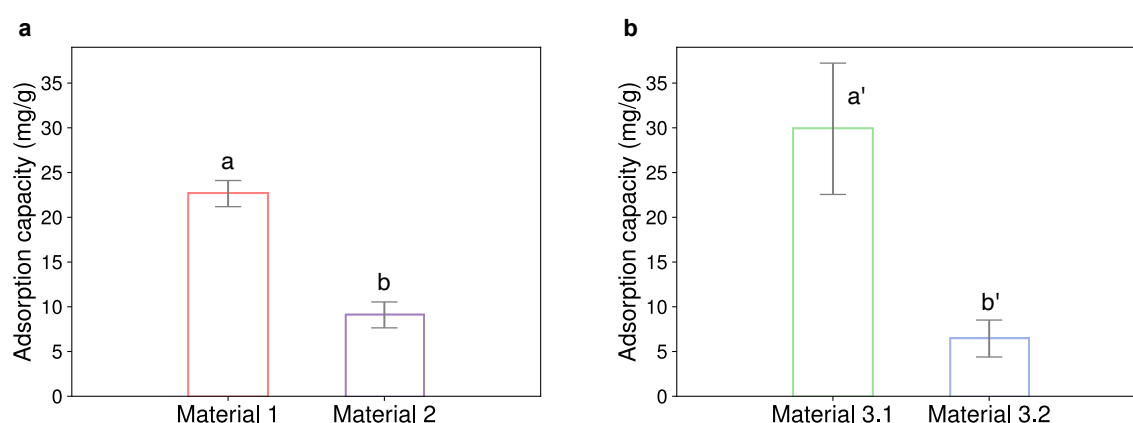


Figure 2: Adsorption capacity of 10 mg of materials 1 and 2 (**a**) and materials 3.1 and 3.2 (**b**) in suspension in a solution of hexanal in ethanol (concentration: $2.49 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) for 24 h at 22 °C, as determined by GC-MS. Error bars represent the standard deviation obtained from 3 replicates. Letters represent the significantly different values ($p < 0.05$) for each set of data according to a one-way ANOVA statistical analysis.

3.3. FLUORESCENCE OF THE MATERIALS

The developed materials were intended to be used as fluorescent sensors. The impact of hexanal on the fluorescence property of the materials was thus investigated. **Figure 3** depicts the fluorescence emission spectrum of each material before and after interaction with hexanal. Each material exhibited a high fluorescence intensity that was quenched by hexanal, probably due to the formation of an imine after nucleophilic addition of hexanal onto the accessible amino groups of the materials. Prior to the study of the modification of the fluorescence property of the materials upon hexanal adsorption, the fluorescence emission spectrum of all the initial reactants used for the synthesis of each material were recorded. As shown in **Figure 3** (a', b', c', d'), only the fluorescent probes (4-trimethoxysilylaniline, 1-naphthyltrimethoxysilane, anthracene and fluorescein) exhibited significant fluorescence intensity. TEOS, 3-aminopropyltrimethoxysilane and CTAB were not fluorescent whether they were excited at 290, 270, 360 or 494 nm. The maximum emission wavelengths of each material were in line with the characteristic peaks of the fluorescent core used for each synthesis. Material 1 had a maximum emission wavelength in the UV region at 337 nm (**Figure 3a**), corresponding to the fluorescence of the aniline group. Material 2 exhibited a maximum emission wavelength in the beginning of the visible region at 400 nm (**Figure 3b**), as it can be observed for 1-naphthyltrimethoxysilane. Material 3.1 showed four emission wavelengths in the UV-visible region at 377, 399, 423 and 448 nm (**Figure 3c**), attributed to anthracene. Finally, material 3.2 had a strong fluorescence in the visible region at 521 nm (**Figure 3d**), due to the presence of fluorescein. It is noteworthy that, although the same quantity of material was used to record the fluorescence emission spectra of materials 1 and 2, material 1 was 2.5 times more fluorescent than material 2. Yet, the quantum yield of aniline (0.08 in cyclohexane)¹⁹ is lower than the one of naphthalene (0.23 in cyclohexane)¹⁹. Both materials 3.1 and 3.2 displayed a similar fluorescence intensity. Although all materials (1, 2, 3.1 and 3.2) should all contain 1 mmol of fluorescent probe, materials 3.1 and 3.2 were 1000 times more fluorescent than materials 1 and 2. Accordingly, the quantity of materials 3.1 and 3.2 was divided by 1000 to obtain similar fluorescence intensity levels compared to materials 1 and 2, as displayed in **Figure 3**. This could be attributed to the higher quantum yields of anthracene¹⁹ and fluorescein¹⁹ compared to aniline or naphthalene. This could also be linked to the fact that the fluorophore compound is under its free form and not covalently linked to the structure.

Therefore, materials 3.1 and 3.2 appeared to be more performant considering the ratio of material used/resulting intensity of fluorescence.

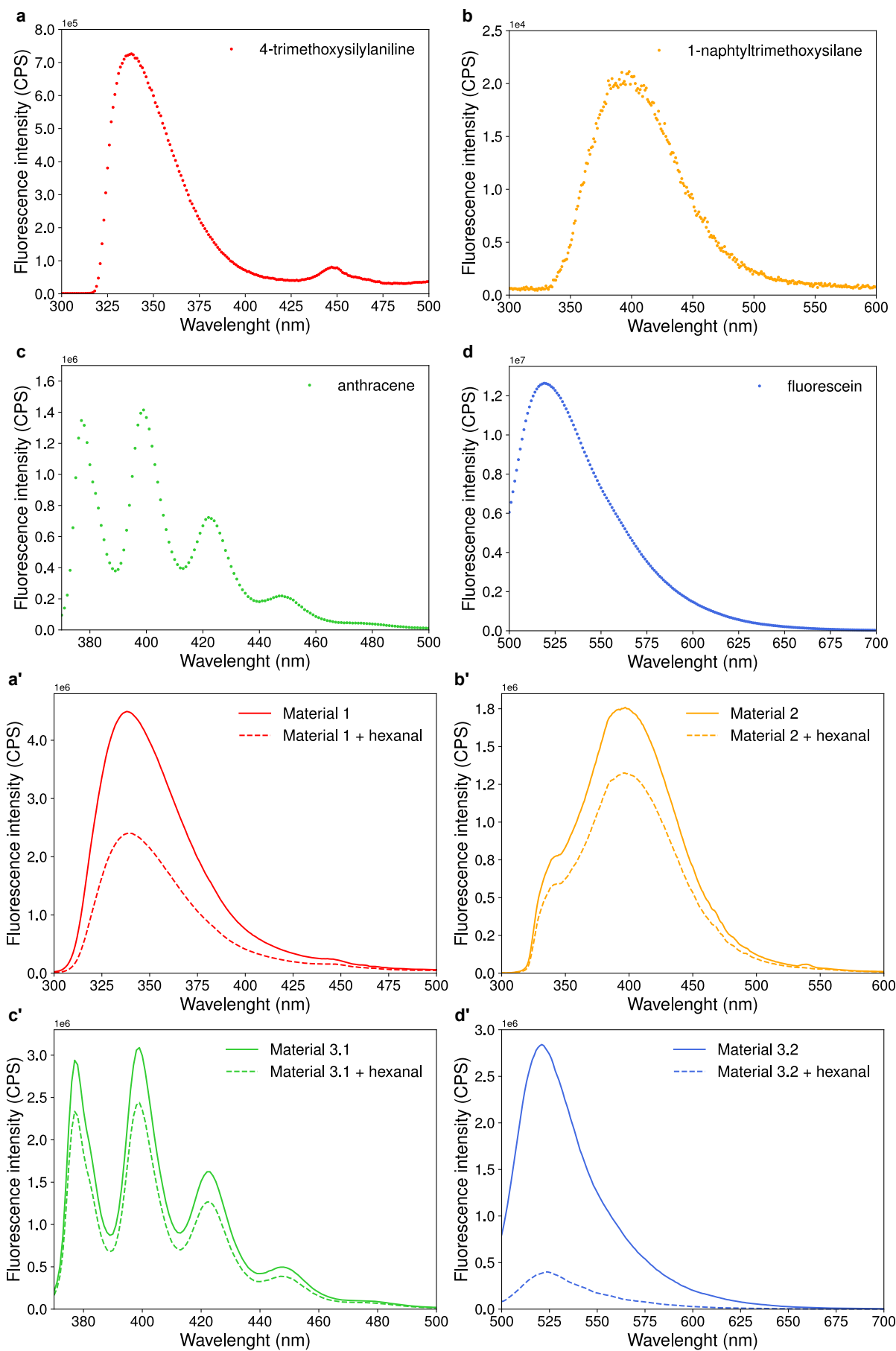


Figure 3: Fluorescence emission spectrum of the fluorescent monomers/the fluorophores (a, b, c, d). Fluorescence emission spectrum of the materials (a', b', c', d') in suspension in ethanol before (straight line) and after (dashed line) interaction with hexanal (at a concentration of $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) for 15 min at 22 °C. Concentration of materials 1 and 2 was $5 \text{ g}\cdot\text{L}^{-1}$, and concentration of materials 3.1 and 3.2 was $5 \times 10^{-3} \text{ g}\cdot\text{L}^{-1}$. The excitation wavelengths were $290 \pm 2 \text{ nm}$ for material 1, $270 \pm 2 \text{ nm}$ for material 2, $360 \pm 2 \text{ nm}$ for material 3.1 and $494 \pm 2 \text{ nm}$ for material 3.2.

As shown in **Figure 4**, the percentage of fluorescence quenching after interaction with identical concentration of hexanal for the same time was clearly different for all the materials. The fluorescence intensity of material 1 was more quenched by hexanal than for material 2 ($47.2 \pm 1.9 \%$ for material 1 vs $24.7 \pm 0.5 \%$ for material 2). This can be explained by the higher spatial proximity between the fluorescent core and the amino group in material 1 compared to material 2. As the fluorescence of a compound is strongly influenced by its near environment, the closer the adsorption sites of the material for hexanal and the fluorescent core are, the stronger will be the impact on the fluorescence of the material. The fluorescence intensity of material 3.2 was four times more quenched by hexanal than material 3.1 ($86.5 \pm 0.4 \%$ for material 3.2 vs $21.4 \pm 1.3 \%$ for material 3.1). This could be explained by the fact that the fluorescence loss observed was not only due to the formation of an imine. An additional phenomenon responsible for the loss of fluorescence intensity of the material 3.2 has also to be considered. As previously mentioned, when the material was placed in suspension in ethanol, fluorescein leaked out of the material. In such a case, fluorescein could also interact with hexanal through hydrogen bonds in the dispersing phase. These intermolecular hydrogen bonds would stabilize the ground state^{20,21}, resulting in a loss of fluorescence intensity of the material 3.2. Material 3.1 also suffered from leakage of the anthracene fluorescent probe. However, due to its hydrophobic nature ($\log P$: 4.4), material 3.1 will not interact with hexanal through hydrogen bonds. To sum up on the fluorescence property of the materials, they can be ranked according to the intensity of fluorescence as follows: materials 3.1 and 3.2 > material 1 > material 2. Nevertheless, if materials are ranked according to the percentage of quenching of the fluorescence intensity, the order changed as follows: material 3.2 > material 1 > material 2 > material 3.1.

