UNIVERSITÉ DU QUÉBEC À MONTRÉAL

CARBOXYMÉTHYL AMIDON ET SON COMPLEXE AVEC DU CHITOSANE COMME EXCIPIENTS POUR DES FORMULATIONS PHARMACEUTIQUES

THÈSE

PRÉSENTÉE

COMME EXCIGENCE PARTIELLE

DU DOCTORAT EN BIOCHIMIE

PAR

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À mes très chers parents

AVANT-PROPOS

Cette thèse préparée comme exigence partielle du programme de Doctorat en Biochimie à l'Université du Québec à Montréal (UQAM) avait trois objectifs principaux : i) montrer l'importance des excipients multifonctionnels à base de biopolymères dans le transport et la libération de différents types d'agents actifs, ii) présenter et discuter de l'ensemble des travaux de recherche réalisés et iii) présenter une vue prospective de la préparation de différents types de formulations avec les excipients multifonctionnels utilisés dans nos travaux de recherche.

Le projet réalisé fait partie de l'ensemble des projets du laboratoire du professeur M. A. Mateescu, Laboratoire d'Enzymologie et des Polymères Biocompatibles, au département de chimie de l'UQAM. Ce projet vise à concevoir de nouveaux excipients à base de biopolymères pouvant être utilisés dans des matrices hydrophiles (comprimés) pour le transport et la libération contrôlée de différents types d'agents actifs.

Les matrices hydrophiles obtenues par compression directe d'un mélange d'un polymère, utilisé comme excipient, et d'une substance active, nommée aussi agent actif ou principe actif, sont les plus faciles à fabriquer au niveau industriel, les plus économiques et les plus préférées par les patients. Le polymère utilisé devrait pouvoir développer une couche d'hydrogel au contact d'un milieu aqueux, influençant ainsi la vitesse de libération de l'agent actif formulé et, par conséquence, l'efficacité thérapeutique du médicament. Le rôle de l'excipient est particulièrement crucial dans le cas de la formulation des protéines et de certains types de bactéries, surtout à cause des obstacles physiologiques que représentent l'estomac et l'intestin pour ces agents administrés par voie orale (dégradation des protéines par les enzymes, sensibilité des bactéries à l'acidité).

Dans le présent projet nous avons utilisé le carboxyméthyl amidon (CMA), le chitosane et le complexe polyélectrolyte CMA-chitosane pour formuler différents types d'agents actifs (des petites molécules, une protéine et un probiotique) dans des matrices hydrophiles. L'importance des travaux menés réside dans l'utilisation des polymères d'origine naturelle, la préparation d'un nouveau complexe polyélectrolyte, les simples méthodes de préparation des excipients et des formulations, la facilité de modifier les propriétés physico-chimiques des excipients, et la capacité des formulations à protéger l'agent actif formulé dans des conditions simulant la résidence gastrique. Le temps de libération a été contrôlé en fonction des modifications apportées au polymère, simulant ainsi l'acheminement de l'agent formulé aux différentes parties de l'intestin.

Cette thèse est divisée en deux grandes parties, la Partie Introductive (partie I) et la Partie Expérimentale (partie II).

La partie I est formée de quatre chapitres. Le premier chapitre présente des informations générales de la littérature sur la formulation, la libération et l'absorption des substances actives administrées par voie orale. L'accent a été mis en particulier sur la libération prolongée et sur les matrices hydrophiles. Le deuxième chapitre est une revue des travaux publiés sur l'utilisation du CMA dans des matrices hydrophiles à libération contrôlée, permettant de tracer l'historique de cet excipient et de montrer la nouveauté apportée spécifiquement par le présent projet et généralement par les projets réalisés au Laboratoire d'Enzymologie et des Polymères Biocompatibles à l'UQAM. Le troisième chapitre est une revue bibliographique sur le chitosane et sur les complexes polyélectrolytes du chitosane, ainsi que sur leurs utilisations dans la formulation pharmaceutique. La description du projet et les contributions à la recherche sont présentés au quatrième chapitre.

Bien que le travail expérimental ait été orienté vers l'utilisation de comprimés obtenus par compression directe de poudres, nous avons abordés dans la partie introductive d'autres types de formulations. Ceci visait à montrer que le CMA, le

chitosane et le complexe CMA-chitosane pourraient servir à la préparation des différents types de formulations et des formes galéniques.

La partie II intitulée « Partie Expérimentale » est formée de quatre chapitres présentés en articles et d'un chapitre de discussion. Les articles représentent la majorité des travaux de recherche réalisés. Ils sont consacrés en grande partie à l'étude des propriétés physico-chimiques du CMA, à la préparation et caractérisation du complexe CMA-chitosane, et à l'étude des cinétiques de libération des différents agents actifs (petites molécules, ovalbumine et *Lactobacillus rhamnosus*) formulés dans des matrices hydrophiles. Le chapitre « Discussion et Conclusions » à la fin de cette partie présente une synthèse de l'ensemble des résultats du projet.

Les « Annexes » contiennent un article sur la caractérisation des matrices par imagerie RMN, des contributions aux congrès et aux conférences, et deux articles publiés. La section « Bibliographie » contient toutes les références de la partie introductive (partie I), du chapitre de la présentation du projet et du chapitre « Discussion et Conclusions ».

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CHAS	Cross-linked high amylose starch
CMA	Carboxyméthyl amidon
CMS	Carboxymethyl starch
CPE	Complexe polyélectrolyte
50% CMS:50% chitosan	Mixture of 50% of CMS and 50% of chitosan
DC	Dry-coated
DDA	Degree of deacetylation
DRX	Diffraction de rayons-X
DS	Degree of substitution
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
HPMC	Hydroxypropylmethyl cellulose
NMR imaging	Nuclear magnetic resonance imaging
Ova	Ovalbumin
PEC	Polyelectrolyte complex
PR	Protonation ratio
$S_{control}$	Starch treated with NaOH, used as a control
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
S_g	Gelatinized starch
SGF	Simulated gastric fluid
SGF (Pe)	Simulated gastric fluid containing pepsin
SIF	Simulated intestinal fluid
SIF (Pan)	Simulated intestinal fluid containing pancreatin
$t_{90\%}$	Time (h) for the release of 90% of drug
TBST	Tris buffer solution containing Tween
TGA	Thermal gravimetric analysis
XRD	X-ray diffraction

RÉSUMÉ

Le recours à l'utilisation des polymères naturels ou d'origine naturelle dans le développement de différentes formes galéniques solides est très commun dans l'industrie pharmaceutique. Un de ces polymères est l'amidon, un polysaccharide biocompatible et abondant, qui provient de sources renouvelables. Dans le présent projet, le carboxyméthyl amidon (CMA), un des dérivés de l'amidon, a été testé *in vitro* comme excipient pour le transport de substances actives avec des comprimés obtenus par compression directe de poudres. L'objectif principal consistait à préparer des matrices qui permettent des libérations contrôlées simulant le transport des substances actives à différentes parties du tractus gastro-intestinal.

En utilisant l'acétaminophène comme substance active modèle, la première étude a montré que la vitesse et le mécanisme de libération à partir des matrices à base de CMA dépendent du degré de carboxyméthylation du CMA, du ratio de protonation (RP) des groupements carboxylates et du pH du milieu de dissolution.
La libération la plus lente dans un fluide intestinal simulé a été obtenue avec un CMA de DS 0,11. Avec des DS plus petits, les matrices n'ont pas développé un hydrogel suffisant pour contrôler la libération. Avec des DS plus élevés, les CMA sont devenus très solubles et ont favorisé l'érosion des matrices.
La protonation des excipients de CMA avant leur utilisation dans la formulation peut, selon les cas, avoir deux effets inverses sur les temps de libération à partir des matrices. Lorsque les groupements carboxyles du CMA sont libres (non-liés), ils interagissent avec les molécules d'eau du milieu de dissolution pour développer un hydrogel qui permet une libération plus lente que celle obtenue avec la forme sodium du CMA. Par contre, lorsque les groupements carboxyles du CMA sont liés via des liaisons d'hydrogène inter- et intra-chaîne, le taux d'hydratation du polymère et sa capacité à développer un hydrogel deviennent très faibles. Dans ce dernier cas, la diffusion du liquide à l'intérieur des matrices s'accélère et la libération de l'acétaminophène devient très rapide. Les interactions carboxyles-carboxyles ou carboxyles-hydroxyles au sein du polymère via des liaisons d'hydrogène augmentent avec l'augmentation du DS et du RP.

Afin d'assurer une libération prolongée à partir des matrices, les études subséquentes ont été consacrées à l'élaboration et à l'étude d'un excipient moins soluble que le CMA dans un fluide intestinal simulé et, par conséquence, moins sensible à l'action de l'α-amylase. Ainsi, un complexe polyélectrolyte (CPE) de CMA et de chitosane a été préparé par une simple coprécipitation des deux polymères. Les matrices à base de CPE ont permis des libérations plus lentes de l'acétaminophène, de l'aspirine et de l'ovalbumine que celles obtenues avec les matrices à base de CMA.

Ces matrices ont aussi permis une très bonne protection de l'ovalbumine formulée contre la dégradation par la pepsine. Le mélange physique CMA:chitosane contenant la même proportion de chitosane que celle du complexe n'a pas montré une amélioration en termes de protection et de temps de libération par rapport au CMA. Ceci a permis de constater que l'association du CMA et du chitosane au niveau moléculaire favorise plus d'interaction entre ces deux polymères que dans le cas du mélange physique. Cette conclusion a été confirmée par imagerie RMN en évaluant les vitesses de diffusion des milieux de dissolution dans des comprimés monolithiques non chargés et en mesurant les taux de gonflement de ces comprimés. Les propriétés de mucoadhésion des comprimés, la bonne protection de l'ovalbumine formulée contre la dégradation par la pepsine et les différents temps de libération assurés par les matrices sont importants pour le développement des formulations convenables pour des vaccinations par voie orale.

La pertinence et l'originalité du présent projet réside dans l'utilisation d'un excipient d'origine naturelle et économique, le CMA, et d'une simple méthode de formulation pour préparer des matrices pouvant assurer des libérations contrôlées et des différents temps de libération des substances actives. La mise au point d'un nouveau complexe polyélectrolyte CMA-chitosane a permis la préparation des matrices qui peuvent assurer des libérations prolongées des substances actives sans avoir recours à la réticulation des polymères avec des agents de réticulation.

Mots clés : carboxyméthyl amidon, chitosane, complexe polyélectrolyte, excipient, formulations pharmaceutiques, comprimés monolithiques, voie orale, libération contrôlée, libération prolongée.

PARTIE I – PARTIE INTRODUCTIVE

CHAPITRE I

FORMULATION, LIBÉRATION ET ABSORPTION DES SUBSTANCES ACTIVES ADMINISTRÉES PAR VOIE ORALE SOUS FORME GALÉNIQUE SOLIDE

L'amélioration de la thérapie médicamenteuse ne concerne pas seulement le développement de nouvelles entités chimiques, mais elle implique aussi une association convenable entre une substance active et un système de relargage. Dans ce sens, les caractéristiques physico-chimiques et les propriétés des excipients, ainsi que la formulation dans son ensemble contribuent à l'efficacité de la thérapie d'un médicament. La sélection de la voie d'administration et la conception d'une formulation appropriée pour une substance active nécessitent des expertises interdisplinaires dans différents domaines tels que la chimie, la biochimie et la pharmacie. L'administration des médicaments pourrait être intraveineuse, intraartérielle, intramusculaire, sous-cutanée, transdermique, vaginale, rectale, nasale, ophtalmique, sublinguale, orale etc. Cependant, l'admisnistration par voie orale des formes galéniques solides, particulièrement des comprimés et des gélules, reste la plus fréquemment utilisée. Nous nous intéressons dans ce chapitre à la formulation des substances actives, aux formes galéniques solides, aux modes de libération et à l'absorption intestinale. Particulièrement, l'utilisation des biopolymères dans des matrices et la libération prolongée seront présentées.

1. Les excipients

Les excipients représentent les produits utilisés dans la formulation pharmaceutique, autres que la substance active. Les excipients les plus utilisés dans

les formes galéniques solides sont des diluants (glucose, cellulose microcristalline, lactose, etc.), lubrifiants (chlorure de sodium, stéarate de magnésium, etc.), liants (dérivés de l'amidon, dérivé de la cellulose, gélatine, polyvinyl pyrrolidone, etc.), désintégrants (amidon, carboxyméthyl amidon réticulé, etc.), colorants (pigments naturels, oxyde de fer, etc.), agents antioxydants (acide gentisique, hydroquinone, etc.), agents gustatifs (manitol, aspartame, etc.) (US *Pharmacopeia*, 2000).
Les excipients sont utilisés dans les formulations dans le but d'assurer plusieurs fonctions, parmi lesquelles (US *Pharmacopeia*, 2000) :

- favoriser la stabilité de la substance active;
- moduler la dissolution et la biodisponibilité de la substance active;
- prévenir l'agrégation;
- maintenir un pH convenable.

En général, les excipients utilisés pour transporter une substance active sont des polymères synthétiques ou d'origine naturelle. Parmi les nombreux polymères synthétiques utilisés comme excipients, il y a l'alcool polyvinylique, l'acide polyacrylique, le polyéthylène, le polypropylène, etc. Les polymères d'origine naturelle les plus souvent utilisés sont la cellulose et ses dérivés, l'amidon et ses dérivés, le chitosane et ses dérivés, la gélatine, l'agar et d'autres.

2. La formulation des substances actives

Il existe différents processus pour formuler une substance active dans une forme galénique solide. Ces processus nécessitent généralement un simple mélange, une granulation (sèche, humide) ou un séchage (atomisation, lyophilisation) et aboutissent à une formulation finale sous forme de comprimés ou de gélules (Fig. 1.1) (Zhang *et al.*, 2004). La préparation des comprimés par compression directe reste la plus simple.

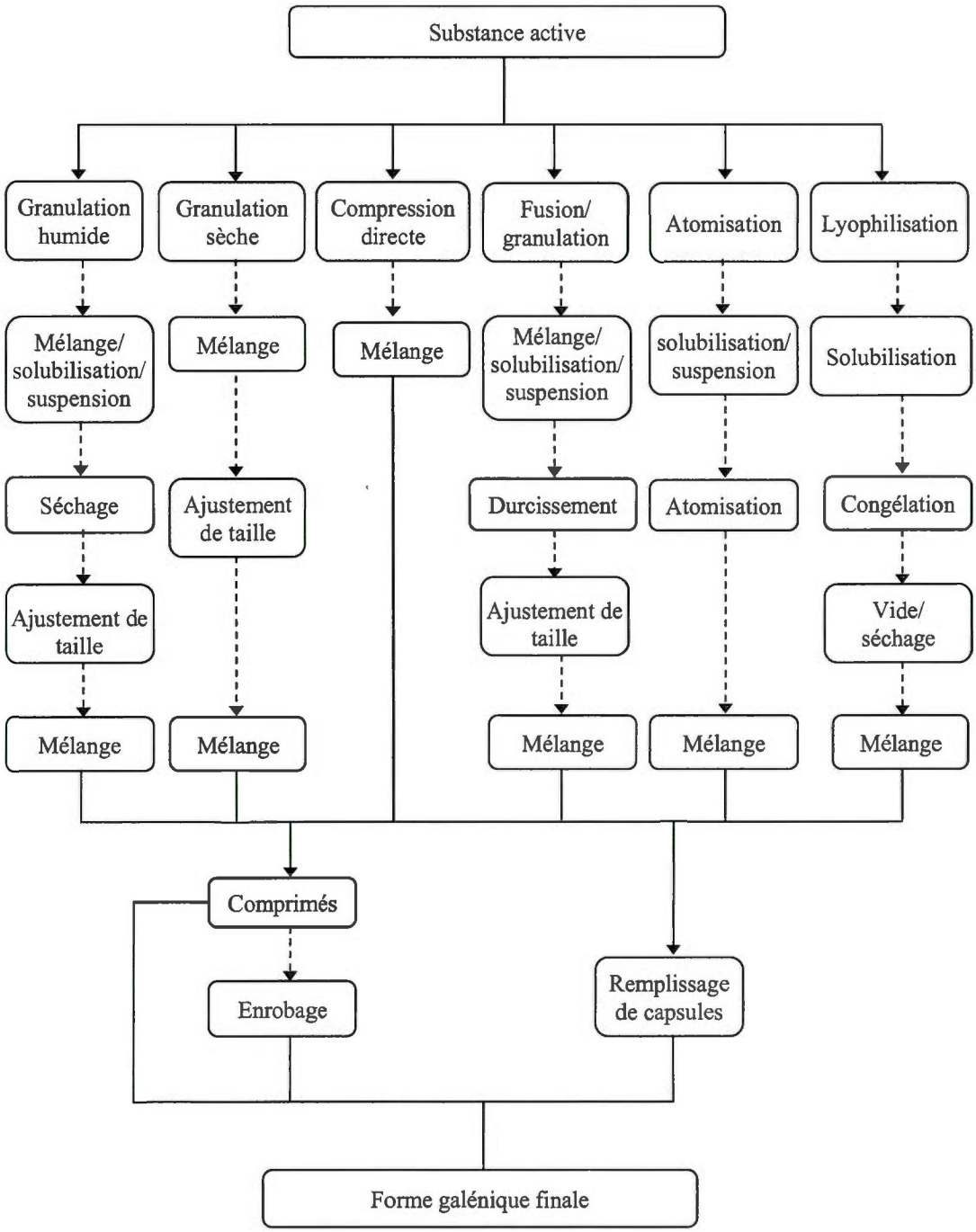

Figure 1.1 : Processus communs pour la préparation des formes galéniques solides. (Adapté d'après Zhang *et al.*, 2004).

La compression directe consiste en un simple mélange physique d'une substance active et d'un (des) excipient(s), suivi par une compression à une force déterminée pour donner des comprimés. La granulation consiste à mélanger une substance active avec un agent liant afin de former des granules. Dépendamment de la stabilité de la substance active et des excipients, la granulation peut être sèche ou humide. Les granules peuvent être utilisées ensuite dans la préparation des comprimés ou dans le remplissage des gélules. La lyophilisation consiste à sécher un échantillon congelé à une faible pression pour obtenir ensuite une poudre. L'atomisation consiste à pulvériser un échantillon (solution, suspension ou émulsion) en fines gouttelettes au contact d'un courant d'air/azote chaud afin d'évaporer le liquide. Cette technique permet l'obtention des particules homogènes qui seront ensuite compressées ou remplies dans des gélules (Zhang *et al.*, 2004).

Les comprimés

La compression directe est la méthode la plus économique pour préparer des comprimés monolithiques, mais elle ne peut pas être appliquée dans tous les cas (ex. dose très élevée, faible compressibilité des poudres, etc.). La compression peut être aussi effectuée après une granulation. Certains critères sont exigés pour les comprimés, tels que l'uniformité du contenu en substance active, la stabilité de la substance active, des propriétés organoléptiques accéptables, une dissolution et une biodisponibilité optimales de la substance active.

Les comprimés sont souvent enrobés dans le but de protéger les ingrédients de l'environnement externe, de modifier le profil de libération, d'améliorer les propriétés mécaniques, de masquer le goût, de faciliter l'administration, d'améliorer les qualités esthétiques, etc. L'enrobage peut être effectué par application d'un film de polymère, par encapsulation ou par d'autres méthodes (Zhang *et al.*, 2004).

Les matériaux les plus communément utilisés dans l'enrobage sont le polyéthylène glycol, l'acétate de cellulose, l'hydroxypropylméthyl cellulose, la méthyl cellulose, l'éthyl cellulose, la povidone, le glycérol, etc. (Chourasia et Jain, 2003).
Il existe aussi des comprimés à double noyau. Ce type de comprimé consiste à préparer un comprimé monolithique, puis à l'entourer par une couche d'excipients appropriés et de le compresser de nouveau. En général, cette forme de formulation est préparée dans le but d'assurer une libération retardée et une protection de la substance active contre l'acidité et la digestion dans l'estomac.

Les comprimés ont plusieurs avantages tels que une manipulation simple, une production en grande quantité, un prix abordable, des différentes méthodes de préparation, une préparation plus facile que celle des gélules, une charge précise en substance active, une administration facile, une plus grande stabilité microbiologique que les formes liquides.

Les gélules

Certaines substances actives ou excipients pourraient être altérés sous l'effet de la compression. À titre d'exemple, des substances comme la caféine, la sulfabenzamide et le chlorhydrate de maprotiline risquent de subir une transformation polymorphique durant la compression. Dans ce cas, l'utilisation des gélules peut être une alternative, puisque les effets thermiques et mécaniques sont moindres que dans le cas d'une compression (Chan et Doelker, 1985; Zhang *et al.*, 2004).

Les micro- et nanoparticules

Le développement des technologies de formulations novatrices a permis la préparation des nanoparticules et leur incorporation dans des formes galéniques solides (comprimés, granules ou microparticules) (Gelperina *et al.*, 2005). Une des approches physiques pour modifier les propriétés pharmacocinétiques et pharmacodynamiques d'une substance active est de la transformer en des micro- ou

nano-particules. Les particules peuvent être incorporées dans la forme galénique solide soit sous une forme solide soit sous une forme liquide. La suspension des particules peut être séchée (ex. atomisation ou lyophilisation) ou co-séchée avec un excipient, puis la poudre sera incorporée dans des billes, comprimés ou gélules. Alternativement, la dispersion aqueuse des particules colloïdales peut être incorporée directement dans la forme galénique solide en effectuant, par exemple, une granulation avec des excipients pour former des granules/comprimés. À la rigueur un enrobage avec un polymère pourrait être effectué (Schmidt et Bodmeier, 1999).

3. Le transport dans le tractus gastro-intestinal : effets des facteurs physiologiques

En pratique, le transport d'une forme galénique solide sous l'effet du mouvement du tractus gastro-intestinal la fait exposer à différents pH durant différentes périodes (Fig. 1.2 et tableau 1.1) (Kendall et Basit, 2006). En effet, la variation du pH la plus prononcée dans le corps humain a lieu au niveau du tractus gastro-intestinal. De plus, le pH dans une région donnée du tractus peut varier chez le même individu après le repas (tableau 1.2) (Hörter et Ressman, 1997).

Maintenir une vitesse constante de libération et d'absorption ainsi qu'un taux optimal d'une substance active dans le sang reste un objectif difficile à atteindre, car de nombreux paramètres peuvent interférer tout au long du transit gastro-intestinal. Parmi les facteurs physiologiques qui influencent la libération et l'absorption, et dont il faut tenir compte lors de la formulation, il y a (Brögmann et Beckert, 2001) :

- Le pH est le facteur physico-chimique et physiologique le plus important. En général, le pH ne change pas beaucoup avec l'âge, mais il pourrait changer sous l'effet de certaines pathologies qui affectent l'intestin. Par exemple, la colite ulcéreuse active entraîne une diminution du pH au niveau du gros intestin (Nugent *et al.*, 2001). En se basant sur la variation du pH dans le tractus gastro-intestinal, des films d'enrobages à base de polymères ont été développés pour différentes formes

galéniques. Ces films se solubilisent à un pH prédéterminé, permettant ainsi la libération de la substance active dans un segment spécifique. Il est recommandé de choisir un polymère qui ne se solubilise pas à pH inférieur à 5, afin d'assurer une protection gastrique de la substance active.

- La présence d'ions dans le milieu peut influencer la dissolution, puisqu'en général les matrices et les enrobages sont à base de polymères anioniques.
- Les aliments augmentent le pH et changent l'hydrophylicité du milieu gastrique. De plus, le temps de transit du médicament dans le tractus gastro-intestinal sera modifié, en général prolongé.
- La vidange gastrique est un facteur important à prendre en considération lors de la conception de l'enrobage entérique. Normalement, les comprimés enrobés gardent leur intégrité dans l'estomac et ils partent avec la vidange gastrique. Les granules avec un diamètre inférieur à 1,4 mm et une densité inférieure à 2,4-2,6 g/cm^3 passent vite à traves l'estomac. Une grande densité et un grand diamètre de la forme galénique pourraient retarder le transit.
- La protéolyse des peptides et des protéines commence dans l'estomac sous l'effet de la pepsine et elle continue dans l'intestin sous l'effet des enzymes pancréatiques telles que la trypsine et la chymotrypsine. En moyenne, 94 à 98 % de protéines totales sont complètement digérées et absorbées. Avec la tendance croissante de développer des médicaments à base de protéines (hormones, enzymes, antigènes), la prévention contre la protéolyse serait probablement une des principales raisons de l'enrobage entérique.

D'autres paramètres peuvent aussi avoir de l'influence sur l'action thérapeutique de la substance active, tels que la dégradation chimique, la complexation (en particulier avec les sels biliaires), les variations des surfaces d'absorption et la spécificité d'un site d'absorption.

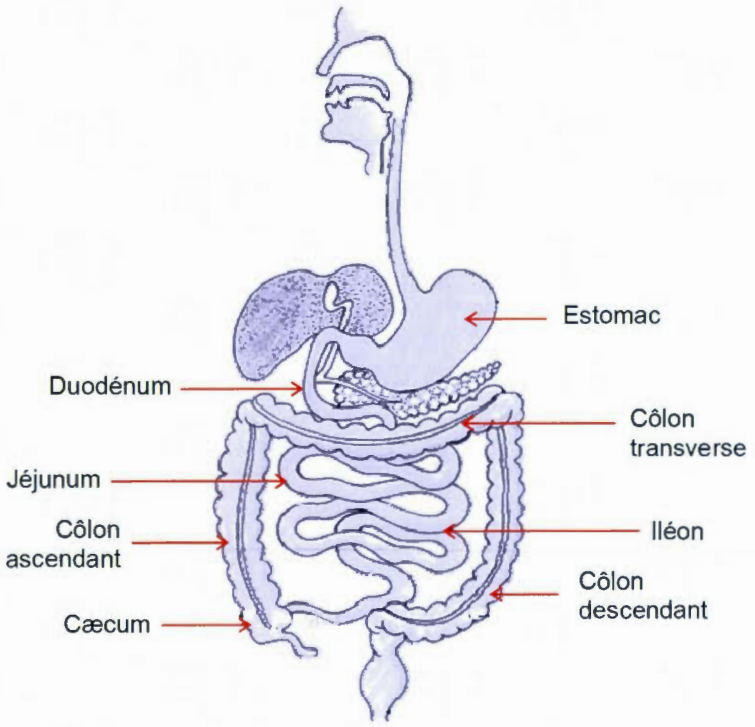

Figure 1.2 : Schéma du tractus gastro-intestinal chez l'Humain.

Tableau 1.1 : Physiologie du tractus gastro-intestinal chez l'Humain.
(Tiré de Kendall et Basit, 2006).

	Longueur (m)	**Superficie (m^2)**	**pH**	**Temps de résidence (h)**
Estomac	0,20	0,10	1,0-2,5	0-2 à jeûne Très variable après repas
Intestin grêle				3-5
Duodénum	0,25	1,9	5,5-6,0	
Jéjunum	2,80	184	6,0-7,0	
Iléon	4,20	276	7,0-7,5	
Gros intestin				20-30
Cæcum	0,20	0,05	6,4-7,0	
Côlon	1,50	0,25	7,0-7,5	

Tableau 1.2 : pH approximatifs dans l'estomac et dans le petit intestin chez l'Humain. (Tiré de Hörter et Dressman, 1997).

	pH (à jeûne)	pH (après repas)
Estomac	1,3	4,0
Duodénum	6,5	5,4
Jéjunum	6,6	5,2-6,0
Iléon	7,4	7,3

4. La libération des substances actives

4.1. La libération classique et la libération contrôlée

Après l'administration par voie orale d'une forme galénique solide, la substance active doit être libérée et absorbée pour avoir un effet thérapeutique. Ceci suit les étapes classiques du système LADMER (libération, absorption, distribution, métabolisme, excrétion, réponse thérapeutique) (Aiache *et al.*, 2007). Dans les formulations classiques (conventionnelles), la libération assez vite de la substance active permet d'obtenir une activité thérapeutique dans un délai court et ainsi un soulagement rapide du patient. Toutefois, cette libération pourrait créer un risque de toxicité lorsque la concentration de la substance active dans le sang dépasse le seuil maximal de tolérence (Bruck, 1983). D'un autre côté, une substance active n'a pas un effet thérapeutique lorsque sa concentration est inférieure au seuil minimal d'efficacité. D'où la nécessité de contrôler la concentration d'une substance active dans une fenêtre thérapeutique entre ces deux seuils (Fig. 1.3). Le recours à une libération contrôlée permet de garder une concentration convenable de la substance active dans le sang durant une durée déterminée, et ensuite de prolonger la durée de l'action thérapeutique.

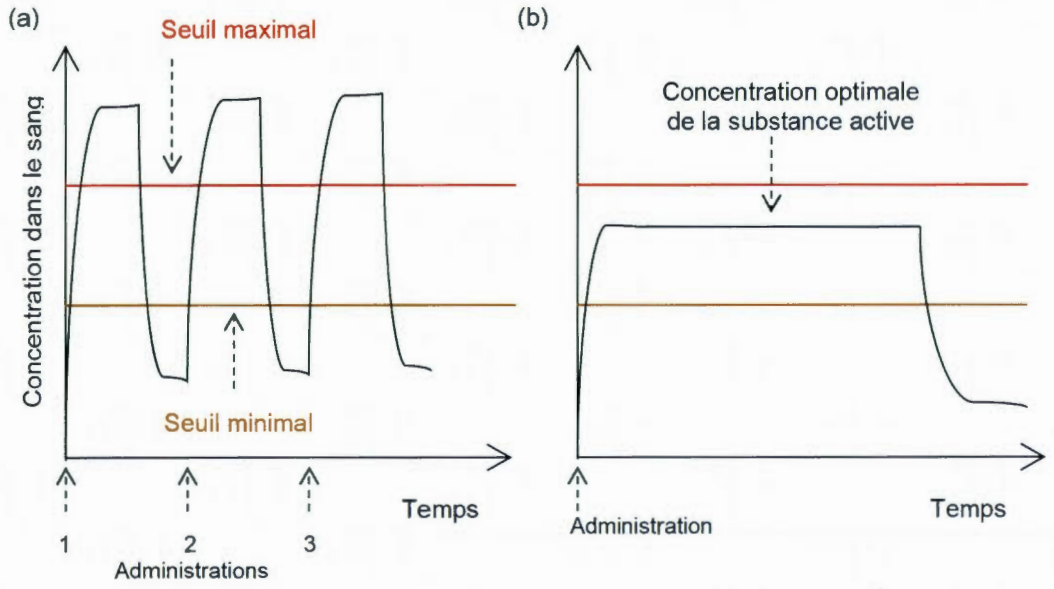

Figure 1.3 : Schéma représentant la variation de la concentration d'une substance active dans le sang suite à une administration d'une forme galénique par voie orale. Libération immédiate (conventionnelle) (a), libération contrôlée (prolongée ou ralentie) (b).

La libération contrôlée fait référence, entre autres, aux formes à libération prolongée et aux formes à libération retardée. En général, des matrices polymériques peuvent être utilisées pour modifier la libération. Ces matrices peuvent être enrobées avec des polymères sensibles au pH tels que des produits à base de cellulose (carboxyméthyléthyl cellulose, phtalate d'hydroxypropylméthyl cellulose, phtalate/acétate de cellulose, acétate/ succinate d'hydroxypropylméthyl cellulose), le phtalate/acétate de polyvinyle et les copolymères méthacryliques (Eudragit®) (Chourasia et Jain, 2003). La solubilité ou le gonflement de ces polymères permet la libération de la substance active en fonction du pH du milieu gastro-intestinal. Ce type de ciblage est très important pour traiter certaines maladies telles que la maladie du Crohn et la colite ulcéreuse affectant le côlon.

4.2. Les formes galéniques à libération prolongée

La libération prolongée par des formes galéniques destinées à l'administration par voie orale peut être principalement obtenue par division de la dose unitaire totale en fractions ou par rétention de la dose unitaire totale au sein d'un système (Aiache *et al.*, 2007).

4.2.1. Division de la dose unitaire totale en fractions

Les formes galéniques avec la dose divisée en fractions, appelées aussi formes à libération séquentielle, permettent la libération d'une dose initiale puis d'une ou de plusieurs doses à des intervalles de temps définis (Aiache *et al.*, 2007). Ainsi, la concentration de la substance active dans le sang reste dans la zone des concentrations efficaces. La libération est contrôlée soit par destruction de l'enrobage imperméable, soit par diffusion de la substance active à travers le film enrobant semi-perméable.

a. Enrobages imperméables destructibles

La dose de substance active est divisée en plusieurs fractions enrobées individuellement. La destruction de l'enrobage dans le tractus gastro-intestinal sous l'effet des différents facteurs (pH, activités enzymatiques, etc.) permet une libération modifiée de type «répétée» ou «retardée». Parmi les polymères entéro-solubles les plus utilisés, il y a l'acéthylphtalate de cellulose, les phtalates d'hydroxypropylméthyl cellulose, les copolymères de l'acide maléique, les copolymères méthacryliques (Eudragit®).

b. Enrobages semi-perméables

Les fractions de substance active sont enrobées en utilisant des techniques classiques, de microencapsulation ou de coacervation. La diffusion dépend de la

perméabilité du film d'enrobage et de sa porosité, ainsi que de la solubilité de la substance active. De nombreuses substances peuvent être utilisées pour préparer les films, telles que l'éthylcellulose, la gélatine, les copolymères méthacryliques (Eudragit®), les alcools gras, etc.

4.2.2. Rétention de la dose unitaire totale au sein d'un système

En générale, la totalité de la dose de substance active est retenue par une matrice sous forme d'un comprimé monolithique à libération prolongée (soutenue). Il existe plusieurs sortes de systèmes (Aiache *et al.*, 2007).

a. Matrices inertes

Ces types de matrices sont constitués d'un réseau poreux, solide, non digestible et insoluble dans le tractus gastro-intestinal. La libération a lieu par diffusion à travers les pores de la matrice et elle dépend de la nature de l'excipient, de la concentration et de la solubilité de la substance active, de la surface totale de la matrice, de la porosité du système, etc. Cette libération est peu influencée par le pH, la concentration ionique et la motilité intestinale. Ces matrices ne sont utilisables que pour formuler des substances actives qui présentent une solubilité relativement élevée et qui n'ont pas une fenêtre d'absorption définie. L'excipient le plus utilisé pour cette forme de formulation est l'éthylcellulose, en raison de sa facilité d'emploi (compression directe, granulation sèche, etc.). D'autres excipients sont aussi utilisés, comme le chlorure de polyvinyle, le polyéthylène, les copolymères acryliques, les copolymères d'acétate et de chlorure de polyvinyle.

b. Matrices lipidiques

Différents excipients lipidiques sont utilisés pour ce type de matrice, comme des mono-, di- et triglycérides, des acides et alcools gras, des cires, des esters divers, etc. La libération a lieu par deux mécanismes concomitants, à savoir la diffusion dans

le réseau poreux et l'érosion de la matrice par hydrolyse enzymatiques des glycérides ou par solubilisation lente des acides gras.

c. Matrices hydrophiles

Les matrices hydrophiles sont parmi les systèmes les plus utilisés pour le transport des substances actives, en raison de la simplicité de la formulation, de la production facile et économique, de la bonne corrélation *in vitro-in vivo* et de la possibilité de formuler des substances actives de masses moléculaires élevées (Huang et Brazel, 2001; Miranda, Millán et Caraballo, 2006; Maderuelo, Zarzuelo et Lanao, 2011). Elles sont constituées d'un mélange physique d'une substance active avec un excipient (macromolécule) capable de développer un hydrogel. L'excipient s'hydrate au premier contact avec le milieu de dissolution et forme une couche d'hydrogel qui constitue un obstacle à la libération rapide de la substance active.

De nombreux polymères sont utilisés comme excipients dans ces types de matrices, tels que des dérivés cellulosiques (méthyl cellulose, hydroxyéthyl cellulose, hydroxypropylméthyl cellulose (HPMC), carboxyméthyl cellulose, etc.), des polysaccharides non cellulosiques (amidon riche en amylose réticulé (Contramid®)), des gommes (gomme arabique, gomme de sterculia, gomme de guar, gomme de caroube), les alginates, l'agar-agar, le carraghénane, des polymères de l'acide acrylique comme le Carbopol®, etc. L'hydroxypropylméthyl cellulose a été et continue d'être parmi les polymères hydrophiles les plus utilisés dans les matrices hydrophiles (Rowe, Sheskey et Owen, 2006).

d. Type réservoir

L'enrobage de la substance active par un polymère plus ou moins perméable, permet d'assurer une vitesse de libération constante. Toutefois, avec ces systèmes de type réservoir il y a le risque de libérer d'un coup toute la substance active.

e. Association des matrices

Un exemple de ce type de matrice est l'association d'une matrice lipidique à une matrice hydrophile. L'avantage est que dans ce cas il se crée un véritable réseau possédant les caractéristiques des deux matrices.

f. Systèmes bioadhésifs

La bioadhésion est définie comme étant un phénomène interfacial entre le polymère bioadhésif et le mucus qui recouvre les tissus naturels. La bioadhésion permet d'immobiliser une forme galénique dans un site particulier et constitue ainsi une alternative récente au prolongement du temps de résidence dans une partie donnée du tractus gastro-intestinal. Dans ce type de formulation, les principaux polymères utilisés sont des polymères cellulosiques, acryliques (réticulés ou non) ou naturels (gommes, protéines de lait).

4.3. Les avantages et les inconvénients de la libération prolongée

Les formulations à libération prolongée, malgré qu'elles présentent plusieurs avantages par rapport aux formulations conventionnelles, ne peuvent être toujours utiles. Par exemple, une libération prolongée de la riboflavine est désavantageuse, puisque l'absorption de cette molécule s'effectue spécifiquement dans la première partie du petit intestin. De plus, une libération très lente limiterait la biodisponibilité des substances qui présentent une constante d'absorption intestinale faible (Aiache *et al.*, 2007). Voici quelques avantages et inconvénients des formes à libération prolongée (Brannon-Peppas, 1997; Aiache *et al.*, 2007).

Avantages

- Réduction du nombre d'administrations par jour avec une diminution du risque des erreurs dans l'application de la posologie et une meilleure complaisance chez le patient;

- Amélioration des conditions de traitement par suppression ou diminution dans les profils plasmatiques de la succession des pics et des vallées faisant suite à chaque dose administrée (Fig. 1.3 a);
- Diminution ou suppression des effets secondaires indésirables provoqués par de fortes concentrations des substances actives libérées rapidement;
- Le contrôle de la concentration de la substance active dans le sang permet d'éviter le risque d'une toxicité (Fig. 1.3 b);
- Le maintien des taux sanguins efficaces durant un temps prolongé pour des substances actives ayant une demi-vie relativement courte;
- Maximisation de l'utilisation de la substance active par rapport aux formulations conventionnelles;

Inconvénients

- Accumulation possible de la substance active si sa présence dans l'organisme est nécessaire 24 heures par jour et si sa vitesse d'élimination est lente;
- Le traitement est difficile à interrompre rapidement en cas d'intoxication grave ou d'intolérance.

4.4. Les mécanismes de libération à partir des matrices hydrophiles

L'hydratation des matrices hydrophiles au contact avec le milieu de dissolution entraîne une augmentation de la taille des molécules du polymère utilisé comme excipient, une relaxation des chaînes de ce polymère et la formation d'une couche d'hydrogel dans laquelle le polymère a un état caoutchouteux (Maderuelo, Zarzuelo et Lanao, 2011). Cette hydratation et cette gélification rapides du polymère empêchent les matrices hydrophiles incubées dans un milieu aqueux de se désintégrer et permettent une libération contrôlée de la substance active formulée.

L'épaisseur de la couche d'hydrogel augmente au fur et à mesure que l'eau pénètre dans la matrice. En même temps, les chaînes du polymère les plus exposées au milieu

de dissolution se relâchent graduellement jusqu'elles perdent leur consistance, entraînant ensuite une érosion de la matrice. Ainsi, la pénétration de l'eau dans la matrice est accompagnée par la formation d'une série des zones (Fig. 1.4) qui disparaissent ultérieurement durant le processus de dissolution (Colombo, Bettini et Peppas, 1999; Colombo *et al.*, 2000) :

- La zone du gonflement : cette zone sépare le polymère en état cristallin (vitreux) du polymère hydraté (état caoutchouteux).
- La zone d'érosion ou de dissolution : elle sépare l'hydrogel du milieu de dissolution.
- La zone de diffusion : elle se trouve entre la zone de gonflement et la zone d'érosion et elle sépare la partie de la matrice qui contient la substance active non encore solubilisée de l'hydrogel qui contient la substance active dissoute.