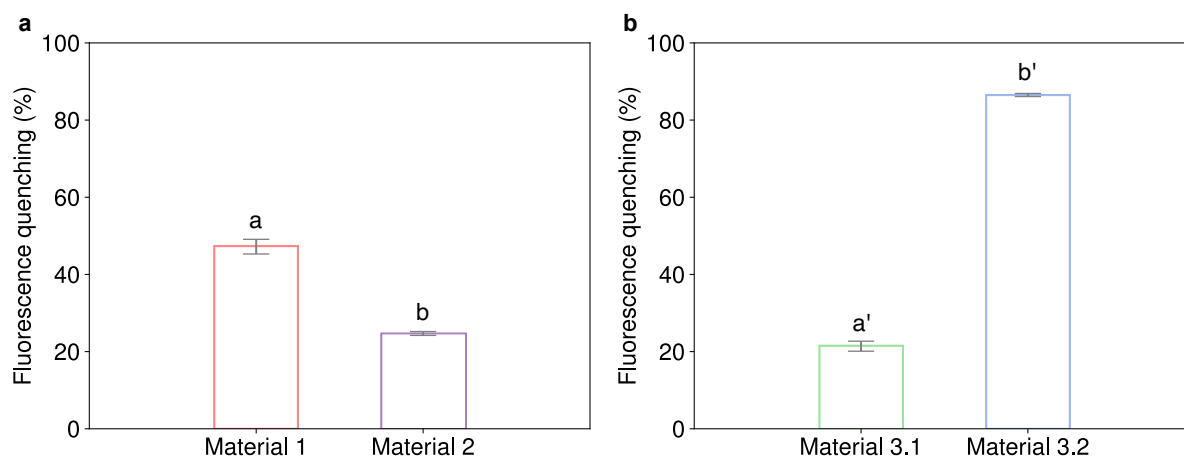


Figure 4: Percentage of fluorescence intensity quenching of the materials in suspension in ethanol after 15 min of interaction with hexanal (concentration: $9.98 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$) at 22 °C. Concentration of materials 1 and 2: $5 \text{ g}\cdot\text{L}^{-1}$, concentration of materials 3.1 and 3.2: $5 \times 10^{-3} \text{ g}\cdot\text{L}^{-1}$. Letters represent the significantly different values ($p < 0.05$) for each set of data according to a one-way ANOVA statistical analysis.

3.4. TOXICOLOGICAL CHARACTERIZATION

According to the targeted application, the toxicity of the sensing material developed has also to be considered. When used in contact with food or for medical applications, such sensors must be safe. The mutagenicity of all four materials was thus assessed according to the Ames' test performed on two bacterial strains: *Salmonella typhimurium* TA98 and TA100. Materials 1 and 2 were not mutagenic, whatever the amount of material used. However, materials 3.1 and 3.2 were mutagenic above 0.01 mg and 0.05 mg, respectively. This result again opposes two categories of materials: materials 1 and 2, into which all reactants are covalently linked to the mineral silica structure and materials 3.1 and 3.2, for which the fluorescent molecule was just solubilized into micelles before polymerization. In such a case, leakage of toxic substances consequently happens, resulting in mutagenic effects. Therefore, in order to be able to use this micellar strategy for applications requiring safe sensors, the surfactant and the fluorescent probe should be replaced and chosen carefully.

4. CONCLUSION

To conclude, this work reported on the performance assessment of four materials, obtained according to three different strategies for their potential use as sensors for carbonyl monitoring using fluorescence. Different strategies were considered in order to have more choice in the use of reactants and to more easily tune the adsorption and fluorescence

properties of the resulting materials. The adsorption capacity and the fluorescence property of the materials after interaction with hexanal were carefully evaluated. The adsorption capacity mainly depended on the accessibility of hexanal to the amino groups within the polymeric structure. Fluorescence emission of the materials either occurred in the UV or in the visible region, depending on the probe selected. Materials 3.1 and 3.2 appeared to be 1000 times more fluorescent compared to materials 1 and 2. The fluorescence intensity of all of the materials was quenched by hexanal. The percentage of fluorescence decrease was related to the nature of the interaction of the materials with hexanal: hexanal could only interact with the materials through the formation of an imine or by forming additional hydrogen bonds with the fluorescent probe, as it was suggested in the case of material 3.2 (based on fluorescein). In order to consider the use of these materials for applications requiring safe sensors, such as in the food or medical fields, the toxicity of the materials was also evaluated. Materials 1 and 2, in which functional monomers were covalently linked to the silica structure, were not mutagenic according to the Ames' test. Materials 3.1 and 3.2 were mutagenic above a given amount of material. This difference was explained by the leakage of the CTAB surfactant and of the fluorescent probe (either anthracene or fluorescein), as revealed by GC-MS analysis of the dispersing phase in which the material powders were placed in suspension. Therefore, a compromise must be found favoring the most important feature of the sensor according to the targeted application (wide choice of reactants, adsorption capacity, fluorescence in the UV or visible region, fluorescence intensity, percentage of fluorescence quenching, toxicology). The costs of the reactants and the energetical cost of the synthesis should also be considered.

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CONCLUSION AND PERSPECTIVES

CONCLUSION AND PERSPECTIVES

The aim of this PhD thesis was to develop an intelligent packaging to monitor food spoilage, and more precisely lipid oxidation, in real-time. It was the first time this new theme was addressed in UMR PAM. It dealt with the design, synthesis, and performance assessment of fluorescent materials for carbonyl sensing. Both acrylate-based materials, synthesized by radical polymerization and silicate-based materials, obtained by the sol-gel process, were investigated. All materials were designed following the same principle: they had to possess an amino group, for the reaction with carbonyl compounds, and a fluorescent core, which properties should be modified by the interaction with the analytes. One of the sol-gel materials tested, based on aniline, was found to be particularly suitable and was thus more deeply studied. Compared to the synthesized acrylate-based materials, its synthesis was highly reproducible. It also exhibited a higher carbonyls adsorption capacity as well as a higher fluorescence intensity and these two last properties were stable over time. Synthesized by a cost-effective and straightforward process, this material was of micrometer sized particles and exhibited a high porosity. Since the sensor is intended for multi-analytes detection, the fluorescence response of the material after interaction with various carbonyl compounds, qualified as trackers of lipid oxidation (propanal, hexanal, nonanal, hexan-2-one, 1-penten-3-one, 1-octen-3-one), was investigated. Its fluorescence was rapidly quenched (less than one min) by carbonyls. Real-time monitoring was possible since the fluorescence loss correlated with the concentration of carbonyls. The sensor exhibited high sensitivity and a broad range of detection (0.5 mmol/L – 0.5 mol/L), allowing to monitor the entire shelf-life of a food product. The sensor performances were primarily assessed in the liquid phase, using a model solution (ethanol), a food simulating medium (ethanol/water mixture) and real foods (rapeseed oil and fish oil). The influence of the nature of the liquid phase and of its pH revealed that the developed sensor could be applied to a large variety of food products, with an optimal pH range (7 – 9) corresponding to the pH of the majority of lipid-rich products. The sensor was successfully applied to the monitoring of the oxidation of a commercial fish oil. It was indeed possible to correlate the fluorescence response with the results provided by classical methods used to determine the oxidation level of foods (PV, TBARS and SPME-GC-MS). This study demonstrated the possibility of using this new sensor instead of classical methods for the fast and simple determination of the lipid oxidation level of food products. Integrated into PLA films,

the material was furthermore applied to the detection of carbonyl in the gas phase. It could thus be used either in direct contact with the food or be integrated into usual food packaging to monitor modifications in the environment surrounding the product. The toxicity of the material was assessed in order to apply this sensor to the agri-food sector. Neither the powder nor the PLA film containing the material were mutagenic according to the Ames' test. Finally, in order to shift the fluorescence emission of the material in the visible range and thus get rid of the need of a detection device, new synthesis strategies were investigated to extend the choice in the fluorescent component of the sensor. All these sensors were synthesized following cost-effective and straightforward procedures, provided fast response and were simple to use, with little sample preparation and no manipulation of any toxic reactant required. These advantageous features make these sensors suitable for a wide range of applications.

The successful design of such fluorescent sol-gel materials constitutes a breakthrough for the detection and quantification of carbonyl compounds. Carbonyls are good quality trackers of lipid oxidation but also of microbiological degradation¹ of food products, and could thus be used to monitor the quality of cosmetics or pharmaceutical products, which are also sensitive to lipid oxidation. In other fields, carbonyls provide information about the level of air pollution or can be used as biomarkers in medical diagnoses of diseases or cancers. Their fast detection and easy quantification, with more user-friendly sensors, are thus highly relevant.

The developed sensors were designed to target a family of molecules. Modifying the detection system using the molecularly imprinted technology could make the sensor selective of one single component. Such MIP-based sensors could greatly benefit the agri-food industry ensuring food safety, reducing food waste and fighting against food fraud by the easy and fast detection of contaminants (*e.g.*, antibiotic residues, heavy metals, pesticides, mycotoxins, adulterants, packaging contaminants, allergens, biogenic amines) all along the food chain² (see **Appendix B** for more details).