Une zone de pénétration (interface vitreuse sèche/vitreuse hydratée du polymère) a été récemment décrite par Ferrero et *al.* (2008), montrant que la concentration du liquide n'est jamais nulle au-delà de l'interface vitreuse/caoutchouteuse.

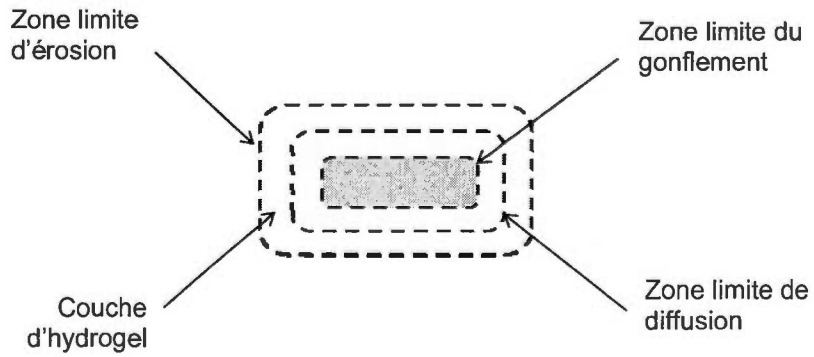

Figure 1.4. : Schéma d'une matrice hydrophile hydratée dans un milieu de dissolution. (Adaptée d'après Maderuelo, Zarzuelo et Lanao, 2011).

Plusieurs stades peuvent être identifiés durant la libération d'une substance active à partir d'une matrice hydrophile (Aiache *et al.*, 2007) :

- pénétration du milieu de dissolution dans le comprimé avec libération simultanée d'une faible quantité de la substance active se trouvant à la surface de la matrice (burst effect);
- formation d'une barrière d'hydrogel favorisée par l'absorption du liquide et par le gonflement de l'excipient gélifiant;
- diffusion progressive du liquide à l'intérieur du comprimé à travers la couche d'hydrogel et dissolution de la substance active;
- diffusion de la substance active à l'extérieur à travers la couche d'hydrogel;
- érosion de la matrice.

Une augmentation de la couche d'hydrogel, une diminution de la taille du cœur de la matrice et une augmentation du diamètre de la matrice en fonction du temps sont trois phénomènes observés durant l'hydratation de la matrice (Ranga Rao et Padmalatha Devi, 1988).

Dépendamment du processus qui contrôle la libération de la substance active à partir de la matrice, l'équation de Peppas (1) permet de distinguer quatre types de mécanismes de libération :

$$\frac{M_t}{M_\infty} = kt^n \qquad (1)$$

où M_t/M_∞ est la fraction de la substance active libérée au temps t. La constante cinétique (k) incorpore les caractéristiques géométriques de la forme galénique et les propriétés de la matrice et de la substance active. L'exposant (n) détermine le mécanisme de libération.

a) n = 0,5 implique une diffusion de type I ou « Fickian ». La libération est contrôlée par un mécanisme de diffusion.

b) $0,5 < n < 1$ correspond à une diffusion anormale ou « non-Fickian ». La libération dépend à la fois du gonflement de la matrice et du phénomène de diffusion et elle implique des cinétiques du premier-ordre.

c) $n = 1$ correspond à un transport du type II. La libération est contrôlée par le gonflement du polymère et elle implique des cinétiques d'ordre zéro (indépendants du temps).

d) $n > 1$ correspond à un transport de type « Supra II » ou « super case-II ». C'est le cas lorsque la matrice qui entre en contact avec le milieu de dissolution forme une couche complètement hydratée susceptible à une érosion continue à la surface (érosion/dégradation du polymère).

4.5. Les facteurs influençant la libération à partir des matrices hydrophiles

De nombreux facteurs peuvent influencer la vitesse et le mécanisme de libération d'une substance active à partir d'une matrice hydrophile. Ces facteurs concernent la substance active (solubilité, masse moléculaire, pourcentage dans la matrice (charge), taille des particules, etc.), le polymère (type de polymère, masse moléculaire, fonctionnalisation, viscosité, pourcentage dans la matrice, taille des particules, mélange des polymères, etc.), le milieu de dissolution (pH, force ionique, etc.) et la formulation (teneur en eau, processus de fabrication, caractéristiques des comprimés, etc.) (Maderuelo, Zarzuelo et Lanao, 2011).
Une brève description de l'influence du polymère utilisé comme excipient sur la libération de la substance active formulée est présentée ci-après.

Type de polymère

La structure et les caractéristiques physico-chimiques du polymère utilisé comme excipient jouent des rôles importants dans les mécanismes de libération. Le polymère est choisi en fonction des propriétés de la substance active à formuler et du mode de libération désiré. Les dérivés de cellulose, particulièrement le HPMC, sont

les polymères les plus utilisés dans la préparation des matrices. Le HPMC est un excipient hydrophile, non ionique, soluble dans l'eau, stable à des pH de 3 à 11 et résistant aux enzymes gastro-intestinales. La matrice de HPMC en contact avec l'eau développe un hydrogel qui joue un rôle essentiel dans le contrôle de la libération (Rowe, Sheskey et Owen, 2006).

Masse moléculaire

La masse moléculaire influence la vitesse de formation et l'épaisseur de la couche d'hydrogel qui contrôle le passage de l'eau. Une masse moléculaire élevée favorise le gonflement de la matrice et la formation d'un hydrogel plus consistant, réduisant ainsi l'érosion.

Fonctionnalisation

Le type de substituant, la longueur des chaînes latérales et le degré de substitution influencent le taux d'hydratation du polymère et la diffusion de la substance active dans le milieu de dissolution.

Par exemple, la substitution du chitosane avec des acides gras (C_6 à C_{16}) a augmenté l'hydrophobicité du polymère et a entraîné une modification importante de sa structure et de ces propriétés physico-chimiques (Le Tien *et al.*, 2003). Les chaînes les plus longues stabilisent mieux la matrice par des interactions hydrophobes et, par conséquence, prolongent le temps de libération de l'acétaminophène. Dans le cas du carboxyméthyl amidon (CMA), le degré de substitution (DS) devrait être dans un intervalle déterminé pour avoir une libération contrôlée de l'acétaminophène à partir des matrices. Un DS faible ne peut pas assurer une hydratation suffisante et homogène, tandis qu'un DS élevé entraîne une solubilisation rapide de la matrice et donc une libération accélérée (Lemieux, Gosselin et Mateescu, 2009; Assaad et Mateescu, 2010). Dans le cas du HPMC, l'hydratation augmente avec l'augmentation du nombre de groupements hydroxyles. Pour assurer une libération prolongée, le type et le degré de substitution devraient être choisis de façon qui permette la formation

d'une couche d'hydrogel consistante pouvant limiter la diffusion de la substance active dans le milieu de dissolution.

Viscosité

La viscosité est un des paramètres qui contrôlent la libération de la substance active et qui déterminent le mécanisme de libération. La viscosité d'un polymère en solution dépend de la structure chimique du polymère, de sa masse moléculaire et de ses interactions avec le solvant. Normalement, un polymère de masse moléculaire élevée engendre la formation d'un hydrogel de grande viscosité, permettant une plus grande résistance du polymère à l'érosion et une libération plus lente. Une augmentation de la viscosité du HPMC de 15 à 30 000 cP entraîne une diminution de la vitesse de libération de métronidazole, tant que le pourcentage du polymère dans la matrice ne dépasse pas le 20% (Campos-Aldrete et Villafuerte-Robles, 1997).

Pourcentage du polymère

En général, l'augmentation du pourcentage du polymère réduit la porosité de la matrice et, par conséquence, la vitesse de libération (Reza, Quadir et Haider, 2003). La variabilité des cinétiques de libération devient plus faible lorsque le pourcentage du polymère est élevé, puisque l'effet des autres facteurs sur la libération diminue. L'augmentation du pourcentage du mélange d'hydroxypropylméthyl cellulose (HPMC) et d'hydroxypropyl cellulose (HPC) dans la matrice de 3,5 % à 19,2 % a entraîné une diminution de la vitesse de libération de l'acétaminophène (Ebube et Jones, 2004). Ce même phénomène a été observé avec des matrices contenant 200 mg de chlorhydrate propranolol et différentes quantités d'éthyl cellulose (95, 140 et 285 mg), en mentionnant que le mécanisme de libération (exposant n) n'a pas été affecté. Contrairement, le changement de la quantité de HPMC (110, 150 et 200 mg) dans la matrice n'a pas modifié le profil de libération du chlorhydrate tramadol (charge de 200 mg) qui est très soluble dans l'eau (Tiwari *et al.*, 2003). Le profil de libération de l'acétaminophène à partir des matrices à base de CMA n'a pas changé en augmentant

le pourcentage du polymère de 60% à 80% (Assaad et Mateescu, 2010). Il semble qu'au-delà d'un certain pourcentage de polymère, l'hydratation de la matrice devient contrôlée et l'hydrogel formé devient complètement homogène et cohérent. Ce pourcentage représente un point critique au-delà duquel le polymère peut assurer des profils de libération assez similaires pour des substances actives solubles.

Taille des particules

La taille des particules est un facteur qui pourrait influencer la vitesse de libération, parce qu'elle influence l'entrée de l'eau dans la matrice. Les particules sont plus proches les unes des autres dans une matrice lorsque leur taille est petite, favorisant dans ce cas une formation rapide de l'hydrogel et une vitesse de libération plus lente que dans le cas des particules de grande taille.

Avec 10 % en HPMC dans la matrice et des moyennes des diamètres des particules de 163, 213, 335 et 505 μm, la vitesse de libération de métronidazole augmente avec l'augmentation des diamètres (Campos-Aldrete et Villafuerte-Robles, 1997). Par contre, avec des pourcentages plus élevés en HPMC (20% et 30%), les vitesses de libération n'ont pas été influencées par les diamètres des particules. Ces phénomènes sont explicables par le fait que les particules de plus grande taille capturent l'eau et gonflent plus lentement pour former l'hydrogel qui constitue une barrière contre la diffusion de la substance active formulée. De plus, la couche d'hydrogel est plus poreuse lorsque la taille des particules est grande. L'augmentation du pourcentage de HPMC dans la matrice réduit la porosité de l'hydrogel et diminue significativement l'effet de taille des particules. En conclusion, la taille des particules ne représente pas un facteur critique lorsque le pourcentage de polymère dans la matrice est suffisant pour former une couche d'hydrogel uniforme et consistante (Maderuelo, Zarzuelo et Lanao, 2011).

Mélange de polymères

L'utilisation de plus qu'un polymère dans la même formulation est une alternative commune pour moduler les profils de libération et pour assurer une libération prolongée (soutenue). Le mélange de polymère permet de contrôler la vitesse d'hydratation et d'érosion. Une des combinaisons la plus utilisée est celle du HPMC (soluble dans l'eau) avec de l'éthyl cellulose (insoluble dans l'eau) (Maderuelo, Zarzuelo et Lanao, 2011). Récemment, le mélange du CMA avec du chitosane a été proposé dans le but d'augmenter la protection des *Lactobacillus rhamnosus* contre l'acidité gastrique et pour prolonger le temps de libération (Calinescu et Mateescu, 2008).

5. L'absorption intestinale des substances actives

Pour avoir une action thérapeutique par un médicament administré sous forme galénique solide, la substance active doit : i) être solubilisée et libérée de la forme galénique, ii) franchir un certain nombre de barrières et iii) atteindre la circulation générale sans qu'elle soit métabolisée.

La libération de la substance active doit être plus lente que l'absorption intrinsèque. Dans ce cas, la vitesse de libération devient le facteur limitant qui contrôle l'arrivée de la substance active au site d'absorption. Le transit intestinal est un facteur important dans le processus d'absorption et la substance active qui atteint la circulation portale sera véhiculée jusqu'à son site d'action (Aiache *et al.*, 2007).

5.1. Les sécrétions enzymatiques présentes dans le tractus gastro-intestinal

Les enzymes présentes dans le tractus gastro-intestinal peuvent influencer le transport et l'absorption des substances actives. Ces enzymes proviennent des sécrétions gastro-intestinales et des sécrétions du pancréas (Vatier et Lamarque, 2007) :

- sécrétion gastrique : les cellules pariétales sont responsables de la sécrétion aqueuse de protons qui se combinent aux chlorures échangés contre de bicarbonates par une pompe échangeuse à proton. Ceci permet la formation du HCl qui est responsable du pH acide de l'estomac. Les cellules principales synthétisent la lipase et le pepsinogène. L'activation autocatalytique du pepsinogène, aboutissant à la formation de la pepsine active, débute à un pH d'environ 6 et devient complète à pH inférieur à 3,5. Les cellules mucipares synthétisent les mucines qui sont des glycoprotéines pouvant servir comme agents protecteurs de la muqueuse.
- sécrétion intestinale : l'entérokinase sécrétée au niveau du duodénum proximal permet l'activation du trypsinogène en trypsine. L'acidité gastrique qui entre dans le duodénum est neutralisée par la sécrétion de bicarbonate dans l'intestin. Dans l'intestin, il y a également une sécrétion électrolytique alcaline, une sécrétion de mucus et une sécrétion enzymatique (lactase, invertase).
- sécrétion pancréatique : les cellules acineuse secrètent plusieurs types d'enzymes qui sont véhiculées par la sécrétion de bicarbonate. Parmi ces enzymes, il y a celles à activité amylolytique (amylase), protéolytique (carboxypeptidase A et B, trypsine, chymotrypsine, lucine-amino peptidase), lipolytique (lipase) et nucléolytiques (ribonucléase, déoxyribonucléase). La neutralisation des ions H^+ qui commence avec la sécrétion alcaline duodénale continue avec la sécrétion exocrine du pancréas pour maintenir un pH intraduodénal entre 6,5 et 7. Ce pH est optimal pour les activités des enzymes sécrétées à ce niveau.

5.2. Le passage à travers l'épithélium intestinal

L'épithélium intestinal, avec une surface d'environ 500 m^2 chez l'Humain, fonctionne normalement comme une barrière sélective. Il permet l'absorption des éléments nutritifs, des électrolytes et de l'eau, mais il restreint le passage des grosses molécules et des composés potentiellement toxiques. Cette restriction empêche

l'entrée des protéines (ex. toxines) et des bactéries qui pourraient être responsables des infections systémiques (Usami *et al.*, 2003). L'épaisseur de la muqueuse n'est pas uniforme dans les différents segments du tractus gastro-intestinal. Elle est d'environ 50-500 µm au niveau de l'estomac, puis elle diminue pour atteindre 15-150 µm au niveau du côlon. La muqueuse la plus mince se trouve au niveau des plaques de Peyer (Norris et Sinko, 1997; Ponchel et Irache, 1998).

L'absorption dans le tractus gastro-intestinal dépend du type de l'épithélium. Par exemple, l'épithélium moins serré au niveau du petit intestin qu'au niveau du fundus gastrique et du côlon permet au petit intestin une plus grande perméabilité. Approximativement, 90 % de l'absorption totale s'effectuent au niveau du petit intestin (Balimane et Chong, 2005; Lee *et al.*, 2005).

Les forces positives qui règlent l'absorption intestinale consistent en le gradient de concentration, la différence du potentiel électrique et le gradient de la pression hydrostatique. Cependant, il y a des facteurs qui peuvent s'opposer à l'absorption, tels que la barrière physique et la barrière biochimique de la muqueuse.

La barrière physique est le résultat des jonctions serrées et de la composition lipidique des membranes cellulaires. La barrière biochimique est représentée principalement par la présence sur la muqueuse des enzymes qui peuvent métaboliser la substance active. Ces enzymes sont essentiellement des enzymes métaboliques des entérocytes, telles que les aminopeptidases, la dipeptidyl peptidase IV, le lysozyme, les estérases, le phénol sulfotransférase, etc. (Grassi *et al.*, 2007).

Pour qu'une substance active soit absorbée par l'intestin, elle doit traverser l'épithélium intestinal formé d'une couche unicellulaire d'entérocytes et des cellules à mucus. Ces cellules sont liées les unes aux autres du côté luminal de l'intestin pour former des jonctions serrées. Ceci crée une barrière continue entre la surface luminale de l'intestin et l'espace intercellulaire (Grassi *et al.*, 2007).

Le passage à travers l'épithélium intestinal a lieu par différents modes de transport : transport transcellulaire passif, transport paracellulaire passif, transport transcellulaire médié par des transporteurs et transport par des vésicules, (Fig. 1.5).

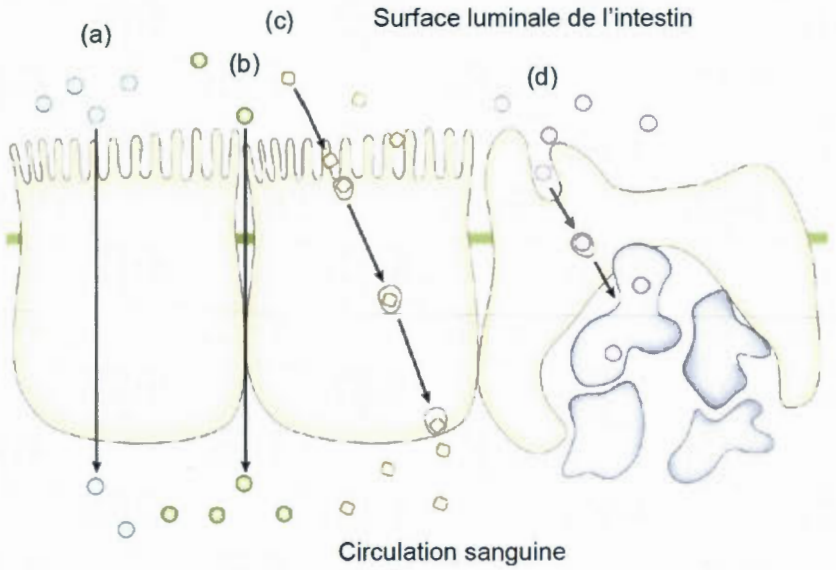

Figure 1.5 : Schéma descriptif de l'épithélium intestinal et des voies d'absorption d'une substance active : (a) voie transcellulaire, (b) voie paracellulaire, (c) endocytose médié par des récepteurs, (d) absorption via des cellules membraneuses de plaques de Peyer. (Adaptée d'après Goldberg et Gomez-Orellana, 2003).

Transport transcellulaire passif

C'est le mode de transport principal pour l'absorption des médicaments. Les molécules solubles passent à travers la membrane apicale et le cytoplasme pour atteindre la membrane basolatérale. Le transport se fait selon un gradient de concentration et il dépend des propriétés physico-chimiques de la molécule, notamment de sa solubilité dans les membranes lipidiques. La surface qui permet le transport transcellulaire (ex. membrane cellulaire) est beaucoup plus large (> 99 %) que celle responsable du transport paracellulaire (jonctions serrées). Ceci suggère que

les composés capables de traverser la membrane cellulaire ont une absorption plus grande que ceux dont la perméabilité est restreinte au transport paracellulaire. Toutefois, la présence de transporteurs peut affecter les taux de ces absorptions.

Le transport paracellulaire passif

En général, les composés hydrophiles sont majoritairement transportés via la voie paracellulaire qui est une voie extracellulaire. La diffusion est réglée par des gradients de potentiels électrochimiques provenant des différences de concentration, par de potentiel électrique et par une pression hydrostatique des deux côtés de l'épithélium. Les jonctions serrées sont les principales barrières à ce mode de transport.

Chez l'Humain, le petit diamètre des pores des jonctions serrées, entre 4 et 8 Å, limite le passage des agents actifs à travers ces jonctions. De plus, la très petite surface des jonctions par rapport à la surface totale de l'intestin contribue à la limitation du transport. Pour favoriser l'abosption des peptides, qui à cause de leurs tailles ne peuvent pas traverser les jonctions serrées, certains agents comme l'aprotinin et la bacitracin peuvent être utilisés pour augmenter les diamètres des jonctions (Fix, 1996). L'action de l'agent doit être rapidement réversible, car une altération significative de la fonction de la barrière gastro-intestinale ouvrirait une fenêtre pour une absorption non spécifique des toxines et d'autres composants qui pourraient entraîner des effets secondaires indésirables.

Transport par l'intermédiaire des transporteurs

Le petit intestin possède un grand nombre de transporteurs qui sont responsables de l'absorption des nutriments, des vitamines et des substances actives des formulations pharmaceutiques. Des transporteurs des tripeptides, des acides aminés, des nucléosides, des acides monocarboxyliques, des acides biliaires, des hormones thyroïdiennes, des oligopeptides, des monosaccharides, des vitamines, etc. ont été déjà identifiés (Akira et Tamai, 1996; Grassi *et al.*, 2007). Toutefois, l'impact

de ces transporteurs sur l'absorption des substances actives reste à déterminer. Les transporteurs peuvent lier des composés solubilisés dans le fluide intestinal et les transférer de l'autre côté de la membrane apicale des entérocytes, facilitant ainsi l'absorption de la substance active. Les composés qui sont des substrats pour ces transporteurs montrent une absorption intestinale plus élevée que ce qui pourrait être obtenu par une simple diffusion à travers la membrane cellulaire.

Transport par des vésicules

Ce mode de transport facilite l'absorption des substances actives des formulations pharmaceutiques. Ceci inclut l'endocytose en phase fluide et l'endocytose médié par des récepteurs. Dans l'endocytose en phase fluide, les molécules solubles dans le fluide luminal sont incorporées dans le fluide des vésicules. Ce processus commence lorsque la membrane plasmique s'invagine pour former une vésicule intracellulaire. Les molécules se trouvant dans les vésicules sont ensuite transportées aux endosomes. Ce processus permet l'absorption de certains peptides et protéines. L'endocytose médié par des récepteurs implique la liaison des macromolécules sur des récepteurs de la membrane, suivie par la formation des vésicules.

5.3. Cas des peptides et des protéines administrés par voie orale

L'action thérapeutique des peptides et des protéines administrés par voie orale est affectée par plusieurs paramètres (Bravo-Osuna, Vauthier et Ponchel, 2007). La masse moléculaire élevée et le caractère hydrophile de ces molécules réduisent grandement leur absorption par l'épithélium. De plus, les conditions non favorables (pH et enzymes) dans le tractus gastro-intestinal entraînent la dégradation des peptides et des protéines.

Afin de mieux comprendre les défis du développement des formulations convenables au transport des peptides et des protéines thérapeutiques administrés par voie orale, nous présentons les principaux obstacles physiologiques et biochimiques auxquels font face ces produits ainsi que certaines stratégies qui permettent de faciliter leur absorption (Bravo-Osuna, Vauthier et Ponchel, 2007).

5.3.1. Obstacles physiologiques et alternatives

Autre que la couche de muqueuse, la barrière principale pour l'absorption d'une substance active est l'épithélium intestinal. Puisque la taille et l'hydrophilicité des peptides et des protéines empêchent leur transport transcellulaire, leur absorption est en grande partie limitée au transport paracellulaire à travers une surface qui représente une petite portion de la surface totale de l'intestin (Tenhoor et Dressman, 1992).

Une des approches pour surmonter la restriction du transport paracellulaire de ces substances est de les coadministrer avec des agents qui favorisent l'absorption en agissant sur l'intégrité des jonctions serrées par des interactions physico-chimiques. L'agent idéal doit être non toxique et il doit agir d'une façon réversible sur les jonctions en les maintenant ouvertes juste durant l'absorption. Par exemple, les formulations mucoadhésives sont faites pour adhérer à la muqueuse, permettant ainsi une transition plus lente de la forme galénique et un temps de contact plus long avec les sites d'absorption. Lorsque la forme galénique est engloutie dans la mucine, le gradient de concentration de la substance active favorise son absorption (Huang *et al.*, 2000; Martinac *et al.*, 2005; Morishita et Peppas, 2006).

Plusieurs polymères cationiques ou anioniques ont été suggérés comme agents qui favorisent l'absorption, même si le mécanisme d'action de ces polymères n'est pas encore complètement élucidé (Borchard *et al.*, 1996; Kriwet et Kissel 1996; Jung *et al.*, 2000). Le mécanisme proposé pour les polymères cationiques (ex. chitosane,

polylysine, etc.) est relié à leur capacité d'interagir avec les protéines des jonctions serrées. Ces interactions permettent la réorganisation des jonctions et ensuite leur ouverture (Ewan *et al.*, 1993; Ranaedi *et al.*, 2002).

5.3.2. Obstacles enzymatiques et alternatives

Le rôle physiologique des protéases gastro-intestinales est de dégrader les protéines en di- et tripeptides et en acides aminés assimilables par le petit intestin. Évidemment, ces protéases représentent un risque sur l'activité biologique des peptides et des protéines thérapeutiques administrés par voie orale.
Selon leur localisation, les protéases intestinales peuvent être divisées en trois groupes principaux : luminales, liées à la membrane et intracellulaires (cytosoliques et lysosomales) (Lueβen *et al.*, 1996; Langguth *et al.*, 1997).

Enzymes luminales

Dans le tractus gastro-intestinal, la première enzyme impliquée dans la dégradation est la pepsine. Les enzymes pancréatiques (trypsine, chymotrypsine, élastase, et carboxypeptidases A et B) sont très actives dans le petit intestin et représentent elles aussi un grand facteur de risque pour les peptides et les protéines (Lueβen *et al.*, 1996).

Enzymes membranaires et intracellulaires

Les enzymes membranaires et intracellulaires sont présentes dans tout le tissu mucosal. Les peptidases liées à la membrane jouent un grand rôle dans la dégradation enzymatique des peptides (Quan *et al.*, 1999; Ameye *et al.*, 2000). Parmi ces peptidases, il y a les aminopeptidases A et N, les amino-oligopeptidases, et la dipeptidyl aminopeptidase IV (Tenhoor et Dressman, 1992). Les peptides absorbés par voie paracellulaire ne sont pas affectés par les protéases intracellulaires.

Il est important de mentionner aussi l'activité métabolique de la microflore microbienne dans le côlon. La microflore est formée de plus de 500 espèces et elle est capable de nombreuses réactions métaboliques qui peuvent influencer la stabilité des peptides (Pinto Reis *et al.*, 2006).

Les stratégies les plus importantes qui ont été adoptées pour surmonter la barrière enzymatique sont les modifications structurales des peptides formulés pour prévenir les attaques protéolytiques, l'utilisation des inhibiteurs de protéases, l'utilisation des systèmes bioadhésifs et l'élaboration de nouveaux systèmes de transport capables de cibler un segment spécifique dans l'intestin où l'activité protéolytique est relativement faible (typiquement le côlon).

Une autre approche pour favoriser l'administration des peptides et des protéines par voie orale est l'utilisation des microparticules ou des nanoparticules. Les nanoparticules pourraient être absorbées non seulement via les cellules membraneuses de la plaque de Peyer, mais aussi via les entérocytes qui représentent la majorité des cellules de l'épithélium intestinal. Ces systèmes de transport peuvent être utilisés aussi pour la vaccination par voie orale qui présente plusieurs avantages par rapport à la vaccination par voie parentérale (par injection). Parmi ces avantages, il y a le fait que la vaccination par voie orale :

- exige des coûts de fabrication plus économiques que ceux des vaccins injectables qui nécessitent une pureté très élevée et des conditions stériles durant la fabrication et la conservation.
- se fait par administration non invasive qui ne nécessite pas l'utilisation d'aiguilles;
- permet d'éviter les problèmes des infections transmissibles par le sang;
- réduit les effets secondaires;
- ne nécessite pas de personnels spécialisés.

CHAPITRE II

LE CARBOXYMÉTHYL AMIDON COMME EXCIPIENT POUR LE TRANSPORT DES SUBSTANCES ACTIVES PAR DES FORMES GALÉNIQUES SOLIDES ADMINISTRÉES PAR VOIE ORALE - UNE REVUE

Résumé

Le carboxyméthyl amidon riche en amylose (CMA) utilisé comme excipient pour le transport des agents actifs par des comprimés administrés par voie orale a été le sujet de nombreuses études durant la dernière décennie. Ces études ont montré que les matrices à base de CMA ont le potentiel d'assurer une protection et une libération contrôlée des petites molécules, des enzymes, des protéines et des microorganismes formulés. Cette revue présente les influences des caractéristiques du CMA (degré de substitution, degré de protonation, etc.) et des paramètres expérimentaux (pH du milieu de dissolution, présence de l'α-amylase, type de la substance active, charge en substance active, etc.) sur les mécanismes et les cinétiques de libération des agents actifs à partir des comprimés.

Mots clés : carboxyméthyl amidon, amidon de maïs riche en amylose, excipient, matrice hydrophile, comprimé, transport des agents actifs, libération contrôlée.

The carboxymethyl starch as excipient for oral drug delivery by solid dosage forms - A review

Abstract

The carboxymethyl high-amylose corn starch (CMS) used as drug carrier for oral administration has been the subject of several studies in the last decade. These studies showed that CMS used as excipient have a good potential to afford protection and controlled release of small molecules, enzymes, proteins and microorganisms formulated as tablet dosage forms. The influences of CMS characteristics (degree of substitution, protonation degree, etc.) and the effects of experimental parameters (pH of dissolution medium, presence of α-amylase, drug type, drug loading, etc.) on drug release mechanisms and on release kinetics from tablets forms are discussed.

Keywords: Carboxymethyl starch, high-amylose corn starch, excipient, hydrophilic matrix, tablet, drug carrier, controlled release.

1. Introduction

The oral route of drug administration is preferred by patients, principally because it is simple and painless, and by pharmaceutical, since the manufacturing process for oral drug delivery systems is easier to conduct and less expensive when compared to that of various parenteral systems. In fact, the monolithic tablet forms obtained by direct compression are considered the most economical at industrial production level. Generally, the tablets are based on polymeric materials as excipients, preferentially polysaccharides. These polysaccharides used as carriers for active ingredients should be pharmaceutically and pharmacologically inactive substances.

The most abundant polysaccharide storage in plants and one of the main natural polymers is the starch. It is a low-cost, renewable and biodegradable polymer. Generally, starch consists in two macromolecular components: the amylose and the amylopectin. The amylose has essentially a non-ramified structure with glucose units joined by α-1,4 linkages. The amylopectin has a branched-chain structure, where the chain is constituted in sequences (α-1,4 linkages) of glucose units joined at branching points by α-1,6 linkages. The molecular weight of amylopectin is about 100 times higher than that of amylose and the ratio of amylose/amylopectin depends upon the source, the age and the extraction process of the starch (Liu et al., 2009). Waxy starch consists of only amylopectin molecules, whereas normal maize starch consists of 25% amylose and 75% amylopectin. The amylose content was increased from 25% to 65% by genetic modifications of maize (Schwartz and Whistler, 2009). The commercially common high-amylose starches present approximately 55% to 70% amylose content.

Starch can be chemically, physically or enzymatically modified. Certain modifications can improve the functional and physicochemical properties of starch intended to industrial applications. Physical modifications can lead to gelatinized

starch, while chemical modifications can lead to various starch derivatives depending on the used reagent.

Among the starch derivatives, the anionic carboxymethyl starch (CMS) has extended applications, such as in pharmaceutical industries. It is used as disintegrant under trade names such as Primojel®, Explotab® and Vivastar® which are cross-linked sodium starch glycolate prepared from potato starch (Edge et al., 2002). Later, the CMS prepared form corn starch was proposed as an excipient for controlled drug delivery in cross-linked form (Mulhbacher et al., 2001) and uncross-linked form (Mulhbacher et al., 2004). Recently, several studies showed multifunctional properties of CMS as an efficient drug carrier for small molecules (Brouillet et al., 2008; Lemieux et al., 2009; Assaad and Mateescu, 2010), proteins (Calinescu et al., 2007; Assaad et al., 2011a) or microorganisms (Calinescu and Mateescu, 2008). The influences of different parameters on the drug release rates and release mechanisms were also widely investigated.

In this review, only the uncrosslinked form of the carboxymethyl starch prepared from corn starch is considered. If not specified, CMS refers to uncrosslinked sodium form of carboxymethyl corn starch.

2. Gelatinization of starch

Gelatinization is a general term which includes several events occurring during starch processing, without precisely defining the conditions of the treatment. Starch gelatinization generally occurs in an excess of water and can be defined as the disruption of molecular orders by breaking hydrogen bonds within the granule (Blanshard, 1987).

When insoluble starch granules are heated in water excess, a number of phenomena take place: diffusion of water inside the starch granule with limited swelling, changes in the extent of molecular entanglements and rearrangements in the granules, phase transitions, double helix unwinding, partial demixing of amylose and amylopectin,

loss of birefringence and disruption of semicrystalline structure, amylose leaching from granules, partial fragmentation of the swollen granules, starch solubilization and increasing of viscosity (Douzals et al., 1996). Different methods have been used to investigate the advancement of starch gelatinization, such as: light microscopy (Biliaderis et al., 1986), viscometry (Okechukwu and Rao, 1996), ^{1}H-NMR (Lelievre and Mitchell, 1975) and calorimetric analysis (Burt and Russell, 1983).

Gelatinization may occur in alkali medium which favors the swelling of starch granules. Moreover, gelatinization can be carried out in organic solvents, with the advantage that the granular structure and physical properties of starch granules may be preserved (Tijsen et al., 2001b). Gelatinization of starch depends on many experimental parameters such as: temperature, presence of alkali and the ratio of aqueous/organic in the solvent. These parameters determine the advancement of gelatinization and then whether the starch is partially or completely gelatinized.

Due to the irreversible disruption of the ordered structures, gelatinized starch absorbed water markedly faster than the native starch. This causes considerable changes in the rheological properties of starch and has a major influence on the behavior and functionality of starch-containing systems. Gelatinization is generally a prerequisite for starch utilization, especially because most starch applications require dispersion in water and partial or complete solubilization.

3. Preparation of carboxymethyl starch

The preparation of carboxymethyl starch (CMS) was described by Chowdhury in 1924 (Sloan et al., 1962). The reaction was performed in aqueous medium with chloroacetic acid or sodium chloroacetate in the presence of sodium hydroxide. The degree of substitution (DS) of CMS is defined as the average number of carboxymethyl groups per glucose unit. Since each glucose unit has one primary and two secondary hydroxyl groups, the DS should be between zero and three (Tijsen et

al., 2001a). In 1952, Filbert proposed preparation of CMS in medium containing ethanol to prevent solubilization (Sloan et al., 1962). By using organic solvent, the granular structure could be preserved up to high degree of substitution (Tijsen et al., 2001b).

The CMS is prepared by starch etherification via an SN_2 reaction according to Williamson ether synthesis. First, the starch (ROH) is treated with a base, generally NaOH, to obtain starch alkoxide (Volkert et al., 2004):

$$ROH + NaOH \longrightarrow RO^{-}(Na^{+}) + H_2O$$

where, R is the starch backbone.

This starch alkoxide subsequently reacts with sodium chloroacetate to form CMS:

$$RO^{-}(Na^{+}) + ClCH_2COONa \longrightarrow ROCH_2COO^{-}(Na^{+}) + NaCl$$

Side reactions can occur, such as the reaction of NaOH with the sodium chloroacetate, producing sodium glycolate:

$$ClCH_2COONa + NaOH \longrightarrow HOCH_2COONa + NaCl$$

Reaction of sodium chloroacetate with water and reaction of sodium glycolate with itself or with sodium chloroacetate to give sodium diglycolate can also occur (Tijsen et al., 2001a).

When chloroacetic acid is used for the substitution, the reaction pH may be reduced and then a pH adjustment should be necessary.

After neutralization of the reaction medium, the CMS obtained in salt form should be washed to eliminate residual salts and then air or spray dried. Protonated form can be

obtained by solubilization/dispersing CMS in water or in organic medium at acidic pH (Stojanovic et al., 2005; Assaad and Mateescu, 2010).

Several studies have been undertaken to evaluate the yield and the efficiency of the carboxymethylation under the influence of starch/solvent ratio, kind of solvent, sodium hydroxide concentration, amount of sodium chloroacetate, temperature, and time of the reaction (Hebeish, Khalil, 1988). Triple reaction sequence was proposed to obtain high carboxymethyl substitution, up to DS 2.2 (Tijsen et al., 2001a). Moreover, a continuous process of CMS production was described by Tijsen et al. (2001b). Recently, a reproducible reaction was carried out at 30 °C to obtain 100% efficiency for carboxymethylation by using a multifunctional catalyst (undisclosed) to accelerate the reaction (El-Sheikh, 2010).

4. Characterization of carboxymethyl starch

The characteristics of CMS influence the properties of dosage forms and then the rate and the mechanism of drug release. We summarize herein some aspects of the characterization of CMS and of matrices based on CMS.

Characterization of excipient

The fuctionalization of starch and the presence of carboxylate (–COONa) and/or carboxyl groups (–COOH) in CMS can be confirmed by FTIR spectra.
One of the main features of CMS is the amout of functional groups (carboxymethyl groups). This parameter can influence the properties of the matrix when the CMS is used as excipient and can be expressed as (i) the mequivalents of –COONa/g of CMS, (ii) the percentage of –COONa groups to the initial –OH groups in starch, or (iii) the average number of carboxymethyl groups per glucose unit, known and used in this paper as the degree of substitution (DS).

The degree of functionalization can be determined by: direct titration (pH-meter or potentiometer), back-titration, Cu (II) salt precipitation (Stojanovic et al., 2005), atomic absorption, inductively coupled plasma (Volkert et al., 2004; Tüting et al., 2004), NMR, HPLC or capillary electrophoresis (Heinze et al., 1999; Lazik et al., 2002). The most common methods for the determination of the substitution pattern are ^{1}H-NMR spectroscopy and ^{13}C-NMR spectroscopy which provide information on the substitutions at the 2-, 3- and 6-positions of the repeating unit (Heinze et al., 1999). The back-titration, first applied for determination of the DS of carboxymethyl cellulose in 1947, is still the most accurate and economic method (Stojanovic et al., 2005).

To determine the morphology and the granulometry of CMS, the scanning electron microscopy technique has been used. No noticeable difference was found between sodium and protonated forms of CMS, whereas the DS can have a major influence (Lemieux et al., 2009). The size of particles increased with the substitution from DS 0.09 to DS 1.74. The CMS particles were partially agglomerated at DS 0.89 and were fully agglomerated at DS 1.23 and DS 1.74. This should be due to the enhancement of CMS solubility when the DS increase.

The X-ray spectra (Fig. 2.1) showed B-type pattern for Hylon VII, with the strongest peak at 5.2 Å, indicating the presence of double-stranded helix (Lemieux et al., 2010; Assaad and Mateescu 2010). After starch gelatinization in alkali medium, the structure became more amorphous with a reduction in the intensity of peak at 5.2 Å. After carboxymethylation, the peak at 5.2 Å disappeared and a new V-type organization appeared with the two characteristic peaks at 4.5 and 6.9 Å. The change of structure from double to single helices after carboxymethylation suggests that carboxymethyl groups enhance the starch granules swelling. The drying process (lyophilization, washing with acetone or spray-drying) did not influence the peaks positions but influenced their relative intensities due to the differences in water content.

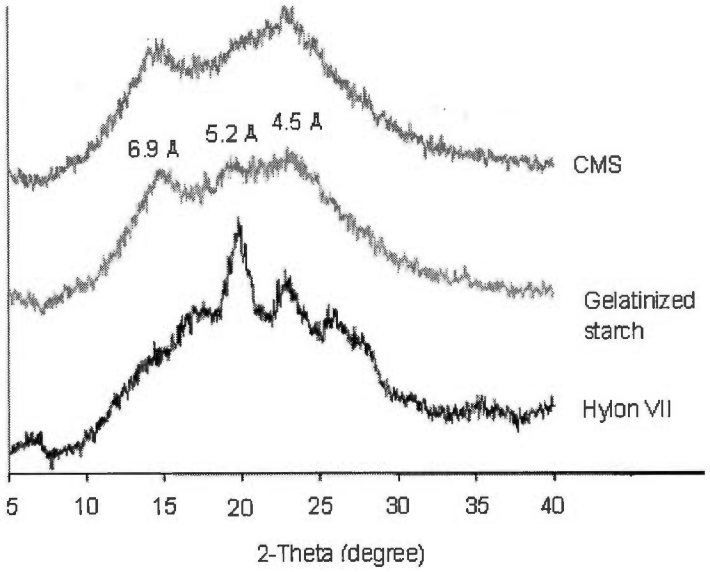

Figure 2.1: X-ray diffraction patterns of native starch (Hylon VII), gelatinized starch and CMS. (From Assaad and Mateescu, 2010).