The present work opens on many interesting perspectives. The most studied sol-gel material in this thesis was only optimized with respect to temperature and solvent volume during synthesis, and only two different functional monomer/cross-linker molar ratios were tested. These synthesis parameters could further be improved to find the optimum molar ratio between all reactants in order to maximize the yield of polymerization, the adsorption capacity,

the fluorescence intensity, and to reduce the limit of detection; all of this using the lowest amount of reactants. The nature of interactions between the material and carbonyls could also be more in depth explored: both chemical reactions and physical interactions were conjectured with the help of ^1H NMR spectroscopy analysis. These could be further confirmed using 2D NMR spectroscopy correlating the ^1H and ^{13}C spectra in order to investigate the modifications induced by the formation of an imine in the case of chemical reaction. The mechanisms of interaction (chemical VS physical) for each carbonyl compound could also be compared. This will help to understand the differences in reactivity observed between aldehydes and ketones. The mechanisms responsible for fluorescence quenching when both chemical reaction and physical interaction occur could be studied. Better solution for the incorporation of the material in a support matrix such as plastics packaging should be explored because the distribution of the material powder inside PLA films is a key parameter to control and was difficult to achieve properly. Other polymers could be considered to replace PLA such as polycaprolactone. Shaping the sensor directly in the form of a film could solve the problem but would require modifications and optimizations of the synthesis procedure. In-situ polymerization of the sensor directly on the surface of plastics packaging or inside bottles could simplify the integration step. Grafting or electropolymerization could be considered but this would require to completely reconsider the choice of reactants and syntheses procedures. The studies of the performances of the sensor after interaction with carbonyls in the gas phase could be more deeply explored in partnership with the Interdisciplinary Carnot laboratory of Burgundy (ICB), UMR CNRS 6303. It would follow a preliminary collaboration in order to study the adsorption phenomenon by exposing the material to pure carbonyl vapors and controlling the pressure and temperature applied. Such a collaboration could also help to understand the impact of oxygen and carbon dioxide on the response of the sensor. Oxygen is a quencher of fluorescence and carbon dioxide could interfere with carbonyls. In the context of this thesis, it was chosen not to control these parameters to observe the response of the sensor in real conditions of use. However, to study the impact of these two compounds could help to optimize the amount of material required to track the entire shelf-life of food. The impact of the temperature and the relative humidity on the behavior of the sensor should also be studied to verify its suitability to monitor the shelf-life of cold-stored products or monitor high temperatures processes such as frying.

For an application of the sensor to packaging, it could be required to shift the emission of fluorescence in the visible region for two main reasons: 1) get rid of any detection device to perceive the spectroscopic modifications of the sensor more easily 2) most of the plastics used in the packaging industry are fluorescent in the UV region, which will hinder the reading of the response of the sensor emitting in the same wavelength range. The two strategies investigated during the thesis did not give enough satisfying alternatives. When using two distinct functional monomers, the modification of fluorescence induced by the interaction of carbonyls was not intense enough. When solubilizing fluorophores inside micelles, leakage of the fluorescent compound and the surfactant was observed, leading to toxicity issues. Safe and eco-friendly surfactants (*e.g.*, provided by SurfactGreen (Rennes, France)) associated with food-grade fluorophores could help limiting the toxicity of the material. But the strategy relying on one single monomer bearing both amino groups and fluorescent core seemed to be the most performing for a food-related application. However, the commercial choice in terms of such silicate-based monomer is restricted. To cope with this, it could be considered to synthesize tailor-made functional monomers based on silica bearing amino groups and fluorophore emitting in the visible region. Such tailor-made compounds were little investigated in the context of this thesis, by functionalizing the cyclam platforms with the required chemical groups. However, other strategies based on commercial reactants were favored to spare some time of synthesis.

A life cycle assessment would help to estimate the benefit brought by such an innovation in the agri-food sector. The economic and environmental impact of food waste should be compared with the consequences of the addition of such a sensor on the packaging. A compromise has to be found between the social and reglementary injunctions favoring a reduction of the packaging weight and the use of more recyclable plastics with the safety and waste issues it creates. Getting completely rid of packaging seems inappropriate in the view of the microbial contaminations implied. When the packaging is strictly required, it should be possible to find eco-friendly alternatives to plastic materials. For the commercialization of such an application, a cost estimation of the sensor should be performed to evaluate if the innovation would be commercially viable or not. The cost of the sensor should be limited as the number of reactants was restrained to its minimum. Moreover, they were chosen to be affordable, and the synthesis is conducted at room temperature within only 36 h. In view of the

performance of the sensor, only a small amount of the material should be necessary to cover the entire shelf-life of the products. Finally, recyclability and reuse of the sensor could be considered as the chemical reaction, between the material and carbonyl compounds, is reversible. As a first step in the industrial application of the developed technology, a patent is pending since December 13th, 2021 (n° FR2113403). A PCT international extension was also filed on December 12th, 2022 (n° PCT/EP2022/085460).

To improve the technology developed, the targeted user should also be properly identified. An application of the sensors on primary or secondary packaging would be dedicated to distributors and retailers. Regulatory issues should therefore be taken care of in order to obtain the food contact approval of the sensor. Preliminary results already demonstrated that the materials were not mutagenic. Further tests should be performed so that the sensors comply with the EC n° 1935/2004 regulation, which applies to materials and articles intended to come into contact with food as well as the EC n° 450/2009 regulation, specific to active and intelligent materials. The impact of the sensor on the recyclability of the packaging should be assessed. A miniaturized device for the on-site excitation and detection should also be considered.

If the sensor is intended for consumers, the modification of the transducer from a fluorophore to a chromophore should be favored. The response of the sensor would thus be easily readable by a change of color. However, misused, the sensor could be responsible for even more food waste and become counterproductive. Moreover, current consumers tend to wish for less packaging and less confusing labels. An alternative use of such colorimetric sensor at the consumer level would be the application of the sensor on tabs, similar to those used to determine the pH of pools. It would allow to monitor the secondary shelf-life of food and cosmetics products intended to be kept open for several weeks or months (such as oils, mayonnaises, creams, butters) directly at home. For such a use case, a reading chart will have to be established according to the food product, which will depend on the amount of lipid and the physical state of the product. It could, for example, rely on the development of a smartphone software that could convert the color given by the sensor into a time to consume/use the product.

In the context of the strong regulatory changes promoting alternative packaging to conventional plastic materials, the developed sensors could be used to find new solutions

better suited to the requirements of each individual product and to optimize the use of food additives. Integrated into a pilot equipment able of controlling the oxygen level, the response of the sensor could thus be used to model the shelf-life of different food products according to their storage conditions.

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APPENDIX

A. APPENDIX: VOLATILE COMPOUNDS PRODUCED BY LIPID OXIDATION

A.A. MAIN VOLATILE ALDEHYDES PRODUCED BY LIPID OXIDATION

OXIDATION TRACKER MOLECULE	ORIGINAL FATTY ACID	FOOD MATRIX IN WHICH THESE MOLECULES WERE IDENTIFIED
Ethanal	Omega 3	Perilla oil ¹ , palm oil ¹
Propanal	Omega 3	Salmon ^{2,3} , mackerel ³⁻⁵ , whiting ⁶ , anchovy ³ , hake ³ , trout ³ , fish oil, perilla oil ¹ , palm oil ¹
Butanal	Omega 3	Perilla oil ¹ , dried fish ⁷ , sand eel oil enriched mayonnaise ⁸
Pentanal	Omega 6	Mackerel ⁹ , whiting ⁶ , anchovy ³ , mackerel ^{3,4} , hake ³ , salmon ³ , trout ³ , milk ¹⁰ , pork ⁹ , chicken ⁹ , turkey ⁹ , yak jerkey ¹¹ , dried fish ⁷ , perilla oil ¹ , palm oil ¹ , sunflower oil ¹ , olive oil ¹²
Hexanal	Omega 6	Menhaden fish oil ¹³ , salmon ² , cod ² , saithe ² , redfish ² , mackerel ² , whiting ⁶ , anchovy ^{3,14} , mackerel ^{3,4} , hake ³ , salmon ³ , trout ³ , herring ¹⁵ , sea bass ¹⁶ , sea bream ¹⁷ , dried fish ⁷ , pork ⁹ , chicken ⁹ , turkey ⁹ , yak jerkey ¹¹ , perilla oil ¹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹ , olive oil ¹²
Heptanal	Omega 6	Cod ² , saithe ² , redfish ² , anchovy ^{3,14} , mackerel ²⁻⁴ , hake ³ , salmon ^{2,3} , trout ³ , whiting ⁶ , pork ⁹ , chicken ⁹ , turkey ⁹ , Iberian ham ⁹ , yak jerkey ¹¹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹ , milk ¹⁰
Octanal	Omega 9	Cod ² , saithe ² , redfish ² , mackerel ²⁻⁴ , hake ³ , salmon ^{2,3} , trout ³ , whiting ⁶ , sea bass ¹⁶ , milk ¹⁰ , beef ¹⁸ , yak jerkey ¹¹ , perilla oil ¹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹
Nonanal	Omega 9	Salmon ² , cod ² , saithe ² , redfish ² , mackerel ² , sea bream ¹⁷ , whiting ⁶ , pork ⁹ , chicken ⁹ , turkey ⁹ , Iberian ham ⁹ , yak jerkey ¹¹ , perilla oil ¹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹ , milk ¹⁰ , milk powder ¹⁹

(E)-2-butenal	Omega 3	Menhaden fish oil ¹³ , anchovy ^{3,14,20} , sardines ²⁰ , mackerel ²⁻⁴ , whiting ⁶ , cod ² , saithe ² , redfish ² , hake ³ , salmon ^{2,3} , trout ³ , sand eel oil enriched mayonnaise ⁸ , camelia oil ¹ , sunflower oil ¹ , olive oil ¹²
(E)-2-hexenal	Omega 3	Red seabream ²¹ , skipjack ²¹ , mackerel ^{2-4,21} , whiting ⁶ , anchovy ^{3,4,14} , hake ^{3,4} , salmon ²⁻⁴ , trout ^{3,4} , saithe ² , sand eel oil enriched mayonnaise ⁸ , herring ¹⁵ , yak jerkey ¹¹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹ , perilla oil ¹ , olive oil ¹²
(Z)-4-heptenal	Omega 3	Sand eel oil ²² , cod roe ²³ , anchovy ^{3,4,14} , hake ^{3,4} , salmon ^{3,4,24} , trout ^{3,4} , mackerel ^{3,4,21} , whiting ⁶ , red seabream ²¹ , skipjack ²¹ , sand eel oil enriched mayonnaise ⁸ , milk powder ¹⁹
(E)-2-octenal	Omega 6	Menhaden fish oil ¹³ , salmon ² , saithe ² , redfish ² , mackerel ^{2,21} , red seabream ²¹ , skipjack ²¹ , yak jerkey ¹¹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹ , perilla oil ¹
(E)-2-nonenal	Omega 6	Salmon ²⁴ , sea bass ¹⁶ , pork ⁹ , Iberian ham ⁹ , yak jerkey ¹¹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹ , perilla oil ¹
(E,E)-2,4-hexadienal		Menhaden fish oil ¹³
(E,E)-2,4-heptadienal	Omega 3	Salmon ^{2-4,24} , cod ² , saithe ² , redfish ² , mackerel ^{2,4,8,21} , whiting ⁶ , red seabream ²¹ , skipjack ²¹ , menhaden fish oil ¹³ , anchovy ^{3,4,14} , hake ^{3,4} , trout ^{3,4} , herring ²² , milk ¹⁰
(E,Z)-2,4-heptadienal	Omega 3	Mackerel ⁴ , sand eel oil ²² , sand eel oil enriched mayonnaise ⁸ , anchovy ¹⁴ , milk powder ¹⁹
(Z,E)-2,4-heptadienal	Omega 3	Sand eel oil enriched mayonnaise ⁸
(E,E)-2,4-octadienal		Salmon ²⁴ , menhaden fish oil ¹³
(E,E)-2,4-nonadienal	Omega 3	Salmon ² , cod ² , saithe ² , redfish ² , mackerel ² , Iberian ham ⁹ , perilla oil ¹
(E,Z)-2,6-nonadienal	Omega 3	Salmon ^{3,24} , lobster ²⁵ , menhaden fish oil ¹³ , cod ² , mackerel ² , sand eel oil enriched mayonnaise ⁸ ,

		anchovy ^{3,14,20} , sardines ²⁰ mackerel ³ , hake ³ , trout ³ , cod roe ²³ , turkey ⁹ , milk powder ¹⁹
(E,E)-2,4-decadienal	Omega 6	Salmon ^{2-4,24} , cod ² , saithe ² , redfish ² , mackerel ^{2,4,8,21} , anchovy ¹⁴ , menhaden fish oil ¹³ , sand eel oil enriched mayonnaise ⁸ , turkey ⁹ , Iberian ham ⁹ , liver ⁹ , perilla oil ¹ , milk ¹⁰
(E,Z)-2,4-decadienal	Omega 6	Salmon ²⁴ , sand eel oil enriched mayonnaise ⁸ , sunflower oil ^{1,26}
(E,Z,Z)-deca-2,4,7-trienal		Menhaden fish oil ¹³
(E,E,Z)-deca-2,4,7-trienal		Menhaden fish oil ¹³ , seal blubber oil ²²
(Z,Z,Z)-4,6,9-nonadecatriene		Anchovy ²⁰ , sardines ²⁰

A.B. MAIN VOLATILE KETONES PRODUCED BY LIPID OXIDATION

OXIDATION TRACKER MOLECULE	ORIGINAL FATTY ACID	FOOD MATRIX IN WHICH THESE MOLECULES WERE IDENTIFIED
Pentan-2-one		Mackerel ⁴ , sardine oil ²⁷ , blue crab ²⁸
Hexan-2-one	Omega 6	Sardine oil ²⁷ , blue crab ²⁸ , crab ²⁹ , whiting ⁶ , lamb ³⁰ , crayfish ²⁹ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹
Heptan-2-one	Omega 6	Cod ³¹ , mackerel ³¹ , whiting ³¹ , yak jerkey ¹¹ , beef ¹⁸ , black carp ⁷ , blue crab ²⁸ , herring ¹⁵ , lamb ³⁰
Octan-2-one	Omega 9	Beef ¹⁸ , dairy products ¹⁹ , crab ²⁹
Nonan-2-one	Omega 9	Salmon ² , cod ² , saithe ² , redfish ² , mackerel ^{2,31} , cod ³¹ , whiting ³¹ , turbot ³² , sand eel oil enriched mayonnaise ⁸ , blue crab ²⁸
Pentan-3-one		Black carp ⁷ , blue crab ²⁸
Heptan-3-one		Salmon ²
Octan-3-one	Omega 9	Blue crab ²⁸ , pork ³³ , camelia oil ¹ , sunflower oil ¹ , palm oil ¹

1-penten-3-one	Omega 3	Menhaden fish oil ¹³ , sand eel oil enriched mayonnaise ⁸ , anchovy ²⁰ , sardines ²⁰ , mackerel ^{3,4,34} , hake ^{3,4} , salmon ^{3,4} , trout ^{3,4} , herring ²² , whiting ⁶ , sardines ³⁵ , lamb ³⁰ , olive oil ¹²
1-octen-3-one	Omega 6	Whitefish ²⁵ , cod ² , saithe, redfish, mackerel, sand eel oil enriched mayonnaise ⁸ , seabass ³⁶ , turkey ⁹ , liver ⁹ , beef ¹⁸ , sea bass ³⁷ , mushrooms ³⁸ , sardines ³⁵ , lamb ³⁰
2,3-butanedione		Lobster ²⁵ , salmon ³⁹ , mackerel ^{4,31} , cod ³¹ , beef ¹⁸ , anchovy ²⁰ , pork ³³
2,3-octanedione	Omega 6	Red seabream ²¹ , skipjack ²¹ , mackerel ²¹ , bream ⁴⁰ , anchovy ^{14,20} , whiting ⁶ , sea bream ¹⁷ , yak jerkey ¹¹
(Z)-1,5-octadien-3-one	Omega 3	Lobster ²⁵ , cod ² , salmon ² , sand eel oil enriched mayonnaise ⁸ , seal blubber oil ²²
(Z,Z)-3,5-octadien-2-one	Omega 3	Menhaden fish oil ¹³ , skipjack ²¹ , mackerel ^{4,21} , whiting ⁶ , anchovy ^{14,20} , blue crab ²⁸ , sand eel oil enriched mayonnaise ⁸ , yak jerkey ¹¹ , dairy products ¹⁹ , milk powder ¹⁹
(E,Z)-3,5-octadien-2-one	Omega 3	Sand eel oil enriched mayonnaise ⁸

A.C. MAIN VOLATILE ALCOHOLS PRODUCED BY LIPID OXIDATION

OXIDATION TRACKER MOLECULE	ORIGINAL FATTY ACID	FOOD MATRIX IN WHICH THESE MOLECULES WERE IDENTIFIED
Pentan-1-ol	Omega 6	Red seabream ²¹ , skipjack ²¹ , mackerel ²¹ , whiting ⁶ , sea bream ¹⁷ , milk ¹⁰ , yak jerkey ¹¹ , olive oil ¹²
Hexan-1-ol	Omega 6	Sea bass ¹⁶ , whiting ⁶ , sea bream ¹⁷ , red seabream ²¹ , skipjack ²¹ , mackerel ²¹ , yak jerkey ¹¹ , olive oil ¹²
Heptan-1-ol	Omega 6	Red seabream ²¹ , skipjack ²¹ , mackerel ²¹
Heptan-2-ol	Omega 9	Yak jerkey ¹¹
Octan-1-ol		Mackerel ⁹ , yak jerkey ¹¹

1-penten-3-ol	Omega 3	Salmon ²⁴ , herring ¹⁵ , sand eel oil enriched mayonnaise ⁸ , mackerel ⁴ , sea bass ¹⁶ , milk powder ¹⁹ , anchovy ^{14,20} , sardine ²⁰ , whiting ⁶ , olive oil ¹²
1-octen-3-ol	Omega 6	Mackerel ⁴ , sea bass ^{16,37} , sand eel oil enriched mayonnaise ⁸ , sea bream ¹⁷ , sardine ¹⁷ , whiting ⁶ , cod ^{2,23} , yak jerkey ¹¹
(Z)-2-penten-1-ol		Whiting ⁶ , sand eel oil enriched mayonnaise ⁸
(E)-2-penten-1-ol		Anchovy ²⁰ , sardines ²⁰ , sand eel oil enriched mayonnaise ⁸ , olive oil ¹²
(Z)-2-octen-1-ol		Sea bass ³⁷ , yak jerkey ¹¹
(E)-1,5-octadien-3-ol	Omega 3	Sand eel oil enriched mayonnaise ⁸

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B. APPENDIX: REVIEW ON MIP APPLICATIONS IN FOOD SCIENCE

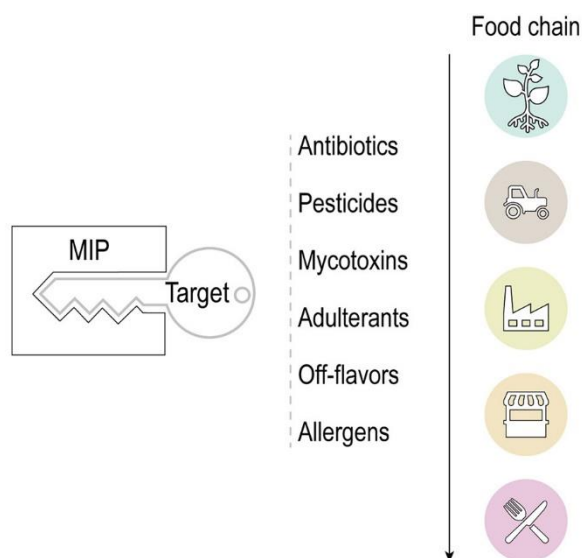
Applications of Molecularly Imprinted Polymers and perspectives for their
use as food quality trackers

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Chem **8**, 2330–2341 (2022)

doi: 10.1016/j.chempr.2022.05.021

GRAPHICAL ABSTRACT



SUMMARY

Molecularly Imprinted Polymers (MIPs), often referred to as ‘artificial’ or ‘plastic’ antibodies, are synthetic alternatives to natural receptors. Based on the ‘key–and–lock’ principle, a template is imprinted into a polymer, which is then able to selectively bind to a target molecule. Over the past 50 years, MIP have continued to attract attention in both academic research and commercial applications. MIPs have a wide range of applications in analytical chemistry, medicine and pharmaceuticals, as well as in the environment. This paper highlights the new perspectives offered by molecularly imprinting technology to food science. Food analysis is challenging because of the complexity of food matrices and the low concentration of analytes. MIPs are ideally suited to overcome both of these issues because of their high selectivity and sensitivity. Furthermore, MIPs could greatly benefit the entire food chain as a tool to ensure food safety, quality and traceability, while reducing food waste.

INTRODUCTION

The greatest challenge facing the food industry today is to promote the accessibility of affordable food while ensuring product safety, traceability and nutritional quality, to guarantee a balanced diet for all. Although food safety is a major concern for the agro–food industry, health scandals are recurrent, and they affect all types of products. A few examples of the scandals that have shaken the food industry in recent decades include the following: Minamata disease caused by mercury–infected fish in the 1950s, mad cow disease caused by the neurodegenerative Creutzfeldt–Jakob disorder in 1986 and 1996, bird flu in 2004, melamine found in milk in 2008 and the insecticide fipronil identified in eggs in 2017. The agro–food sector is always looking for better analytical tools to ensure the safety of its products.⁴² In addition, reducing food waste remains one of today’s greatest challenges to be overcome.