The thermogravimetric method was used to determine the water content and the decomposition temperature. Moisture of about 11% and decomposition temperature of about 290 °C were found for CMS (Massicotte et al., 2008; Lemieux et al., 2010; Assaad et al., 2011b). The differential scanning calorimetry (DSC) was used to determine the fusion temperature, the crystalline domains and the decomposition temperature (Massicotte et al., 2008; Assaad et al., 2011a).

The solubility of CMS increased with DS and was higher in neutral medium than in acid medium (Lemieux et al., 2010; Assaad et al., 2011b). For the same DS, protonated CMS had similar or lower solubility to that of sodium CMS. These differences in solubilities can be explained by the enhancement of polymer hydration by carboxylate groups.

Characterization of tablets

With acetaminophen as active agent, no significant differences were observed between crushing strengths of tablets based on sodium CMS or on protonated CMS

up to DS 0.89 (Lemieux et al., 2009). Moreover, the ratio –COONa/–COOH has no effect (Assaad and Mateescu, 2010). The crushing strength may depend on the DS and on the drying method due to change in water content and in particles size and morphology (Lemieux et al., 2009 and 2010). Data on crushing strength indicated good compression properties for CMS powders.

At DS < 0.66, the tablets friability was lower than 1% and no differences were observed between the sodium and the protonated forms (Lemieux et al., 2009).

The CMS tablets were found to be susceptible to enzymatic erosion by α-amylase, depending on the degree of substitution (Calinescu et al., 2005). The DS can influence the hydrophilicity and the steric hindrance of CMS chains into the tablet and thus the accessibility of α-amylase. The erosion of tablets can also be related to the solubilization which was higher in simulated intestinal fluid (SIF) than in simulated gastric fluid (SGF) and generally it increases with DS (Assaad and Mateescu, 2010). The fluid diffusion into the unloaded tablet (Fig. 2.2) and the tablet swelling were faster in neutral fluids (water, SIF) than in acidic fluid (SGF) as showed by imaging NMR analysis (Assaad and Mateescu, 2011b; Wang et al., 2011).

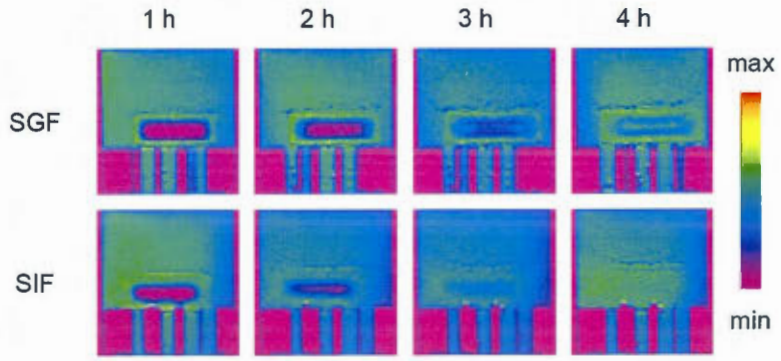

Figure 2.2: NMR images of unloaded CMS tablets in SGF and in SIF at 37 °C for 1, 2, 3 and 4 h. (From Wang et al., 2011).

5. *In vitro* drug release from carboxymethyl starch matrix

Since CMS was proposed as an excipient for oral drug delivery by Mulhbacher et al. (2004), several studies have been conducted to evaluate its efficiency in the delivery of drugs and/or bioactive agents. In these studies the preparation of CMS was not necessarily done under the same conditions (i. e. in water or organic phase). Unless other indication, the tablets are monolithic.

5.1. Release of small molecules

Release of acetaminophen

Acetaminophen is often used as a model drug in various studies concerning formulation. It was chosen as tracer for CMS excipients, mainly due to its pK_a (9.5) which is higher than the pH of commonly used intestinal media (SIF, 6.8-7.2). The acetaminophen solubility in aqueous medium is about 21 mg/ml at pH 1.2 and 18 mg/ml at pH 6.8 (Takahashi et al., 2005). This makes the differences in release rates in SGF and in SIF mostly dependent on the characteristics of the excipient rather than on acetaminophen solubility.

One of the most important characteristics of CMS is its degree of substitution (DS), because it can influence the solubility, the polymorphism, the inter- and intra-chain interactions and consequently the drug release rate and the mechanism of release.

For CMS prepared in 90% isopropanol, DS between 0.1 and 0.2 are preferred for sustained release, whereas DS between 0.9 and 1.2 are preferred for delayed release (Lemieux et al., 2009).

Assaad and Mateescu (2010) showed that CMS prepared in aqueous medium presented a non-monotonous dependency of release profiles upon the DS (0.07-0.2), a DS of about 0.11 being the most convenient for sustained release. Lower and higher DS resulted in faster release. The tablets made of low gelatinized starch were quickly

disintegrated, whereas those made of higher gelatinized starch showed prolonged release times although cracks appeared in the tablets. Cracks may appear also in tablets of CMS at relatively low DS (Lemieux et al., 2009; Assaad and Mateescu, 2010). It seems that the gelatinization degree and the DS should be high enough to permit sufficient and uniform hydration of tablets. To prevent tablet cracks and to ensure sustained release with CMS at very low DS 0.045, a solide electrolyte (NaCl) was added to the formulation (Nabai et al., 2007; Brouillet et al., 2008, 2010). It is believed that adding an appropriate amount of NaCl maintains equilibrium between hydrogen bonds, which maintain matrix structure, and swelling of the polymer chains, which gives the matrix its elasticity. Increasing the acetaminophen loading to 40% also permit to prevent tablet cracks (Nabai et al., 2007).

The drying procedure may influence the drug release properties of CMS (Lemieux et al., 2010). Fast release has been observed with tablets based on lyophilized CMS due to the particle microporosities which favors a rapid water penetration into the matrix. Contrarily, CMS (DS 0.14 or 0.25) dried either by treatment with acetone or by spray-drying appeared suitable for controlled release from matrices due to the formation of an outer gel layer, with the mention that similar profiles were obtained for each DS.

A low protonation of CMS can prolong the drug release time due to the stabilization of matrix by hydrogen bonds (Assaad and Mateescu, 2010). The inter- and intra-chain attractive interactions increase at high DS and high protonation ratio, leading to lower solubility of CMS particles and faster diffusion of fluid into the matrix. Fast tablet disintegration can occur when the CMS used as excipient presents sufficient hydrogen bonded carboxyl groups.

Compression forces between 1 and 2.5 tonnes/cm^2 showed low influence on drug release from CMS matrices at DS 0.045 (Brouillet et al., 2008). A linear relationship between tablet weight and release time was observed for formulations

with 27% NaCl and 40% acetaminophen loading. An increase in drug loading from 10% to 40% led to an increase in release time (Brouillet et al., 2008). Contrarily, faster release was observed with CMS at DS 0.2 when the loading was increased from 20% to 40%, and no difference in the release profile was observed with CMS at DS 0.11 for these loadings (Assaad and Mateescu, 2010). It seems that the influence of acetaminophen loading on release kinetics depends on the DS of CMS and/or on the presence of an electrolyte in the matrices.

The rate and the mechanism of drug release depend on pH of the dissolution medium, because CMS is a hydrophilic anionic polymer. The acetaminophen release from gelatinized starch matrix showed no dependency on pH (Lemieux et al., 2009; Assaad and Mateescu, 2010). Moreover, the drug release profile from matrix based on CMS at very low DS 0.045 and containing NaCl was not affected by acidic pH value and by preincubation time of tablets in the acidic medium (Nabai et al., 2007). Contrarily, at higher DS, the tablet preincubation in acidic medium made the release longer (Assaad and Mateescu, 2010). The release rate with CMS (DS 0.07-0.20) in SGF was lower than that in SIF for each excipient. This is due to the interaction between hydroxyl and carboxyl groups of CMS and to the lower solubility of CMS in acidic medium compared to that in neutral medium. The sensitivity to the pH increases with the increase of DS (Assaad and Mateescu, 2010).

The presence of α-amylase in SIF accelerates the drug release rate due to the enzymatic hydrolysis of polymer chains. The release of acetaminophen was accelerated (time reduced by about 50 - 60%) in presence of pancreatin in SIF (Ispas-Szabo et al., 2007; Assaad and Mateescu, 2008). Accelerated release was also observed with protonated CMS form and with dry-coated tablets (Ispas-Szabo et al., 2007). Furthermore, this acceleration of the release in presence of pancreatin was not directly proportional to DS (Lemieux et al., 2009).

Due to the solubilization of CMS in neutral medium, chitosan can be added as a coexcipient to ensure longer release (Leonida and Mateescu, 2006). This prolongation in release time depends on the molecular weight of chitosan (Assaad and Mateescu, 2010). The use of chitosan can also reduce the sensitivity of the matrix to hydrolysis by pancreatic enzymes. In order to ensure drug delivery until the colon and at the same time to keep CMS as the main excipient in the formulation, a CMS-chitosan polyelectrolyte complex was proposed as carrier for oral drug delivery (Assaad et al., 2011b).

Release of aspirin and other small molecules

The aspirin or acetylsalicylic acid has a pK_a of about 3.5 (Schrör, 2009). The aspirin was released slower than acetaminophen from tablets based on CMS (DS 0.14), maybe due to its lower solubility (Assaad et al., 2011b). When the aspirin loading was increased from 20% to 60%, the release time was increased twice (15 h to 30 h) (Assaad et al., 2008). The same phenomenon was previously observed with cross-linked CMS (Mulhbacher et al., 2001). Moreover, a longer release time was observed with the protonated CMS form than with the sodium form and delayed release was observed when dry-coating with same CMS excipient was used (Ispas Szabo et al., 2007). These longer release times can be explained by the aspirin-aspirin and excipient-aspirin interactions via hydrogen bonds between carboxyl groups.
Matrices based on (CMS : aspirin : polyvinylpyrrolidone, in the ratios 10 : 1 : 1) with silicon-di-oxide and magnesium stearate (in the ratio 2 : 1) in amount not exceeding 3-5% were tested *in vitro* for aspirin release (Sen and Pal, 2009). By using CMS prepared in aqueous medium with DS 0.5, the release time decreased by increasing the pH of the dissolution medium (pH 4, 7 and 10) due to the higher swelling of CMS at alkaline pH. We can mention that in this study the release was incomplete (about 70%), irrespective of the pH value. Although the release times from matrices based on CMS were relatively short (8 h at pH 4), they were longer than those obtained with matrices based on starch (Sen and Pal, 2009).

These results showed that CMS can be a good excipient for the delivery of drugs similar to aspirin which need to be preferentially released in the intestine rather than in the stomach.

The presence of pancreatin in the dissolution medium accelerated the release, regardless of the CMS form (sodium or protonated) and of the dosage form (monolithic or dry-coated) (Ispas Szabo et al., 2007).

Chitosan used as a coexipient with CMS accelerated the aspirin release, acting in this case as a disintegrant. Contrarily, sustained release was obtained with CMS-chitosan polyelectrolyte complex and it was 2.5 fold longer than that obtained with CMS (Assaad et al., 2011b).

Metformin hydrochloride and chlorpheniramine maleate showed fast release from monolithic tablets based on CMS, due to their high solubilities in acidic medium (Assaad et al., 2008). The release of metformin can be delayed for 1-2 h by using double coated tablets (Assaad et al., 2011b). This delayed relase can be useful *in vivo*, because it can reduce secondary effects due to the release of metformin in stomach and at the same time can carry the entire formulated drug to intestine where it can be absorbed. Delayed release was observed for sodium diclofenac formulated with CMS even without coating (Ispas Szabo et al., 2007). This may due to CMS-diclofenac or diclofenac-diclofenac interactions via carboxyl groups at acidic pH.

5.2. Release of proteins

The main limitation to the administration of proteins by oral route and to their use as oral vaccines is the digestion of the proteins by pepsin and by pancreatic enzymes. Consequently, the first condition to ensure the desired benefits is to protect the bioactive agents against gastric acidity and pepsinolysis during their residence in stomach. In this context, CMS has been proposed as a drug carrier for proteins as F4 *fimbriae*, alfalfa protein extract and ovalbumin.

F4 *fimbriae* is the most prevalent type of *fimbriae* in Enterotoxigenic *Escherichia coli* strains and it enables the bacteria to adhere to the small intestinal epithelium. Oral vaccination with F4 *fimbriae* can be an approach to prevent post-weaning diarrhea caused by *Escherichia coli* in pigs.

F4 *fimbriae* formulated with CMS in monolithic tablet preserved high stability after incubation in simulated gastric fluid (SGF) containing pepsin, whereas free (unformulated) F4 *fimbriae* were almost completely degraded (Calinescu et al., 2007). A controlled release of F4 *fimbriae* formulated with CMS was observed in SIF containing pancreatin. The F4 *fimbriae* inside the tablets retain their structural stability and their receptor binding activity that are essential for the induction of an intestinal mucosal immune response.

De Koninck et al. (2010) proposed the formulation of alfalfa protein extract (30% loading) with CMS in monolithic tablet containing protease inhibitors. Almost complete protection of the protein against pepsinolysis was obtained when 1.6 % (w/w) of Pefabloc SC inhibitor was used. The protein was then released within 6 h in SIF containing pancreatin at time-points corresponding to the small intestine transit. The structural and functional properties, that are important to induce mucosal immunity, were maintained.

The CMS showed also its potential as carrier for oral administration of ovalbumin. More than 70% of the loaded ovalbumin (50 % loading) still was stable after 1 h of incubation in SGF containing pepsin. The complete release occurred over a period of 6 h in SIF containing pancreatin (Assaad et al., 2011a). Improved protection against pepsin and longer release time were obtained when chitosan was added as a coexipient and when the ovalbumin was formulated with CMS-chitosan polyelectrolyte complex.

CMS was also proposed as carrier for pancreatic enzymes (α-amylase, lipase and trypsin) formulated together or individually (Massicotte et al., 2008). The

formulated pancreatic enzymes retained an overall (average of the three enzymes) activity of 72% after 1 h of incubation in SGF containing pepsin. CMS showed a high loading capacity (up to 70-80%) which is of interest for pancreatic enzymes replacement therapy in the case of pancreatitis.

5.3. Release of microorganisms

CMS was proposed as a carrier for microorganisms, specifically for non-pathogen *Escherichia coli* (*E. coli*) acting as an oral vaccine that prevents post-weaning diarrhea caused by these bacterial strains and for *Lactobacillus rhamnosus* as a probiotic for preventing therapy (Calinescu et al., 2005; Calinescu and Mateescu, 2008).

The viability of microorganisms was almost unaffected by tableting with CMS, indicating that the pressure, the heat in tablet during the compression and the water activity within the tablet were still convenient to keep the microorganisms alive.

When formulated with CMS, the *E. coli* can be protected against acidity of SGF and released gradually in SIF. Tablets with CMS at low DS showed capping, whereas CMS with DS at 0.11 (denoted as 11 carboxylic functional groups per 100 glucosidic units) seemed to be the most appropriate for bacterial transit through the gastrointestinal tract (Calinescu et al., 2005). The bacteria in the tablets were almost completely preserved alive after six months of storage at 4 °C.

The oral administration of probiotics is expected to improve the host intestinal microbial balance and can represent a promising alternative to control infections and disequilibrium in bacterial populations without using antibiotics. Among probiotic bacteria, *Lactobacilli* strains are largely present in humans. To be effective, orally administered probiotics should survive during the passage through stomach and should be able to establish themselves in intestine. In this context, Calinescu and Mateescu (2008) suggested CMS associated with chitosan as carrier for *Lactobacillus*

rhamnosus probiotic. Increasing the ratio of the CMS in the CMS:chitosan matrix enhanced the erosion and the release of higher amount of living bacteria. The increase of chitosan molecular weight generated a delayed release. Therefore, release of the bacteria can be controlled by an adequate choice of the amount and of the molecular weight of chitosan in the tablet (Calinescu and Mateescu, 2008).

6. Factors influencing the release rate and mechanism

Different factors can influence the release rate of drugs and the mechanisms controlling the release from tablets using CMS as the main excipient. Although the preparation of CMS or the dissolution tests were not necessary carried out in the same conditions for all the reported studies, it can be concluded that the DS is the major factor that can influence the drug delivery by CMS. At low DS the drug release is mostly controlled by diffusion through the matrix, whereas at high DS the release is mostly controlled by swelling and solubilization/erosion of the matrix. The mechanism of the drug release form CMS matrices is mostly controlled by diffusion at acid pH, and by solubilization/erosion at neutral pH (Lemieux et al., 2009; Assaad and Mateescu, 2010). Other factors such as the particle size and the solubility of the active agents or the degradation of CMS by α-amylase can also have an important impact on release rate. We present in table 2.1 the main reported factors with their effects on the active agent release from CMS matrix.

7. *In vivo* studies

Only few *in vivo* studies with carboxymethyl starch have been published, but the available data showed the safety (non-toxicity) of this product. A study with albino rats fed with pellets coated with CM-potato-starch or intubated with CM-potato-starch solution showed no significant changes in gain of weekly body weight, in weight of vital organs and in the haematological and histopathological parameters (Ramesh et al., 2004).

Table 2.1: Factors influencing the drug release from tablets based on CMS.

Factor	Active agent and effect	Reference
DS	[a]*Escherichia coli* and [b]acetaminophen: - DS (< 0.07): tablet cracks/capping. - 0.07 < DS < 0.20 controlled release.	[a]Calinescu et al., (2005) [b]Lemieux et al., (2009, 2010) [b]Assaad and Mateescu (2010)
Protonation (–COOH)	Acetaminophen: release time depend on protonation ratio, on DS and on storage time of CMS.	Assaad and Mateescu (2010)
Drying method	Acetaminophen: - Freeze-drying: relatively fast release. - Solvent precipitation and spray drying: controlled/sustained release and similar release rates.	Lemieux et al., (2010)
Loading	Acetaminophen: faster release when loading increases (20% - 60%).	Assaad and Mateescu (2010)
	Aspirin: slower release when loading increases (20% - 60%).	Assaad et al., (2008)
Electrolyte (NaCl)	Acetaminophen: tablet integrity maintained and cracks prevented (optimal amout of NaCl is 27%).	Nabais et al., (2007)
Compression force	Acetaminophen: (1 - 2.5 tonnes/cm^2) no significant influence on the release.	Brouillet et al., (2008)
Tablet weight	Acetaminophen: linear relationship of release time with tablet weight.	Brouillet et al., (2008)
Dry coating	[a]Acetaminophen, [b]aspirin, [b]sodium diclofenac and [c]metformin hydrochloride: delayed release.	[a,b]Ispas-Szabo et al., (2007) [a]Assaad and Mateescu (2010) [c]Assaad et al., (2011b)
Enzymatic degradation (α-amylase)	[a]F4 *fimbriae*, [b]acetaminophen, [c]aspirin and [c]sodium diclofenac: bioerosion and acceleration of the release rate.	[a]Calinescu et al., (2007) [b,c]Ispas-Szabo et al., (2007) [b]Assaad et al., (2008) [b]Lemieux et al., (2009)

In vivo drug absorption is an important step in the dosage form development process. An exploratory *in vivo* study was realized using tablets of 600 mg prepared by direct compression of dry mixture of acetaminophen (40 % loading), CMS, and NaCl (Nabais et al., 2007). The pharmacokinetic (*in vivo* concentration-time profiles) study conducted in 5 healthy volunteers indicated that the matrices maintained their integrity while traversing the stomach and they did not show burst-effect during the first 3-5 h after administration of the formulation. Moreover, this study demonstrated extended and continuous drug absorption, but it appeared that the release rate was faster *in vivo* than *in vitro*.

8. Conclusion

Carboxymethyl starch (CMS) used as drug carrier permits the delivery of small molecules as well as proteins and microorganisms. The drug release rate from monolithic tablets depends essentially on the degree of substitution (DS) of CMS, on the molecular size and solubility of drug, and on the CMS-drug interactions. A DS of about 0.1 is needed to ensure relatively prolonged drug release, whereas high DS lead to fast drug release due to the solubilization of the matrix at neutral and basic pH. With very low DS, the tablets show cracks/capping which can be prevented by adding NaCl to the formulation. Highly protonated CMS with hydrogen bonded carboxyl groups are not able to generate hydrogel when are used as excipients in matrices, leadind to fast matrices disintegration and fast drug release.

The CMS is sensitive to the pH of the dissolution medium due to its anionic character. The tablets based on CMS are compact at acid pH due to the protonation and the stabilization by hydrogen bonds, whereas they are more hydrated and erodible at neutral pH. To favor sustained release, CMS-chitosan polyelectrolyte complex can be used as excipient or chitosan can be added as a coexcipient in formulation based on CMS. These modifications reduce the solubility of the matrix at neutral pH and the sensitivity of the excipient to degradation by α-amylase.

CHAPITRE III

LE CHITOSANE ET LES COMPLEXES À BASE DE CHITOSANE

Ce chapitre est une étude bibliographique sur la chitine et le chitosane, ainsi que sur les complexes de chitosane avec d'autres polymères. Nous mettons plus d'accent sur les polymères non modifiées chimiquement (natifs). En particulier, les applications du chitosane et de ses complexes dans la formulation des substances actives administrées par voie orale sont abordées.

1. La source de la chitine et l'obtention du chitosane

La chitine

La chitine est un hétéropolysaccharide binaire et non-ramifié composé d'unités de *N*-acétyl-D-glucosamine et de D-glucosamine liées par des liaisons glycosidiques β-(1-4), où les unités *N*-acétyl-D-glucosamine sont prépondérantes dans la chaîne polymérique.

La chitine est, après la cellulose, le deuxième biopolymère le plus abondant dans la nature (Muzzarelli, 1999). Elle a été découverte en 1811 par H. Braconnot durant ses études sur les champignons et elle a été identifiée comme étant la fraction resistante au traitement alcalin (Labrude et Becq, 2003). Le terme *chitine* a été proposé pour la première fois par C. Odier (1823) qui a trouvé ce polymère dans la cuticule des coléoptères. Ce terme provient du mot grec *chiton* qui signifie «tunique» et qui fait référence à la cuticule.

La chitine est présente dans des champignons, des diatomées, des arthropodes, des nématodes et dans des différentes plantes, mais elle est absente chez les mammifères

(Hayes *et al.*, 2008). Les principales sources de chitine se trouvant sur le marché sont les déchets de l'industrie marine (Teng *et al.*, 2001).

Bien que la chitine ait été identifiée avant la cellulose, elle a initialement suscitée moins d'attention dans la recherche scientifique que cette dernière (Labrude et Becq, 2003). La chitine assure une fonction similaire à celle de la cellulose dans les plantes en agissant comme un matériau de soutien et de protection. Avec sa structure cristalline et son association avec des protéines de l'exo- et l'endo-cuticule, elle empêche les organes internes des crustacés de se gonfler dans l'eau de mer (Raabe *et al.*, 2006).

L'extraction de la chitine à partir des carapaces des invertébrés aquatiques s'effectue par des méthodes chimiques, par des méthodes enzymatiques ou par d'autres procédés. L'extraction chimique (Fig. 3.1) consiste en deux étapes principales :

- enlèvement des protéines des carapaces broyées par traitement avec une solution d'hydroxyde de sodium ou d'hydroxyde de potassium à une température élevée (Shahidi et Synowiecki, 1991).
- enlèvement des sels minéraux, comme le carbonate de calcium et le phosphate de calcium, par traitement avec un acide dilué (No et Meyers, 1995).

La décoloration pourrait être effectuée à température ambiante en utilisant des réactifs, comme l'acétone, l'éthanol, l'éther ou l'hypochlorite de sodium (No et Meyers, 1995). La proportion de la chitine dans les exosquelettes dépend des espèces et des saisons. En général, les exosquelettes contiennent 15-40 % de chitine, 20-40 % de protéines, 20-50 % de carbonate de calcium et d'autres composants comme des lipides, des pigments, etc. (Kurita, 2006). La chitine extraite est blanche, dure et non élastique. La production de la chitine par la biomasse est une des plus abondantes, avec une production annuelle estimée à 10^{10}-10^{12} tonnes.

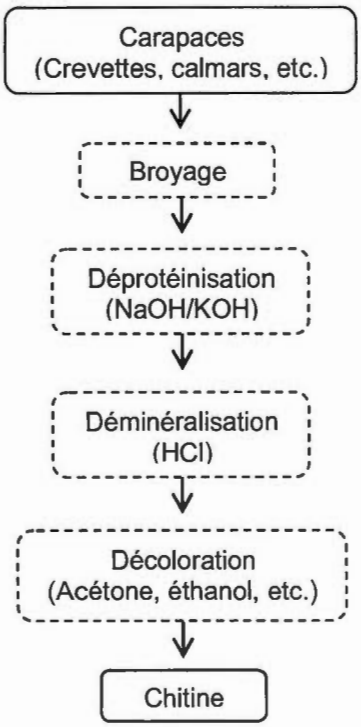

Figure 3.1 : Principales étapes de l'extraction de la chitine.

Le chitosane

Après avoir traité la chitine avec une solution chaude et concentrée de KOH, Rouget a découvert en 1859 un produit qui a des caractéristiques différentes des celles de la chitine et a logiquement nommer ce produit *chitine modifiée*. En 1894, Hoppe-Seyler qui ignorait les travaux de Rouget a proposé de donner à ce dérivé le nom *chitosane* (McKay, 1995). Présentement, ce nom est largement utilisé dans le langage scientifique. En fait, ce qui différencie le chitosane de la chitine est le degré de désacétylation (DDA). En général, le polymère prend le nom *chitosane* lorsque le DDA est supérieur à 60 %.

Le chitosane, très peu disponible dans la nature, est majoritairement obtenu par désacétylation de la chitine à une température élevée (environ 100 °C) en présence

d'une solution concentrée de NaOH ou de KOH (environ 45 %). Cette méthode pourrait entraîner une réduction de la masse moléculaire du chitosane. Pour éviter une hydrolyse du polymère, l'utilisation du thiophénol comme catalyseur de la réaction de désacétylation a permis l'obtention des DDA élevés dans des conditions plus douces (Domard et Rinaudo, 1983). De plus, une désacétylation en présence du thiophénol permet l'obtention d'un chitosane avec une structure plus ordonnée (Harish Prashanth, Kittur et Tharanathan, 2002). Les conditions expérimentales d'extraction et l'ordre des différentes étapes (déminéralisation, déprotéinisation, désacétylation) pourraient affecter les caractéristiques du chitosane.

Le chitosane peut être purifié par solubilisation dans de l'acide dilué suivie d'une filtration. Il peut être ensuite précipité avec une solution de NaOH et lavé avec de l'eau distillée, puis séché (Hirano, 1996). Le chitosane est disponible sur le marché sous différentes formes (poudres, flocons, fibres, etc.) et sa couleur dépend de la source et des conditions expérimentales d'obtention (Fig. 3.2).

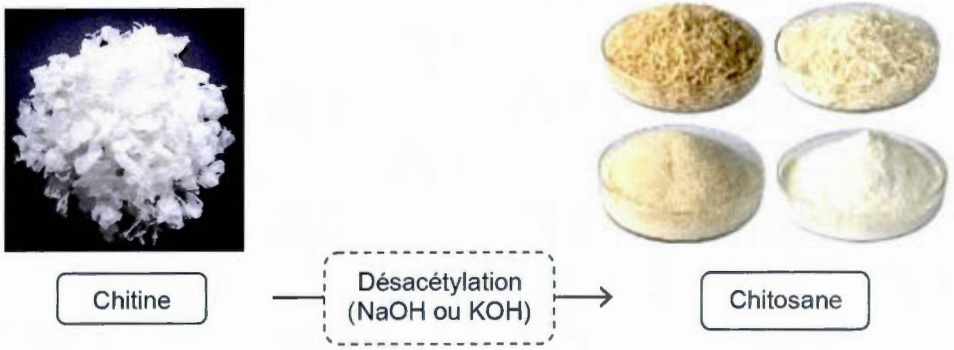

Figure 3.2 : Obtention du chitosane à partir de la chitine.

2. La structure et les caractéristiques de la chitine et du chitosane

La structure moléculaire, le polymorphisme, le DDA et la masse moléculaire ont un effet majeur sur les propriétés du chitine/chitosane telles que l'hydrophillicité, la solubilité et la susceptibilité à la dégradation par des enzymes (Kittur, Vishu Kumar et Tharanathan, 2003; Alves et Mano, 2008; Kasaai, 2009).

Structure moléculaire

La chitine et le chitosane peuvent être représentés par une structure chimique unique (Fig. 3.3) qui correspond à des séries de copolymères liés par des liaisons β-(1-4) où l'un ou l'autre des deux groupements $-NH_2$ et $-NH-CO-CH_3$ est présent sur le carbone-2 de chaque unité glycosidique.

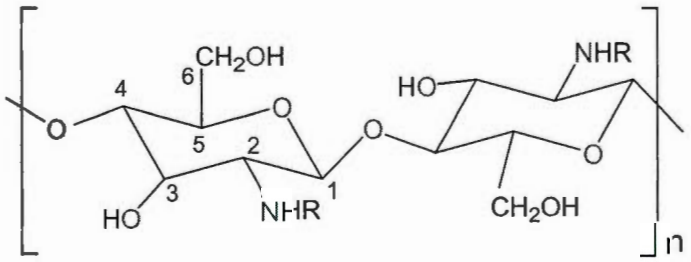

Figure 3.3 : Structure chimique de la chitine et du chitosane. R = H ou $COCH_3$.

Le spectre infrarouge (IR) du chitosane (Fig. 3.4) permet d'identifier la présence de différents groupements chimiques (tableau 3.1). La large bande à 3100-3500 cm^{-1} correspond aux vibrations d'élongation de –NH et –OH, incluant les liaisons d'hydrogène. Normalement, cette bande est plus intense chez la chitine, en raison des fortes liaisons d'hydrogène et de l'état cristallin.

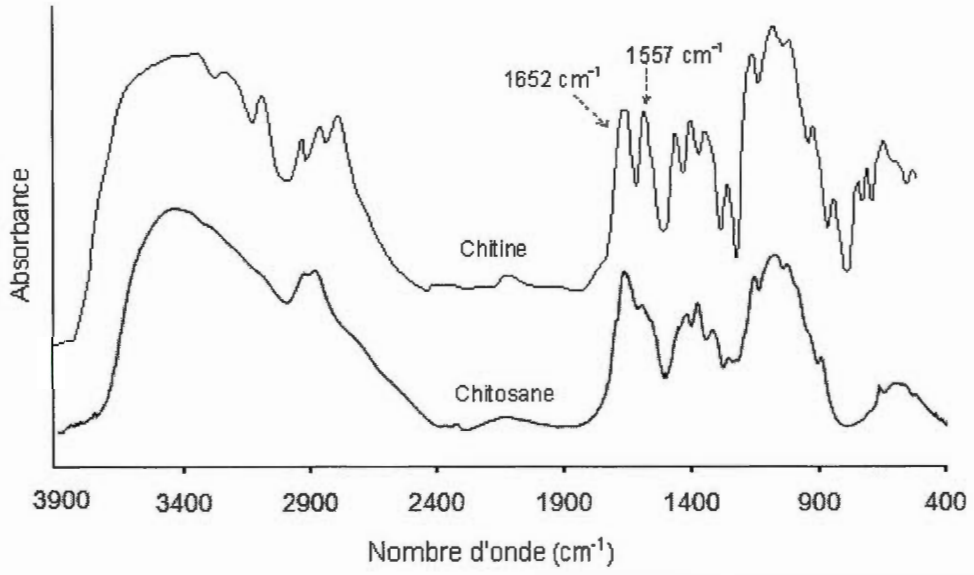

Figure 3.4 : Spectres infrarouge de la chitine et du chitosane.
(Tirée de Assaad, 2006 et de Assaad *et al.*, 2011b)

Tableau 3.1 : Principales bandes du spectre infrarouge du chitosane. (Tirées de Cho *et al.*, 2000; Kasaai, 2008).

Nombre d'onde (cm^{-1})	Bandes d'absorption
890-1030	Liens glycosidiques
1160	Liens glycosidiques/ponts C–O–C (élongation asymétrique)
1254	Vibration de distorsion de –O–H
1423	Déformation symétrique de $–CH_3$ et $–CH_2$
1557 et 1652	Amide
1590-1630	Liaisons NH et NH_2
523, 741 et 1652	Identifient la chitine
2880 et 2923	Élongation de –CH et $–CH_2$
3105	Liaisons d'hydrogène intermoléculaires C(2)NH...O=C(7)
3246	Liaisons d'hydrogène intermoléculaires C(6)OH...HOC(6)
3100-3500	Élongation de –NH et –OH, incluant les liaisons d'hydrogènes

Les absorbances à 3105 cm^{-1} et 3246 cm^{-1}, correspondant aux liaisons d'hydrogène intermoléculaires C(2)NH...O=C(7) et C(6)OH...HOC(6), sont normalement plus intenses pour la chitine que pour le chitosane (Cho *et al.*, 2000). La diminution de l'intensité dans cette région reflète une réduction des liaisons d'hydrogène intermoléculaires et montre que les arrangements antiparallèles des chaînes de chitine se transforment en arrangements parallèles avec l'augmentation du degré de désacétylation. Les deux bandes caractéristiques qui apparaissent à 1557 et 1652 cm^{-1} sont dues aux vibrations d'élongation de la fonction amide. Ces bandes sont plus facilement identifiables dans le cas de la chitine, car cette dernière est plus acétylée que le chitosane. La région entre 1590 et 1630 cm^{-1} correspond au groupement amine du chitosane. Une diminution progressive des intensités des bandes correspondantes aux liaisons d'hydrogène et à l'amide a lieu au fur et à mesure que la désacétylation devient plus avancée (Harish Prashanth, Kittur et Tharanathan, 2002).

Polymorphismes

Dépendamment de son origine, la chitine a deux formes polymorphiques, la forme α et la forme β (Blackwell, 1973). Une troisième forme, la γ-chitine, a été aussi raportée, mais elle n'est en réalité qu'un mélange des α- et β-chitines (Takai *et al.*, 1989). L'α-chitine est la forme la plus abondante dans les carapaces de crustacées, les insectes et les champignons. La β-chitine se trouve dans les diatomées et les membranes internes des calmars, tandis que la γ-chitine se trouve dans les calmars et les parois des estomacs des seiches (Muzzarelli, 1977; Gorovoj et Burdukova, 1996). L'α-chitine a une structure cristalline formée de feuillets antiparallèles compacts avec des liaisons d'hydrogène inter- et intra-feuillet très fortes (Kurita, 2001; Rudrapatnam Kittur et Kittur, 2002). La β-chitine est formée de feuillets parallèles caractérisés par de faibles forces intermoléculaires, tandis que la γ-chitine est formée d'une combinaison de feuillets parallèles et antiparallèles (Fig. 3.5) (Lamarque, Viton et Domard, 2004a, 2004b). La forme β peut se transformer en forme α sous l'effet de la chaleur de vapeur (Kurita, 2001).

L'extraction de la β-chitine se fait dans des conditions plus douces que celles nécessaires à l'extraction de l'α-chitine (Minke et Blackwell, 1978). De plus, la β-chitine a plus de réactivité et plus d'affinité pour les solvants que l'α-chitine (Kurita *et al.*, 1993, 1994).

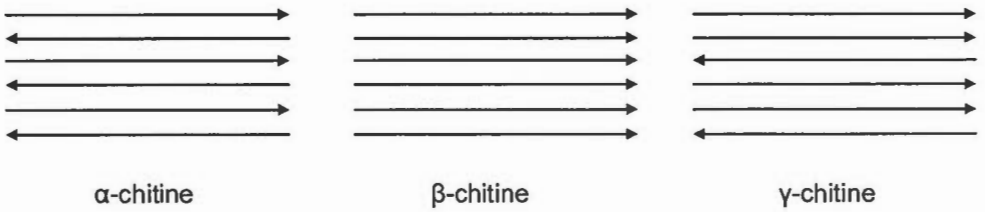

Figure 3.5 : Représentation schématique de l'arrangement polymorphique de chitine.

Le chitosane a différentes formes polymorphiques et une structure semi-cristalline due aux liaisons d'hydrogène inter- et intra-moléculaire. Ces formes polymorphiques ont toutes une structure de double hélice, mais qui se différencient par le degré d'entassement et la quantité d'eau retenue. Trois formes polymorphiques principales ont été rapportées pour le chitosane : *tendon* pour le chitosane provenant des exosquelettes de crabes, *recuit* pour le chitosane obtenu par la déshydratation de la forme tendon, et *L-2* pour le chitosane provenant des exosquelettes de crevettes (Saitô, Tabeta et Ogawa, 1987; Ogawa, 1991; Ogawa et Yui, 1993; Okuyama *et al.*, 1997, 1999).

Ces différentes formes de polymorphismes de la chitine et du chitosane peuvent être identifiées, entre autres, par résonnance magnétique nucléaire (RMN), spectroscopie IR et diffraction de rayons-X (Focher *et al.*, 1992a, 1992b).

Degré de désacétylation

Le degré de désacétylation (DDA) est une des caractéristiques les plus importantes du chitosane. Il peut influencer les propriétés physiques, chimiques et biologiques du chitosane, telles que la cristallinité, la solubilité, la biodegradabilité et

l'activité antimicrobienne (Tan *et al.*, 1998; Cho *et al.*, 2000). En effet, c'est cette caractéristique, particulièrement, qui permet de faire la différence entre la chitine et le chitosane.

Le DDA peut être contrôlé en sélectionnant les conditions convenables durant le processus d'obtention du chitosane, ou même après avoir l'obtenu en procédant à une deuxième désacétylation ou à une réacétylation (Sorlier *et al.*, 2001). La réacétylation peut être effectuée en présence de l'anhydride acétique.

En général, la désacétylation se fait à une température élevée dans une solution concentrée de base. Le désavantage de cette méthode et qu'elle pourrait entraîner une réduction de la masse moléculaire du polymère. Dans ce contexte, Weska *et al.* (2007) qui ont utilisé un système factoriel et une méthode de surface de réponses pour optimiser la désacétylation de la chitine ont rapporté que l'obtention d'un DDA élevé implique la formation du chitosane de masse moléculaire réduite. Différents paramètres peuvent jouer un rôle dans la réaction de désacétylation, tels que le temps de la réaction, la concentration de chaque réactif et le nombre d'étapes. Une désacétylation complète peut être obtenue, sans trop réduire la masse moléculaire du chitosane, en utilisant le thiophénol comme catalyseur (Domard et Rinaudo, 1983). Cette désacétylation effectuée en présence du diméthylsulphoxide et du thiophénol à 100 °C durant 1 h permet de prévenir la dégradation du polymère. La réaction peut être répétée, deux ou trois fois selon la masse moléculaire initiale du polymère, afin d'augmenter le DDA.

Différentes techniques ont été proposées pour déterminer le DDA. Parmi celles-ci, il y a la spectroscopie infrarouge (Sannan *et al.*, 1978; Domard et Rinaudo, 1983), la spectroscopie à résonance magnétique nucléaire (RMN) (Hirai, Odani et Akio, 1991), la conductimétrie (Domard et Rinaudo, 1983), la spectroscopie ultraviolet (UV) (Aiba, 1986, Tan *et al.*, 1998), la chromatographie (Aiba, 1986), des analyses enzymatiques (Nanjo, Katsumi et Sakai, 1991) et des analyses élémentaires (Kim, Kim et Lee, 1996).

Les méthodes les plus utilisées sont le dosage pH-métrique ou potentiométrique qui est une méthode économique, le RMN qui est une méthode précise et l'infrarouge qui est une méthode flexible où plusieurs bandes peuvent servir à faire les mesures selon le DDA (Kasaai, 2008). Pour une analyse quantitative avec la spectroscopie infrarouge, il est nécessaire d'établir une courbe de calibration avec des échantillons qui ont des DDA connus.