Food waste can be defined as food originally produced for human consumption that is lost at some point in the food chain.⁴³ According to the United Nations Food and Agriculture Organization (FAO), 1.3 billion tons of food is wasted each year worldwide; this represents one–third of the global production. Food waste is an environmental, economic and social issue. In addition to the consumption of natural resources for the production, manufacturing, packaging and transport, the carbon footprint of food waste is as high as that of the third most polluting country in the world.⁴⁴ The FAO estimates the economic loss of food waste at \$750 billion per year. According to a 2019 study by the United Nations World Food Program, more than 800 million people, i.e., 1 in 9 people worldwide, suffer from hunger today.⁴⁵ The need to feed more than 9 billion people by 2050 forces the food industry to find sustainable methods of producing and consuming. Maximizing product shelf–life and reducing waste is thus a top priority. Food waste occurs throughout the food chain from the producers to consumers. In developing countries, most losses occur at the beginning of the food chain because of storage and distribution limitations, while in medium– and high–income countries most food is wasted at the end of the food chain by the consumer because of high quality standards and health precautions.⁴³ The agro–food sector could thus greatly benefit from a reliable and easy–to–use tool capable of monitoring food quality all along the chain to ensure food safety and traceability and increase product shelf–life while reducing waste. Hence, MIPs could be a solution of choice.

MIPs AND THEIR APPLICATIONS

MIPs are synthetic alternatives to natural receptors. Based on the 'key-and-lock' principle, a template, which is the molecule targeted or a structural analogue, is imprinted into a polymer. In the three-step process for the synthesis of MIPs (**Figure 1**), a host-guest complex is first formed between the template and the monomer(s). Then, the cross-linker is copolymerized with the monomer around the previously formed complex. The final step is the removal of the template by solvent extraction. At the end of the process, the polymer material is able to bind to the target in a selective manner. As shown in **Figure 1**, the selectivity is the ability of the MIP to bind only to the target molecule compared with other compounds with a similar chemical structure.⁴⁶ MIPs have functional groups located in cavities, offering a unique chemical and steric complementarity with the target molecule. Their synthesis can be conducted by either the so-called covalent or non-covalent approaches. This refers to either the chemical bonds or physical interactions governing the formation of the template-monomer complex prior to polymerization, which must be stable enough to withstand the polymerization step, but easily cleavable afterward. In the 'covalent' approach⁴⁷⁻⁴⁹, the template is chemically bound to the monomer(s); this offers the benefit of providing homogeneous binding cavities, and strong chemical and physical stability for the synthesis. However, template removal occurs under drastic conditions and the kinetics of target binding and release are relatively slow. In the widely used 'non-covalent' approach⁵⁰, the template and monomer physically interact through attractive forces, such as hydrogen bonding, π - π stacking or van der Waals forces. This route is easy to implement and the template can be removed under mild conditions. Binding and release kinetics are fast. Finally, the 'semi-covalent approach'⁵¹ integrates the two previously mentioned principles. Imprinting is based on chemical bonds, while binding to the target relies on physical interactions. This approach combines the respective advantages of the two previously mentioned ones.

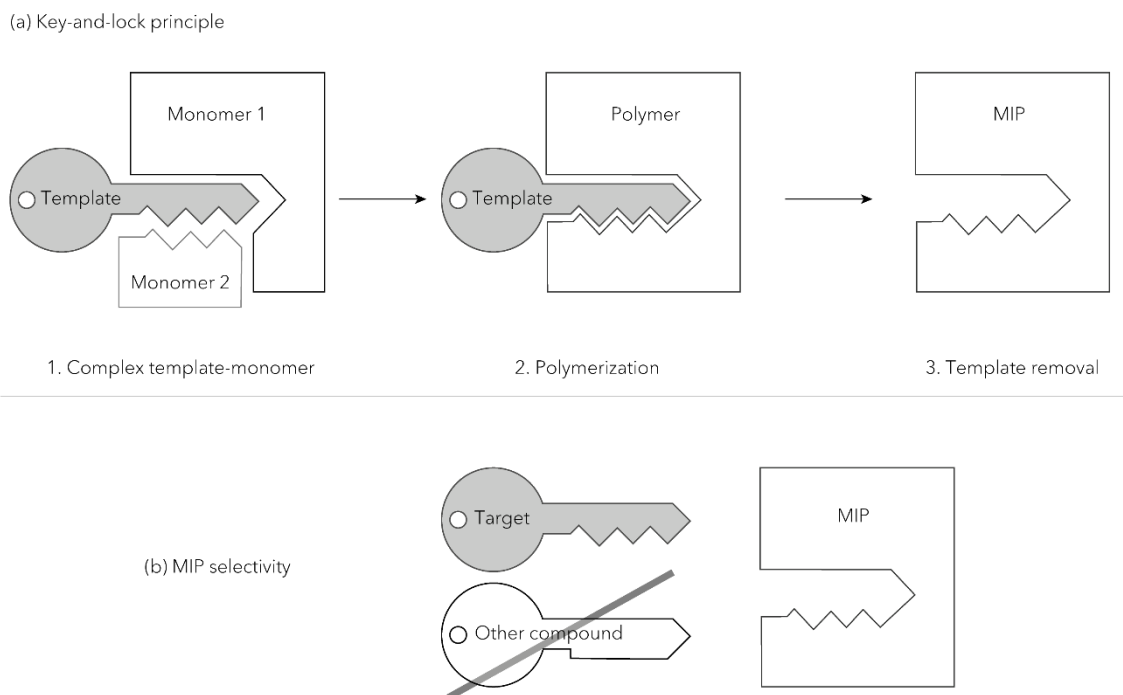


Figure 1: Schematic illustration of the synthesis route and operating principle of MIPs.

- (a) Key-and-lock principle for the synthesis of a MIP.
- (b) MIP selectivity is defined by the ability of the MIP to bind only with the target molecule compared with other compounds of similar chemical structure. Binding cavities in the polymer provide chemical and steric complementarity with the target.

Following the imprinting, polymerization can be achieved either by radical polymerization or by a sol-gel process. The radical polymerization route requires a template, one or more organic or inorganic functional monomers, a cross-linker, a polymerization initiator and a solvent. The solvent plays two major roles: solubilization of the reactants and pore formation within the structure of the polymer material. Radical polymerization is usually performed either thermally or photo-initiated. There are different types of radical polymerization leading to polymers of various morphologies and performances. Bulk polymerization is the oldest and the simplest. The resulting block polymers must be ground and sieved prior to use, affording particles of irregular size and shape. Mass transfer is relatively slow because some binding cavities remain trapped within the structure. Precipitation polymerization requires large amounts of solvent in which the growing polymer is insoluble and gradually precipitates.⁵² The resulting monodispersed spherical particles (ranging in size from

nanometers to millimeters^{53,54}) show better mass transfer properties due to the increase in the specific surface area. Although more difficult to realize, suspension and emulsion polymerizations lead to similar particles. *In situ* polymerization, e.g., electropolymerization⁵⁵, allows the formation of a MIP as a thin layer on the outer surface of a material by grafting. MIPs can also be obtained as a stand-alone film. Hence, the polymerization process strongly influences the morphology of the MIP and thus its final performance.

The sol-gel route, first use in the 1930s⁵⁶ and developed in the 1950s⁵⁷⁻⁶⁰, relies on the generation of a sol, a colloidal fluid containing the template and inorganic monomers, which is then transformed into a gel. Molecularly imprinted silica obtained by the sol-gel route shows better physical stability and higher selectivity compared with a MIP synthesized by radical polymerization.⁶¹ The reactants used are also less toxic.⁶² However, the sol-gel synthesis is conducted in an aqueous medium, which limits the choice of reactants, due to solubility limitations. This makes the sol-gel route challenging, hence less explored.

Among the synthesis parameters, the molar ratio between the different reactants is the most critical parameter for the polymer material's performance because it influences its morphology and its selectivity as well as the number of binding cavities and their accessibility.⁵² Other synthesis parameters are also crucial and hence require optimization. For example, the selectivity of MIPs is higher when using UV polymerization compared with thermally initiated polymerization.⁶³ The selectivity of MIPs is also higher when binding experiments are conducted in the same solvent as that used for the synthesis⁶⁴, a phenomenon known as the 'memory effect' of the solvent.

Since the first publication by Wulff and Sarhan in 1972, MIPs have attracted a great deal of attention within the scientific community⁶⁵, leading to the development of applications in many fields, from analytical chemistry to medicine and pharmaceuticals, as well as in the environment and food science, as displayed in **Figure 2**. Molecular recognition is one of the most important features of natural receptors because it underlies many biological processes. Similar to antibody-antigen, enzyme-substrate and hormone-receptor pairs, MIPs can distinguish chiral molecules and were first used for this purpose.⁶⁶ This field of research is still active⁶⁷ because this property is very important in the pharmaceutical and flavor industries. MIPs have the advantage over their biological analogues of being easy to synthesize at low cost. Furthermore, they benefit from being more stable over a wide variety of physical and chemical

conditions. Therefore, MIPs are used in enzyme-linked immunosorbent assay⁶⁸ tests, as biomimics, to investigate the mechanisms occurring in natural systems⁶⁸, as well as for bioimaging⁶⁹, cell culture⁷⁰, drug development⁷¹, cancer diagnosis⁷² and therapy.⁷³ In addition, based on stimuli-responsive MIPs, which have a switchable binding and release capacity in response to an external physical or chemical stimulus (e.g., pH, temperature, light, electric fields)⁷⁴, drug delivery can be optimized. Lastly, their reusability also makes MIPs particularly interesting for catalysis.⁷⁵

MIPs are also widely used as sensors. MIP sensors possess a transducer capable of translating the target-MIP interaction into a measurable signal. Here, the main categories include optical, electrochemical, mass-sensitive, thermal and acoustic sensors. MIP sensors combine the advantages of their two components: the MIP recognition unit and the signal transducer. This leads to technologies with high stability, sensitivity, selectivity, fast detection and miniaturization capacities.⁷⁶ Optical sensors are based on chemiluminescence, fluorescence, surface plasmon resonance, surface-enhanced Raman scattering or resonance light scattering. Electrochemical sensors rely on potentiometry, amperometry, conductometry, impedimetry or voltammetry measurements. Recently, the first combination of MIPs with microfluidic devices was even proposed.⁷⁷ Thus, the development of MIP-based sensors has increasingly been pursued over the last 10 years, a trend that is likely to continue.