Masse moléculaire

La masse moléculaire moyenne de la chitine varie entre 1000 et $2500x10^3$ kDa (Muzzarelli, Lough et Emanuelli, 1987). Cette masse pourrait être réduite lors de l'obtention du chitosane dans des conditions fortement basiques. Afin d'élargir les applications du chitosane, différentes méthodes ont été proposées pour réduire la masse moléculaire du polymère d'une façon contrôlée (Allan et Peyron, 1995a).

La masse moléculaire peut être réduite par hydrolyse chimique avec de l'acide nitreux (Allan et Peyron, 1995b). La dégradation du chitosane peut être réalisée aussi par des rayons ultraviolets et par de peroxyde d'hydrogène avec un effet de synergie entre ces deux paramètres (Wang, Huang et Wang, 2005). La dégradation peut aussi avoir lieu avec des enzymes, comme la papaïne et le lysozyme. La susceptibilité à la papaïne immobilisée qui agit sur les liaisons entre les unités glucosamine et N-acétyle glucosamine a été plus grande lorsque le DDA a été plus faible (Terbojevich, Cosani et Muzzarelli, 1996). Par contre, le lysozyme agit plus sur le chitosane que sur la chitine.

De plus, l'hydrolyse chimique ou enzymatique permet l'obtention des oligosaccharides de chitine et de chitosane. Ces oligosaccharides ont fait l'objet de plusieurs études afin d'évaluer leur activités antibactérienne, antifongique et anticancérigène (Wang *et al.*, 2008)

La masse moléculaire moyenne de chitine/chitosane peut être déterminée par différentes techniques telles que la viscosimétrie (Wang, Huang et Wang, 2005), la chromatographie d'exclusion stérique (Chen et Hwa, 1996) et la diffusion de lumière

(Terbojevich *et al.*, 1996). La séparation du chitosane des oligomères du chitosane peut être effectuée par électrodialyse (Aider *et al.*, 2006; Aider, Brunet et Bazinet, 2008).

3. Les propriétés et les applications du chitosane

3.1. Les propriétés du chitosane

Le chitosane est un polymère cationique d'origine naturelle avec des propriétés multifonctionnelles. Il est biocompatible (Tharanathan et Kittur, 2003), biodégradable et mucoadhésif (Henriksen, Green et Smart, 1996; He, Davis et Illum, 1998). Il présente aussi des propriétés d'échange d'ions et antimicrobienne (Yadav et Bhise, 2004).

Solubilité

La solubilité de chitine/chitosane dépend de la structure cristalline du polymère et de son DDA. C'est un des critères qui permettent de faire la différence entre la chitine et le chitosane, même si la frontière entre ces deux entités n'est pas précisément définie. La structure cristalline très ordonnées de l'α-chitine est due aux liaisons d'hydrogène qui impliquent les deux groupements hydroxyle et le groupement amide de chaque unité glycosidique (Minke et Blackwell, 1978; Salmon et Hudson, 1997). Particulièrement, les liaisons d'hydrogène intramoléculaires qui lient l'hydroxyle du carbone-3 d'une unité à l'oxygène-5 (dans le cycle) d'une unité adjacente se traduisent par une rigidité de la chaîne linéaire du polymère (Fig. 3.6) (Cho *et al.*, 2000). À cause de cette structure très cristalline, l'α-chitine a une faible solubilité dans la majorité de solvants organiques dans des conditions douces.

Il est connu que le polymère (chitine/chitosane) devient soluble dans un acide dilué à partir de DDA supérieur à 50 % (Roberts, 1992). Aiba (1991), de sa part,

rapporte que la chitine avec un DDA de 40 %, obtenue par une N-acylation homogène, est soluble dans l'acide dilué. Une étude plus récente a évalué la solubilité des polymères obtenus par désacétylation homogène d'une α-chitine de DDA 8,6 % (Cho *et al.*, 2000). Dans cette étude, la solubilité a été interprétée en fonction du polymorphisme. Il a été constaté que le polymère devient soluble dans l'acide acétique dilué à un DDA supérieur à 28 % avec la forme α-chitine retenue, et il est soluble dans l'eau à un DDA de 49 % avec un polymorphisme similaire à celui de la β-chitine. Une partie des liaisons d'hydrogène a été rompue lorsque le DDA a passé de 28 % à 49 % (Cho *et al.*, 2000).

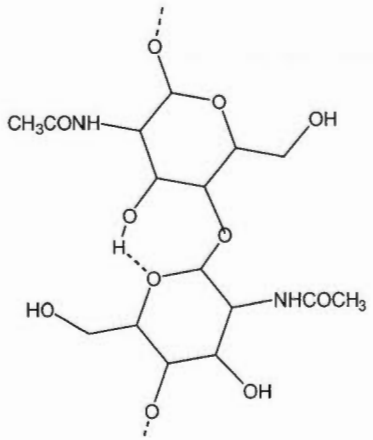

Figure 3.6 : Liaisons d'hydrogène intramoléculaires entre deux unités glycosidiques de chitine. (Adaptée d'après Cho *et al.*, 2000).

Le pK_a du chitosane dépend du DDA et de la masse moléculaire, mais en général il est autour de 6,5 (Krayukhina, Samoilova et Yamskov, 2008; Werle, Takeuchi et Bernkop-Schnürch, 2009). Dans un acide dilué (acide chlorhydrique, acide acétique, acide lactique, etc.), la protonation des groupements amines produit une rupture de la structure cristalline, un gonflement et une solubilisation du polymère. À un pH critique, qui dépend du DDA et de la masse moléculaire, les molécules dans la solution développent suffisamment de liaisons d'hydrogène pour former un gel (Matthew, 2002).

Biodégradation

Le chitosane est susceptible à l'activité hydrolytique de divers systèmes enzymatiques tels que la chitinase, la chitosanase, la cellulase, l'hemicellulase, la dextranase, la pectinase, la lipase, la pepsine, la papaïne, la pancréatine et le lysozyme (Hirano *et al.*, 1988; Aiba, 1992; Terbojevich, Cosani et Muzzarelli, 1996; Muzzarelli, 1997), malgré que juste la chitinase, la chitosanase et le lysozyme sont considérés spécifiques. Le chitosane est plus vulnérable à l'action non spécifique de certaines enzymes que ce qu'il été supposé. Le chitosane peut être dégradé par les α-amylases des plantes, des champignons et de l'Humain. Il est aussi sensible à l'action des lipases des animaux et des plantes (Muzzarelli, 1997).

L'action de la chitosanase sur les liens glycosidiques du chitosane conduit à la production des glucosamines et des hétérooligosaccharides qui consistent en de glucosamines et de N-acétyl-D-glucosamines (Fukamizo *et al.*, 1994).

Biocompatibilité

Le chitosane utilisé en quantités ou à des concentrations pas très élevées est considéré non toxique. Des poules et des lapins soumis respectivement à des doses de 1,4 g chitosane/kg corps et de 0,7 g chitosane/kg corps par jour durant 239 jours n'ont pas montré des symptômes anormaux (Hirano *et al.*, 1990). La chitine et le chitosane ont été digérés à 88-98 % par les poules et à 35-83 % par les lapins.

Une étude récente a montré que l'exposition des cellules hépatiques (bi-potential human liver cells) à une solution de chitosane de 0,5 % (m/v) durant 4 h a favorisé la prolifération cellulaire, en mentionnant que le chitosane en solution n'a pas pénètré dans les cellules (Loh *et al.*, 2010). Par contre, l'exposition de ces cellules à des nanoparticules de chitosane de même concentration a réduit la prolifération et la viabilité cellulaire, parce que les nanoparticules du chitosane ont passé à l'intérieure des cellules. L'entrée des nanoparticules dans les cellules a été suivie d'une

libération de l'alanine amino transférase dans l'espace extracellulaire, entraînant ainsi l'endommagement des membranes cellulaires.

La toxicité du chitosane en solutions de concentrations entre 0,003 et 0,025 % sur des cellules Calu-3 peut être considérablement diminuée suite à la fonctionnalisation du chitosane avec des groupements méthoxy-polyéthylène glycol (Casettari *et al.*, 2010). Ceci phénomène a été expliqué par l'encombrement stérique créé par la fonctionnalisation qui réduit les interactions des groupements amines chargés positivement avec les membranes cellulaires. La toxicité a été directement liée à la densité de charge et à l'arrangement spatial des résidus cationiques du chitosane.

Bioadhésion

Une des propriétés intéressantes du chitosane est sa mucoadhésivité conférée par sa capacité d'établir des liaisons ioniques ou des ponts d'hydrogène avec la mucine. La mucoadhésion permet une plus longue résidence des matrices à base de chitosane dans la région ciblée, et ensuite une meilleure stabilité et assimilation de la substance active (Lehr, 1994). L'interaction du chitosane avec deux différents types de mucines, un obtenu des glandes submaxillaires des bovins et l'autre de l'estomac du porc, a été étudiée dans l'eau distillée et dans HCl 0,1 M par Rossi *et al.*, (2000). Cette interaction a été trouvée dépendante des ratios chitosane : mucine, du milieu d'hydratation et du type de la mucine, suggèrant des interactions de différentes stœchiométries. Une proportion plus élevée de la mucine de l'estomac du porc que celle des glandes submaxillaires de bovins a été nécessaire pour avoir la même force d'interaction entre mucine et chitosane.

3.2. Les applications du chitosane et de ses dérivés

L'insolubilité du chitosane à pH neutre a limité son utilisation sous une forme native. Des alternatives sont disponibles pour élargir les champs d'applications du chitosane, comme le mélange physique avec d'autres polymères, la modification

chimique ou la formation des complexes avec d'autres polymères. Cette dernière alternative sera discutée dans la section suivante. Présentement, les produits à base de chitine/chitosane ont trouvé des applications dans des domaines assez différents (médical, pharmaceutique, environnemental, etc.) (Fig. 3.7). Nous présentons dans cette section quelques exemples sur la libération des substances actives à partir des matrices à base de chitosane modifié ou non modifié.

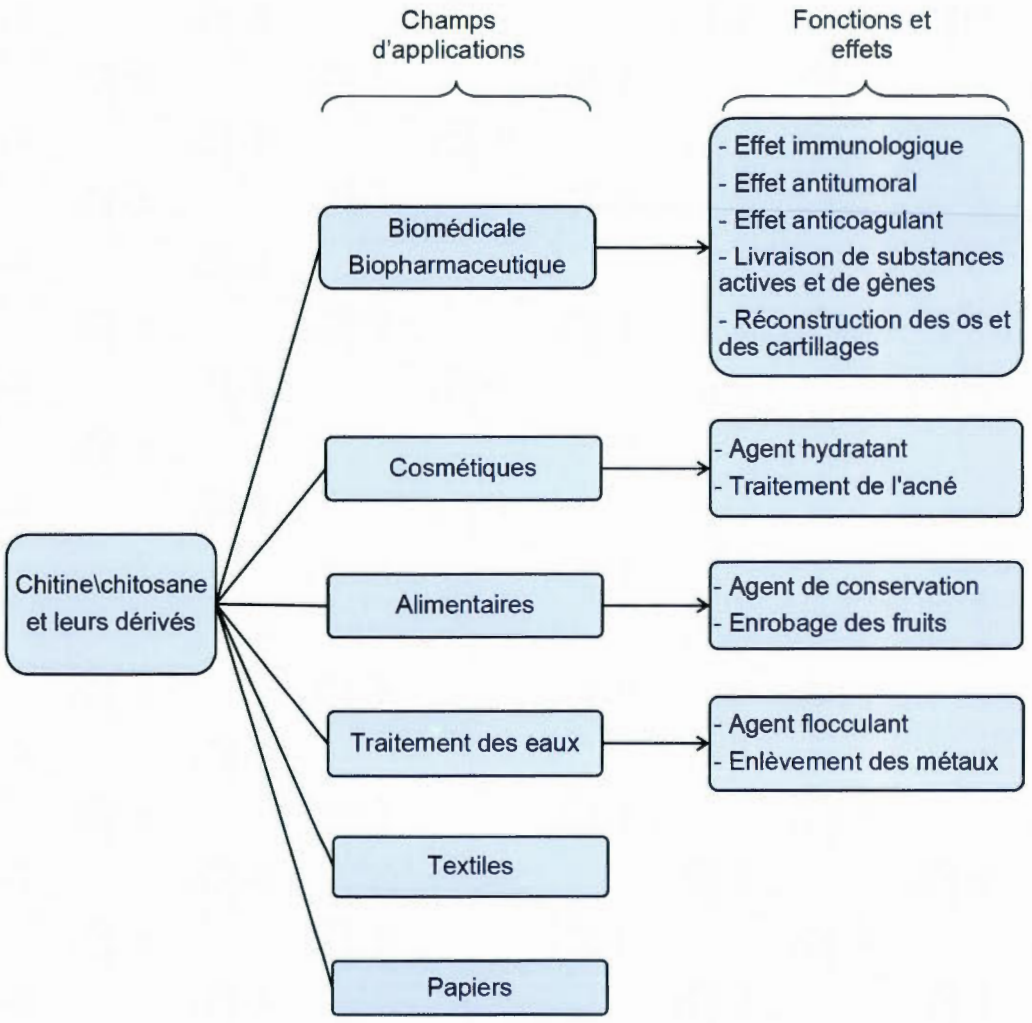

Figure 3.7 : Principales applications du chitosane et de ses dérivés.
(Adaptée d'après Ravi Kumar, 2000 et Rinaudo, 2006).

La chitine et le chitosane ont des bonnes propriétés de compressibilité qui sont comparables à celle de la cellulose microcristalline (Mir *et al.*, 2008). Le chitosane a aussi une bonne stabilité mécanique et une déformation plastique combinée à une élasticité élevée durant la compression (Picker-Freyer et Brink, 2006).

Pour des comprimés formés du chitosane natif comme excipient et de la théophylline comme substance active, une désintégration rapide a été observée dans un milieu acide (Mi *et al.*, 1997). Ilium (1998) a rapporté que le chitosane joue le rôle d'un désintégrant dans les comprimés. Lorsqu'il est ajouté à la formulation à un pourcentage supérieur à 5 %, il a une efficacité de désintégration plus grande que celles de l'amidon de maïs et de la cellulose microcristalline. Toutefois, cette efficacité dépend de la cristallinité, du DDA, de la masse moléculaire et de la taille des particules du chitosane. Le Tien *et al.* (2003) ont rapporté que les comprimés à base de chitosane natif ont montré une faible résistance mécanique et une libération très rapide de l'acétaminophène à pH 7,2. Une libération contrôlée de l'acétaminophène (Leonida et Mateescu, 2006; Assaad *et al.*, 2011b), de l'aspirine (Assaad *et al.*, 2011b) ou de l'ovalbumine (Assaad *et al.*, 2011a) peut être obtenue lorsque les comprimés à base de chitosane sont préincubés dans un fluide gastrique simulé (FGS, pH 1,2) avant d'être transférés dans un fluide intestinal simulé (FIS, pH 6,8-7,2). Cependant, la cohésion entre les particules de chitosane qui n'entrent pas en contact avec l'acidité reste faible. Il semble qu'il n'est pas convenable d'utiliser le chitosane natif seul comme excipient pour assurer une libération contrôlée des substances actives, puisque la libération est très dépendante du pH du milieu. Dans un milieu neutre, l'infiltration de l'eau dans les comprimés favorise leur désintégration.

Le chitosane mélangé avec d'autres excipients, en particulier avec de polymères, s'avère performer mieux que lorsqu'il est utilisé seul. Des comprimés préparés avec un mélange physique de chitosane et d'alginate de sodium comme coexcipients et avec le diltiazem comme substance active ont montré une bonne

mucoadhésivité et un temps de libération qui dépend des ratios de deux polymères (Miyazaki, *et al.*, 1995).

Un chitosane faiblement réticulé a été utilisé comme co-excipient avec l'alginate de sodium, la cellulose microcristalline et la carboxyméthyl cellulose de sodium dans des formulations destinées à la libération vaginale du métronidazole (El-Kamel *et al.*, 2002). Les comprimés préparés ont montré une bonne propriété de mucoadhésion et une libération contrôlée.

Calinescu et Mateescu (2008) ont étudié la libération des bactéries de type *Lactobacillus rhamnosus* à partir des comprimés monolithiques en utilisant le carboxyméthyl amidon (CMA) et le chitosane comme coexcipients. L'augmentation du pourcentage ou de la masse moléculaire du chitosane dans la formulation a entraîné une libération retardée et plus lente. Avec du chitosane de masse moléculaire d'ordre 2,2 x 10^6 kDa, un pourcentage de CMA d'ordre 80 % dans la formulation a été nécessaire pour permettre la libération des bactéries probiotiques.

La présence, en général, de deux groupements hydroxyles et d'un groupement amine sur chaque unité glycosidique de la chaîne du chitosane rend ce polymère susceptible à de nombreuses modifications chimiques (Ravi Kumar, 2000; Rudrapatnam, Kittur et Kittur, 2002). Quelques exemples des études sur l'utilisation des dérivés du chitosane dans des comprimés pharmaceutiques sont rapportés ci-après.

L'efficacité de l'acétate de chitosane comme agent liant dans des comprimés pharmaceutiques a été le sujet d'une étude menée par Nunthanid *et al.* en 2004. La granulation de la théophylline avec de l'acétate de chitosane séché par atomiseur a permis l'obtention des granules ayant un bon écoulement et une bonne compressibilité. Les résultats obtenus ont été comparables à ceux obtenus en utilisant le polyvinyle pyrrolidone (PVP) K30 qui est un liant pharmaceutique commercial connu. Une libération contrôlée à partir des comprimés a été obtenue dans

HCl 0,1 M, dans un tampon de pH 6,8 et dans l'eau. Les temps de libération étaient de 6 h, 16 h et 24 h, respectivement. Au contraire, les comprimés avec PVP k30 se sont désintégrés rapidement.

La N-acylation du chitosane avec des acides gras (C_6-C_{16}) favorise un caractère hydrophobe du polymère et induit un changement important au niveau de sa structure (Le Tien *et al.*, 2003). L'acylation avec des chaînes courtes a rendu le polymère plus amorphe et elle a favorisé une augmentation significative du gonflement des comprimés. L'acylation avec des chaînes plus longues (C_8-C_{16}) a favorisé la formation d'une structure plus cristalline du polymère et un faible gonflement et une résistance mécanique plus grande pour les comprimés (Fig. 3.8). Ces phénomènes ont été expliqués principalement par la destruction des liaisons d'hydrogène initialement présentes dans le chitosane et par la création des interactions hydrophobes par les chaînes de l'acide gras. Plus les chaînes sont longues, plus la stabilisation par des interactions hydrophobe est forte.
Avec l'acétaminophène comme substance active, les comprimés préparés avec du chitosane palmitoylé (C_{16}) ont montré les meilleures propriétés mécaniques et la libération la plus lente.

D'autres dérivés du chitosane, le succinate de chitosane et le phtalate de chitosane, ont été préparés dans le but de développer des matrices destinées au transport des substances actives jusqu'au côlon (Aiedeh et Taha., 1999). Des comprimés ont été préparés avec du diclofénac de sodium granulé avec l'un ou l'autre de ces deux dérivés du chitosane en présence du lactose. La libération du diclofénac a été pH dépendante, avec une très faible libération dans un milieu gastrique simulé et une libération plus accélérée à des pH plus élevés. Dans un milieu acide, les groupements carboxyliques sont protonés et donc non chargés. Cette forme protonée est moins hydrophile que la forme carboxylate et restreigne la libération. Dans des conditions neutres ou basiques, les groupements carboxyliques sont sous forme

carboxylate qui favorise l'hydratation, la solubilisation et la libération du diclofénac. Les comprimés préparés avec du chitosane natif ont été complètement solubilisés dans le fluide gastrique simulé. Pour une charge de 33 % en diclofénac, une augmentation de la proportion du dérivé du chitosane dans la formulation de 10 % à 30 % a entraîné une réduction de la vitesse de libération. Le phtalate de chitosane a permis une libération plus lente que celle obtenue avec le succinate de chitosane, parce que la fonction phtalate possédant un noyau aromatique est moins hydrophile que la fonction succinate (Aiedeh et Taha., 1999).

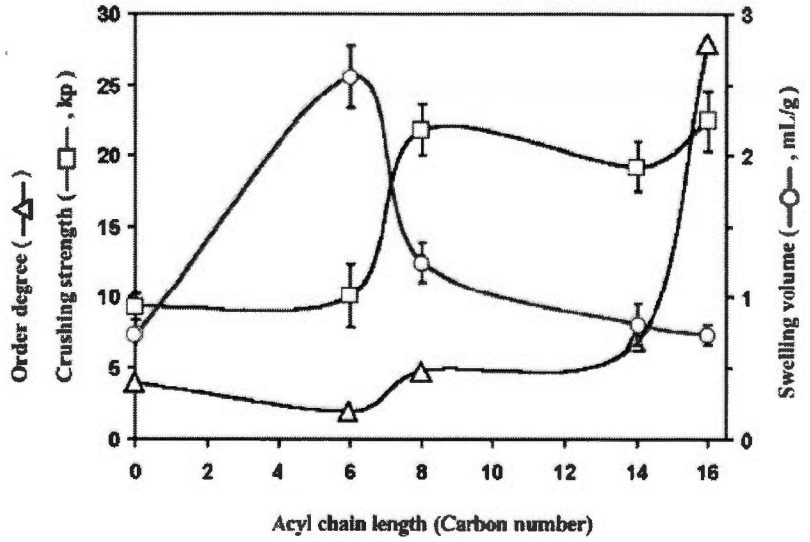

Figure 3.8 : Degré de l'ordre des chaînes (polymorphisme), dureté des comprimés et gonflement des comprimés du chitosane natif et N-acylé. (Tirée de Le Tien *et al.*, 2003).

4. Les complexes polyélectrolytes à base de chitosane

Définition du complexe polyélectrolyte

L'Union Internationale de Chimie Pure et Appliquée (UICPA, 2006) définit le complexe polyelectrolyte (CPE) de la manière suivante « Neutral polymer-polymer complex composed of macromolecules carrying charges of opposite sign causing the

macromolecules to be bound together by electrostatic interactions ». Toutefois, cette définition stricte n'est pas toujours respectée dans la littérature, où les CPE pourraient présenter certaine solubilité, certaine charge et des interactions non exclusivement électrostatiques. Généralement, le complexe formé des macromolécules stabilisées principalement par des liaisons ioniques intermoléculaires est appelé CPE (Krayukhina, Samoilova et Yamskov, 2008). Ces interactions électrostatiques peuvent coexister avec des interactions hydrophobiques, dipôle-dipôle ou Van der Waals, ainsi qu'avec des ponts d'hydrogène. Un tel système obtenu avec des polymères est considéré comme un système « intelligent ». Il a attiré l'attention des chercheurs grâce à ses propriétés uniques et à sa méthode de préparation facile.

L'obtention d'un CPE nécessite la présence de deux macromolécules de charges opposées en solution. Une formation effective du CPE a lieu juste à un pH dans l'intervalle des pK_a de deux polymères. En général, la densité des charges, le ratio des charges cationique/anionique, le degré de neutralisation et la flexibilité des chaînes des polyélectrolytes jouent des rôles importants dans la formation et les propriétés du CPE (Kekkonen, Lattu et Stenius, 2001; Gamzazade et Nasibov, 2002). D'autres paramètres peuvent aussi jouer un rôle, tels que la force d'agitation, la force ionique et la valence des ions présents dans le milieu. Les polymères qui s'ionisent complètement ont tendance à former des CPE forts (Denuziere, Ferrier et Domard, 1996). La méthode la plus simple pour préparer des CPE est le mélange des solutions aqueuses de polycations et de polyanions. La complexation a lieu par une réaction d'échange d'ions à une vitesse élevée, même si les solutions de départ sont diluées. En général, la réaction des polyélectrolytes forme un équilibre où des CPE solubles et insolubles coexistent.

Quelques approches ont été proposées pour une classification des CPE (Krayukhina, Samoilova et Yamskov, 2008) :

- CPE basés sur des polymères seulement d'origine naturelle ou seulement d'origine synthétique et CPE basés sur un mélange des polymères synthétiques et des biomacromolécules;
- CPE formés par des polyélectrolytes faibles ou forts;
- CPE stœchiométriques ou non stœchiométriques, selon leurs compositions;
- CPE solubles ou insolubles, dépendamment des conditions.

Le chitosane, étant donné son caractère cationique, a suscité beaucoup d'intérêts pour la préparation des CPE avec des différents polymères d'origine naturelle, des polymères synthétiques ou de l'ADN. La protonation des groupements amines du chitosane lui permet de former spontanément des CPE avec une large variété de polyanions. À titre d'exemple, des microsphères des CPE ont été obtenues par Muzzarelli *et al.*, (2004) en atomisant des solutions contenant du chitosane et des polyuronans tels que l'acide alginique, l'acide polygalacturonique, l'acide hyaluronique, le xanthane, la pectine, le k-carraghénane, la gomme d'acacia, la gomme de guar, le carboxyméthyl de gomme de guar, et la carboxyméthyl cellulose. Différents facteurs peuvent influencer la formation et les propriétés des CPE à base de chitosane, notamment : le DDA du chitosane, la masse moléculaire du chitosane, la distribution des groupements fonctionnels (amino et acetamido) sur les chaînes, la concentration des polymères, les ratios des différentes composantes formant le CPE, les propriétés du milieu de préparation (pH, température, force ionique) etc. Une représentation schématique (Fig. 3.9) des interactions entre le chitosane (pK_a 6,3) et l'alginate (pK_a 3,5) à pH 5,4 et à pH 2 a été proposée par Huguet *et al.*, (1994). Les caractéristiques du produit qui fait le complexe avec le chitosane peuvent elles aussi influencer les propriétés du CPE. Tous ces facteurs amènent à une large variété de CPE à base de chitosane qui ont des propriétés et des applications très diversifiées.

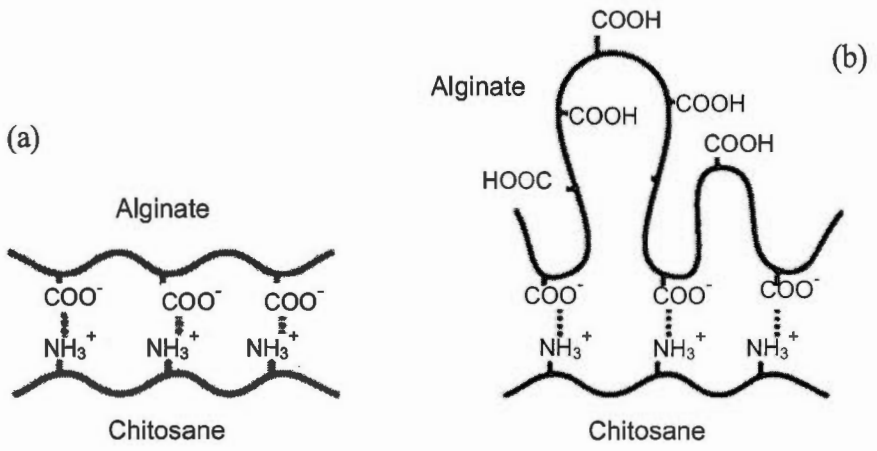

Figure 3.9 : Représentation schématique des interactions ioniques entre le chitosane et l'alginate à pH 5,4 (a) et à pH 2 (b). (Adaptée d'après Huguet *et al.*, 1994).

Exemples des complexes polyélectrolytes à base de chitosane

De nombreuses études ont été effectuées sur la complexation du chitosane avec d'autres produits. Nous présentons ci-après quelques exemples des complexes de chitosane et leurs applications biomédicales (tableau 3.2).

Le complexe chitosane-alginate est un des CPE le plus étudié. L'alginate est un polysaccharide obtenu à partir des algues brunes. Il est formé de deux types de monomères, le mannuronate ou acide mannuronique dont certaines unités sont acétylées et le guluronate ou acide guluronique. L'interaction des groupements amines du chitosane avec les groupements carboxylates de l'alginate permet une forte complexation entre ces deux polymères. La préparation du complexe chitosane-alginate se fait par un mélange des solutions aqueuses des deux polymères. Du $CaCl_2$ peut être utilisé dans la préparation, afin d'induire une réticulation de l'alginate par des interactions ioniques avec les cations bivalents Ca^{2+}.

Tableau 3.2 : Exemples des applications biomédicales des complexes à base de chitosane.

Contre-partie polyanionique du chitosane	Forme physique	Application	Référence
ADN	Nanoparticles	Transfère des gènes	Lavertu *et al.* (2006)
Alginate	Éponge	Pansement pour blessures	Kim *et al.* (1999)
	Gel injectable	Stimulation de la formation osseuse avec des cellules souches	Park *et al.* (2005)
Carboxyméthyl amidon	Comprimé	Transport des substances actives	Assaad *et al.*, (2011a, 2011b)
Carboxyméthyl cellulose	Pastille	Anticoagulant du sang	Fukuda (1980)
	Échafaudage	Régénération cellulaire	Chen et Fan (2007)
Héparine	Gel	Re-épithélialisation des plaies	Krutz *et al.* (1997)
	Nanoparticules	Transport des substances actives (protéines)	Liu *et al.* (2007)
Hyaluronate	Film	Réparation tissulaire	Denuziere *et al.* (1998)
Kératine	Film	Culture cellulaire	Tanabe *et al.* (2002)
Polyaspartate	Nanoparticules	Transport des substances actives	Zheng *et al.* (2007)
Sulfate de dextran	Pastille	Anticoagulant du sang	Fukuda et Kikuchi (1977)
	Nanoparticules	Transport (petites molécules, peptides et protéines)	Chen, Mohanraj et Parkin (2003) Chen *et al.* (2007)
Xanthane	Gel	Immobilisation des enzymes	Dumitriu *et al.* (1994) Dumitriu et Chornet (1998)

L'influence de différents paramètres sur la taille des particules des complexes chitosane-alginate de sodium (quelques nm à quelques µm) a été étudiée par Sæther *et al.*, (2008). Des chitosanes de 50 à 400 kDa (DDA 86-98 %), des alginates de 40 à 220 kDa (différentes fractions d'acide guluronique 46-69 %) et des solutions de 0,1 % ont été utilisés. Chaque solution d'alginate (pH 6,5) a été ajoutée goutte-à-goutte à une solution de chitosane (pH 4), ou inversement, sous une homogénéisation continue. La taille des particules des CPE formées a diminué avec l'augmentation de la vitesse et de la taille de l'élément dispersant de l'homogénéisateur.

Les ratios des charges des deux polymères et leurs masses moléculaires sont les deux facteurs les plus influençant sur la taille des particules, sur le potentiel zêta et sur le pH. L'ordre de mélange des deux polymères influence la taille des particules des CPE, mais pas le potentiel zêta et le pH.

Les chitosanes et les alginates de faibles masses moléculaires donnent les particules les plus fines. Le DDA et la fraction de l'acide guluronique n'ont qu'une faible influence sur les propriétés du CPE. L'augmentation du pH du CPE au dessus de 7 entraîne une augmentation remarquable de la taille des particules.

Dans le but de développer des pansements pour blessures, des films ont été préparés avec des CPE de chitosane (130, 530 et 1000 kDa) et d'alginate (100 kDa) (Yan, Khor et Lim, 2001). Le chitosane de faible masse moléculaire a réagit d'une façon plus complète avec l'alginate que le chitosane de masse moléculaire élevée. Le complexe avec le chitosane de faible masse moléculaire a formé des films moins épais et plus transparents que ceux préparés avec le chitosane de masse moléculaire élevée. De plus, un seuil de masse moléculaire a été exigé, puisque les chitosanes de 530 kDa et de 1000 kDa ont donné des films qui ont les mêmes propriétés physiques. Les films préparés étaient biocompatibles avec des fibroblastes humains où 91 % des cellules ont resté vivantes après une exposition de quatre jours.

Des éponges de CPE à base de chitosane et d'alginate de sodium contenant un agent antibactérien (sulfadiazine d'argent) ont été préparées par Kim *et al.* (1999) afin d'évaluer leurs effets dans la réparation tissulaire. La capacité antibactérienne de ces éponges utilisées comme des pansements a été testée contre des *Pseudomonas aeruginosa* et des *Staphylococcus aureus*. Il a été déduit que ce type de pansement peut protéger la blessure de l'invasion des bactéries et peut inhiber la prolifération bactérienne. Le contrôle de la vitesse de libération de l'agent antibactérien était dépendant du degré de complexation entre les deux polymères.

La dégradation des particules du complexe chitosane-alginate par du lysozyme en solution aqueuse dépend du degré de complexation entre les deux polymères (Li *et al.* 2009a). Le chitosane qui a un DDA relativement faible (62 %) a été effectivement dégradé par le lysozyme, tandis que celui qui a un DDA élevé (95 %) a été moins sensible à l'action de lysozyme. Les CPE, indépendamment du DDA du chitosane utilisé, ont montré une résistance à la dégradation par le lysozyme à cause de la forte interaction entre le chitosane et l'alginate et à cause de l'encombrement crée par l'étroite adsorption du lysozyme sur les particules du complexe.

Un taux d'immobilisation de 85-98 % a été retrouvé pour l'endo-1,4-β-xylanase de *Trichoderma viride* et pour une protéase de l'*Aspergillus sojae* en utilisant un complexe chitosane-xanthane (Dumitriu *et al.*, 1994). Ces deux enzymes ont été utilisées individuellement ou ensemble. L'activité de la xylanase immobilisée a été plus élevée que celle de la xylanase libre et le système binaire a favorisé l'activité de la protéase.

Des études de biocompatibilité du complexe chitosane-xanthane ont été effectuées par Chellat *et al.*, (2000). Des particules de l'hydrogel du complexe ont été utilisées pour évaluer *in vitro* les effets cytotoxiques sur des fibroblastes L-929 et les effets inflammatoires sur des macrophages J-774. De plus, une étude *in vivo* à été effectuée en procédant à des implantations sous-cutanées des pastilles du complexe

au niveau dorsal de rats *Wistar*. Ces études ont montré que le complexe et ses produits de dégradation ne provoquent pas des effets cytotoxiques ou des dommages cellulaires.

5. Les complexes à base de chitosane comme excipients : exemples

Dans cette section nous nous intéressons au transport des petites molécules, des peptides et des protéines formulés avec des complexes de chitosane. Plus particulièrement, des exemples sur le transport par des comprimés monolithiques seront présentés.

5.1. Le transport des petites molécules

En général, les CPE sont utilisés dans les formulations dans le but de réduire la libération de la substance active dans le milieu gastrique, de prolonger le temps de libération et certainement pour assurer une libération contrôlée. Ceci permet l'acheminement de la substance active dans l'intestin de façon qui aide à mieux profiter de ces effets thérapeutiques.

Ainsi, pour réduire la désintégration des comprimés formés du chitosane comme excipient et de la théophylline comme substance active, Mi *et al.* (1997) ont procédé à la complexation du chitosane avec de l'alginate. Ceci a permis de réduire le taux de gonflement et d'érosion des comprimés, et ensuite d'assurer une libération contrôlée de la théophylline.

Le système chitosane-alginate a été trouvé plus efficace que celui du chitosane-carraghénane pour prolonger le temps de libération du chlorhydrate de diltiazem à partir des comprimés préparés par compression directe du mélange de poudres (Tapia *et al.*, 2004). Le temps de libération et le profil de dissolution peuvent être contrôlés en modifiant la forme (mélange physique ou complexe) et le ratio du chitosane et

d'alginate. La libération à partir des matrices à base de chitosane-alginate a été contrôlée par le gonflement des polymères. La diffusion de l'eau dans les matrices à base de chitosane-carraghénane a été favorisée par le carraghénane et a été le facteur principal qui a contrôlé la libération.

Une matrice à base de complexe chitosane-pectine à été proposée afin de favoriser le transport de la vancomycine jusqu'au côlon (Bigucci *et al.*, 2008). Des microsphères du complexe ont été obtenues par atomisation, puis la forme galénique a été préparée par compression du mélange microsphères:vancomycine. Parmi les différents ratios chitosane : pectine, le ratio 1:9 a permis la mucoadhésivité la plus élevée et un gonflement pH-dépendant. Ce ratio a permis aussi la libération la plus lente, puisque la présence d'une proportion élevée de pectine favorise le développement d'un gel visqueux qui limite la diffusion de la substance active. En effet, pour un ratio élevé de pectine, les interactions entre les deux polymères est relativement faible. Ceci laisse des charges négatives libres favorisant la formation d'un gel, particulièrement à pH neutre. La libération en milieu acide a été très limitée, tandis qu'à pH neutre elle a été plus rapide à cause de l'hydratation et de la dégradation de la matrice par la β-glucosidase.

Des films du complexe chitosane-sulfate de chondroïtine ont montré une grande efficacité lorsqu'ils été utilisés pour enrober des comprimés chargés de budesonide. L'efficacité de cet enrobage est due aux interactions entre les groupements $-NH_3^+$ du chitosane et les groupements $-OSO_3^-$ et $-COO^-$ du chondroïtine qui favosisent la stabilité du complexe à pH acide (Kaur *et al.*, 2010). L'enrobage a empêché complètement la libération de la substance active à pH 1,2 *in vitro*, ensuite la libération dans un milieu alcalin a eu lieu durant 19 h avec une cinétique d'ordre zéro. Les résultats *in vivo* sur des rats *Sprague-Dawley* ont montré que la concentration de la substance active dans le sang reste stable durant une bonne période.

Un complexe chitosane-carboxyméthyl de gomme de guar a été utilisé comme liant dans la formulation de fluticasone et comme enrobant des comprimés (Kumar, Tiwary et Kaur, 2010). Avec un ratio 1:1 des deux polymères dans le complexe, les comprimés ont été capables d'empêcher la libération de la substance active dans l'estomac et le petit intestin et de la libérer au niveau du côlon. L'administration de tels comprimés a permis une réduction significative ($p < 0.05$) des colites ulcéreuses chez des rats *Sprague Dawley*. Cette étude a montré l'utilité du complexe utilisé pour assurer une libération sélective du fluticasone au niveau du côlon et pour développer une thérapie effective des inflammations en général.

Différentes études ont montré l'utilité des billes à base de complexe chitosane-alginate pour le transport des petites molécules. À titre d'exemple, des billes obtenues par gélation ionotropique en présence de calcium ont assuré des libérations prolongées du maléate de timolol (Sezer et Akbuğa, 1999) et du chlorhydrate de vérapamile (Pasparakis et Bouropoulos, 2006). Des billes de chitosane-alginate réticulés avec de génipine ont été aussi évaluées pour le transport de l'indométhacine (Mi, Sung et Shyu, 2002). De plus, des microcapsules, des microsphères et des nanoparticules préparées avec des complexes de chitosane ont été évaluées pour le transport des petites molécules. À titre d'exemple, l'encapsulation du nitrofurantoïne dans des microparticules du complexe chitosan-alginate de calcium a permis une libération contrôlée avec une faible libération dans un fluide gastrique simulé (Hari, Chandy et Sharma, 1996). Des nanoparticules du complexe chitosane-sulfate de dextran ont été considérées promettant pour le transport des petites molécules d'après des résultats obtenus en utilisant la rhodamine R6G comme traceur (Chen *et al.*, 2007).

5.2. Le transport des protéines

De nombreux travaux ont été entrepris afin d'évaluer l'efficacité des complexes à base de chitosane dans la formulation et le transport des peptides et des protéines. Les principals objectifs étaient de protéger ces produits contre la degradation par des enzymes, de retarder ou prolonger la libération et d'assurer plus d'absorption.

Des billes de 1-2 mm à base de complexe chitosane-alginate ont été testées dans l'encapsulation de l'albumine du sérum bovin (Xu *et al.*, 2007). La libération dans un fluide gastrique simulé (FGS) a été très limitée et remarquablement plus lente avec des billes doublement réticulées (avec Ca^{2+} et SO_4^{2-}) qu'avec des billes réticulées juste avec du Ca^{2+}. Dans un fluide intestinal simulé (FIS), la structure des billes réticulées avec Ca^{2+} a été détruite et la libération a été rapide. Avec la double réticulation, la vitesse de libération a diminué en réduisant le ratio citosane:alginate de 9:1 à 5:5. Une augmentation du pH du milieu de dissolution a entraîné une augmentation de la vitesse de libération.