In analytical chemistry, MIPs are used as stationary phase in flow analytical techniques, including chromatography and electrophoresis. Sample preparation is a critical step to extract the analyte of interest from the matrix prior to analysis. However, the process usually suffers from two major limitations: the lack of selectivity of the sorbent (the so-called matrix effect) and the trace level of analytes. MIPs allow the selective separation of the analyte from complex matrices and sensitive determination of its concentration.⁷⁸ MIPs have been mainly designed for sample preparation via solid-phase extraction (SPE), solid-phase microextraction (SPME), dispersive SPE (d-SPE), magnetic SPE (MSPE), and stir bar sorptive extraction (SBSE).

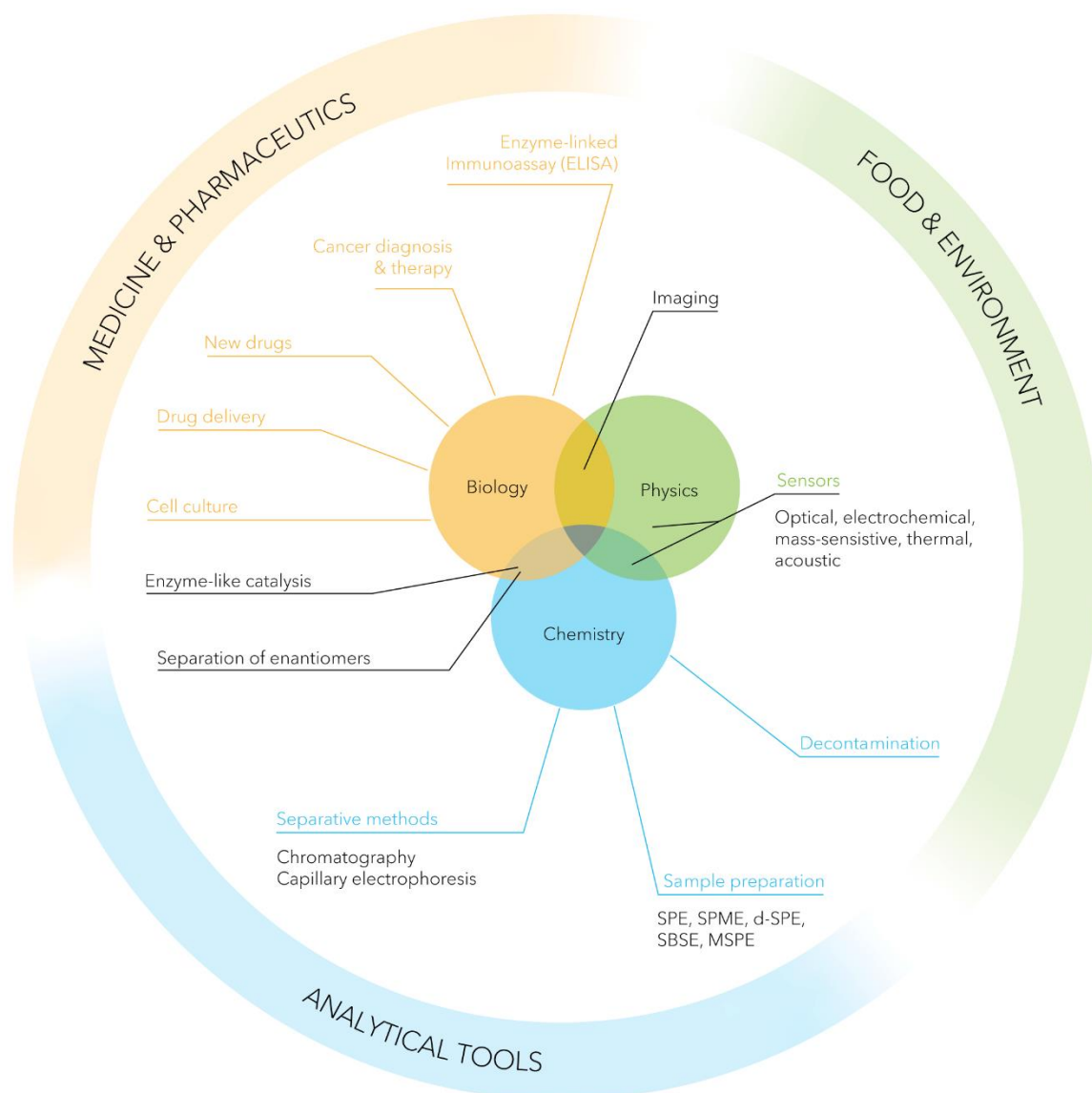


Figure 2: Overview of the application areas with their related fields of science covered by developments based on MIPs.

EMERGING TRENDS IN THE USE OF MIPs TO ADDRESS FOOD ISSUES

The analysis of food samples is particularly challenging due to the complexity of the matrices and the low concentration of analytes.⁷⁶ The presence of contaminants in food products is usually determined by liquid or gas chromatography coupled with mass spectroscopy, which requires the use of expensive facilities by highly trained professionals as well as time-consuming and complex sample preparation. MIPs have the advantage of being easily synthesized at low cost and they are simple to use. MIPs are synthetic receptors with high selectivity and they have been shown to be very sensitive materials as detection limits can

be as low as the trace level (down to ng/L levels).⁷⁹ MIPs can also be tailor-made for any target molecule, and they are chemically and physically very stable, which opens up a wide range of applications. Moreover, MIP-based sensors tend to be portable, which facilitates their rapid application in any product.

As shown in **Figure 3**, MIPs can be used as a powerful analytical tool for quality control in food science because they allow the detection, quantification and even extraction of many target compounds along the food chain, from agro-resources to wastes. The targets can be the contaminants present in water and soil, such as antibiotic residues⁸⁰ (e.g., chloramphenicol, tetracycline) and heavy metals⁸¹ (e.g., arsenic, mercury). MIPs can also be designed for the decontamination of food and beverages at each step of the food chain from production to consumption. Various molecules can be targeted, such as pesticides⁸², like herbicides (e.g., atrazine, glyphosate) and insecticides (e.g., λ -cyhalothrin, diazinon), adulterants⁸³ (e.g., melamine, ractopamine), mycotoxins⁸⁴ (e.g., aflatoxin B1, ochratoxin A), substances migrating from packaging⁸⁵ (e.g., acrylamide, bisphenol A), allergens⁸⁶ (e.g., α -casein; genistein, an isoflavone from soy) and even food spoilage substances that can be dangerous in the event of human consumption, such as histamine.⁸⁷ Finally, MIPs can be used as analytical or treatment tools during the waste management process, such as for the extraction of dye residues⁸¹ (e.g., Orange II, Sudan I) from food packaging to favor their recyclability.

Hence, opportunities are constantly emerging, leading to the development of new applications of MIPs in food science. MIP-based sensors are particularly interesting to ensure food safety and reduce food waste. In terms of food safety, MIPs could indeed be interesting tools to fight food adulteration, prevent allergic reactions, ensure sustainable production methods, avoid antibiotic resistance and protect health. As an example, to fight against food adulteration, a potentiometric sensor based on MIPs has been developed for the rapid detection of melamine in milk in as short a time as 16 seconds.⁸³ In comparison, classical chromatographic analysis takes several minutes, not including the time required for sample preparation. To prevent allergic reactions, an electrochemical MIP-based sensor has recently been proposed to selectively track the presence of the genistein isoflavone, a molecule identified as a marker for soy allergens.⁸⁶ This sensor allows in-field detection and it has been tested on a selection of dishes offered by a restaurant. The results obtained within 5 minutes showed excellent selectivity. Even if the detection limit remains higher than that achievable by

mass spectroscopy, it is still 100 times lower than the allergic threshold. In addition to the restrictions on pesticide use imposed by regulations, the increasingly systematic detection of pesticides residues in food products forces the producers to shift towards sustainable production methods to protect nature's biodiversity. A recently reported portable MIP-based electrochemical sensor allows real-time detection and quantification of glyphosate in beverages with a limit of detection of 2 µg/L (or 10 nM).⁸² The portability of such a sensor, a feature that cannot be offered by any currently used analytical tool, extends its applicability. However, to date, its efficiency has only been proven in orange juice and rice beverages spiked with glyphosate; it has not yet been tested in complex solid food matrices. To avoid antibiotic resistance, a sensor combining magnetic MIPs and surface plasmon resonance has been developed to detect tetracycline down to 1 ng/L (or 2 pM) in milk.⁸⁰ The detection limit is comparable to that obtained with chromatographic devices.⁸⁸ However, this sensor was not tested in contaminated milk, but only in tetracycline-spiked milk. Such sensors, made available to retailers and distributors, should assist in preventing the overuse, by farmers, of antibiotics in animal feeding. At the consumer level, to protect health by warning of mycotoxin contaminations, a MIP-based sensor has recently been developed to detect *Fusarium* in cereals using a smartphone⁸⁴, therefore making it usable at home.

MIPs could also be reliable tools to reduce food waste, either by extending or by monitoring the shelf-life of products. The decrease in organoleptic properties and nutritional value as well as the potential generation of compounds toxic to humans during food degradation processes are the main reasons for the need to monitor product quality throughout the whole food chain.⁸⁹ To address these food-related issues, active and intelligent materials are considered recent and promising improvements in food preservation. On the one hand, active materials can interact with food or its environment through a controlled sorption or release of compounds to extend the shelf-life of a product (EC 450/2009). Such active materials could be advantageously used when packaging with optimal barrier properties or modified atmosphere packaging are not sufficient. A few MIPs have already been designed with preservatives that are gradually released into the food to prevent spoilage, such as ferulic acid as a potent antioxidant to prevent lipid oxidation⁹⁰ or allyl isothiocyanate as an antibacterial agent to preserve meat.⁹¹ On the other hand, intelligent materials can monitor food or its environment and provide information to the user (EC 450/2009). Such intelligent packaging

could be of great value to monitor product quality throughout the food chain by indicating the dynamic shelf-life of the product for the retailer or consumer. The field of intelligent packaging has attracted increasing interest in recent years with the development and commercialization of time-temperature indicators, leakage indicators, cold chain break indicators or radio frequency identification tags. However, none of these innovations exploit the analytical benefits of MIPs. Photoresponsive and fluorescent MIPs are ideally suited for the design of intelligent packaging. These MIPs are obtained via monomers possessing photosensitive chromophores (e.g., azobenzene, spiropyran) or fluorophores (e.g., fluorescein, rhodamine), allowing the optical properties of the material to be modified once the MIP-target interaction occurs. Obviously, the compromise between the benefits brought by the addition of such MIP-based sensors and their contribution to overpackaging has to be carefully considered, as well as the end-of-life management of the material and its recycling.