Afin d'augmenter la charge des billes en albumine du sérum bovin ou en insuline, un système de complexe de trois polymères (chitosane, glucomannane de la gomme de konjac et alginate) à été suggéré (Wang et He, 2002). L'augmentation de la charge en substances actives a été expliquée par l'interaction électrostatique entre le glucomannane et l'alginate. À savoir, le glucomannane est un polysaccharide ramifié composé d'unités de D-mannose et de D-glucose liées par des liaisons β-(1-4) dans la chaîne principale (Katsuraya *et al.*, 2003). Les ramifications sont liées à la chaîne principale par des liaisons glycosidiques β-(1-6).

Dans le but de développer un système permettant une libération retardée de l'ovalbumine, une microencapsulation de cette protéine a été effectuée par complexation de l'alginate avec du chitosane en présence du $CaCl_2$ (Polk *et al.*, 1994). L'augmentation de la concentration de l'alginate de 1,5 à 2,5 % dans la solution de départ ou l'augmentation de la masse moléculaire du chitosane de 250 à

1250 kDa a entraîné une diminution de la vitesse de libération de l'ovalbumine. Le pH du milieu de l'encapsulation affecte lui aussi la libération, par exemple à pH 3 la libération a été plus lente qu'à pH 8.

Un complexe chitosane-alginate a été utilisé pour la microencapsulation de l'immunoglobuline de jaune d'œuf (IgY) dans le but de développer une formulation permettant l'administration de cet anticorps par voie orale (Li *et al.*, 2007). L'IgY joue le rôle d'un vaccin permettant le traitement ou la prévention des infections entériques causées par des virus ou par des bactéries. La formulation idéale devrait protéger l'IgY de l'acidité et de la pepsine dans l'estomac, ensuite elle devrait assurer sa libération dans l'intestin afin de profiter de son effet thérapeutique. Les anticorps IgY ont été ajoutés à la solution d'alginate qui à son tour a été ajoutée goutte-à-goutte sur la solution d'encapsulation contenant le chitosane et du $CaCl_2$. La charge des microcapsules en IgY n'a pas été altérée par le pH du milieu d'encapsulation (pH 3 à pH 6), tandis que l'efficacité d'encapsulation la plus élevée (74 %) a été obtenue à pH 3,5. L'activité de l'IgY n'a pas été affectée par le pH du milieu d'encapsulation et sa stabilité dans FGS (pH 1,2) a été grandement améliorée après encapsulation. De plus, l'IgY encapsulée a été efficacement protégée contre l'hydrolyse par la pepsine. Dans FIS (pH 6,8), la libération a été plus lente lorsque le pH de l'encapsulation a été plus élevé.

L'efficacité protectrice des IgY microencapsulées contre la diarrhée induite par *Escherichia coli* (K88 positive) chez des porcelets de 40 jours d'âge a été évaluée (Li *et al.*, 2009b). Le traitement avec les IgY microencapsulées a réduit significativement la diarrhée 24 h suite à l'infection, tandis que le traitement avec IgY non encapsulées a réduit la diarrhée 72 h suite à l'infection. Les témoins ont continué de souffrir de la diarrhée et de la déshydratation. De plus, le gain de poids a été plus évident chez les porcelets qui ont reçu le traitement que chez les autres groupes. Cette étude a montré que la microencapsulation des IgY avec le complexe chitosane-alginate protège

efficacement l'anticorps contre la digestion gastrique et pourrait contribuer à la prévention et le contrôle de certaines maladies.

Pour prolonger son activité biologique, un hexapeptide a été incorporé dans des nanoparticules (environ 220 nm) du complexe chitosane-sulfate de dextran par coacervation (Chen, Mohanraj et Parkin, 2003). Le ratio des masses chitosane : dextran (0,5:1 à 1:1) a été le facteur déterminant pour contrôler la taille des particules, l'efficacité de l'encapsulation et la libération. L'encapsulation maximale (75 %) et une libération soutenue ont été obtenues avec un ratio de masse 0,59:1. Cette étude a montré que les caractéristiques physico-chimiques du complexe et la vitesse de libération de la substance active peuvent être modulées en modifiant le ratio de deux polymères.

Des nanoparticules du complexe chitosane-sulfate de dextran ont été évaluées pour le transport de l'albumine du sérum bovin (Chen *et al.*, 2007). L'encapsulation maximale (98 %) et une libération soutenue ont été obtenues avec un ratio de charge amine/sulfate entre 1,2 et 1,9. Le mécanisme de libération a été basé sur un échange d'ions.

CHAPITRE IV

PRÉSENTATION DU PROJET ET CONTRIBUTIONS À LA RECHERCHE

L'amidon est un produit naturel, biocompatible et économique. Ceci justifie son utilisation courante dans le domaine pharmaceutique sous forme gélatinisée ou après une modification chimique. Un des dérivés de l'amidon est le carboxyméthyl amidon (CMA) qui était proposé en 2004 comme excipient hydrophile pour le transport de substances actives avec des comprimés (matrices) (Mulhbacher, Calinescu et Mateescu, 2004). Depuis, de nombreuses études ont été effectuées afin d'étudier les propriétés du CMA et ses avantages dans le transport et la libération d'une variété de substances actives (Calinescu *et al.*, 2005; Calinescu *et al.*, 2007; Nabais *et al.*, 2007; Calinescu et Mateescu, 2008; Massicotte, Baille et Mateescu, 2008; Brouillet, Bataille et Cartilier, 2008; Lemieux, Gosselin et Mateescu, 2009; Lemieux, Gosselin et Mateescu, 2010; De Koninck *et al.*, 2010; Assaad et Mateescu, 2010).

Ces études *in vitro* ont montré que le CMA utilisé comme excipient dans des matrices peut réduire les effets de l'acidité gastrique et de la pepsine sur les agents actifs formulés, tout en assurant une libération contrôlée. Toutefois, la solubilité élevée du CMA en milieu neutre et la sensibilité de ce polymère à la dégradation par l'α-amylase pancréatique constituent des facteurs limitants pour la libération prolongée (soutenue). Dans ce contexte, le chitosane a été utilisé comme coexcipient dans des comprimés à base de CMA afin d'augmenter le temps de libération (Leonida et Mateescu, 2006; Calinescu et Mateescu, 2008). Cependant, l'utilisation d'une proportion importante de chitosane a été nécessaire pour atteindre l'amélioration visée, en particulier avec de petites molécules. Étant donné le coût du chitosane

relativement élevé par rapport à celui du CMA, nous avons envisagé une alternative qui permet de réduire la quantité du chitosane utilisé, tout en préservant les avantages potentiels des matrices. Nous décrivons ci-après les principaux travaux effectués et les principales étapes du présent projet de thèse (Fig. 4.1).

La première étude (chapitre V) avait comme objectif d'évaluer les variations des propriétés physico-chimiques du CMA en fonction de différents paramètres : le degré de carboxyméthylation du CMA, le degré de protonation des groupements carboxyméthyles et le pH du milieu de dissolution.

Deux raisons principales ont encouragé l'entreprise de cette étude, à savoir le caractère anionique du CMA et la variation du pH dans le tractus gastro-intestinal. La variation du pH, acide dans l'estomac et relativement neutre dans l'intestin, affecte l'ionisation du polymère et la solubilisation/érosion de la matrice, ainsi que la vitesse de libération de la substance active formulée. L'acétaminophène a été utilisé comme substance active modèle, principalement en raison de son pK_a de 9,5 qui est supérieur au pH du fluide gastrique simulé (FGS, pH 1,2) et à celui du fluide intestinal simulé (FIS, pH 6,8), réduisant ainsi la différence de solubilité de cette molécule dans les deux milieux et l'interférence de cette différence avec les résultats. Cette étude qui a permis une meilleure compréhension des cinétiques de libération à partir des matrices hydrophiles à base de CMA a été le sujet d'un article publié en 2010 dans *International Journal of Pharmaceutics* sous le titre «The influence of protonation ratio on properties of carboxymethyl starch excipient at various substitution degrees: Structural insights and drug release kinetics».

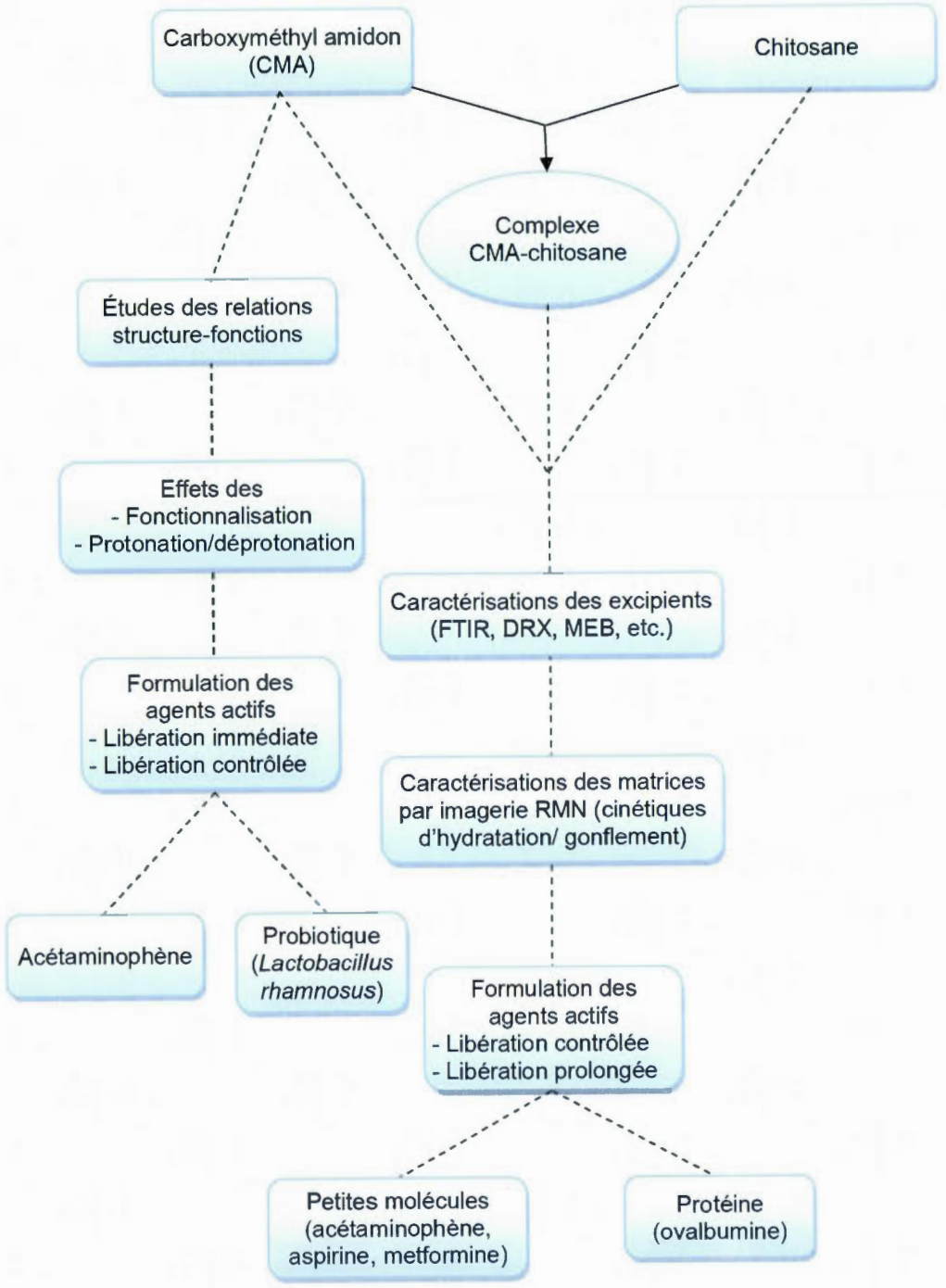

Figure 4.1 : Présentation schématique du projet.

Le CMA a été ensuite comparé à l'hydroxypropylméthyl cellulose (HPMC) qui est un des excipients les plus utilisés dans les matrices hydrophiles à libération contrôlée. Cette comparaison a permis d'évaluer le seuil du pourcentage du polymère nécessaire pour assurer une libération contrôlée de l'acétaminophène utilisé comme molécule modèle. Dans FGS, les matrices à base de CMA ont montré qu'elles peuvent assurer une protection partielle des *Lactobacillus rhamnosus* formulés, malgré la grande sensibilité de ces probiotiques à l'acidité. Cette étude présentée au chapitre VI a été réalisée en collaboration avec la société *Gestion Valéo* (Montréal) et avec la compagnie *Grain Processing Corporation* (États unis).

Afin d'obtenir des libérations prolongées avec des matrices à base de CMA, le CMA a été complexé avec du chitosane, permettant d'avoir un complexe polyélectrolyte (CPE) moins soluble à pH neutre et moins dégradable par l'α-amylase pancréatique que le CMA. Les propriétés et la performance de ce nouveau complexe dans la libération des agents actifs à partir des comprimés ont été comparées à celles du CMA et du mélange physique CMA:chitosane.

Des analyses par imagerie RMN sur des matrices non chargées ont été effectuées au département de chimie de l'Université de Montréal dans le cadre d'une collaboration avec professeur X. X. Zhu. Après 2 h d'incubation, le FGS n'a diffusé que partiellement dans les matrices du CMA et du CPE, indiquant que les deux excipients utilisés ont la capacité de protéger des agents actifs formulés. La diffusion du FIS dans les matrices du CPE et le gonflement de ces matrices ont été plus faibles que dans le cas des matrices du CMA, montrant qu'à pH neutre le CPE s'hydrate plus lentement que le CMA et il pourrait ainsi favoriser un temps de libération plus longue. Les résultats des analyses par imagerie RMN ont été publiés en 2011 dans *International Journal of Pharmaceutics* sous le titre «NMR imaging of chitosan and carboxymethyl starch tablets: Swelling and hydration of the polyelectrolyte complex». Cet article est présenté dans la section « Annexes ».

Trois petites molécules (acétaminophène, aspirine et chlorhydrate de métformine) présentant des propriétés chimiques différentes ont été choisies comme modèles pour évaluer les cinétiques de libération à partir des matrices. Il a été conclu que le CPE peut assurer une libération plus lente que celle obtenue avec CMA ou avec un mélange physique CMA:chitosane contenant la même proportion de chitosane. Cette étude présentée au chapitre VII a été le sujet d'un article publié en 2011 dans *Carbohydrate Polymers* sous le titre «Polyelectrolyte complex of carboxymethyl starch and chitosan as drug carrier for oral administration».

Le CMA et le CPE ont été aussi utilisés pour formuler l'ovalbumine qui est une protéine qui se dégrade rapidement sous l'action de la pepsine gastrique et qui sert comme antigène modèle dans des études sur la vaccination par voie orale. L'étude a été menée en collaboration avec Monsieur M. Lessard d'Agriculture et Agroalimentaire Canada (Lennoxville). Cette étude présentée au chapitre VIII a été publiée en 2011 dans *Journal of Biomaterials Science, Polymer Edition* sous le titre «Polyelectrolyte complex of carboxymethyl starch and chitosan as protein carrier: Oral administration of ovalbumin».

Les matrices à base de CMA ont offert une très bonne protection pour l'ovalbumine dans FGS et ont assuré une libération contrôlée. La protection a été améliorée et la libération a été prolongée en utilisant des matrices à base de CPE. Les résultats obtenus sont très intéressants, puisqu'ils peuvent entrer dans le cadre de développement des vaccins administrés par voie orale qui représentent une alternative économique et pratique à la vaccination parentérale (par injection).

En résumé, nous avons montré que l'utilisation du CMA comme excipient principal dans des comprimés permet l'obtention des formulations pharmaceutiques pouvant assurer des durées de libération assez différentes des agents actifs formulés, simulant ainsi le transport à différentes parties du tractus gastro-intestinal.

Principales contributions à la recherche durant le projet de doctorat

Brevets/droits de propriété intellectuelle

Elias Assaad et Mircea Alexandru Mateescu, Carboxymethyl starch and chitosan polyelectrolyte complexes. 2011, US Patent application, 61/434142.

Articles avec comité de lecture

(Présentés dans la partie expérimentale ou en annexes)

Assaad, E. et M. A. Mateescu. 2010. «The influence of protonation ratio on properties of carboxymethyl starch excipient at various substitution degrees: Structural insights and drug release kinetics». *International Journal of Pharmaceutics*, vol. 394, p. 75-84.

Assaad, E., Y. J. Wang, X. X. Zhu et M. A. Mateescu. 2011. «Polyelectrolyte complex of carboxymethyl starch and chitosan as drug carrier for oral administration». *Carbohydrate Polymers*, vol. 84, p. 1399-1407.

Assaad, E., L. Blemur, M. Lessard et M. A. Mateescu. 2011. «Polyelectrolyte complex of carboxymethyl starch and chitosan as protein carrier: Oral administration of ovalbumin». *Journal of Biomaterials Science, Polymer Edition*, en impression, JBS-D-11-00080.

Wang, Y. J., E. Assaad, P. Ispas-Szabo, M. A. Mateescu et X. X. Zhu. 2011. «NMR imaging of chitosan and carboxymethyl starch tablets: Swelling and hydration of the polyelectrolyte complex». *International Journal of Pharmaceutics*, vol. 419, p. 215-221.

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(Présentés en annexes)

Assaad, E., A. Azzouz et M. A. Mateescu. 2008. «Influence of bentonite on drug release by sodium carboxymethyl starch excipients». Transaction of the *35th Annual Meeting & Exposition of CRS*, New York, Extended abstract, # 512.

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Assaad, E. et M. A. Mateescu. 2010. «Effet du ratio de protonation et du degré de substitution sur les propriétés du carboxyméthyl amidon utilisé comme excipient». *78e Congrès de l'Acfas*, Université de Montréal, Montréal.

Assaad, E., L. Blemur et M. A. Mateescu. 2011. «Carboxyméthyl amidon et chitosane comme excipients pour le transport d'agents bioactifs par des comprimés administrés par voie orale». *79e Congrès de l'Acfas*, Université de Sherbrooke, Sherbrooke.

Assaad, E., Y. J. Wang, X. X. Zhu et M. A. Mateescu. 2011. «Complexe carboxyméthyl amidon-chitosane comme nouvel excipient pour le transport des substances actives : Analyse par imagerie RMN». *3e Colloque de Pharmaqam*, Université du Québec à Montréal, Montréal.

Assaad, E., Y. J. Wang, X. X. Zhu et M. A. Mateescu. 2011. «Complexe carboxyméthyl amidon-chitosane comme nouvel excipient : Cinétiques d'hydratation analysées par imagerie RMN». *1ère journée scientifique du Réseau Québécois de Recherche sur les Médicaments*, Université de Montréal, Montréal.

Contributions des auteurs des articles

Article 1 : *E. Assaad et M. A. Mateescu, 2010, International Journal of Pharmaceutics, vol. 394, p. 75-84.*

L'étude a été proposée par les deux auteurs de l'article. E. Assaad a planifié les expérimentations et a mené le travail expérimental au laboratoire du professeur M. A. Mateescu (Laboratoire d'Enzymologie et des Polymères Biocompatibles) au département de chimie de l'Université du Québec à Montréal (UQAM). Des équipements du département de chimie de l'UQAM ont été également utilisés pour la réalisation d'une partie du travail expérimental. Le suivi des travaux a été assuré par professeur Mateescu d'une façon continue. L'article a été rédigé par E. Assaad, ensuite les révisions ont été effectuées conjointement par les deux auteurs.

Article 2 : *E. Assaad, Y. J. Wang, X. X. Zhu et M. A. Mateescu. 2011. Carbohydrate Polymers, vol. 84, p. 1399-1407.*

L'idée de l'association du carboxyméthyl amidon et du chitosane dans des matrices hydrophiles a été proposée par professeur M. A. Mateescu et par E. Assaad. Suite à une contribution du docteur A. Richard de la société *Gestion Valéo*, cette étude a fait l'objet d'une demande de brevet à *US Patent* en 2011. La partie pratique a été principalement réalisée au Laboratoire d'Enzymologie et des Polymères Biocompatibles à l'UQAM. Des appareils du département de chimie et du département des sciences de la terre et de l'atmosphère de l'UQAM ont aussi servi à la réalisation de certaines analyses. Les analyses par imagerie RMN ont été effectuées à l'Université de Montréal dans le cadre d'une collaboration avec professeur X. X. Zhu qui dirige un laboratoire de recherche en chimie des polymères au département de chimie de l'Université de Montréal et qui détient la chaire de recherche du Canada en biomatériaux polymères.

La majorité du travail (préparation et caractérisation des excipients et des formulations, tests de dissolution, présentation et analyse des résultats) a été effectuée par E. Assaad. Les analyses par imagerie RMN ont été réalisées par Y. J. Wang et par E. Assaad au département de chimie de l'Université de Montréal. Le suivi de tout le travail a été assuré par professeur Mateescu. L'article a été rédigé par E. Assaad et révisé par professeur Mateescu, ensuite il a été révisé de nouveau par tous les auteurs.

Article 3 : *E. Assaad, L. Blemur, M. Lessard et M. A. Mateescu. 2011. Journal of Biomaterials Science, Polymer Edition, en impression, JBS-D-11-00080.*

Cette étude fait partie d'un large projet financé par CRIP (Centre de recherche en infectiologie porcine - un regroupement stratégique FQRNT, Faculté de médecine vétérinaire de l'Université de Montréal) et mené par collaboration entre docteur M. Lessard (Agriculture et Agroalimentaire Canada, Lennoxville), professeur M. A. Mateescu (département de chimie de l'UQAM), professeur D. Archambault

(Département des sciences biologiques de l'UQAM) et professeur M. Subirade (Département des sciences des aliments et de nutrition de l'Université Laval).

L'article a été le fruit d'un travail réalisé principalement au Laboratoire d'Enzymologie et des Polymères Biocompatibles à l'UQAM. Des équipements du département de chimie de l'UQAM ont aussi servi à la réalisation de certaines analyses. Les tests de mucoadhésion ont été effectués chez *Corealis Pharma* à Laval. Une grande partie du travail expérimental (tests de dissolution, électrophorèse sur gel de polyacrylamide et transfert de protéines (Western blot), et tous les autres tests et analyses) a été menée par E. Assaad qui a aussi planifié les expérimentations et rédigé l'article. L. Blemur a planifié et a contribué efficacement aux expérimentations de l'électrophorèse et de Western blot. Le suivi des travaux et la révision de l'article ont été assurés par professeur Mateescu. L'article a été ensuite révisé par tous les auteurs.

Article 4 : *Wang, Y. J., E. Assaad, P. Ispas-Szabo, M. A. Mateescu et X. X. Zhu. 2011. International Journal of Pharmaceutics, vol. 419, p. 215-221.*

Cet article a été le fruit d'une collaboration entre le laboratoire du professeur X. X. Zhu au département de chimie de l'Université de Montréal et le laboratoire du professeur M. A. Mateescu (Laboratoire d'Enzymologie et des Polymères Biocompatibles, département de chimie, UQAM).

Les analyses par imagerie RMN et la rédaction de l'article ont été effectuées par Y. J. Wang à l'Université de Montréal. P. Ispas-Szabo a préparé l'amidon réticulé et E. Assaad a réalisé différentes tâches (purification et caractérisation du chitosane, préparation et caractérisation du carboxyméthyl amidon (CMA) et du complexe CMA-chitosane, et l'étude des gonflements apparents des comprimés). La révision de l'article a été effectuée par tous les auteurs, principalement par professeur Zhu.

PARTIE II - PARTIE EXPÉRIMENTALE

CHAPITRE V

INFLUENCE DU RATIO DE PROTONATION ET DU DEGRÉ DE SUBSTITUTION SUR LES PROPRIÉTÉS DU CARBOXYMÉTHYL AMIDON : APERÇUS DE LA STRUCTURE ET DES CINÉTIQUES DE LIBÉRATION DES SUBSTANCES ACTIVES

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Résumé

Du carboxyméthyl amidon (CMA) ayant une sensibilité au pH modulée par le ratio de protonation (0-100%) et par le degré de substitution (DS 0,07-0,20) a été préparé dans un milieu aqueux. Les propriétés du CMA utilisé comme excipient et les mécanismes de libération de l'acétaminophène à partir des comprimés monolithiques dans un fluide gastrique simulé (FGS, pH 1,2) et dans un fluide intestinal simulé (FIS, pH 6,8) ont été étudiés. La forme protonée du CMA a permis une libération plus lente que celle obtenue avec la forme sodium. Le temps de libération a augmenté avec l'augmentation du pourcentage de protonation. Avec le temps, les CMA qui ont des ratios de protonation et des DS élevés sont devenu de moins en moins solubles et des modifications progressives de leurs structures ont été observées. Simultanément, une accélération de la libération de l'acétaminophène formulé avec ces excipients a été observée. Ce phénomène a été expliqué par l'implication des groupements carboxyles dans des liaisons d'hydrogène inter- et intra-moléculaire. Le CMA de DS 0,11 avec une protonation allant jusqu'au 50% a montré une faible sensibilité au pH du milieu de dissolution et une libération relativement prolongée quasiment indépendante de la préincubation du comprimé dans FGS et de la charge en substance active (20% et 40%). Le CMA de DS 0,20 a été plus sensible au pH et a montré une libération accélérée dans FIS. La libération de l'acétaminophène à partir des comprimés de CMA a été contrôlée par un mécanisme de diffusion dans FGS et par le gonflement et l'érosion dans FIS.

Mots clés : carboxyméthyl amidon, excipient, ratio de protonation, degré de substitution, comprimé monolithique, transport des subastances actives.

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The influence of protonation ratio on properties of carboxymethyl starch excipient at various substitution degrees: Structural insights and drug release kinetics

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Abstract

Carboxymethyl starch (CMS) with pH sensitivity modulated by the protonation ratio (PR 0–100%) and the degree of substitution (DS 0.07–0.20) was synthesized in aqueous medium. The properties of CMS excipient and the mechanism of acetaminophen release from monolithic tablets in simulated gastric fluid (SGF, pH 1.2) and in simulated intestinal fluid (SIF, pH 6.8) were investigated. Compared to sodium CMS, the protonated CMS provided a longer release time which increases with PR. Over storage time, the highly protonated CMS showed decrease of solubility and progressive structural alteration due to hydrogen bonded carboxyl groups. Simultaneously, the release rate of formulated drug was accelerated. The CMS(DS 0.11) with PR up to 50% showed relatively low sensitivity to dissolution medium pH and sustained release pattern almost independent of tablet preincubation in SGF and of drug loading (20% and 40%). The CMS(DS 0.20) was more sensitive to pH and showed an accelerated release rate in SIF. For the CMS formulations, a diffusion mechanism was suggested in SGF, whereas in SIF the release was mostly controlled by swelling and erosion.

Keywords: carboxymethyl starch, excipient, protonation ratio, degree of substitution, monolithic tablet, drug delivery.

Abbreviations: CMS, carboxymethyl starch; SGF, simulated gastric fluid (pH 1.2); SIF, simulated intestinal fluid (pH 6.8); DS, degree of substitution (the average number of carboxymethyl groups per glucose unit); PR, protonation ratio as percentage of carboxyl groups (–COOH) in the sum of carboxylate (–COONa) and carboxyl groups; $S_{control}$, starch treated with NaOH to obtain the control of CMS samples; S_g, gelatinized starch; $t_{90\%}$, time (h) for the release of 90% of drug.

1. Introduction

Despite the advent of synthetic biodegradable polymers, the use of natural biodegradable polymers as excipients in drug formulation is still prefered. One of the most available, renewable and versatile natural polymers is starch which is composed of two polysaccharides: amylose (unbranched-chain structure with glucose units joined by α-1,4 linkages) and amylopectin (branched-chain structure with glucose sequences joined at branching points by α-1,6 linkages). Starch is susceptible to physical, enzymatic and chemical modifications that can modulate its properties according to intended applications. For instance, chemical modifications of starch involve esterification, etherification or oxidation of the hydroxyl groups of glucose units in aqueous or organicmedium. Although the latter procedure permits a higher substitution, salts and modifying reagent by-products can still remain in the final material (Chiu and Solarek, 2009).

Carboxymethyl high amylose starch (CM-HAS) was proposed as a novel pH sensitive excipient for oral delivery of bioactive agents (Mulhbacher et al., 2004b). Carboxymethyl starch is not a new product. Indeed, it was known in reticulated form and mostly used as disintegrant (e.g. Primojel®, Explotab®) (Edge et al., 2002). In acid (gastric) fluid the carboxyl groups seem to enhance the stability of tablet by dimerization and by hydrogen bonds. When tablet is transferred in neutral (intestinal) fluid, the matrix becomes more hydrated due to the exchange of protons with cations. This hydration facilitates the swelling of the matrix and the release of bioactive agent (Mulhbacher et al., 2004a; Calinescu et al., 2005; Calinescu et al., 2007). The increase of the degree of substitution (DS 0.03–0.25) of CM-HAS synthesized in aqueous medium favors a longer drug release time form matrices based on this CM-HAS (Lemieux et al., 2007).

Three main processes were identified in tablets based on swellable polymers: swelling, diffusion and erosion. These processes correspond respectively to fronts

generated at the interface between the dry central core and the hydrated gel region, at the locus where the drug concentration forms a maximal gradient, and at the contact region between tablet and dissolution medium (Colombo et al., 1999; Barba et al., 2009).

It was shown that a protonated carboxymethyl starch presented a better gastro-resistance than its sodium salt form (Ispas-Szabo et al., 2007). However, no investigations were carried out on the influence of percentage of protonation at different degrees of substitution. Recently, it was reported that the sodium salt form of CM-HAS synthesized in non-aqueous medium is preferred for controlled release, whereas the protonated form presented a fast release (Lemieux et al., 2009).

Considering the effect of carboxyl/carboxylate groups on matrix stabilization, on water uptake, on swelling and gel forming, and on tablets erosion, it is of interest to elucidate the role of protonation/deprotonation of carboxymethyl starch (CMS) in drug delivery. Hence, the aims of the present study on CMS synthesized in aqueous medium are (i) to evaluate the effect of substitution and of protonation degrees on CMS properties, (ii) to investigate the effect of pH of dissolution medium on the mechanism and the rate of drug release by CMS, and (iii) to establish the advantageous parameters, protonation ratio (PR) and degree of substitution, for the desired rate or mechanism of drug release from CMS formulations.

To our knowledge, this is the first investigation on the effect of protonation degree on the properties of CMS excipient and the first detailed study on the effect of DS on kinetic drug release by CMS synthesized in aqueous medium. Comprehension of the role of PR and DS will facilitate obtaining formulations permitting better drug delivery at various sites of intestinal tract.

2. Materials and methods

2.1. Reagents and chemicals

High Amylose Corn Starch (Hylon VII) was provided by National Starch (Bridgewater, NJ, USA). Sodium chloroacetate (SCA, 98%) and acetaminophen were from Sigma–Aldrich (St-Louis, MO, USA). The other chemicals were of reagent grade and used without further purification. Pepsin-free simulated gastric fluid (SGF, pH 1.2) and pancreatin-free simulated intestinal fluid (SIF, pH 6.8) were prepared following the USP (US *Pharmacopeia*, XXIV, 2000).

2.2. Synthesis of sodium CMS

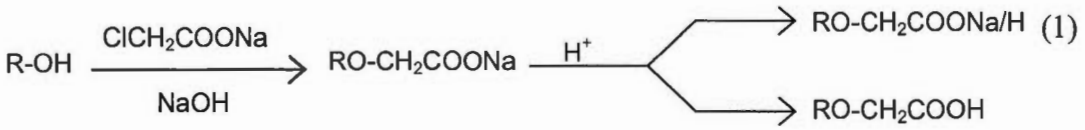
Sodium carboxymethyl starch [CMS(Na) or CMS(PR 0%)] was prepared in aqueous medium from high amylose corn starch. A jacketed beaker (2 L) and a heating circulator bath (*HAAKE*, D1, Berlin, Germany) were used to ensure a constant temperature (55 °C) during the synthesis. An amount of 140 g of starch was dispersed in 340 mL of distilled water under continuous vertical stirring (*Servodyne* Mixer, 50000-40, IL, USA). Then, 470 mL of 1.5M NaOH were added and the stirring was maintained (30 min) for gelatinization. The carboxymethylation (nucleophilic substitution) occurred by adding sodium chloroacetate to the alkaline (0.9M NaOH) mixture. After 1 h of reaction, a volume of 540 mL of distilled water was added, the mixture was cooled-down, and the reaction was stopped by neutralization with acetic acid. The synthesized CMS(Na) was precipitated with methanol and washed repeatedly with 2 L of 80% methanol until a conductivity of about 50 μS/cm was reached. CMS(Na) was then washed with methanol 40%/acetone 60%, dried at 40 °C for 24 h, and sieved on a 300 μm screen. Different amounts (70, 78, 84, 112 and 208 g) of SCA were used in similar conditions to obtain CMS(Na) at

various degrees of substitution. Except CMS with DS(0.20), the CMS presented small granulometry and no grinding was necessary.

The control ($S_{control}$) was prepared following the same procedure as for CMS preparation, but without adding SCA. To prepare gelatinized starch (S_g), 10M NaOH was added until a final concentration of about 2M in the mixture.

2.3. Preparation of CMS with different protonation ratios

Each sample of CMS(Na) with a specific DS was dispersed in 80% methanol and thepHwas then adjusted with acid solutions in order to obtain derivatives with various protonation ratios (up to 100%), as follows:



(1)

The PR(100%) or CMS(H) was obtained by maintaining the pH at 1.5 for 1 h using 10% HCl, whereas the other PR were obtained by adjusting the pH at 5.8, 5.2, 4.1, 3.9 and 3.6 with (4% acetic acid + 8% HCl) solution maintaining overnight agitation. The samples were filtered and the wet slurries were each washed with 400 mL of 80% methanol and then with (methanol 40%/acetone 60%) as mentioned so far. Finally, samples were air-dried overnight at 40 °C and sieved to retain particles smaller than 300 μm. The $S_{control}$ and the S_g were treated in the same way as CMS(Na) in order to obtain $S_{control}$ (PR 50%), $S_{control}$ (PR 100%), S_g (PR 50%) and S_g (PR 100%). All the samples used in this study were kept under the same conditions in screw top containers stored at room temperature.

Conversion of CMS(PR 100%) to sodium salt form was done by solubilization of 40 mg of polymer in 2 mL of 0.1M NaOH under overnight agitation. Then 20 mg

of NaCl were added and solubilized by mixing for 2 min. Finally, the CMS was precipitated and washed as described so far, then dried at room temperature for 2 days.

2.4. Characterization of unmodified and modified starch samples

2.4.1. FTIR analysis

The carboxymethylation of starch and the protonation status of CMS were assessed by Fourier Transform Infrared (FTIR) spectroscopy (*Nicolet* 4700, Madison, WI, USA). The spectra were recorded from 4000 to 400 cm^{-1} at 2 cm^{-1} resolution and with a total of 32 scans. The pellets were made with a homogenous mixture of dried KBr (67 mg) and polymer sample (3 mg). The compression at 3 tonnes was done in flat-faced punches with 12 mm diameter by using a hydraulic *Carver* press (Wabash, IN, USA).

2.4.2. DS, PR and pK_a

Pure CMS(PR 100%), washed until a conductivity of less than 15µS/cm, was used to determine the DS by back-titration. An amount of 300 mg of CMS(PR 100%) was solubilized in 20 mL of 0.05M NaOH (n = 3). Subsequently, the excess of NaOH was determined by titration with 0.05M HCl using phenolphthalein as indicator. Blank (n = 3) consisting of 20 mL of 0.05M NaOH was also titrated. The amount of –COOH groups and the DS were calculated as described by Stojanovic et al. (2005) using the following equations:

$$n_{COOH} = (V_b - V) \times C_{HCl} \qquad (2)$$

$$DS = \frac{(162 \times n_{COOH})}{m - 58 \times n_{COOH}} \qquad (3)$$

where V_b (mL) is the volume of HCl used for the titration of the blank; V (mL) is the volume of HCl used for titration of the sample; C_{HCl} (mol/L) is the concentration of HCl; 162 (g/mol) is the molar mass of glucose unit; 58 (g/mol) is the increase in the mass of glucose unit by substitution with one carboxymethyl group, and m (g) is the mass of dry sample.

The protonation ratios (PRs) were determined by titration of samples by the same method as for DS. The pK_a were determined by direct titration (n = 2) as follows: an amount of 300 mg of CMS(PR 100%) was dispersed in 150 mL of distilled water, and titrated until pH 11 with 0.1M NaOH. Finally, pH was plotted against the volume of added NaOH to obtain the pK_a value at half-equivalence point.

2.4.3. Tapped density, solubility and polymorphism of powders

The tapped densities of the samples were measured according to the USP (616) method by using *Varian's Vankel* tapped density tester (NC, USA). The solubilities were determined by dispersing 150 mg of polymer powders in 6 mL of SGF or of SIF and stirring (*Thermolyne*, 37600 Mixer, Dubuque, IA, USA) at full speed for 3min and 3 times to ensure that all the soluble fractions were solubilized.
The obtained mixture was centrifuged (20 min, 4000 rpm) at room temperature, and then 1.5 mL of supernatant were evaporated at 65 °C until a constant mass was reached. Controls were prepared similarly with SGF or SIF only (without polymer). The weights of dried samples and of controls were used to calculate the solubilities.

The polymorphism of samples was evaluated by X-ray diffractometer (XRD, *Siemens* D5000, Munich, Germany) at 1.789Å wavelength. The original XRD spectra, recorded between 5° and 50° (2-theta), were treated using Excel software (regression type: moving average, period 10).

2.5. Tablet preparation and crushing strength measurement

Monolithic tablets [200 mg, 20% or 40% (w/w) loading] were obtained by direct compression of a homogenous mixture of excipient and acetaminophen powders (flat-faced punches with 9.6 mm diameter, 2.5 tonnes, *Carver* hydraulic press). Unloaded tablets of 200 mg were prepared with excipient only and without acetaminophen. The monolithic tablets of 500 mg with 20% and 40% acetaminophen loading were prepared by the same way using punches of 12 mm diameter. The crushing strength (n = 6) was measured with a Tablet Hardness Tester (*Varian*, VK 200, *Cary*, NC, USA).

2.6. *In vitro* dissolution tests

The *in vitro* dissolution tests were carried out at 100 rpm and 37 °C using an USP dissolution apparatus II (*Distek* 5100, North Brunswick, NJ, USA) coupled with an UV spectrophotometer (*Hewlett Packard* 8452A). The acetaminophen release from tablets (n = 3) in 900 mL of enzymes-free dissolution media was measured at 244 nm. The dissolution was followed: (i) in SGF until complete drug release, (ii) in SIF until complete drug release, and (iii) in SGF for 2 h and then in SIF up to complete release.

The drug release kinetics was evaluated following the equation described by Peppas (1985):

$$\frac{M_t}{M_\infty} = kt^n \qquad (4)$$

where Mt/M_∞ is the fraction of drug released at the time t and k is a kinetic constant incorporating the properties of the matrix, the properties of the drug, and the geometric characteristics of the dosage form. The release exponent (n) is characteristic of the drug release mechanism, where n = 0.45 suggests a diffusion-

controlled release (Fickian diffusion), n = 0.89 a swelling-controlled release, and n between 0.45 and 0.89 indicates an anomalous diffusion (Wei et al., 2009). The $\log(M_t/M_\infty)$ was plotted against log(t) up to 90% of release to obtain the values of n, k and the correlation coefficient (R^2).

2.7. Erosion and fluid uptake

Erosion and fluid (SGF or SIF) uptake by the unloaded tablets were evaluated in the same conditions as for dissolution tests (100 rpm, 37 °C). The tablets (200 mg) were incubated for 2 h in SGF or in SIF. They were then removed from the media, blotted with tissue paper to eliminate the water excess on surface, and weighed before (wet tablets) and after freeze-drying (dried tablets).

The percentage of erosion and the percentage of fluid uptake by unit of remaining polymer were calculated as described per Kavanagh and Corrigan (2004) and Calinescu et al. (2007):

$$\%\,Erosion = \frac{(W_i - W_d)}{W_i} \times 100 \qquad (5)$$

$$\%\,Fluid\,uptake = \frac{(W_w - W_d)}{W_d} \times 100 \qquad (6)$$

where W_i is the initial weight of the tablet, W_w is the weight of wet tablet and W_d is the weight of dried tablet.