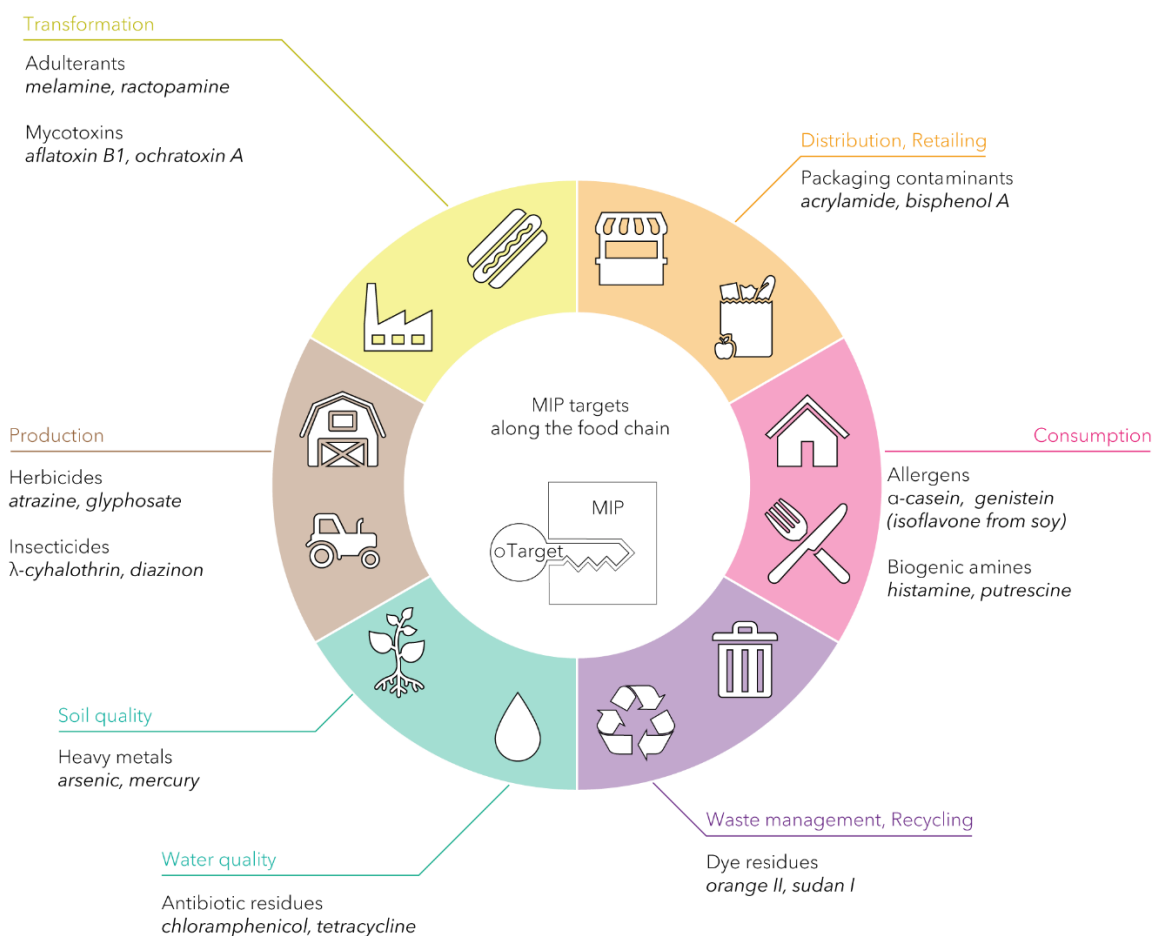


Figure 3: Examples of MIP targets along the food chain, from agro-resources to waste. MIPs can be used as tunable quality trackers for detection, quantification and extraction of various contaminants along the food chain.

(Colour: the part of the food chain involving MIP applications; bold: family of chemical compounds identified as MIP targets; italics: selected examples of MIP targets.)

TRACKING FOOD QUALITY WITH MIPs: CHALLENGES AND SOLUTIONS

Although much research has been carried out in the field of MIPs over the last 50 years, several technical and application aspects remain challenging, particularly regarding their technological transfer to use them as food quality trackers, as shown in **Table 1**.

Technical challenges that must be overcome concern both the synthesis and the use of MIPs. The limited number of suitable commercially available monomers (especially alkoxysilane derivatives) restricts the number of MIPs that can be synthesized and hence the number of

possible applications to track any target compound in food matrices. The organic synthesis of tailor-made monomers is an alternative, but this is often costly and time-consuming. Generally, synthesis optimization is a long process, particularly as there are many parameters that strongly influence MIP performance. To reduce the experimental time, scientists can rely on computer simulations⁹² and experimental design⁹³ to support their choices in terms of reactants and synthesis parameters. The use of automatic reactors for the synthesis and testing of MIPs can also contribute to reducing experimental time.⁹⁴ Complete removal of the template at the final stage of MIP synthesis is also difficult to achieve. If it is not done properly, it will result in inaccessible cavities and potential leakage of the template when using the MIP, which can thus compromise the results of binding experiments or the compliance as a food contact material. The use of a dummy template, which is a structural analogue of the target compound, may solve the problem caused by template leakage. It can also be worthwhile when the template to imprint is expensive or even dangerous, such as in the case of some contaminants of food products such as mycotoxins.⁹⁵ When the structure and/or the conformation of the template can be modified due to radicals generated during radical polymerization, an altered molecule could be imprinted. Yet, controlling the exact imprinting of the conformation of a protein is essential when it is responsible for a food allergy. In this case, the sol-gel polymerization can be an alternative route. Another frequently reported problem is the binding of the target outside the cavities. In imprinted polymers, the cavities responsible for steric complementarity between the target and the polymer contain appropriately oriented binding sites that can interact chemically and physically with the target compound. However, some binding sites, such as functional groups from the monomer can be randomly distributed in the polymer and can be responsible for physical interactions with the target outside the cavities. The cross-linker can also sometimes physically interact with the target through attractive forces and be responsible for binding the target outside the cavities. Optimization of the selectivity of MIPs is crucial to improve their performance, especially for their use as sensors in complex matrices such as food. Such optimization would, moreover, reduce the cost of resulting MIP-based sensors for future commercial applications. Of interest is that, it has already been proven that molecularly imprinted silicas are less affected by these unintended interactions⁶¹, mainly because the use of a mineral cross-linker limits the interactions with organic targets.

Reproducibility of the polymer synthesis, lack of homogeneity of the polymer and surface immobilization of the polymer material are the major problems that hinder the technological transfer and development of commercial applications of MIP-based sensors. First, reproducibility of the synthesis is challenging due to the numerous parameters that influence the MIP performance, and the resulting difficulty in controlling them all perfectly. A standardization of the synthesis protocol is then required.⁹⁶ Second, a lack of homogeneity of the MIP is usually due to a heterogeneous distribution of the binding cavities within the polymer. Such homogeneity is rather difficult to control during the synthesis, especially when bulk polymerization is used. Synthesis of core-shell particles allows for better control of the distribution and access to binding cavities as they are located in the outer layer of the MIP, which favors high specific surface area and mass transfer.⁹⁷ Solid-phase synthesis, which relies on polymerization around a template molecule immobilized by grafting onto a surface, also improves accessibility to the binding cavities, compared with polymers obtained by bulk polymerization.⁹⁸ Third, MIPs are often synthesized as powders, which raises the question of their immobilization to use them as sensors. A homogeneous distribution of a powder on the surface of a sensor is rather difficult to achieve. The synthesis of MIPs directly in the form of stand-alone films is an interesting outcome, but very challenging to achieve. Indeed, MIP films, are brittle when dried and can only be used at high relative humidity. Coating (e.g., spin- or drop-coating) of a MIP solution onto a surface can also be considered. Surface grafting and electropolymerization offer an interesting solution to directly synthesize the MIP on the surface of a material. Addressing these various challenges is required prior to the market launch of simple-to-use and portable MIP-based sensors for real-time and on-site detection and quantification of target compounds in food products.

Concerning application challenges, the analysis of food products represents, in many respects, an underestimated complexity. MIPs as selective materials have still to be improved for their use in aqueous phase, their ability to bind high-molecular-weight molecules, their compliance as food contact materials and their ability to simultaneously detect several targets. First, the synthesis and use of MIPs in aqueous phase remains an issue, for three main reasons. For the synthesis, most reactants are insoluble in water. Moreover, water involves hydrogen bonds between the solvent and the reactants, which can reduce the interactions between the template and the functional monomers during the synthesis and, once polymerization has

taken place, during binding experiments. Finally, the performance of MIPs is low in aqueous media because they are usually hydrophobic. Some proposals have been made to overcome this issue by selecting monomers that are water soluble⁹⁹ or using hydrophilic coatings on the surface of MIPs.¹⁰⁰ This challenge, which has been addressed by the scientific community for many years, is important to overcome when dealing with the use of MIPs in humid foods and beverages.

Second, imprinting high-molecular-weight molecules, such as proteins, is another difficulty due to their conformational flexibility, fragile nature and insolubility in most organic solvents.¹⁰¹ This is an important hurdle to overcome, for example, when targeting food allergens. To deal with this challenge, the imprinting of an epitope (a peptide located on the surface of the targeted protein) could be sufficient to achieve selective binding of the whole protein.¹⁰² This method could also be extended to the imprinting of viruses or bacteria that alter food products. However, the native structure and conformation of the protein surface is not always known and an isolation process could alter it.

Third, MIPs made for food contact applications must comply with the related regulations (EC 1935/2004). The toxicity of a polymer is generally related to the potential release of the functional monomer unit. The latter has thus to be carefully chosen, and its toxicity must be assessed at the outset. Imprinted polymers obtained by the sol-gel process seem to be particularly indicated for food applications because of their lower toxicity compared with the more classical MIPs resulting from radical polymerization.⁶² Moreover, for the application of MIPs in food, a green chemistry approach is highly encouraged to replace organic solvents in the synthesis.¹⁰³ The alternative use of supercritical CO₂ could also be a solution, albeit challenging.¹⁰⁴

Lastly, the high selectivity of MIPs is one of their greatest advantages, but this also limits their application. Indeed, food applications often require the simultaneous detection of several target compounds. For example, the wine industry is seeking a tool to monitor multiresidues of pesticides rather than a single molecule.¹⁰⁵ Three potential solutions can be investigated to address this limitation. The first one is the synthesis of a single MIP capable of binding multiple targets. This is possible if all targets have a similar chemical structure. In this case, a common part of the targets can be selected as a template. This solution has already proven to be successful in the detection of antibiotics from the amphenicol group in shrimp samples.¹⁰⁶ The

second one relies on mixing several MIPs selective for different targets. However, the difference in reactivity of the different MIPs and their response in the presence of interferents must be carefully evaluated. The third attractive alternative based on the imprinting of several targets in a single MIP also requires some technical issues to be addressed, such as the solubility of the targets in the same solvent, the control of the distribution of binding cavities in the resulting polymer and the difference in reactivity between the MIP and the targets. Because of the various difficulties, this route has been little explored.¹⁰⁷

To conclude, molecularly imprinting technology is still quite a young field, but it has already led to significant developments in many different fields. Extending its use to food analysis offers great benefits, but several challenges remain. There are exciting perspectives to be explored in the coming years.

Table 1: Current challenges and potential solutions for using MIPs to track the quality of food.

Challenges	Potential solutions	Interest for food	Ref.
Design			
Commercial availability of monomers	- Synthesis of monomers	Controlled toxicity of the MIP Extension of targeted molecules	-
Optimization of synthesis parameters	- Computational modelling - Experimental design - Use of automatic reactor	Time saving	- 51 - 52 - 53
Complete template removal Template leakage	- Imprinting of a structural analogue of the template - Solid-phase synthesis	Avoiding the migration of a toxic compound in contact with a food product	- 54 - 57
Imprinting of toxic / expensive template	- Imprinting of a structural analogue of the template	Imprinting of mycotoxins	- 54
Template degradation or isomerization caused by radical polymerization	- Imprinting of a structural analogue of the template - Sol-gel synthesis to avoid radical polymerization	Imprinting of proteins, antioxidants, vitamins	- 54 - 19
Accessibility of binding cavities	- Core-shell particles - Solid-phase synthesis	Improvement of the MIP performance	- 56 - 57
Technology transfer			
Reproducibility of the synthesis	- Standardization of the synthesis method	Development of simple-to-use and portable sensors for real-time and on-site detection, and quantification of target compounds in food products	- 55
Lack of homogeneity of the MIP	- Core-shell particles - Solid-phase synthesis		- 56 - 57
Surface immobilization of the MIP	- MIP films		-
	- Coating		-
	- Surface grafting		-
	- Electropolymerization		- 13
Applications			

MIP synthesis and use in aqueous phase	- Use of water-soluble monomers - Hydrophilic coating of the MIP	Use of MIPs in beverages or in humid foods	- 58 - 59
Imprinting of proteins	- Epitope imprinting	Allergen and virus tracking	- 61
Compliance as food contact material	- Choice of low toxicity monomers - Use of silica polymers - Green chemistry approach: synthesis in food-grade solvents or supercritical fluids	Strong regulations for food contact	- 21 - 62, - 63
Simultaneous detection of various targets	- Imprinting of a common structure of the targets - Mixing several MIPs - Imprinting of several targets in a single MIP	Global contamination index of a food product (e.g., pesticides, antibiotics)	- 65 - 66

ACKNOWLEDGEMENTS

This work was financially supported by the French “Investissements d’Avenir” program, project ISITE-BFC (contract ANR-15-IDEX-0003).

AUTHOR CONTRIBUTIONS

E.B.M. and T.K. conceptualized the topic. L.C. wrote the original draft. L.C., T.K., P.C., M.G., N.S., and E.B.M. contributed to the reviews. L.C. and T.K. designed the figures and M.G. drew them. T.K. handled the funding acquisition.

DECLARATION OF INTERESTS

The authors have a patent related to this work (FR2113403).

ADDITIONAL INFORMATION

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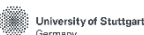
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WORK EXPERIENCE

- Oct. 2019 – July 2023 **PhD thesis**
[Université de Bourgogne Franche-Comté, France](#)
 Development of intelligent packaging to monitor food spoilage and reduce food waste
- Mar. – Sept. 2019 **Master thesis**
[Physico-chemical institute, AK Stubenrauch, Stuttgart, Germany](#)
 Study of emulsions and polymeric foams obtained by membrane process
- Dec. – Feb. 2019 **Research internship**
[Liquid crystals institute, AK Giesselmann, Stuttgart, Germany](#)
 Synthesis and characterization of mesoporous materials for catalytic applications
- May – Aug. 2018 **R&D engineering internship**
[Gascogne flexible, Dax, France](#)
 Eco-design of compostable barrier packaging
- Mar. – Apr. 2018 **Research project**
[SurfactGreen, Rennes, France](#)
 Eco-responsible synthesis and physico-chemical evaluation of new cationic surfactants derived from seaweed
- Dec. – Apr. 2018 **HSE project leader**
[ENSCR, Rennes, France](#)
 Managed a team of 7 people for the construction of a fictive laboratory
- June – July 2017 **R&D technician internship**
[Felor – Algo Paint, Rennes, France](#)
 Raw material equivalence for the formulation of water-based paintings
- 2016 – 2017 **Student mentoring**
[ENSCR, Rennes, France](#)
 Promotion of science and culture to high school students

EDUCATION

- 2018 – 2019 **Master of Science**, [University of Stuttgart](#), Germany
- 2016 – 2019 **Engineering degree**, [Ecole Nationale Supérieure de Chimie de Rennes](#), France

SKILLS

LANGUAGES

French: native speaker
English: C1 (2018 TOEIC 925/985)
German: C1
Spanish: B2
French sign language: basis

COMPUTING

Python, Swift
Microsoft Office

HOBBIES

Dance
Dance conservatory
10 years, Strasbourg, France
Literature & reading
Active member of a writing club

Titre : Développement d’emballages intelligents à base de matériaux sol-gel fluorescents permettant le suivi des composés carbonylés générés par l'oxydation des lipides.

Mots clés : gaspillage alimentaire, durée de vie, emballage intelligent, oxydation des lipides, matériau sol-gel, capteur fluorescent

Réduire le gaspillage alimentaire tout en garantissant la sécurité sanitaire des aliments est un défi majeur auquel fait face l'industrie agroalimentaire aujourd'hui. L'oxydation des lipides est le principal processus de dégradation chimique impliqué dans la dégradation des aliments. Outre la perte en qualité nutritionnelle, l'oxydation des lipides génère des composés malodorants qui contribuent au rejet des produits par les consommateurs. Elle entraîne également la production de composés nocifs pour la santé. Avec la seule information fournie par les dates de péremption, une quantité importante d'aliments est gaspillée au niveau du distributeur et du consommateur en raison des précautions excessives prises pour assurer la sécurité sanitaire. L'industrie agroalimentaire manque d'un outil rapide et facile à utiliser pour contrôler la fraîcheur des aliments en temps réel et tout au long de la durée de conservation des produits. Les matériaux intelligents, destinés à contrôler l'évolution des aliments emballés et à fournir des informations à l'utilisateur, offrent une solution de choix pour pallier ce manque. Cette thèse de doctorat porte sur le développement de matériaux fluorescents pour suivre l'oxydation des lipides. Plus précisément, la fluorescence des matériaux synthétisés a été rapidement atténuée (moins de 1 min) lors de l'interaction avec des composés carbonylés, une famille de molécules dont la concentration augmente lors de l'oxydation des lipides. Leur suivi en temps réel est rendu

possible par la corrélation entre la perte d'intensité de fluorescence du matériau et la concentration en carbonyles. Plusieurs stratégies de synthèse ont été testées. Des matériaux à base d'acrylates, synthétisés par polymérisation radicalaire, ont été comparés à des matériaux à base de silicates, obtenus par procédé sol-gel. La toxicité des matériaux produits a été systématiquement évaluée. Un matériau sol-gel, à base d'aniline, s'est avéré particulièrement prometteur et a donc été étudié plus en détail. Ses performances ont été validées en contact direct avec des carbonyles, en utilisant différentes phases liquides comme simulants alimentaires, ainsi qu'en contact indirect, en exposant le matériau à une phase gazeuse contenant des vapeurs de carbonyles. Le capteur fluorescent permet de couvrir une très large gamme de concentrations de carbonyles (de 0,5 mmol/L à 0,5 mol/L), ce qui rend possible le contrôle de l'état de fraîcheur d'un aliment tout au long de sa durée de vie. Enfin, ce nouveau capteur a été utilisé avec succès pour suivre l'oxydation accélérée d'une huile de poisson commerciale. Tous ces résultats démontrent la possibilité d'utiliser ce nouveau matériau sol-gel intelligent en remplacement de méthodes classiques pour la détermination rapide, simple et sensible du niveau d'oxydation des produits riches en lipides, avec peu de préparation d'échantillon et sans utiliser de réactifs toxiques. Ce nouveau capteur fluorescent est donc prometteur pour une large gamme d'applications.

Title: Development of intelligent packaging based on sol-gel fluorescent materials to monitor carbonyl compounds released by lipid oxidation.

Keywords: food waste, food shelf-life, intelligent packaging, lipid oxidation, sol-gel material, fluorescent sensor

Reducing food waste while ensuring food safety is a major challenge facing the agri-food industry today. Lipid oxidation is the main chemical degradation process involved during food spoilage. In addition to a loss of nutritional value, lipid oxidation generates unpleasant off-flavors contributing to the consumer rejection of food products. It also leads to the production of compounds harmful to human health. With the sole information provided by the expiration dates, a significant amount of food is wasted at the distributor and consumer levels because of excessive precautions taken to ensure food safety. The agri-food industry lacks a rapid and easy-to-use tool to control food freshness in real time throughout the entire shelf-life of the product. Intelligent materials, intended to monitor the evolution of packaged food and to provide information to the user, offer a perfect user-friendly solution. This PhD thesis reports on the development of fluorescent materials to track lipid oxidation. More specifically, the fluorescence of the designed materials was rapidly quenched (less than 1 min) upon interaction with carbonyl compounds, a family of molecules which concentration increases during lipid oxidation. Their real-time monitoring was made possible since the fluorescence loss correlates with the

fluorescence loss correlates with the concentration of carbonyls. Several synthesis strategies were tested. Acrylate-based materials, synthesized by radical polymerization, were compared to silicate-based materials, obtained by the sol-gel process. The toxicity of the so-produced materials was systematically assessed. A sol-gel material, based on aniline, was particularly promising and was thus more deeply investigated. Its performance was validated in direct contact with carbonyls, using different liquid phases as food simulant, as well as in indirect contact, by exposure of the material to a gas phase containing carbonyl vapors. The fluorescent sensor covered a very broad range of carbonyl concentrations (from 0.5 mmol/L to 0.5 mol/L), allowing to track the entire shelf-life of a food product. Finally, this new sensor was successfully applied to monitor the accelerated oxidation of a commercial fish oil. All of these results demonstrated the possibility of using this new intelligent sol-gel material instead of classical methods for the fast, simple, and sensitive determination of the lipid oxidation level of lipid-rich products, with little sample preparation and without use of any toxic reactant. This new fluorescent sensor appears thus promising for a wide range of applications.