3. Results and discussion

The properties of the obtained CMS materials were studied and their efficiencies as excipients for acetaminophen controlled release from monolithic tablets were evaluated. Unless other indications are present, the tablets are of 200 mg with 20% loading.

3.1. Characterization of CMS (carboxymethylation, DS, PR, pK_a)

The FTIR analysis revealed no difference between the spectrum of native starch (Hylon VII) and those of starch after gelatinization ($S_{control}$ and S_g), whereas carboxymethylation and protonation of CMS generated some characteristic bands. The patterns of $S_{control}$ as an example of unsubstituted starch, and that of CMS(DS 0.11) as an example of carboxymethylated starch are presented in Fig. 1. The CMS(PR 0%) presents two new characteristic bands, one at 1417cm^{-1} and one at 1603 cm^{-1} which overlaps that at 1643 cm^{-1}. For totally protonated form (PR 100%), no band was seen at 1603 cm^{-1}, while a new band appeared at 1735 cm^{-1}. The bands at 1417 and 1603 cm^{-1} were attributed to symmetrical and asymmetrical stretching vibration of $-COO^-$ groups, whereas those at 1643, and 1735 cm^{-1} were assigned to –OH groups and –COOH groups, respectively (Yang, 1991; Silverstein et al., 2005; Zoldakova et al., 2005). The CMS(PR 50%) presented the bands corresponding to $-COO^-$ groups and to –COOH groups, but with lesser intensity compared to those of CMS(PR 0%) and CMS(PR 100%), respectively (Fig. 1). The FTIR patterns confirmed that CMS(PR 0%) was under carboxylate form and that CMS(PR 100%) presented no more carboxylate form (as proof of total protonation), whereas CMS(PR 50%) presented both groups.

The DS of CMS samples determined by back-titration were 0.07, 0.09, 0.11, 0.14 and 0.20. These values correspond respectively to 0.42, 0.55, 0.68, 0.83 and 1.15 milliequivalents of functional groups per gram of polymeric powder (meq./g). The determination of pK_a (4.8–5.0) was done in order to facilitate the choice of pH interval for protonation. Thus, the pH values were adjusted at approximately 5.8, 5.2, 4.1, 3.9, 3.6 and 1.5 to obtain PR of about 10%, 20%, 50%, 70%, 85% and 100%, respectively.

3.2. Effect of DS and of PR on drug release time

The times (h) for the release of 90% of acetaminophen ($t_{90\%}$) were obtained from dissolution kinetics in SGF or in SIF (Fig. 2). For all CMS variants, the release was longer in SGF than in SIF. Also, for each CMS with a specific DS, the $t_{90\%}$ increased with protonation ratio in both SGF and SIF and the major increase occurred at PR lesser than 50%. In addition, fast release and disintegration (less than 30 min) were seen in all cases for $S_{control}$ (Fig. 2 – insert). The explanation of this disintegration may be that the temperature (40 °C) and the concentration of NaOH (0.09M) used to prepare $S_{control}$ were not high enough to produce a soluble gelatinized starch.

In SGF, for the same protonation ratio, an increase of DS makes the release faster (Fig. 2A), probably due to a higher solubility at increasing number of carboxymethyl groups. An important output is that a low substitution (DS 0.07) generated the longest release time (10.8–12.8 h), whereas unsubstituted ($S_{control}$) starch was rapidly disintegrated. This suggests that a low DS generates major difference in release behavior. This phenomenon is relatively comparable to that of crosslinked starch when low crosslinking drastically enhances the release time (Ispas-Szabo et al., 2000).

In SIF, the CMS(DS 0.11) showed the longest $t_{90\%}$ and a release rate less affected by protonation than those obtained with DS(0.07) and DS(0.20) (Fig. 2B). Thus, when PR changed from 0% to 100%, the $t_{90\%}$ of CMS(DS 0.07) increased from 2.5 to 6.3 h (a factor of 2.5-fold) and that of CMS(DS 0.20) from 2.7 to 7 h (2.6-fold), while the $t_{90\%}$ of CMS(DS 0.11) increased from 5.3 to 8 h (only 1.5-fold).

For each CMS, the increase of $t_{90\%}$ with protonation ratio is less pronounced in SGF than in SIF, because the acidity of SGF contributes to the protonation of –COONa groups reducing thus the differences between different preprepared PR.

The preparation of CMS from low gelatinized starch ($S_{control}$) permits a better examination of the influence of carboxymethyl groups on the properties of CMS excipients. When CMS was prepared from S_g, even a very low DS(0.03) was enough to achieve controlled release of acetaminophen, but a higher substitution (about 0.07) was still required to prevent tablet cracks (data not shown).

3.3. Effect of storage time on the properties of totally protonated CMS

Although the highly protonated CMS presented the lowest release rates, acceleration of the release was observed in function of samples storage time, especially for high DS. This acceleration was examined over 4 weeks for CMS(DS 0.11, PR 100%) and CMS(DS 0.20, PR 100%) as examples of totally protonated CMS. Thus, after 28 days of storage, a decrease of $t_{90\%}$ by 10% in SGF and by 21% in SIF was found for CMS(DS 0.11), whereas for CMS(DS 0.20) the decrease was about 46% in SGF and 71% in SIF (Fig. 3).

In an attempt to elucidate the origin of this change of $t_{90\%}$ over storage time, the solubility of CMS(PR 100%) was evaluated (Fig. 4) and FTIR spectra were recorded after conversion of CMS(PR 100%) to sodium salt form (Fig. 5). Storage duration and samples chosen for analyses in this study are considered as non-exclusive examples to show the effects of protonation on CMS properties.

From day 1 at 2 weeks and at 8 months after total protonation of CMS, the solubility in SGF and in SIF decreased over time (Fig. 4). This decrease was more pronounced at high DS and was not necessarily proportional to the decrease in $t_{90\%}$. For instance, after 2 weeks of storage, the solubility of CMS(DS 0.11) was decreased by 52% in SGF and by 63% in SIF, whereas the $t_{90\%}$ was reduced only by 4% and 14%, respectively. For CMS(DS 0.20) the solubility was decreased by 68% in SGF and by 71% in SIF, while the $t_{90\%}$ was reduced by 21% and 40%, respectively (Figs. 3 and 4). It is worth to mention that at day 1 after protonation the solubility of CMS

increased with DS, whereas after 2 weeks and 8 months the CMS at high DS became less soluble than CMS at low DS. Thus, after 8 months of storage, a certain solubility is still retained for the CMS at low DS, while the solubility ofCMSat high DS was drastically reduced (Fig. 4). The high solubility in SIF of CMS(PR 100%) freshly prepared suggests that the carboxyl groups are still non-associated and that no crosslinking occurred via ester link after protonation and drying processing of samples. The subsequent decrease of solubility over storage time of totally protonated CMS suggests an augmentation of inter- and intra-chain interactions via carboxyl groups. These results on solubility fit well with data of Heinze and Koschella (2005) on carboxymethyl cellulose, where the treatment with mineral acids leads to water insoluble polymer.

The conversion of totally protonated CMS to sodium salt form at day 1 after protonation or at 8 months after protonation was done in order to investigate by FTIR whether there are any modifications on carboxyl group vibrations. The FTIR patterns of CMS(DS 0.11) and of CMS(DS 0.20) are presented in Fig. 5 as examples of CMS samples. After conversion to sodium salt form the band at 1735 cm^{-1} ascribed to non-associated carboxyl groups disappeared and that at 1603 cm^{-1} corresponding to carboxylate groups reappeared (Figs. 1 and 5). The patterns of samples deprotonated at day 1 after protonation were similar to those of initially unprotonated form (PR 0%), whereas the patterns of those deprotonated at 8 months after protonation showed a new shoulder at around 1705 cm^{-1} (Figs. 1 and 5). This shoulder indicates the presence of hydrogen bonded carboxyl groups which absorb at a lower (1705 cm^{-1}) frequency than non-bonded groups (1735 cm^{-1}) as reported elsewhere (Harada et al., 2004; Silverstein et al., 2005). In addition, a higher relative intensity of peak at 1603 cm^{-1} can be correlated with the increase of DS (Fig. 5a and c). Furthermore, the intensity of this peak was lower for samples deprotonated after 8 months than those converted at day 1 (Fig. 5).

Overall, the decrease in release time (Fig. 3), the reduction of solubility (Fig. 4) and the FTIR data (Fig. 5) suggest an increase of carboxyl–carboxyl and carboxyl–hydroxyl interactions over storage time of CMS(PR 100%) samples. These interactions concern only the protonated form of CMS and not sodium form.

3.4. Crystalline type of unmodified and modified starch samples

The patterns of native starch (Hylon VII) presents prominently a B-type crystalline structure with X-ray diffraction (XRD) peaks at 3.7, 4.0, 4.5, 5.2 (strongest peak), 5.9, 7.1 and 14.5 Å. The $S_{control}$, treated with 0.9 M NaOH, retained a structure almost similar to that of untreated native starch. Differently, the gelatinized starch (S_g), treated with more concentrated NaOH (2 M), presented a clear reduction of the peak at 5.2 Å and an increase of peaks at 4.5 Å and 6.9 Å (Supplementary Fig. 1). These XRD patterns show the effect of NaOH concentration during gelatinization of starch on the subsequent structural organization of chains in dried powders. Thus, in the presence of alkaline solution, the starch becomes negatively charged (alkoxide) with dissociation of the protons of –OH groups. Further negative charges generate extensive swelling and may lead to dissociation of double-helical regions and to break-up of crystalline structure (Chen and Jane, 1994).

After carboxymethylation of $S_{control}$, the initial B-type order was lost and a new V-type organisation appeared with the two characteristic peaks at 4.5 and 6.9 Å (Supplementary Fig. 1). The change of crystalline structure from double to single helices after carboxymethylation, suggests that carboxymethyl groups enhance the starch granules swelling.

To clarify whether the CMS(PR 100%) undergo any crystalline modification over storage time, XRD patterns were recorded for these excipients at day one and after 8 months. Freshly protonated CMS(PR 100%) presented the same XRD patterns as CMS(PR 0%), but a decrease in intensity of the peak at 6.9 Å can be observed for

CMS(PR 100%) over storage time (8 months) (Supplementary Fig. 1). Furthermore, this decrease, indicating more amorphous structure, is particularly evident for CMS with high DS.

Taken together, the results on CMS excipients indicate that the presence of –COOH groups favors progressive inter- and intra-chain interactions via hydrogen bonds. When the DS and the PR are high enough, the carboxyl-carboxyl and carboxyl-hydroxyl interactions can even lead to structural alterations or rearrangements (Supplementary Fig. 1). A further stability study, including accelerated stability, will permit a better understanding of the structural rearrangement of CMS(PR 100%).

3.5. Properties of CMS(PR 0%) and of CMS(PR 50%)

To investigate the influence of partial protonation on the properties of CMS, only sodium CMS and freshly prepared CMS(PR 50%), unless other indications are present, were retained for the subsequent experiments. We supposed that CMS(PR 50%) can be a model for partially protonated samples and then similar interpretations of acquired data can be applicable to PR less than 50%.

3.5.1. Solubility and density of powders and crushing strength of tablets

As shown in Fig. 6, the control ($S_{control}$) is almost insoluble, whereas the solubility of S_g is about 30% in SGF and 40% in SIF. This higher solubility of S_g is probably related to the higher concentration of NaOH (2M) used in treatment for gelatinization liberating more polysaccharide chains from starch granules and inducing crystalline disorder (Supplementary Fig. 1). Since polyhydroxylic S_g is non-ionized in used dissolution media, its higher solubility in SIF may be related to solvation power of the medium.

For the CMS samples, the solubility of powder increases with the DS and it was higher in SIF than in SGF. Similar solubility was found for CMS(DS 0.07, PR 0%)

and CMS(DS 0.07, PR 50%). At higher DS, the solubility of CMS(PR 0%) in SGF was higher than that of CMS(PR 50%). It appears that the partial protonation of –COONa groups (PR 50%) reduces solubility in SGF, due to the augmentation of inter- and intra-chain interactions and to a lesser hydration of polymer chains when the sodium is replaced by proton. In SIF, no differences were found between the solubility of CMS(PR 0%) and CMS(PR 50%), probably because the –COOH groups turn all to unprotonated form in neutral medium.

As an overall behavior of samples, high tapped density was associated with low crushing strength, irrespective to DS and to PR (Supplementary Fig. 2). The $S_{control}$ and CMS(DS 0.20) presented the lowest crushing strength (177–188 N) with the highest tapped density (0.38–0.41 g/cm^3), whereas for the other samples the crushing strength was in the range 236–278N with 0.26–0.34 g/cm^3 tapped density. The relatively high tapped density and low crushing strength of $S_{control}$ are probably due to the low hydration and swelling of starch particles when treated with 0.09MNaOH. In the case of CMS(DS 0.20), high density can be explained by stronger interactions between particles and agglomeration during the precipitation. The obtained values of tapped density and crushing strength are in the normal range compared to common excipients (Rowe et al., 2006).

3.5.2 Erosion of tablets and fluid uptake

Erosion and fluid (SGF or SIF) uptake by the unloaded tablets were examined in the same conditions as for the drug dissolution tests: The tablets were incubated 2 h in SGF to simulate the approximate retention time in stomach, or 2 h in SIF to compare the effect of acid medium to that of neutral medium on tablet erosion and fluid uptake. The $S_{control}$ were not considered for these experiments due to the rapid tablets disintegration.

The S_g showed very low and similar erosion percentages, irrespective of dissolution medium (SGF or SIF) (Fig. 7). For all CMS samples, the erosion in SGF was lower than in SIF (Fig. 7), fitting well with the results of powders solubility (Fig. 6). For the same DS, the erosion of CMS(PR 50%) was lower than that of CMS(PR 0%), due to the network stabilization by hydrogen bonds between carboxyl–carboxyl groups and carboxyl–hydroxyl groups.

The erosion increases with DS for CMS(PR 0%) and CMS(PR 50%) incubated in SGF, and for CMS(PR 50%) incubated in SIF. Differently, the CMS(PR 0%) at DS(0.07, 0.09 and 0.11) incubated in SIF showed almost the same erosion which increased at DS(0.14) and DS(0.20) due to the higher hydration and solubilization of tablets.

The fluid uptake was the lowest (87%) for S_g in both SGF and SIF media (Fig. 7). It increased with CMS due to the presence of –COONa/H groups, and it was lower in SGF than in SIF. In SGF, the fluid uptake by CMS(PR 50%) was similar to that by CMS(PR 0%), because the SGF acidity contributes to the protonation of carboxylate groups and thus the differences between initially non-protonated and protonated CMS diminished.

For all CMS samples in SGF and for CMS(PR 50%) in SIF, increasing DS generated higher fluid uptake, probably due to the higher hydration of –COONa/H groups compared to hydroxyl groups and to the low erosion ensured by the stabilization of the network by hydrogen bonds.

For CMS(PR 0%) in SIF, the CMS(DS 0.09) and CMS(DS 0.11) had the same erosion with lower fluid uptake for DS(0.11), indicating that the gel layer generated by CMS(DS 0.11) is consistent enough to reduce the diffusion of fluid into tablets (Fig. 7). From DS(0.09) to DS(0.14) the fluid uptake decreases in spite of the increase of the number of –COONa groups due to the increasing of tablet erosion (solubilization). At DS(0.20) the fluid uptake by CMS increases due to the difficulty to wipe well this fragile tablet before weighing.

3.5.3. *In vitro* dissolution tests

Dissolution tests in SGF (pH 1.2 lower than CMS pK_a) and in SIF (pH 6.8 higher than CMS pK_a) (Fig. 8) provided useful information on the properties of the CMSmatrix and on the influence of DS and of PR on the kinetics drug release.
The correlation coefficients (R^2) obtained by plotting log(Mt/M∞) against log(t) were higher than 0.996 indicating that Peppas's equation is applicable to the present devices. In both SGF and SIF media, the control ($S_{control}$) showed a fast release of acetaminophen and a rapid disintegration of tablets (less than 30 min) due to its low solubility and its inability to develop hydrogel (Figs. 6 and 8).

In SGF, longer dissolution assays were conducted in order to better understand the influence of acidity on the properties of CMS matrix. It was found that the percentage of acetaminophen released after the first 2 h in SGF was almost similar for all CMS (Fig. 8A and B), that all CMS tablets preserved their shape even after the complete drug release, and that the S_g tablets presented cracks after approximately 2 h. It was also found that S_g and CMS(DS 0.07), due to the low fluid uptake, low erosion and low solubility, afford longer sustained release than CMS at higher DS (Figs. 6, 7, and 8A and B). For the same DS, the PR(50%) showed slightly slower release than the sodium form (PR 0%), since a higher protonation enhances inter-chain associations via hydrogen bonding. The exponent (n) values (0.580–0.669) suggested an anomalous diffusion mechanism in acid medium.

In neutral medium (SIF), the tablets are less compact and the $t_{90\%}$ is lower than in acid medium (SGF) (Fig. 8C and D). The S_g, which is not pH sensitive, presented almost the same release rate in neutral and in acid media. Like in SGF, the CMS(PR 50%) provided a lower release rate than CMS(PR 0%), in agreement with the lower erosion of CMS(PR 50%) tablets (Figs. 7, and 8C and D). The fastest release was found with CMS at lowest DS(0.07) and at highest DS(0.20), irrespective of the protonation ratio (0% and 50%). The fast release of acetaminophen from

CMS(DS 0.07) is almost due to its inability to generate a hydrogel structure compact enough to prevent fast fluid diffusion within the tablet and thus to control the drug release. In the case of CMS(DS 0.20), the fast release is due to the high solubility (Fig. 6) and the high erosion (Fig. 7) of this matrix compared to the others CMS. When protonated (PR 50%), the solubility of CMS(DS 0.20) was reduced and the release became slower. The slowest release of acetaminophen was provided by the middle DS(0.11). In this case, hydration was just enough to generate a low soluble hydrogel layer which can reduce the fluid diffusion and drug dissolution. The exponent (n) values (0.759–0.968) suggest amechanism controlled mostly by swelling of CMS matrices in neutral medium (Fig. 8C and D).

3.5.4. Effect of drug loading and of SGF acidity on drug release rate in SIF

Tablets of CMS (DS 0.11 and 0.20) loaded with acetaminophen (20% and 40%) were first incubated for 2 h in SGF to mimic the gastric residence and then in SIF until complete dissolution. The CMS(DS 0.11) was chosen for its ability to ensure sustained release and the CMS(DS 0.20) was chosen for its high sensitivity to the pH of the dissolution medium.

The drug release fromCMS(DS0.11) showed almost similar rates irrespective of protonation ratio (0% and 50%) and of loading (20% and 40%) (Fig. 9A). In these cases, $t_{90\%}$ (7–7.5 h) are close to that (7.1 h) obtained with CMS(DS 0.11, PR 50%) and higher than that (5.3 h) obtained with CMS(DS(0.11, PR 0%) in SIF only (Figs. 8C, D and 9A). These results indicate that a partial protonation of DS(0.11) makes dissolution kinetics almost independent of time retention in SGF.

For CMS(DS 0.20, PR 50%) at 20% loading, the $t_{90\%}$ (6 h) is close to that in SIF only (Figs. 8D and 9A). For the same loading (20%), the CMS(DS0.20, PR 0%)showedhigher $t_{90\%}$ (5 h) than in SIF only (2.5 h), but with final acceleration of release (late burst) (Figs. 8C and 9A). The CMS(DS 0.20) at 40% loading presented

similar dissolution profiles to that with CMS(DS 0.20, PR 0%) at 20% loading, but the release rate was higher (Fig. 9A). Therefore, the longer dissolution observed with DS(0.20, PR0%) is almost due to the preincubation of tablets in acid medium (SGF) which generates a kind of preconditioning for further intestinal release.

Contrarily to CMS(DS 0.20), the CMS(DS 0.11) did not show a final acceleration of release due to its low erosion in SIF (Fig. 7). Thus, at high DS(0.20), the progressive conversion of carboxyl to salt form in SIF induces a high water absorption by the tablet until the inside pressure becomes enough to disrupt the weak erodible hydrogel and to liberate the drug.

In order to evaluate the effect of tablet size and of storage time on drug release rate, the same experiments as before were done with tablets of 200 mg and 500 mg after 1 year of CMS storage (Fig. 9B).

For tablets of 200 mg, same results were obtained as with freshly prepared samples except for CMS(DS 0.20, PR 50%) where the dissolution becomes faster upon samples storage (not shown). These results indicate that for high DS a protonation ratio less than 50% is necessary to limit the inter- and intra-chain interactions and to ensure maintaining of excipient properties at storage. Interestingly, the CMS(DS 0.11) with protonation ratio up to 50% did not show any modification even after 1 year of storage. Maybe, the DS and the PR are not high enough and thus the number of –COOH groups is not sufficient to permit high interactions between carboxyl and hydroxyl groups. Moreover, the presence of –COONa groups may reduce the inter- and intra-chain interactions.

For tablets of 500 mg, the CMS(DS 0.11) showed almost the same sustained release irrespective of protonation ratio (0% and 50%) and of loading (20% and 40%) (Fig. 9B). The CMS(DS 0.20) showed final acceleration of release as with tablets of 200 mg. Also, the release with CMS(DS 0.20, PR 50%) was faster than with CMS(DS 0.20, PR 0%). The effect of DS and of PR on release profile for 500 mg tablets was similar to that for 200 mg tablets, but the time release was longer with larger tablets.

Except for $S_{control}$ and CMS(DS 0.20), the crushing strength of tablets are close. This suggests that the differences of drug release rate from various formulations are not due to crushing strength effect (Supplementary Fig. 2 and Fig. 8).

It was recently shown (Brouillet et al., 2008) that adding of NaCl (loading 27.5%) to CMS tablet formulation was necessary to maintain the integrity of matrices used for sustained release. In our conditions, formulations with CMS(DS 0.11) can keep integrity and ensure sustained release without adding any electrolyte to the formulation.

Taken together, these results indicate that oral solid dosage forms based on CMS(DS 0.20) are suitable for duodenum and upper intestinal delivery, whereas those based on CMS(DS 0.11) are useful for sustained drug release.

4. Conclusions

This first detailed study on the effect of protonation ratio and of degree of substitution on the properties of carboxymethyl starch synthesized in aqueous medium provides useful information about the delivery mechanisms with this excipient type. The control ($S_{control}$) disintegrated rapidly in the media, whereas the CMS showed fast or slow release of acetaminophen depending on the PR and the DS. To ensure a gel network formation and to maintain a limited solubility of the matrix, the amount of carboxyl groups (DS) must be sufficient and not too high. Protonation of CMS excipients made the drug release rate lower than that provided by sodium CMS. High protonation, especially at high DS(0.20), lead to time-dependant reduction of solubility and alteration of crystalline structure of CMS. The CMS(DS 0.20) was remarkably sensitive to the pH of dissolution medium and showed high release rate in SIF. Longer release time was observed after tablet preincubation in SGF due to the protonation acquired in acid medium. The CMS(DS 0.09–0.11) with PR up to 50% appears as the most suitable excipients for drug sustained release.

When partially protonated, CMS(DS 0.11) was slightly sensitive to pH of dissolution medium and showed almost similar sustained release rate with 20% and 40% loading.

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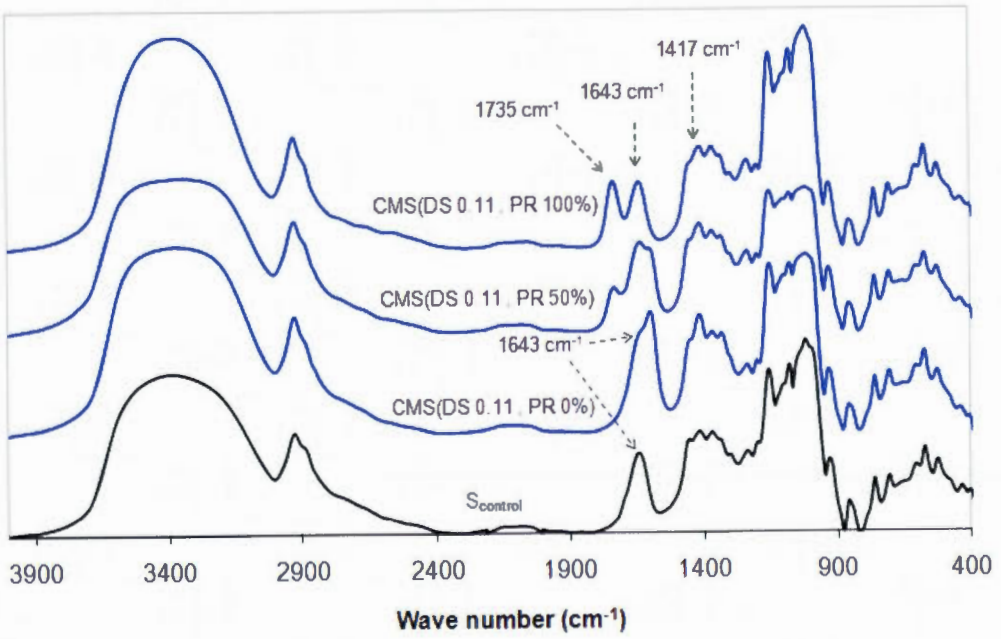


Figure 1: FTIR spectra of $S_{control}$ and of CMS(DS 0.11) at various protonation ratios (0%, 50% and 100%). Pellets (12 mm diameter) of KBr (67 mg) and samples (3 mg) mixture obtained by compression at 3T.

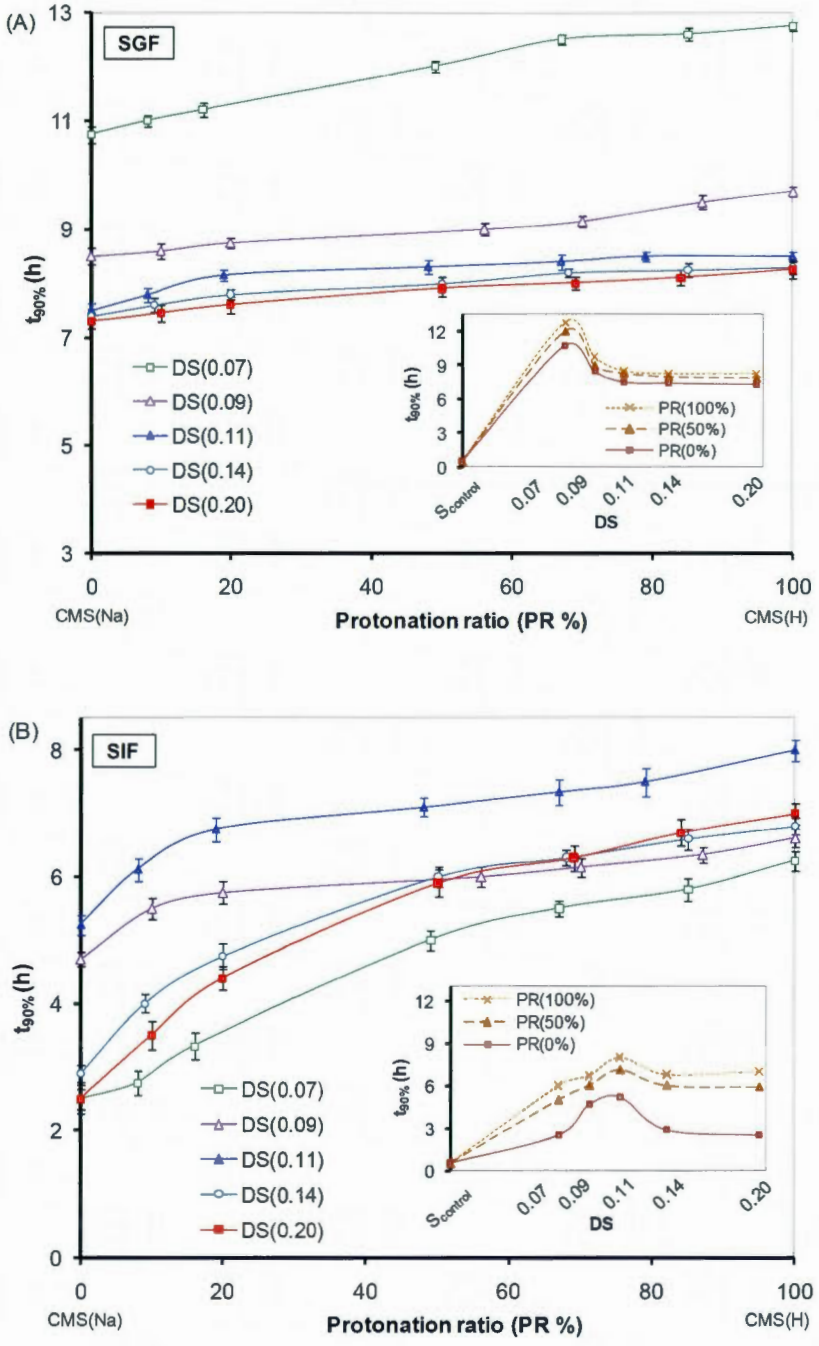


Figure 2: Time of acetaminophen release ($t_{90\%}$) from CMS tablets at various DS in (A) SGF and (B) SIF. Tablets of 200 mg (20% loading) were used for the dissolution tests (900 mL, 37 °C, 100 rpm).

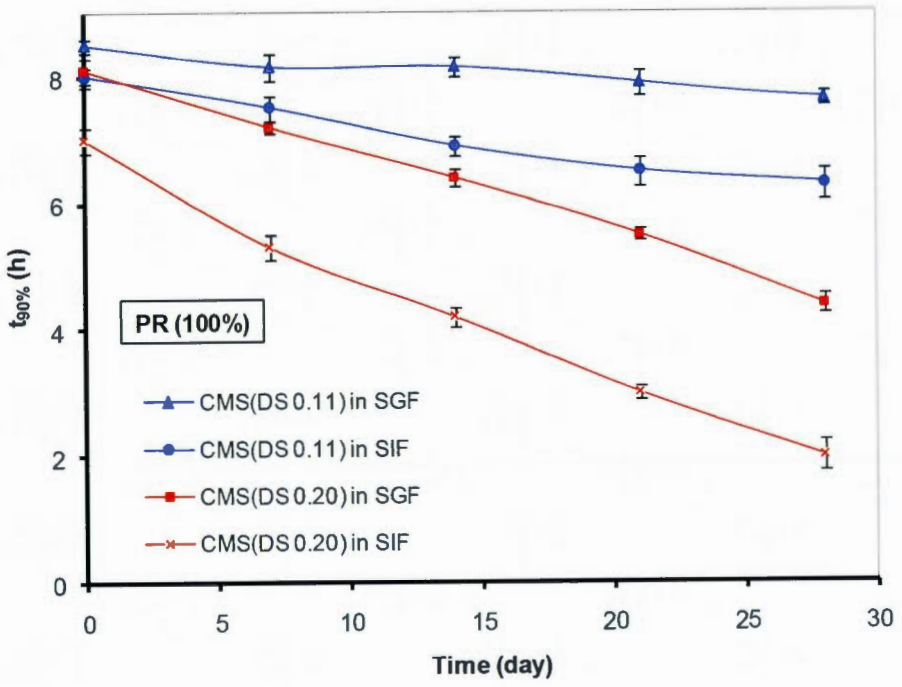


Figure 3: Time of acetaminophen release ($t_{90\%}$) from totally protonated CMS (DS 0.11 and 0.20) over storage times. Tablets of 200 mg (20% loading) were used for the dissolution tests (900 mL, 37 °C, 100 rpm) in SGF and in SIF.

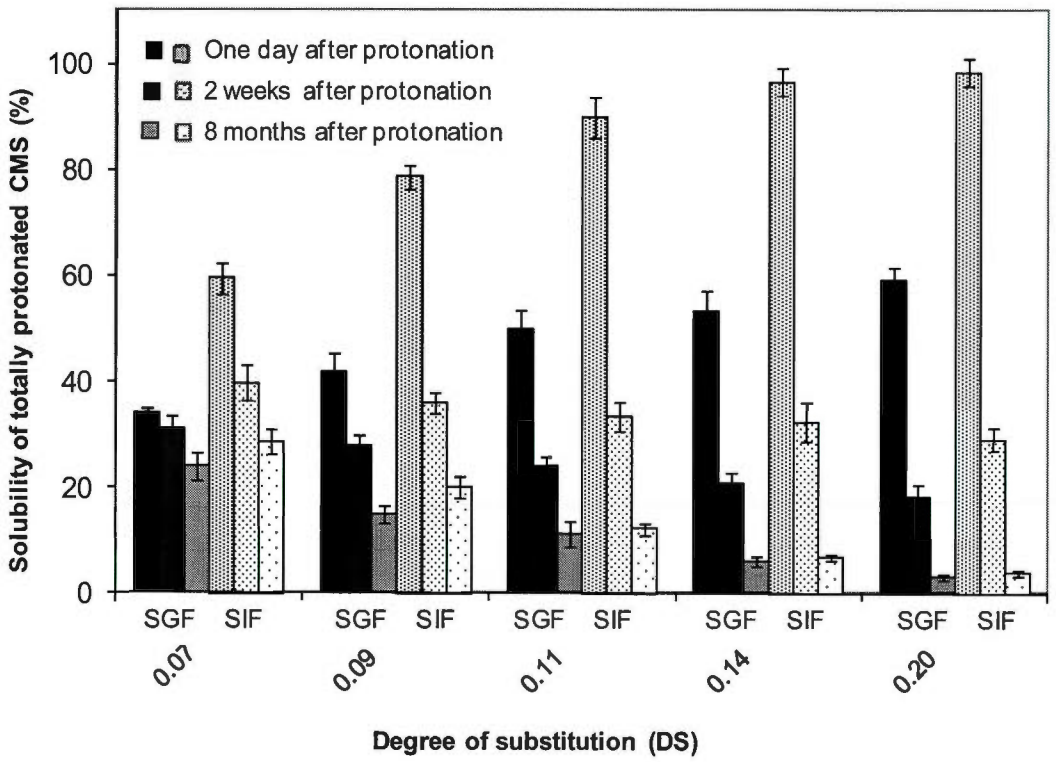


Figure 4: Solubility in SGF and in SIF of totally protonated CMS (PR 100%, different DS, various storage times).

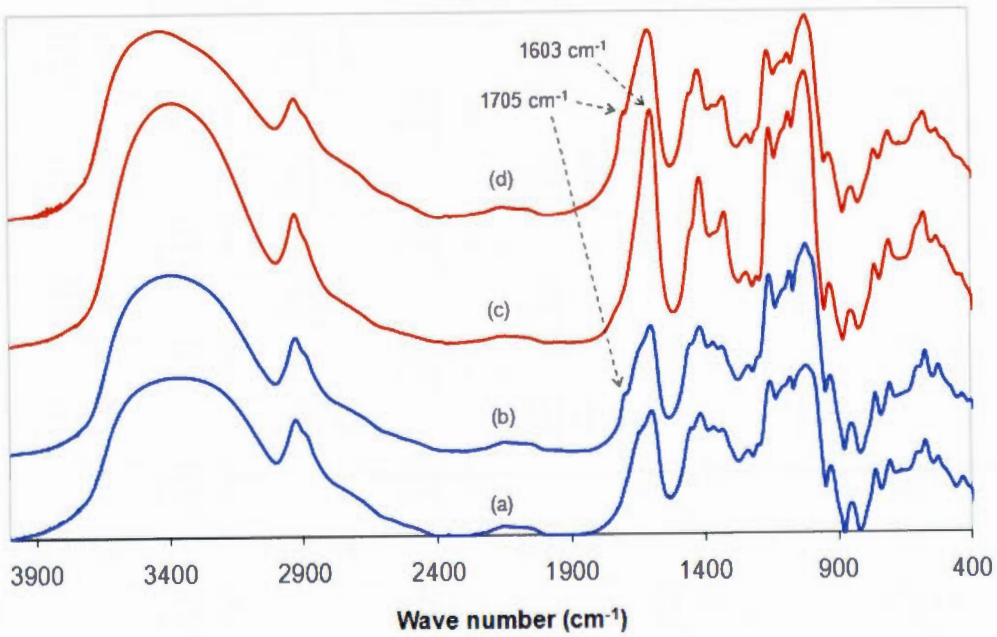


Figure 5: FTIR spectra of CMS obtained by conversion of totally protonated form (PR 100%) at various storage times to sodium salt form: CMS(DS 0.11) deprotonated at day one (a) or at 8 months after protonation (b), and CMS(DS 0.20) deprotonated at day one (c) or at 8 months after protonation (d). Pellets (12 mm diameter) of KBr (67 mg) and samples (3 mg) mixture obtained by compression at 3 tonnes.

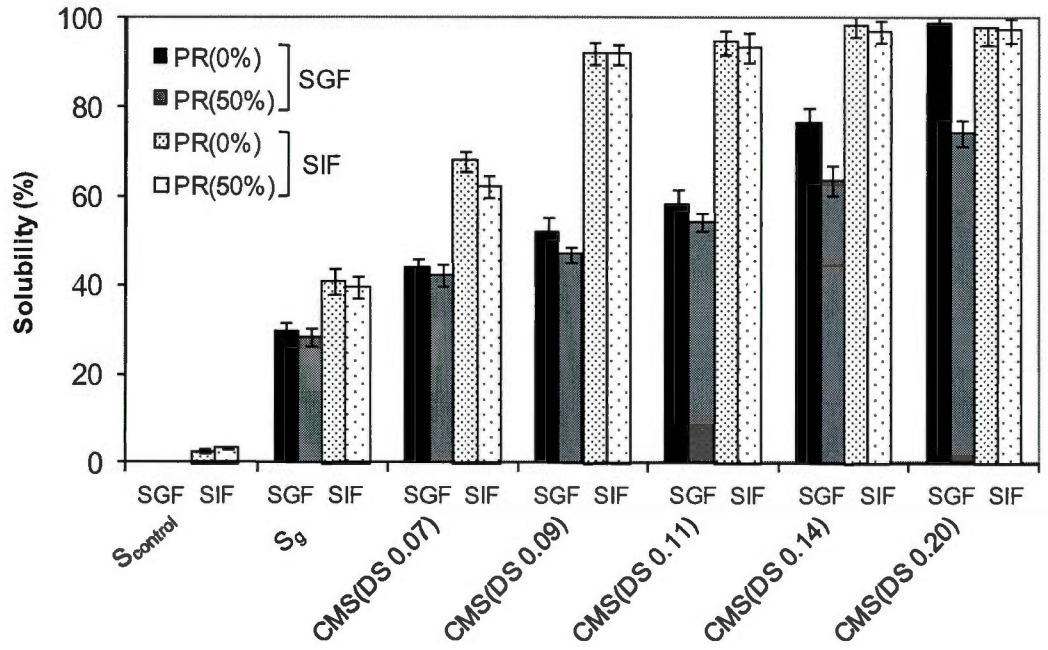


Figure 6: Solubility in SGF and in SIF of powders of $S_{control}$, S_g, sodium CMS, and CMS with 50% protonation ratio.

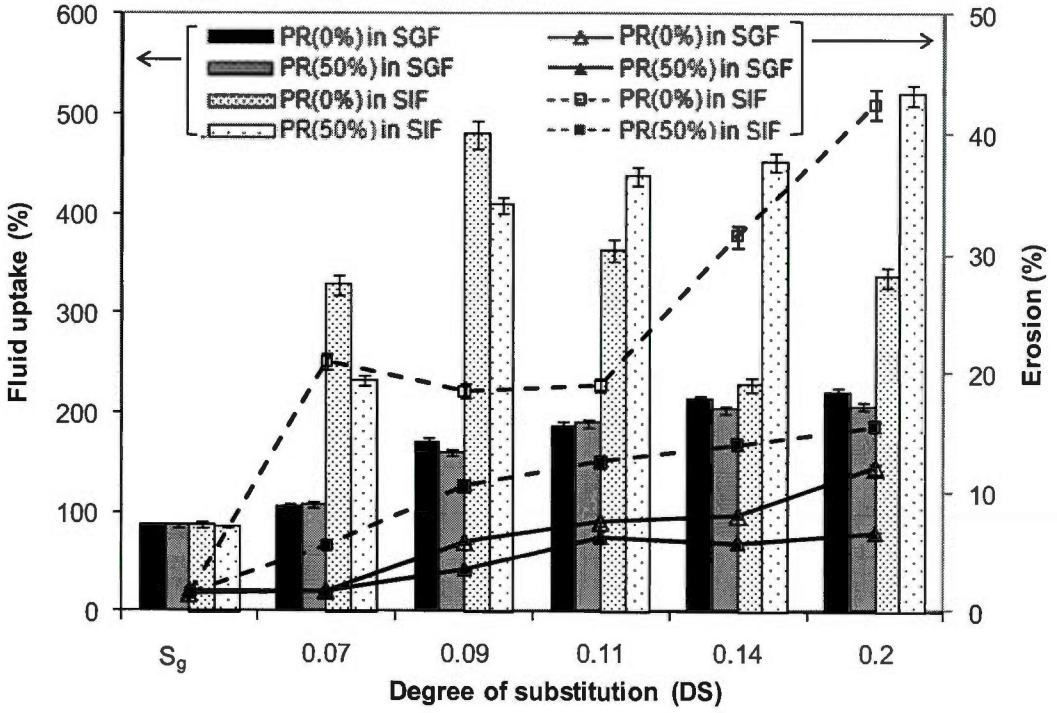


Figure 7: Erosion and fluid uptake by tablets (200 mg) of CMS(PR 0% and PR 50%) after 2 h in SGF or 2 h in SIF (900 mL, 37 °C, 100 rpm).

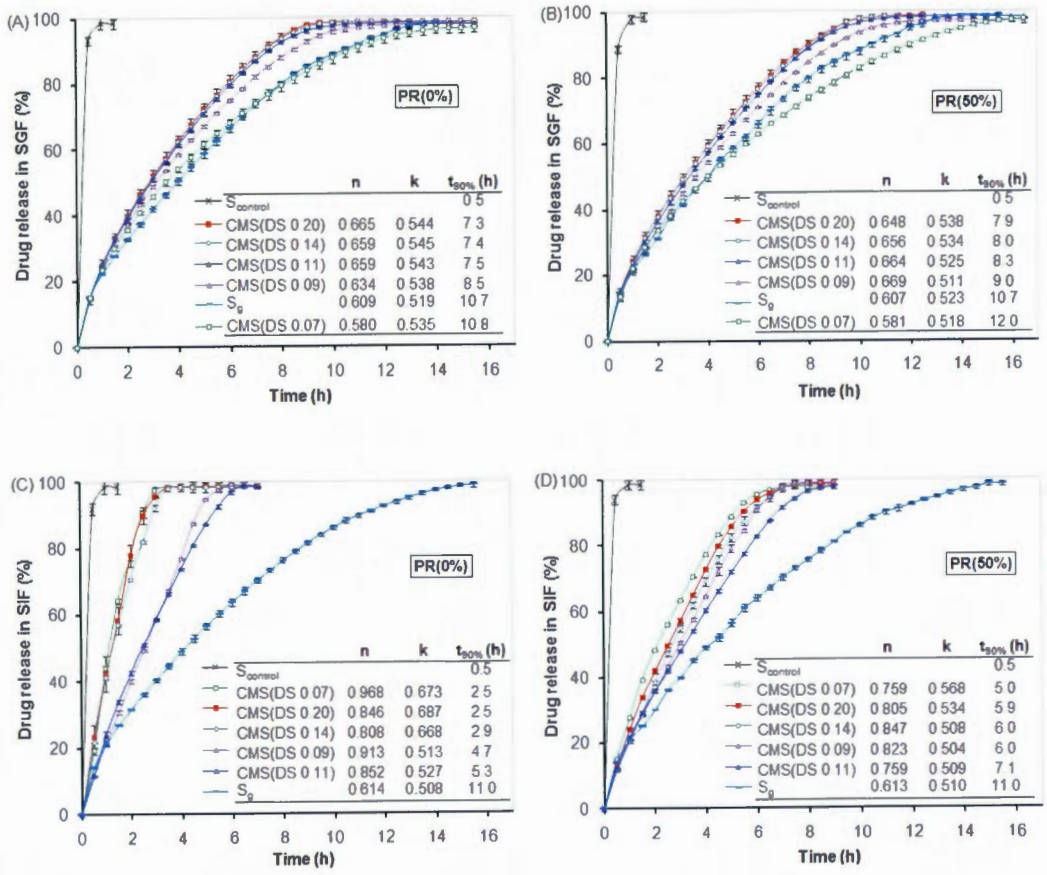


Figure 8: Kinetics of acetaminophen dissolution (900 mL, 37 °C, 100 rpm) from tablets (200 mg, 20% loading) of $S_{control}$ and of CMS. (A) Sodium form in SGF, (B) 50% protonated form in SGF, (C) sodium form in SIF, (D) 50% protonated form in SIF.

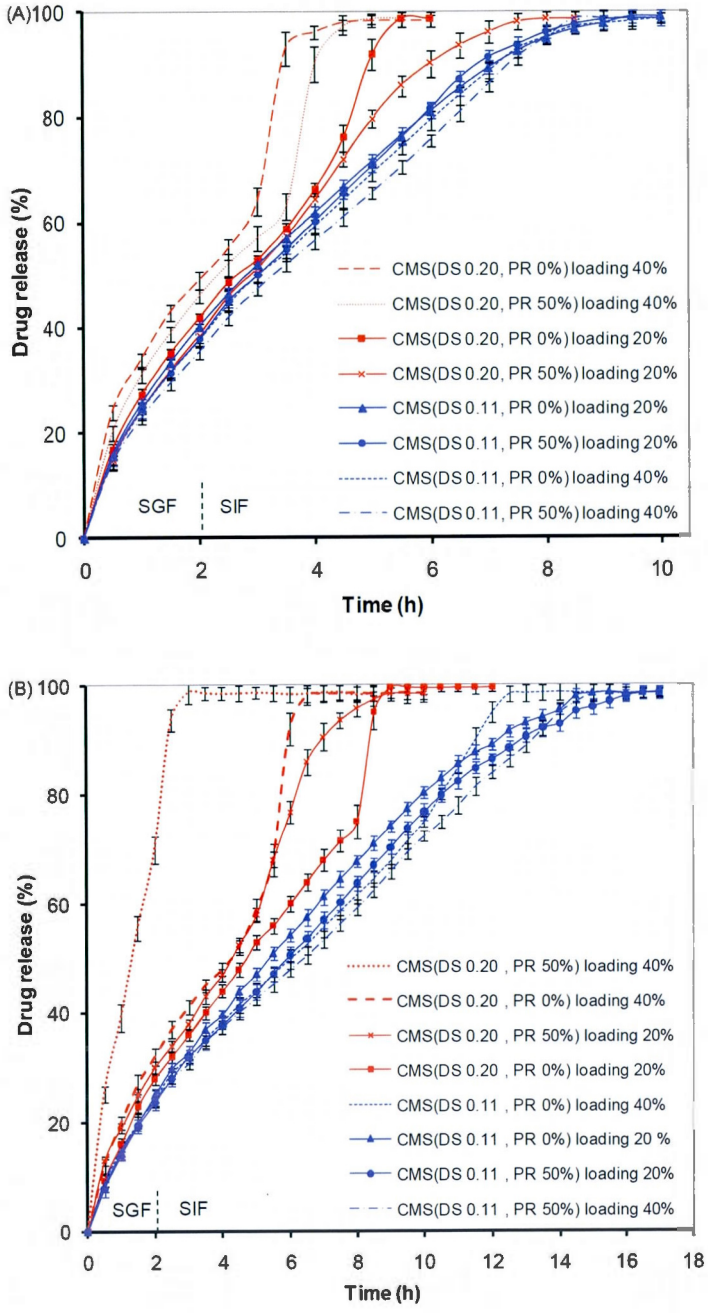
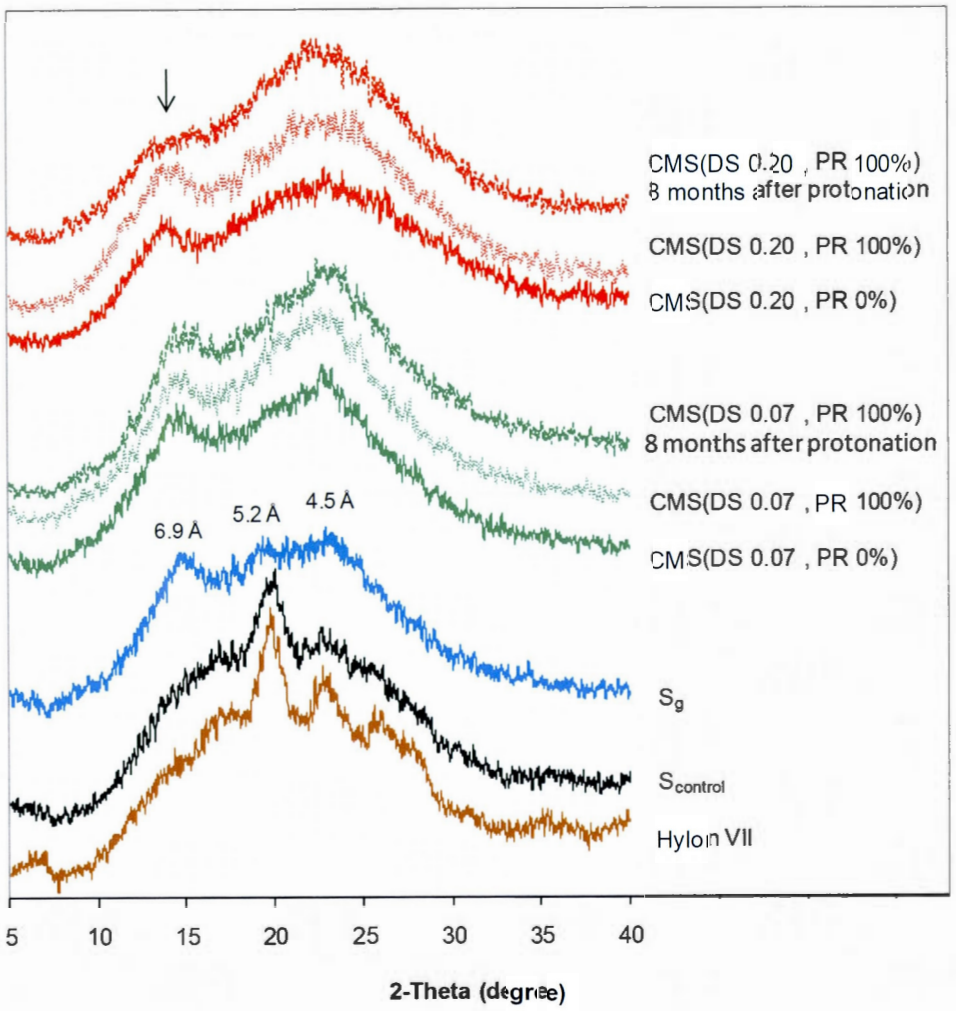
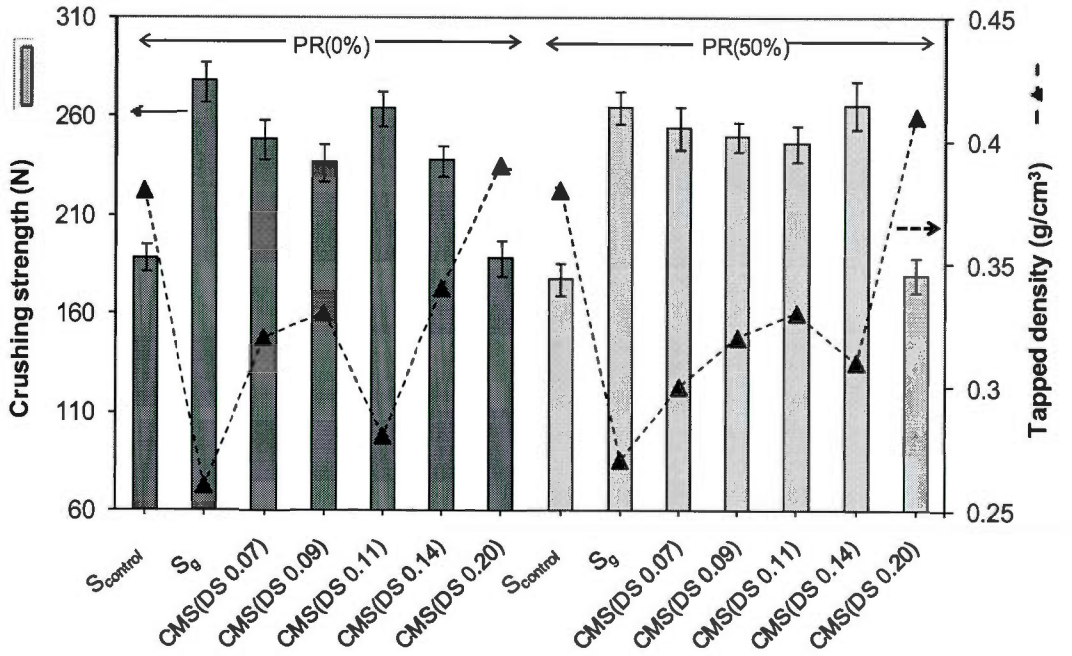


Figure 9: Kinetics of acetaminophen dissolution (900 mL, 37 °C, 100 rpm) from tablets of CMS (DS 0.11 and 0.20, PR 0% and 50%). Tablets of (A) 200 mg or (B) 500 mg (20% or 40% loading) incubated 2 h in SGF and then transferred in SIF until complete dissolution.



Supplementary figure 1: X-ray diffraction patterns of native starch (Hylon VII), $S_{control}$, S_g, sodium CMS, and totally protonated CMS (freshly protonated or after 8 months of storage).



Supplementary figure 2: Crushing strength of tablets and tapped density of powders of $S_{control}$, S_g, sodium CMS, and CMS with 50% protonation ratio.

CHAPITRE VI

ÉTUDE COMPARATIVE DU CARBOXYMÉTHYL AMIDON ET DE L'HYDROXYPROPYLMÉTHYL CELLULOSE COMME EXCIPIENTS POUR LE TRANSPORT DE L'ACÉTAMINOPHÈNE ET DU *LACTOBACILLUS RHAMNOSUS* ADMINISTRÉS PAR VOIE ORALE

Résumé

Les performances des matrices à base de carboxyméthyl amidon (CMA) et à base d'hydroxypropylméthyl cellulose (HPMC) ont été comparées dans des conditions simulant le passage gastro-intestinal. L'acétaminophène et le *Lactobacillus rhamnosus* ont été utilisés comme agents actifs modèles. Cette étude comparative a montré que les matrices de CMA et de HPMC assurent une protection partielle des bactéries contre l'acidité du fluide gastrique simulé (FGS, pH 1,2). Une libération contrôlée de 4 h à 7 h après l'incubation a été observée avec la matrice de CMA dans un fluide intestinal simulé (FIS, pH 6,8). Dans les mêmes conditions, la matrice de HPMC a empêché la libération des bactéries, même après 15 h d'incubation, à cause de la formation d'une épaisse couche d'hydrogel.

Les matrices de CMA et de HPMC ont montré une capacité de charge élevée en acétaminophène (jusqu'à 60 %), avec des temps de libération d'environ 12 h et 34 h, respectivement. Les comprimés à double noyau permettent une libération retardée de l'acétaminophène. La présence de l'α-amylase dans FIS a accéléré la libération à partir des comprimés à base de CMA, tandis que la libération à partir des comprimés à base de HPMC n'a pas été affectée. Le mécanisme de libération peut être contrôlé par le gonflement, la diffusion et la solubilisation dans le cas des deux matrices.

Mots clés : carboxyméthyl amidon, hydroxypropylméthyl cellulose, matrice hydrophile, *Lactobacillus rhamnosus*, comprimé, transport des substances actives.

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Comparative study of carboxymethyl starch and hydroxypropylmethyl cellulose as excipients for oral delivery of acetaminophen and of *Lactobacillus rhamnosus*

Abstract

A comparative study of carboxymethyl starch (CMS) - an anionic carbohydrate - and hydroxypropylmethyl cellulose (HPMC) - a polar non ionic carbohydrate - as excipients in hydrophilic matrices was conducted in conditions simulating the gastrointestinal transit. Small molecules (i.e. acetaminophen) and probiotics (i. e. *Lactobacillus rhamnosus*) were used as models of active agents in monolithic tablets. Both CMS and HPMC afforded partial protection of bacteria against acidity of simulated gastric fluid (SGF, pH 1.2). A controlled release was observed with CMS matrix in simulated intestinal fluid (SIF, pH 6.8) from 4 h to 7 h after incubation. Under the same conditions of SGF-SIF transfer, the HPMC matrix did not permit bacteria release in SIF, even after 15 h of incubation, due to the formation of a thick outer hydrogel layer. This major difference lies in the fact that only CMS is pH sensitive with a high swelling and solubilization in SIF.

Both CMS and HPMC provided controlled relase for relatively high loading of acetaminophen (up to 60%), with release times of about 12 h and 34 h, respectively. Dry-coated tablets permitted delayed release of acetaminophen. The presence of α-amylase in SIF accelerated the release from CMS tablets, whereas the release from HPMC tablets was not affected. The release mechanism may be controlled by swelling, diffusion and solubilization in the case of CMS and HPMC matrices. Bioerosion by α-amylase can also influence the release mechanism from tablets based on CMS.

Keywords: Carboxymethyl starch, hydroxypropylmethyl cellulose, hydrophilic matrix, *Lactobacillus rhamnosus*, tablet, drug carrier.

1. Introduction

The carboxymethyl starch (CMS) (Fig. 1A) was proposed by Mulhbacher et al. (2004) as a pH sensitive excipient for drug delivery. Monolithic formulations based on CMS were able to protect *Escherichia coli* against acidity (Calinescu et al., 2005) and to provide controlled release of small molecules in conditions simulating the gastrointestinal transit (Lemieux et al., 2009; Assaad and Mateescu, 2010). The release of active agents was found to depend on the sensitivity of CMS matrix to pH of dissolution medium. Thus at acid pH of SGF, simulating gastric fluid, the CMS is protonated and the matrix swelling is low. At neutral pH of SIF, simulating intestinal fluid, the CMS keeps its salt form which favors the matrix swelling and solubilization.

To better protect probiotic and to ensure colon delivery, chitosan was added as a coexcipient with CMS. The protection of *Lactobacillus rhamnosus* and their release were dependent on the molecular weight of chitosan (Calinescu and Mateescu, 2008). The *Lactobacillus* species, used as probiotics, are part of the oral and gastrointestinal tract flora (Avlami et al., 2001). Probiotics are considered to be beneficial to intestinal microbial balance and to alleviate some effects of inflammatory bowel disease (Mach, 2006). The suggested mechanisms of action of probiotics in these inflammation conditions can be summarized by inhibition of pathogenic enteric bacteria growth, improvement in epithelial and mucosa barrier function, and downregulation of proinflammatory cytokines secretion (Dotan and Rachmilewitz, 2005; Chermesh and Eliakim, 2006; Bai and Ouyang, 2006; Gionchetti et al., 2006).

Hydroxypropylmethyl cellulose (HPMC) is one of the largely used carriers for oral drug delivery (Fig. 1B). When compressed with an active agent, HPMC forms a water-swellable matrix. A water mobility gradient across the gel layer of hydrated HPMC matrix has been shown by nuclear magnetic resonance imaging analysis (Rajabi-Siahboomi et al., 1994 and 1996). The relaxation time in outer parts of the

gel layer approached that of free water, but it decreased progressively in the inner parts. This hydration of polymer chains and the matrix volume expansion have an important effect on drug release kinetics (Colombo, 1993; Siepmann and Peppas, 2001).

The present study, was aimed to: (i) compare the behavior of carboxymethyl starch (CMS), anionic polymer, and of hydroxypropylmethyl cellulose (HPMC), uncharged polymer, as carriers for small molecules (i.e. acetaminophen) and for large size tracers (*Lactobacillus rhamnosus*), (ii) evaluate the ability of these two hydrophilic polymers to afford protection of active agents against acidity of SGF and (iii) determine the loading capacity of monolithic formulations based on CMS or HPMC.

The acetaminophen and the *Lactobacillus rhamnosus* bacteria were chosen as models for small molecules and for probiotics, respectively. The acetaminophen was chosen because its pK_a (9.5) is higher than the pH of the dissolution media (SGF, pH 1.2 and SIF pH 6.8), making the dissolution rate dependent on the excipient properties rather than on the pH of media. The sensitivity of *Lactobacillus rhamnosus* bacteria to the gastric acidity permits the evaluation of the gastroprotection afforded by the matrices.

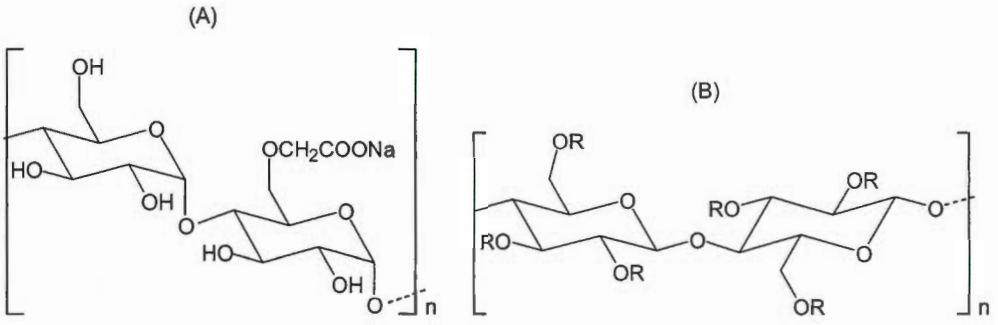

Figure 1: Chemical structures of CMS (A) and HPMC (B). R represents –H, $–CH_3$, or $–CH_2CHOHCH_3$.

2. Materials and methods

2.1. Materials

Hydroxypropylmethyl cellulose (HPMC, Methocel K4M Premium CR EP) was provided by *Dow Chemical Company* (USA). The viscosity of a 2% HPMC solution in water at 20 °C is 3000-5600 cp. *Lactobacillus rhamnosus* bacteria (HA-111 strain) lyophilized powder was from *Harmonium International* (Mirabel, QC, Canada). *Lactobacilli* MRS agar was furnished by *Becton Dickinson and Company* (USA). Pancreatin, eight times strength, from porcine pancreas was provided by *A&C American Chemicals Ltd.* (Montreal, QC, Canada). Acetaminophen and bromocresol green (yellow below pH 3.8 and blue over pH 5.4) were from *Sigma-Aldrich* (St-Louis, MO, USA). Pepsin-free simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) with or without pacreatin were prepared following the USP (US *Pharmacopeia*, 2009). The other chemicals were of reagent grade and used without further purification.

2.2. Preparation of CMS

The sodium carboxymethyl starch (CMS) was prepared in aqueous medium from high amylose corn starch as previously described (Mulhbacher et al., 2001; Calinescu et al., 2005), with certain modifications. Briefly, an amount of 70 g of Hylon VII was suspended in 170 mL of distilled water at 55 °C by using a *Hobart* mixer (Vulcan, Canada). Then, 235 mL of 1.5 M NaOH were added for gelatinization under continuous mixing for 30 min. Subsequently, 55 mL of 10 M NaOH and a freshly prepared solution of monochloroacetic acid (45.5 g in 40 mL of distilled water) were added. After 1 h of reaction, a volume of 130 mL of distilled water was added and the slurry was cooled-down to room temperature and neutralized with acetic acid. The CMS was then precipitated from the slurry by gradually adding

600 mL of acetone and washed by repeated dispersion in volumes of 1 L of 70% acetone and filtration until the final conductivity of filtrate decreased at about 50 µS/cm. The CMS particles were again dispersed in pure acetone and filtrated three times, and finally dried at 40 °C for 24 h. The obtained powder of sodium CMS form was sieved with a 300 µm screen and stored at room temperature.

2.3. Preparation of tablets

Monolithic tablets were prepared by direct compression (2.5 tonnes) of manually mixed dry powders of excipient (CMS or HPMC) and of active agent (*Lactobacillus rhamnosus* or acetaminophen) using a *Carver* hydraulic press (Wabash, IN, USA) and flat-faced punches.

Tablets of 200 mg (9 mm diameter) with 5% *Lactobacillus rhamnosus* loading or with 1.5% bromocresol green pH indicator loading and of 500 mg (13 mm diameter) with acetaminophen as tracer (20%, 40%, 60% and 80% loading) were prepared.

Dry-coated tablets (500 mg) with 20% acetaminophen loading were compressed in two steps: first, a core of 150 mg (9 mm diameter) containing a homogenous mixture of 100 mg acetaminophen and 50 mg of polymeric excipient (CMS or HPMC) was prepared. Then, the core was dry coated with 350 mg of corresponding polymeric excipient and compressed to obtain tablets of 500 mg and 13 mm diameter.

2.4. Evaluation of the diffusion of acidity into tablets

Tablets (n = 2) loaded (1.5%) with bromocresol green were individually agitated with a rotator (*Glas-Col*, USA) for 5 min in 50 mL of distilled water at room temperature. Then they were kept for 2 h in 50 mL of simulated gastric fluid (SGF, pH 1.2) in an incubator shaker (*New Brunswick Scientific*, series 25 D) at 37 °C and

50 rpm. The tablets were then immediately transected to evaluate the advancement of acidity into the tablets by analysing the color modifications.

2.5. Dissolution tests

The dissolution tests were carried out two times in duplicate with CMS or HPMC as excipients and with *Lactobacillus rhamnosus* or acetaminophen as tracer.

2.5.1. Viability and release of *Lactobacillus rhamnosus*

The tests were carried out following Calinescu and Mateescu (2008). The SGF and SIF were sterilized by filtration with 0.22 µm *Millipore* filters before use. Each tablet was incubated in 50 mL of pepsin-free sterile SGF for 2 h (37 °C, 50 rpm). After 2 h, the SGF solution was replaced with 50 mL of sterile simulated intestinal fluid (SIF) containing pancreatin (α-amylase, lipase and trypsin). Pepsin was not used, because neither starch nor cellulose is susceptible to degradation by human pepsin.

Samples of 100 µL were taken at each hour during the dissolution and were spread on MRS agar Petri dishes after preparation of suitable dilutions in 0.1% sterile peptone water. The Petri dishes were then incubated at 37 °C for 48 h, allowing bacteria growth into individual colonies. The counting of bacterial colony forming units (CFU) permitted the calculation of the number of living bacteria released with time.

The total number of CFU per tablet containing 10 mg of probiotic bacteria was determined in sterile phosphate buffered saline (PBS, pH 7.4) at 37 °C.

2.5.2. Dissolution of acetaminophen

The dissolution tests of acetaminophen were carried out with an USP dissolution apparatus II (*Distek* 2100A, USA) at 100 rpm and 37 °C. Tablets were

kept in dissolution cells (1 L of pepsin-free SGF) for 2 h and then in 1 L of SIF with or without pancreatin until complete dissolution. For the tests without pancreatin, samples were automatically pumped and the acetaminophen released was measured with a *Hewlett-Packard* UV-Vis spectrophotometer at 280 nm. In the case of tests containing pancreatin, samples of 1 mL were taken every hour, filtered with filters of 0.42 μm pores diameter after which the absorbances were measured.

3. Results and discussion

3.1. Diffusion of acidity into tablets

After 5 min in distilled water, the tablets of CMS and HPMC, loaded with bromocresol green as a pH indicator, got wet and become completely blue on the surface at pH > 5.4 (Fig. 2A2 and B2). When transferred to SGF, the tablets developed gel networks on surface and the color turned progressively into yellow-orange due to acid pH (pH < 3.8).

The CMS tablets were completely transformed into hydrogel after 2 h in SGF, keeping their inside at neutral pH (blue color) (Fig. 2A3 and A4). Even after 2 h in SGF, some blue spots indicating neutral pH still observed in the outer gel layer of HPMC matrices (Fig. 2B3). The core of the HPMC tablets was still almost dry and hard and almost maintaining its original color (Fig. 2B1 and B4), indicating no diffusion of acidic fluid inside the tablets. This test with the matrices gives an idea about the extent of protection that can be afforded to formulated bacteria against gastric acidity. The observations on tablets shape and color indicate that CMS may afford a good protection at the level of inner core, whereas HPMC may afford higher protection with slower subsequent release.

3.2. Viability and release of *Lactobacillus rhamnosus*

No living bacteria were detected in SGF when the tablets of CMS or HPMC were incubated for 2 h (Fig. 3). Due to the sensitivity of *Lactobacillus rhamnosus* bacteria to acidic medium, no free bacteria are expected to survive at pH 1.2 (Calinescu and Mateescu, 2008), and this is the reason of formulation with excipients forming matrices.

The release of living bacteria from CMS tablets started after about 4 h (2h in SGF + 2h in SIF) of incubation and continued until the complete dissolution. At the end of the test, the CMS tablets disappeared due to the polymer solubilization and erosion by α-amylase at neutral pH. This indicates that CMS matrix provided a partial protection of probiotics during residence in SGF and allowed their controlled release in SIF.

Even after 15 h of incubation (2 h in SGF and 13 h in SIF), no living bacteria were released from HPMC matrix. Due to the formation of a thick hydrogel on the outer layer of HPMC tablets, the release of a large size active agent (*Lactobacillus rhamnosus*) was restrained. In order to determine the percentage of bacteria protected within HPMC matrix after incubation, the solubilization was accelerated by crushing this matrix in PBS solution and about 85% of the initial loaded bacteria. Although the formulation with HPMC afforded high protection of bacteria against acidity, it did not allow their release in SIF.

3.3. Dissolution of acetaminophen

The release patterns of acetaminophen from CMS tablets with 20, 40 and 60% loading were almost similar (Fig. 4). The time corresponding to 50% of release was about 5.5-7 h and that to 90% of release was about 11-12 h. It became apparent that the hydration caused by the increase of acetaminophen loading (20 to 60%) is not enough to create a modification in the dissolution kinetics. The release rate does not decrease above a certain percentage of polymer in the tablet (Maderuelo et al., 2011).

Above this critical point the hydrogel formed is homogeneous and coherent, controlling the hydration of the matrix and the drug release rate. For CMS tablets with 80% acetaminophen loading, the release occurred within few hours. The dry-coating delayed the release for about 4 h when 20% loading was used.

The presence of pancreatin accelerated the release from CMS tablets due to the polymer degradation by α-amylase. For instance, 50% and 90% of acetaminophen were released within 3.5 h and 6 h, respectively. A delayed release was still observed with dry-coated tablets, although the erosion and the release were accelerated by α-amylase.

As in the case of CMS, the release of acetaminophen from HPMC tablets was independent of loading within certain range (20-60%) (Fig. 5). The time corresponding to 50% of release was about 7 h and that corresponding to 90% of release was about 28 h. With 80% acetaminophen loading, the release from HPMC tablets was longer than with CMS tablets. Thus, 50% percent of acetaminophen were released within 4.5 h, while the 90% were released within 16 h. The times of 50% acetaminophen release from HPMC matrices were markedly shorter than those of 90% release, maybe due to a burst release in the first hours of incubation followed by a diffusion-controlled release after the hydrogel layer formation.

Delayed release was observed with dry-coated tablets containing 20% acetaminophen. Fifty percent of acetaminophen were released within 27 h, while the 90% were released within 44.5 h.

Within 6 h for monolithic tablets and 12 h for dry-coated ones, the release rate from HPMC tablets was not affected by the presence of pancreatin in SIF (not shown). This was expected because α-amylase can not break the beta bonds in cellulose.

The drug release from tablets based on CMS may be influenced by the pH of dissolution medium due to the ionic character of CMS, and by the presence of pancreatin due to the degradation of polymer chains by α-amylase. In contrast,

HPMC is non-ionic and not susceptible to degradation by α-amylase. This limits the use of such excipient alone to formulate drugs with specific absorption window. However, HPMC showed ability to control the release of high loading of acetaminophen.

4. Conclusions

In monolithic tablets, the carboxymethyl starch (CMS) used as a carrier for *Lactobacillus rhamnosus* afforded a partial protection against acidity of SGF and controlled release in SIF. Under the same conditions, the monolithic tablets based on HPMC afforded a higher protection of probiotics against acidity, but it did not allow their release in SIF even after 15 h of incubation.

Both CMS and HPMC provided controlled release for relatively high loading of acetaminophen (up to 60%). For the same excipient, similar release profiles were obtained with 20%, 40% and 60% loadings. Delayed releases, 4 h for CMS and 6 h for HPMC, were observed with dry-coated tablets containing 20% acetaminophen. The presence of α-amylase in SIF accelerates the release from CMS tablet due to the enzymatic erosion of polymer chains, whereas the release from HPMC tablet was not affected. Swelling, diffusion and solubilization contribute to the control of release from tablets based on CMS and on HPMC.

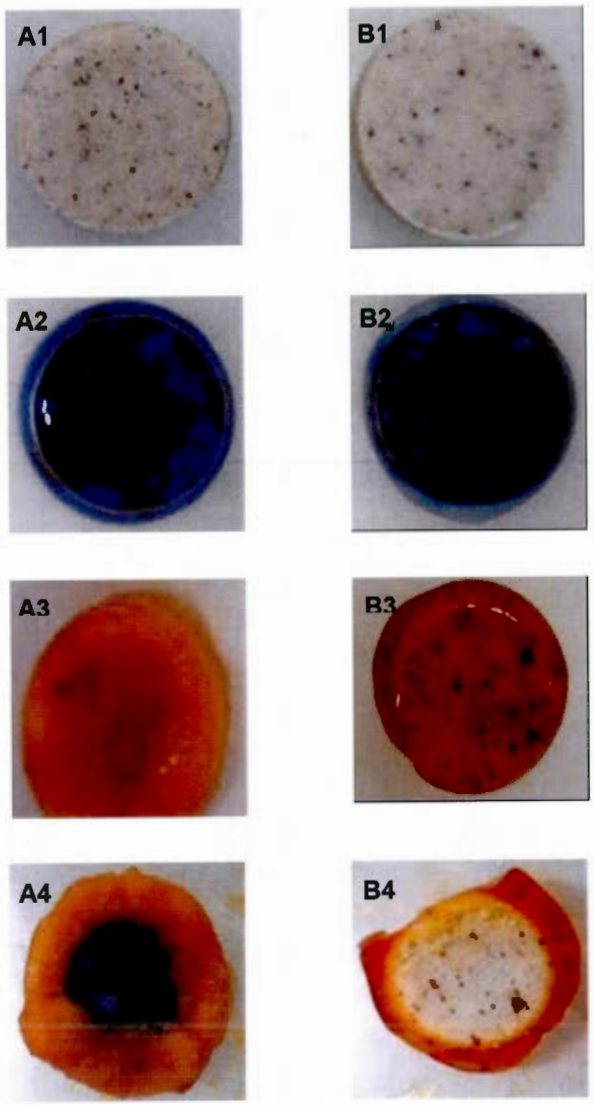

Figure 2: Photographs of CMS (A) and HPMC (B) tablets loaded with bromocresol green (orange at acid pH and blue at neutral pH). Tablets after compression (A1, B1), tablets after 5 min in water (A2, B2), tablets and tablets cross sections after 2 h in SGF (A3-A4 and B3-B4).

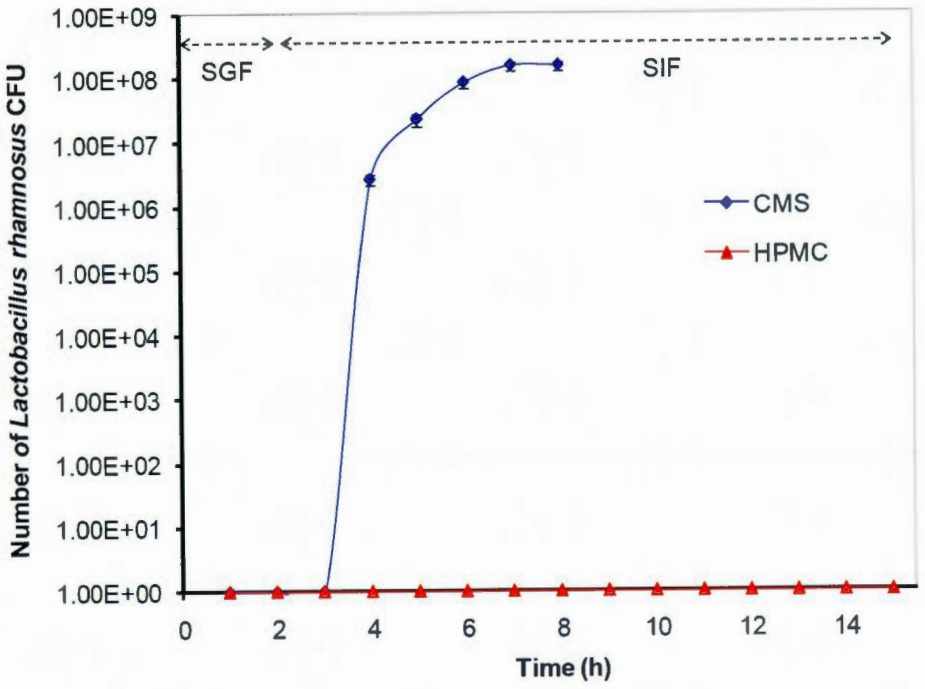

Figure 3: Evaluation of *Lactobacillus rhamnosus* release from CMS and HPMC tablets (200 mg, 5% loading) by measuring the number of colony forming units (CFU). The tablets (n = 3) were incubated (50 rpm, 37 °C) in 50 mL SGF for 2 h and then in 50 mL SIF containing pancreatin.

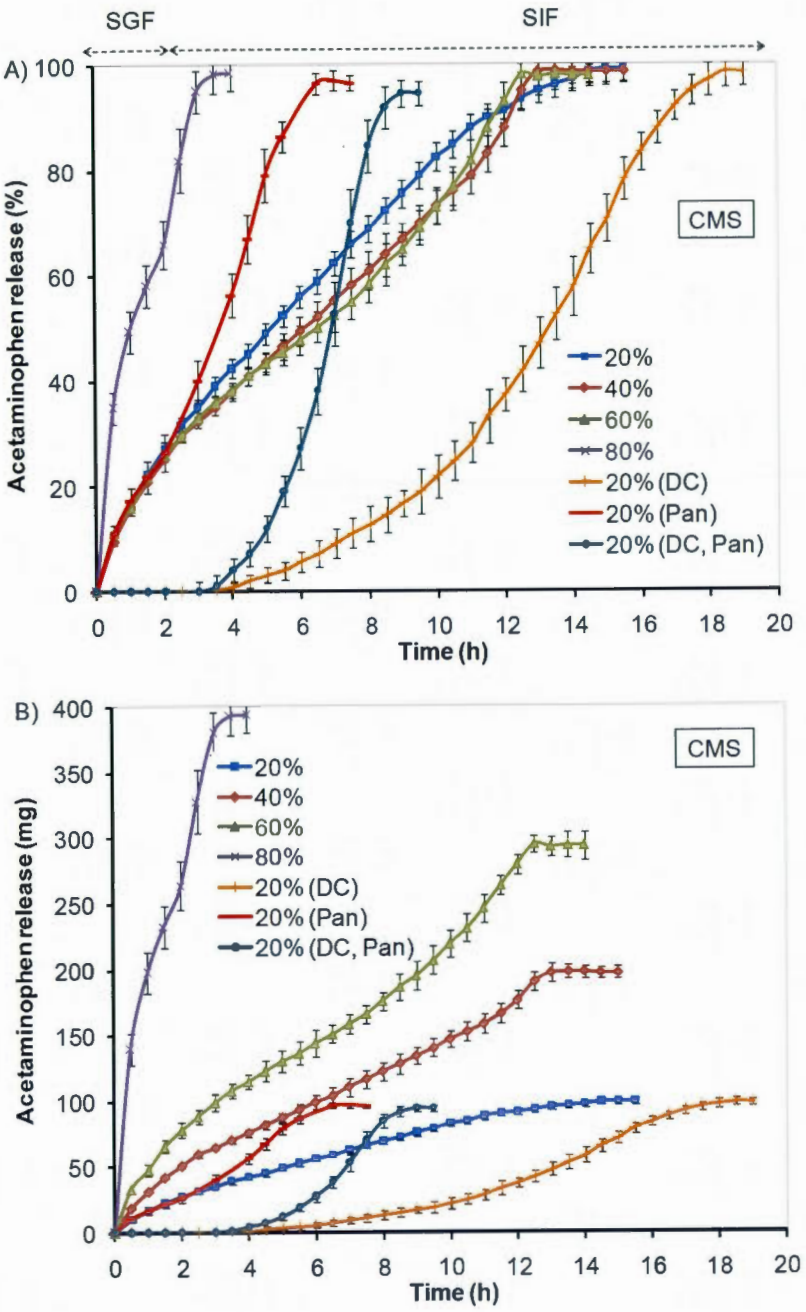

Figure 4: Kinetics of acetaminophen dissolution from monolithic or dry-coated (DC) tablets of CMS (500 mg, 20-80% loading): (A) in %, (B) in mg. The tablets were incubated (1 L, 37 °C, 100 rpm) for 2 h in SGF and then in SIF with or without pancreatin (Pan).

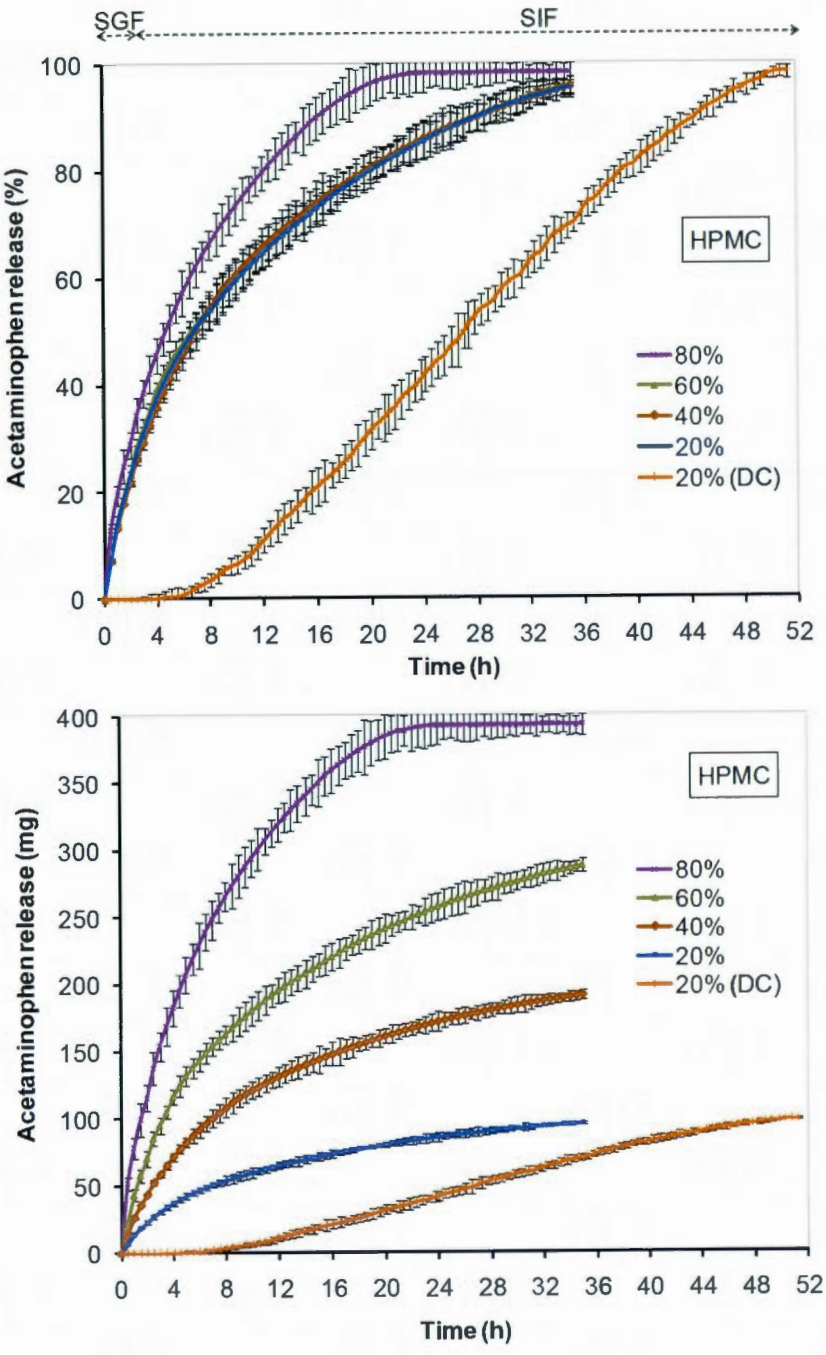

Figure 5: Kinetics of acetaminophen dissolution from monolithic or dry-coated (DC) tablets of HPMC (500 mg, 20-80% loading): (A) in %, (B) in mg. The tablets were incubated (1 L, 37 °C, 100 rpm) for 2 h in SGF and then in SIF without pancreatin.

CHAPITRE VII

COMPLEXE POLYÉLECTROLYTE À BASE DE CARBOXYMÉTHYL AMIDON ET DE CHITOSANE COMME EXCIPIENT POUR LE TRANSPORT DES SUBSTANCES ACTIVES ADMINISTÉES PAR VOIE ORALE

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Résumé

Un nouveau complexe polyélectrolyte (CPE) carboxyméthyl amidon-chitosane a été préparé, caractérisé et testé *in vitro* comme excipient pour le transport des substances actives par voie orale. Ce CPE, contenant 14% en masse de chitosane, a été plus amorphe que le CMA et le chitosane. Dans des conditions simulant le transit gastro-intestinal, l'analyse par imagerie RMN a montré que la diffusion des fluides à l'intérieur des comprimés monolithiques du CPE a été plus lente que dans ceux de CMA. Le CPE semble plus convenable pour la libération prolongée des substances actives que le CMA, puisqu'il peut prolonger la libération de l'acétaminophène de 8 h à 11 h et celle de l'aspirine de 13 h à 30 h. Le chitosane utilisé comme coexcipient accélère la libération de l'aspirine à partir des matrices basées sur un mélange physique CMA:chitosane.

Mots clés : carboxyméthyl amidon, chitosane, complexe polyélectrolyte, coexcipient, comprimé monolithique, transport des substances actives.

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Polyelectrolyte complex of carboxymethyl starch and chitosan as drug carrier for oral administration

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Abstract

A novel polyelectrolyte complex (PEC) of carboxymethyl starch (CMS) and chitosan was prepared, characterized and tested *in vitro* as a carrier for oral drug delivery. This PEC, containing 14% (w/w) of chitosan, showed a structure more amorphous than those of CMS and of chitosan. Under conditions simulating the gastrointestinal transit, NMR imaging analysis showed slower fluid diffusion inside PEC monolithic tablets than inside CMS tablets. The PEC appear to be a more suitable drug carrier for colon targeting than CMS, since it can prolong the acetaminophen release time from 8 h to 11 h and the aspirin release time from 13 h to 30 h. In contrast, chitosan used as a coexcipient accelerated aspirin release from matrices based on a CMS:chitosan physical mixture.

Keywords: carboxymethyl starch, chitosan, polyelectrolyte complex, coexcipient, monolithic tablet, drug delivery.

Abbreviations: CMS, carboxymethyl starch; PEC, polyelectrolyte complex; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; DS, degree of substitution; DDA, degree of deacetylation; DC, dry-coated; 50% CMS:50% chitosan, an excipient containing 50% (w/w) of CMS and 50% (w/w) of chitosan; $t_{90\%}$, time (h) for the release of 90% of drug.

1. Introduction

Since carboxymethyl starch (CMS) was proposed (Mulhbacher, Mateescu, & Calinescu, 2004) as an excipient for controlled drug release from oral solid dosage forms (tablet), several studies have been undertaken in order to investigate the properties and the efficiency of this excipient. The influence of the degree of substitution (DS), of the degree of protonation, and of the formulated drug type and loading on release kinetics of small molecules from CMS matrices has been recently studied (Assaad & Mateescu, 2010; Assaad, Azzouz, & Mateescu, 2008; Ispas-Szabo, De Koninck, Calinescu, & Mateescu, 2007; Lemieux, Gosselin, & Mateescu, 2009). Moreover, the effects of certain formulation parameters, such as compression force and NaCl electrolyte particle size, on drug release rate have been investigated (Brouillet, Bataille, & Cartilier, 2008; Nabais et al., 2007). CMS has also been suggested for the formulation of large size bioactive agents, such as pancreatic enzymes (α-amylase, lipase and trypsin) (Massicotte, Baille, &Mateescu, 2008), *Escherichia coli*, filamentous surface proteins of *Escherichia coli* (F4 *fimbriae*) and *Lactobacillus rhamnosus* probiotic (Calinescu & Mateescu, 2008; Calinescu, Mulhbacher, Nadeau, Fairbrother, & Mateescu, 2005; Calinescu, Nadeau, Mulhbacher, Fairbrother, & Mateescu, 2007).

These studies have shown that CMS can reduce the damaging effect of the acidity of gastric medium on bioactive agents and affords a controlled drug release in intestinal medium. In simulated gastric fluid (SGF, pH 1.2), the CMS in the outer layer of tablet is protonated, making the matrix compact. At higher pH (simulated intestinal fluid, SIF, pH 6.8), the carboxyl groups are deprotonated and ionized, thus favoring hydration, swelling and finally solubilisation of tablet. The solubility of CMS in neutral medium (SIF) and its degradation by pancreatic α-amylase can be limiting factors to effect a sustained drug release (Assaad & Mateescu, 2010; Calinescu & Mateescu, 2008). With the aim to ensure a longer time of drug release and targeting to the colon, chitosan dry powder has been used as a coexcipient in such formulations

(Calinescu & Mateescu, 2008; Leonida & Mateescu, 2006). Chitosan has been shown to interact with unmodified starch via intermolecular hydrogen bonds, leading to the formation of chitosan–starch complex (Xu, Kim, Hanna, & Nag, 2005).

There is a recent growing interest for polyelectrolyte complexes of chitosan due to its cationic character and biocompatibility (Chen & Fan, 2007): some of them have been proposed for delivery of bioactive agents, such as chitosan–xanthan complexes (Chitoxan TM) for controlled drug delivery (Chellat et al., 2000); chitosan–carboxymethyl konjac glucomannan and chitosan–heparin for delivery of albumin (Du et al., 2005; Liu, Jiao, Liu, & Zhang, 2007); chitosan–dextran sulfate and chitosan–alginate for oral delivery of insulin (Sarmento et al., 2006); chitosan–polyaspartate for delivery of 5-fluorouracil (Zheng et al., 2007). A number of polyelectrolyte complexes of chitosan and polyuronans have been prepared and spray-dried as microspheres (Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004).

The objectives of the present study are (i) to prepare CMS–chitosan polyelectrolyte complex (PEC) and to investigate its performance in drug delivery; (ii) to evaluate the influence of chitosan molecular weight on drug release rate; (iii) to compare the drug dissolution from tablets based on anionic water-soluble excipient (CMS) alone, on cationic water-insoluble excipient (chitosan) alone, on physical mixture powder of these two excipients, or on PEC; and (iv) to compare the dissolution profiles of drugs with different charges and solubilities.

2. Materials and methods

2.1. Materials

High amylose corn starch (Hylon VII) was obtained from *National Starch* (Bridgewater, NJ, USA) and crab shell chitosans were from *Marinard Biotech* (Rivière-au-Renard, QC, Canada). Acetaminophen was from *Sigma-Aldrich*

(St-Louis, MO, USA). Metformin (1,1-dimethylbiguanide hydrochloride) was from *MP Biomedicals* (Solon, OH, USA). Aspirin (acetylsalicylic acid) and monochloroacetic acid were from *Fisher Scientific* (Fair Lawn, NJ, USA). The other chemicals were of reagent grade and used without further purification. Pepsin-free simulated gastric fluid (SGF, pH 1.2) and pancreatin-free simulated intestinal fluid (SIF, pH 6.8) were prepared following the USP methods (US *Pharmacopeia*, XXIV, 2000).

2.2. Preparation of CMS and purification of chitosans

Sodium carboxymethyl starch (CMS) was prepared in aqueous medium from high amylose corn starch as previously described (Calinescu et al., 2005; Mulhbacher, Ispas-Szabo, Lenaerts, & Mateescu, 2001), with minor modifications. Briefly, an amount of 70 g of Hylon VII was suspended in 170 mL of distilled water in a Hobart mixer (Vulcan, Canada) at 55 °C. Then, 235 mL of 1.5 M NaOH were added for gelatinization under continuous mixing for 30 min. Subsequently, 55 mL of 10 M NaOH and a freshly prepared solution of monochloroacetic acid (45.5 g in 40 mL of distilled water) were added. After 1 h of reaction, a volume of 130 mL of distilled water was added and the slurry was cooled-down to room temperature and neutralized with acetic acid. The CMS was then precipitated from the slurry by gradually adding 600 mL of acetone. After that, the CMS was washed by repeated dispersion in volumes of 1 L of 70% acetone and filtrations until a final conductivity of filtrate decreased at about 50 μS/cm. The CMS mass was again washed three times with acetone, and then dried at 40 °C for 24 h. The obtained powder of sodium form CMS was sieved with a 300 μm screen and stored at room temperature.

Two chitosans of different molecular weights were each purified by solubilization in acetic acid and by filtration as follows: an amount of 20 g of chitosan was solubilized in 350 mL of 0.35 M acetic acid and the volume was

adjusted to 2 L with distilled water. The acidic solution was filtered under vacuum through Whatman filter papers (medium 40). Subsequently, the chitosan was precipitated with 0.1 M NaOH under continuous stirring. The mass was washed with distilled water, then with nanopure water (volumes of 2 L) until conductivity of about 200 µS/cm and finally with acetone. The chitosan was dried at 40 °C for 24 h, ground and sieved on a 300 µm screen.

2.3. Preparation of CMS-chitosan PEC

A CMS-chitosan polyelectrolyte complex (PEC) was prepared by coagulation of CMS and chitosan-700 in aqueous medium at room temperature. Essentially, 1 g of chitosan-700 was solubilized in 44 mL of 0.1 M HCl, and the volume was adjusted to 150 mL with distilled water. A 1% solution of CMS was prepared by solubilizing 6 g of CMS in 600 mL of distilled water. The precipitation occurred under vigorous mixing by adding the solution of polycation (chitosan-700) to that of polyanion (CMS) at 1 : 1 ratio ($-NH_3^+ : -COO^-$), with a final pH about 5. The PEC, containing 14% (w/w) of chitosan-700, was washed and dried with acetone by the same procedure as for CMS.

2.4. Physical and chemical characterizations of excipients

2.4.1. The degree of substitution

The degree of substitution (DS) of CMS was determined by back-titration as previously described (Assaad & Mateescu, 2010). Briefly, 300 mg of protonated CMS (n = 3) were solubilized in 20 mL of 0.05 M NaOH and then the excess of NaOH was titrated with 0.05 M HCl using phenolphthalein as indicator. The blank (20 mL of NaOH) was also titrated by the same method. The amount of –COOH groups and the DS were calculated by using the following equations (Stojanovic, Jeremic, Jovanovic, & Lechner, 2005):

$$n_{COOH}=(V_b-V)\times C_{HCl} \qquad (1)$$

$$DS=\frac{(162\times n_{COOH})}{m-58\times n_{COOH}} \qquad (2)$$

where V_b (mL) is the volume of HCl used for the titration of the blank; V (mL) is the volume of HCl used for the titration of the sample; C_{HCl} (mol/L) is the concentration of HCl; 162 (g/mol) is the molar mass of glucose unit; 58 (g/mol) is the increase in the mass of glucose unit by substitution with one carboxymethyl group, and m (g) is the mass of dry sample.

2.4.2. The degree of deacetylation

The degree of deacetylation (DDA) of each chitosan was determined by acid-base titration. An amount of 150 mg of chitosan was solubilized in 20 mL of 0.1 M HCl and the volume was completed to 200 mL with distilled water. A titration was done with 0.1 M NaOH and the pH and the conductivity were recorded. The DDA was calculated following the method and the equation given by Broussignac (1968) and Muzzarelli (1977):

$$DDA(\%)=\frac{203\times(v_2-v_1)\times M\times 100}{m+42\times(v_2-v_1)\times M} \qquad (3)$$

where V_1 and V_2 are the volumes of NaOH solutions corresponding to the two inflexion points of the curve obtained by titration; M is the concentration of NaOH (mol/L); m is the weight of chitosan (g); 203 (g/mol) is the molar mass of acetylated unit, and 42 (g/mol) is the difference between molar mass of acetylated unit and that of deacetylated unit.

2.4.3. The molecular weights

The molecular weights of chitosans were determined by viscometric method, using experimental reported viscometric constants data (Kasaai, 2007; Knaul, Kasaai, Bui, & Creber, 1998). Samples were dissolved in a solution containing 0.1 M acetic acid and 0.2 M sodium chloride for chitosan-400 and in a solution containing 0.2 M acetic acid and 0.1 M sodium acetate for chitosan-700. The viscosities of chitosan solutions with different concentrations (0.07-0.7%) were measured by using an electronic viscometer (*Viscosity Monitoring and Control Electronics*, Medford, MA, USA). The temperature was adjusted at 25 °C for chitosan-400 and at 30 °C for chitosan-700 as reported elsewhere (Roberts & Domszy, 1982; Wang, Bo, Li, & Qin, 1991).

The data on viscosities and concentrations were used to calculate the reduced viscosities. Plotting reduced viscosities against chitosan concentrations gives the intrinsic viscosity ([η]) by extrapolation of the straight line obtained by linear regression to zero concentration. The average molecular weight (M) of chitosan was calculated from the intrinsic viscosity by Mark-Houwink-Sakurada's empirical equation:

$$[\eta] = kM^{\alpha} \qquad (4)$$

where k (dL/g) and α (dimensionless) are constants that depend on the solvent-polymer system.

2.4.4. The Fourier Transform Infrared spectra

The Fourier Transform Infrared spectra (FTIR) of samples were recorded from 4000 to 400 cm^{-1} at 2 cm^{-1} resolution with a total of 32 scans by using a *Nicolet* 4700 spectroscopy (Madison, WI, USA). To prepare the pellets, homogenous mixtures of dried KBr (67 mg) and of polymer powders (3 mg) were compressed at 3 tonnes (*Carver*, Wabash, IN, USA) in flat-faced punches with 12 mm diameter.

2.4.5. The polymorphism

The polymorphism of samples was evaluated by X-ray diffractometer (XRD, *Siemens* D5000, Munich, Germany) at 1.789 Å wavelength. The original XRD spectra, recorded between 5° and 50° (2θ), were treated using Excel software (regression type: moving average, period 10).

2.4.6. The thermogravimetric analyses

The thermogravimetric analyses were carried out in platinum crucible at a heating rate of 10 °C/min between 25 and 900 °C under nitrogen atmosphere (flow rate 100 mL/min). A *Seiko* TG/DTA 6200 (Japan) instrument was used and the alumina was taken as reference material.

2.4.7. The morphology

The morphology of the sample particles was examined by a *Hitachi* (S-4300SE/N) scanning electron microscopy with variable pressure (Hitachi High Technologies America, Pleasanton, CA, USA) at voltage of 15 kV and magnifications of 100x and 500x. Samples were mounted on metal stubs and sputter-coated with gold.

2.4.8. The density

The density of the polymer powders was determined according to the (616) USP method, using a Vankel tapped density tester (*Varian*, NC, USA).

2.5. Preparation of tablets

Monolithic tablets (200 mg, 20% (w/w) loading) were obtained by direct compression (2.5 tonnes) of a homogenous mixture of excipient and drug (acetaminophen, metformin or aspirin) powders. The unloaded (drug-free) tablets of

200 mg were prepared with excipient only. Flat-faced punches with 9.6 mm diameter and a *Carver* hydraulic press were used.

Dry-coated (DC) tablets (200 mg, 20 % (w/w) loading) were prepared with a core consisting in a homogenous mixture of drug (40 mg) and excipient (40 mg) and compressed in a 7 mm cylinder outfit. This core was then dry coated with 120 mg of excipient, giving tablet of about 9.6 mm diameter and 2.1 mm thickness after compression.

2.6. Nuclear magnetic resonance (NMR) imaging analysis

NMR imaging analyses were carried out at 37 °C with a *Bruker* Avance-400 NMR spectrometry (Germany) as previously reported (Baille, Malveau, Zhu, & Marchessault, 2002; Malveau, Baille, Zhu, & Marchessault, 2002; Thérien-Aubin & Zhu, 2006, 2009; Thérien- Aubin, Baille, Zhu, & Marchessault, 2005; Thérien-Aubin, Zhu, Ravenelle, &Marchessault, 2008; Wang, Ravenelle, & Zhu, 2010). A standard spin-echo pulse sequence (90-τ-180-τ-Acquisition) was used to obtain spin density images of the unloaded tablets (n = 3) in a NMR tube (20 mm diameter) containing 20 mL of dissolution media (SGF or SIF). A slice of 0.5 mm in thickness was selected either perpendicular or parallel to the main magnetic field (axial axis). Eight scans were accumulated with a field of view of 2 cm and an in-plane resolution of 156 μm. An echo time of 3 ms and a repetition time of 1 s were fixed, leading to an acquisition time of about 17 min for each image. Each tablet was first incubated for 2 h in SGF and then in SIF until the end of the test. The percentage of axial and radial swelling was calculated by comparison to the initial dimension of tablet.

2.7. *In vitro* dissolution tests

The *in vitro* dissolution tests were carried out at 100 rpm and 37 °C in an USP dissolution apparatus II (*Distek* 5100, North Brunswick, NJ, USA) coupled with an

UV spectrophotometer (*Hewlett Packard* 8452A, USA). The tablets (n = 3) were incubated in SGF (1 L) for 2 h and then in SIF (1 L) up to complete release. The drug release from tablets was evaluated by measuring the absorbance at the appropriate wavelength (acetaminophen at 244 nm, metformin at 218 nm, and aspirin at 246 nm).

3. Results and discussion

3.1. Characterization of the excipients

The degree of substitution of carboxymethyl starch (CMS) determined by the back-titration method was about 0.14, representing the average number of carboxymethyl groups per glucose unit. The degrees of deacetylation of chitosans determined by acid-base titration were about 80% and the approximate molecular weights determined by Mark-Houwink-Sakurada method were about 400 kDa for chitosan-400 and 700 kDa for chitosan-700.

The scanning electron microscopy micrographs showed that chitosan particles were compact, whereas those of CMS and PEC were porous (Fig. 1). The morphology of the polyelectrolyte complex appeared homogenous, indicating a uniform distribution and a good compatibility between CMS and chitosan (Figs. 1d1 and 1d2).

Chitosan-400 and chitosan-700 showed the highest tapped densities (0.61 and 0.64 g/mL, respectively) due to their compact morphology, whereas PEC showed the lowest density (0.20 mg/mL) due to its higher granulometry and porosity (Fig. 1). The CMS showed a density of 0.36 mg/L.

3.2. CMS-chitosan interactions and preparation of PEC

When the chitosan-700 solution was added to the CMS solution, immediate coagulation and precipitation occurred. This suggests effective interactions between

functional groups of CMS and of chitosan-700 with possible partial charges neutralization, leading to the formation of a polyelectrolyte complex. To verify this hypothesis, the products were characterized by FTIR spectroscopy, by X-ray diffractometry (XRD) and by thermogravimetry (TGA) (Figs. 2-4).

The FTIR spectrum of CMS (Fig. 2) presents two characteristic bands at 1603 and 1417 cm^{-1}. They were attributed respectively to asymmetrical and symmetrical stretching vibration of $-COO^-$ groups (Silverstein, Webster, & Kiemle, 2005; Zoldakova, Srokova, Sasinkova, Hirsch, & Ebringerova, 2005). The bands at 2930 and 1643 cm^{-1} are assigned respectively to C–H stretching and to O–H groups.

The spectrum of chitosan-700 shows characteristic absorption bands of chitosan at 1653 and 1597 cm^{-1} ascribed to $-CONH_2$ stretching vibrations, and two bands at 2922 and 2876 cm^{-1} due to C–H stretching. The bands at 1417 and 1376 cm^{-1} were assigned to the C–H symmetrical deformation mode as per Mathew and Abraham (2008).

The polyelectrolyte complex (PEC) shows a spectrum similar to that of 50% CMS: 50% chitosan-700, with bands at about 2923-2880, 1636, 1600, 1417 and 1376 cm^{-1}. This indicates the presence of both CMS and chitosan in the PEC. Roughly similar spectra of dry blend polymer powders and polyelectrolyte complexes were reported in other studies, as for chitosan and carboxymethyl cellulose polymers (Fukuda, 1980). The weak shoulders at around 1735 and 1540 cm^{-1} for PEC obtained at pH 5 could be assigned respectively to –COOH and $-NH_3^+$ groups. These shoulders suggest that interactions between CMS and chitosan in the PEC may occur via hydrogen bonds (–OH, –COOH) or ionic interactions ($-COO^-$, NH_3^+).

The XRD pattern (Fig. 3) of CMS shows the two characteristic peaks at 6.9 and 4.5 Å, indicating a V-type single helix structure as previously reported (Assaad & Mateescu, 2010). The pattern of chitosan-700 shows characteristic crystalline peaks

at around 6.9 and 4.4 Å (major one), fitting well with the typical XRD pattern of chitosan (Choi, Kim, Pak, Yoo, & Chung, 2007; Wang et al. 2005).

The structure of PEC is definitely more amorphous that those of CMS and chitosan-700. The suppression of crystalline peak of chitosan-700 at 4.4 Å and the broad amorphous pattern of the PEC indicate a good compatibility between CMS and chitosan with a complete dispersion of chitosan chains. These intermolecular interactions could prevent macromolecules to crystallize individually as reported for some interpolymer complexes (Mathew & Abraham, 2008; Sakurai, Maegawa, & Takahashi, 2000; Xu et al., 2005; Yin, Yao, Cheng, & Ma, 1999).

The TGA results (Fig. 4) show relatively lower moisture content for chitosan-700 than for CMS and PEC, maybe due to higher hydrogen association of chitosan chains. The 50% CMS:50% chitosan-700 shows a nonsymmetrical dTG peak with a weak shoulder at around 287 °C and a maximum at 303 °C, indicating the presence of two components. The difference of decomposition temperatures between CMS (287 °C) and chitosan-700 (308 °C) seems not enough to identify two separate peaks for the dry powder mixture of these two polymers. Differing from 50% CMS:50% chitosan-700, the PEC presented a symmetrical dTG peak and the highest decomposition temperature (313 °C).

Overall, these results suggest a good compatibility between CMS and chitosan, a strong interaction between the chains of these two polymers, and the formation of a homogenous polyelectrolyte complex.

3.3. Examination of tablets hydration and swelling by NMR

Water penetration into unloaded tablets (Fig. 5A) and the axial and radial swelling (Fig. 5B) were followed by NMR imaging in SGF for 2 h and then in SIF to simulate the gastrointestinal transit. The location where the water concentration matches 1/6 of the maximal concentration corresponding to free fluid (SGF or SIF)

was considered as the front of fluid diffusion inside the tablets (Baille et al., 2002; Malveau et al., 2002).

For all tablets, the axial swelling was higher than radial swelling (Fig. 5B). This may be explained by the formation of flat oriented particles in tablet after axial compression of polymer powders. Upon tablet hydration, the stress resulting from compression is released, leading to a higher swelling in the direction where compression force was applied (Le Bail, Morin, & Marchessault, 1999; Malveau et al., 2002; Thérien-Aubin & Zhu, 2006, 2009; Thérien-Aubin et al., 2005, 2008; Wang et al., 2010).

After 2 h in SGF, the CMS tablet still showed a dry core (purple) with a partial penetration of SGF and formation of a gel network in the outer layer (blue and green) (Fig. 5A). In acidic medium (SGF, pH 1.2), the carboxylate groups (–COONa) of the outer layer are converted to carboxyl groups (–COOH), thus reducing the solubility of the CMS excipient and limiting the gastric fluid penetration into the tablets. When tablets were transferred to SIF (pH 6.8), the fluid advanced rapidly to the core which became hydrated within 2 h in this neutral medium. The protonation acquired in SGF is lost and the –COOH groups turn into their salt form (–COOK), increasing thus the solubility of the excipient and accelerating intestinal fluid advancement to the core of the tablet. The axial and radial swelling of CMS tablet increase relatively fast, reaching 150% and 60%, respectively, after 4 h of incubation (Fig. 5B).

The diffusion of fluid (SGF or SIF) into the chitosan (chitosan-400 and chitosan-700) tablets was slower than into the CMS tablets (Fig. 5A). A gel network was developed by chitosan in SGF due to the protonation of amino groups exposed to the acid medium. In SIF, the tablet size was stabilized (Fig. 5B) due to chitosan insolubility in neutral medium while an anisotropic fluid diffusion was observed (Fig. 5A). For chitosan-400 the core was almost completely hydrated after 8 h of incubation, whereas for chitosan-700 the core still showed dry regions even after

20 h. Thus, chitosan-700 with a higher molecular weight seems to provide a thicker (more substantial) outer layer gel than chitosan-400.

The tablets of 50% CMS:50% chitosan-700 mixture showed the fastest fluid diffusion (Fig. 5A) and the highest swelling (Fig. 5B). The gel network formed in SGF was less substantial than that formed with chitosan tablets due to close neighboring of CMS in the mixture. In SIF, the chitosan would be deprotonated, whereas the CMS would be converted to the salt form triggering a higher *in situ* hydration of tablet previously swollen in SGF.

The tablets of PEC presented slower fluid diffusion than CMS and 50% CMS:50% chitosan-700 tablets, particularly in SIF (Fig. 5A). This suggests that association of CMS and chitosan at molecular level as PEC favors more interactions between these two compounds than in physical mixture of powders. A more extensive swelling occurred in the first two hours of incubation in SGF due to the protonation and the hydration of chitosan within the PEC. When SGF was changed to SIF, the size of PEC tablets was reduced due to the deprotonation and dehydration of chitosan chains in neutral medium, indicating a stronger interaction between chitosan and CMS than that between CMS and water. The shape of tablets was after that as stable as those of the chitosan, despite the low ratio (14%) of chitosan in PEC. This is an important aspect and can be related to the insolubility of chitosan in neutral medium and to a lower tendency of CMS to swell when intimately complexed with chitosan.

3.4. *In vitro* dissolution tests

All dissolution tests, except for metformin formulated in monolithic tablets, were followed first in SGF for 2 h and then in SIF until complete drug release, simulating thus the gastrointestinal transit. The shape of tablets and the dissolution

profiles of acetaminophen, metformin and aspririn are presented in Figs. 6 and 7. Unless otherwise specified, the tablets (200 mg) used for dissolution were monolithic.

The release rates of acetaminophen from chitosan-400 and 50% CMS:50% chitosan-400 matrices were higher than from CMS matrix, whereas the release rates from chitosan-700 and 50% CMS:50% chitosan-700 matrices were lower than from CMS matrix (Fig. 7A). That is why the chitosan-700 was chosen to prepare the PEC. In addition, the 75% CMS:25% chitosan-700 matrix showed almost the same release rate as CMS (not shown). It seems that a molecular weight of 700 kDa rather than 400 kDa and an adequate ratio in dry blends are required for chitosan to favor a longer drug release time.

Although the chitosan-700 matrix showed the lowest release rate, chitosan alone does not seem suitable for controlled drug release, because the transformation of the gel developed in SGF (Fig. 6, a2) to a semi-solid form (Fig. 6, b2) that limits the diffusion of SIF into the tablet makes the release slow (Fig. 7A). It is worthwhile to note that the solid core of tablet was still compact and insoluble even after the complete acetaminophen release (Fig. 6, b2).

The faster release from tablets based on CMS:chitosan-700 powder mixture compared to that from those with chitosan-700 as only excipient, indicates that the CMS favors the tablet hydration and accelerates the diffusion of SIF into the tablets. These results are in agreement with those obtained by NMR imaging (Fig. 5A). At the end of the dissolution tests, the tablets based on a mixture of CMS and chitosan powders appeared as a water-insoluble empty shell (Fig. 6, b3) with a crust still containing amixture of these two polymers as confirmed by FTIR analysis (not shown). This indicates that although the tablets are based on dry blend of polymer powders, physical or chemical interactions can occur between CMS and chitosan during the dissolution.

The release rate of acetaminophen from PEC matrix was lower than that from 50% CMS:50% chitosan-700 matrix (Fig. 7A). This is an interesting advantage for PEC

which contains only 14% (w/w) of chitosan-700, considering the higher cost of chitosan compared to that of CMS.

Metformin is a freely soluble drug (US *Pharmacopeia*, 2000) and its release from hydrophilic excipients is usually fast. Neither CMS nor chitosans, separately or in association, were able to control the release of metformin in monolithic dosage form (Fig. 7B). Dry coated (DC) tablets can delay this release in SGF, especially when the outer part of tablet is based on chitosan-700. However, when the fluid reached the inner core of the tablets the release was accelerated. The dissolution profiles of metformin from tablets based on chitosan-400 were similar to those based on chitosan-700, but with higher release rate (not shown). The dry coating formulation can be of interest, considering the very high hydrosolubility of metformin and the fact that the release is undesired in stomach.

For aspirin, which is slightly soluble in water (US *Pharmacopeia*, 2000), the higher the molecular weight of chitosan, the lower was the release rate (Fig. 7C). CMS and chitosan-700 provided a low release of aspirin in SGF and a long sustained release in SIF. Probably, this is due to the interactions of carboxyl groups of aspirin with carboxyl groups and hydroxyl groups of CMS, and to the formation of consistent outer layer gel network in tablets based on chitosan (Fig. 6, c2). As with acetaminophen, the dissolution of aspirin from chitosan-700 matrix after 80% of release was reduced probably due to the formation of chitosan insoluble outer layer in SIF (Fig. 6, d2). The matrices based on CMS:chitosan mixture showed an accelerated release of aspirin compared to those based on individual excipient (CMS or chitosan). It seems that the aspirin–CMS and aspirin–chitosan interactions compete and reduce those between these two polymers, favoring a faster tablet erosion and aspirin release. Similar to what was observed with acetaminophen, a water-insoluble excipient residue was still present after complete release of aspirin (Fig. 6, d3), indicating the presence of CMS–chitosan attractions. A sustained release of aspirin, over more than

30 h, was observed with PEC (Fig. 7C). This time release is markedly longer than $t_{90\%}$ obtained with 50% CMS:50% chitosan-700 (6.5 h), CMS (11 h) or chitosan-700 (11.5 h). This difference is ascribed to the interactions between aspirin, CMS and chitosan within the PEC matrix. These interactions reduce the diffusion of both aqueous fluid and the drug, leading thus to the lower release rates. The remained tablet based on the PEC after the complete release of drug (Fig. 6, d4) further supports the existence of the interpolymer attractions. In this case, aspirin interacts with the hydroxyl, carboxyl and amino groups of the PEC without dissociation of interactions between the chains of CMS and chitosan.

Taken together, these results showed the advantage of PEC for monolithic formulations. The PEC excipients, containing only 14% of chitosan-700, can afford controlled release of acetaminophen and aspirin. Moreover, the tablets of the PEC were homogenous and less swellable than those of 50% CMS:50% chitosan-700 (Figs. 5B and 6).

4. Conclusion

The CMS–chitosan polyelectrolyte complex (PEC) showed a structure more amorphous than those of carboxymethyl starch (CMS) and of chitosan-700. The fluid (SGF or SIF) diffusion and the swelling were lower with PEC tablets than with those based on CMS:chitosan-700 powder mixture. The PEC provided a controlled release of acetaminophen and a markedly slower sustained release of aspirin than that provided by CMS or chitosan-700, making this excipient favorable to colon targeting.

Chitosan at molecular weight of about 400 kDa (chitosan-400) did not afford a long release time for any of the three tracer drugs (metformin, acetaminophen and aspirin). CMS and chitosan-700 matrices showed a fast release of metformin, a controlled release of acetaminophen and a sustained release of aspirin. This indicates that the drug solubility has a major influence on release rate irrespective of the charge

of drug and of excipient. The low hydration of chitosan and its insolubility in neutral medium can be a limitation for drug delivery with this excipient alone, but it can be an advantage in the case of PEC. Adding an adequate amount of chitosan with an appropriate molecular weight to the formulations based on CMS can prolong the release time of acetaminophen. Contrarily, the aspirin release from CMSmatrix was accelerated when chitosan was added as coexcipient.

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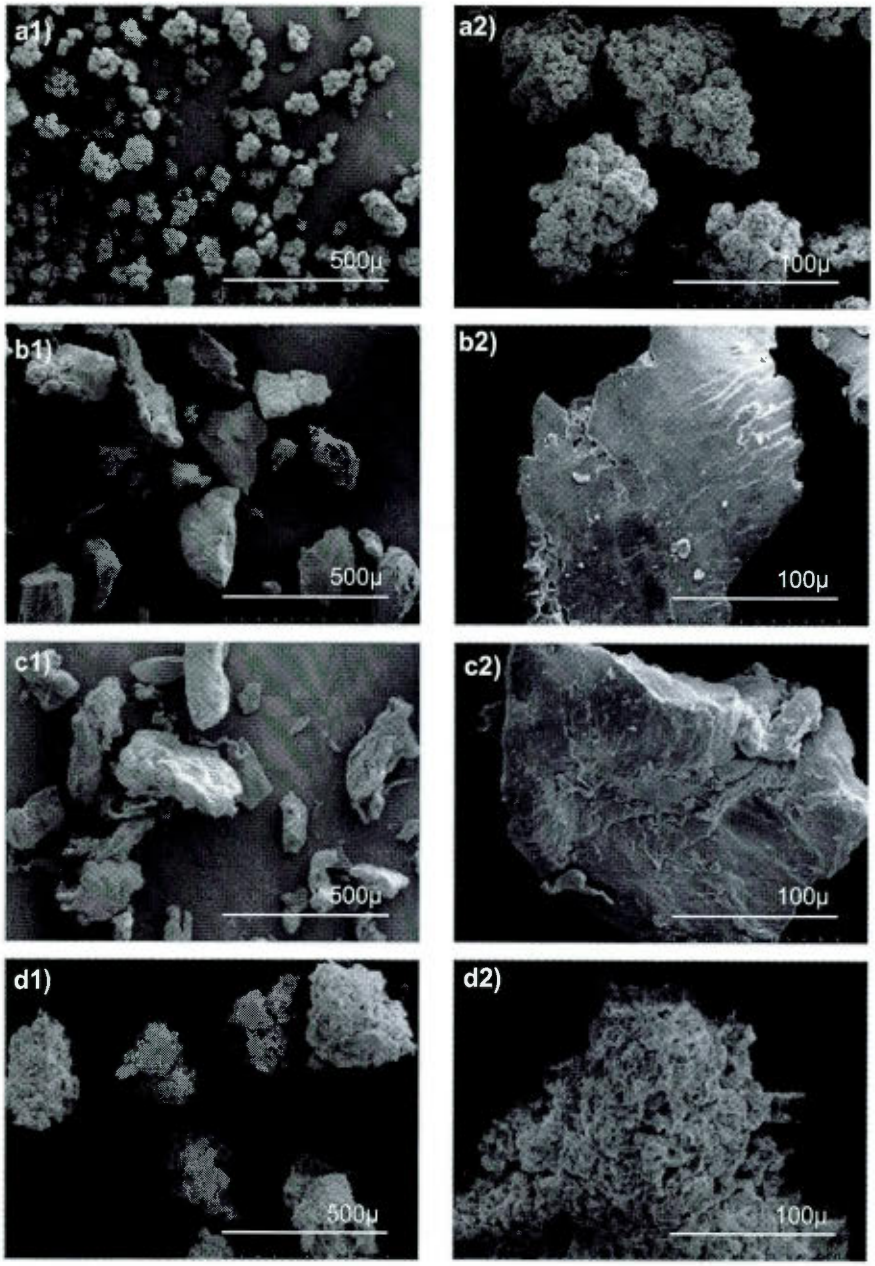

Figure 1: Scanning electron microscopy micrographs of (a) CMS, (b) chitosan-400, (c) chitosan-700 and (d) PEC at magnification of 100x and 500x and voltage of 15 kV.

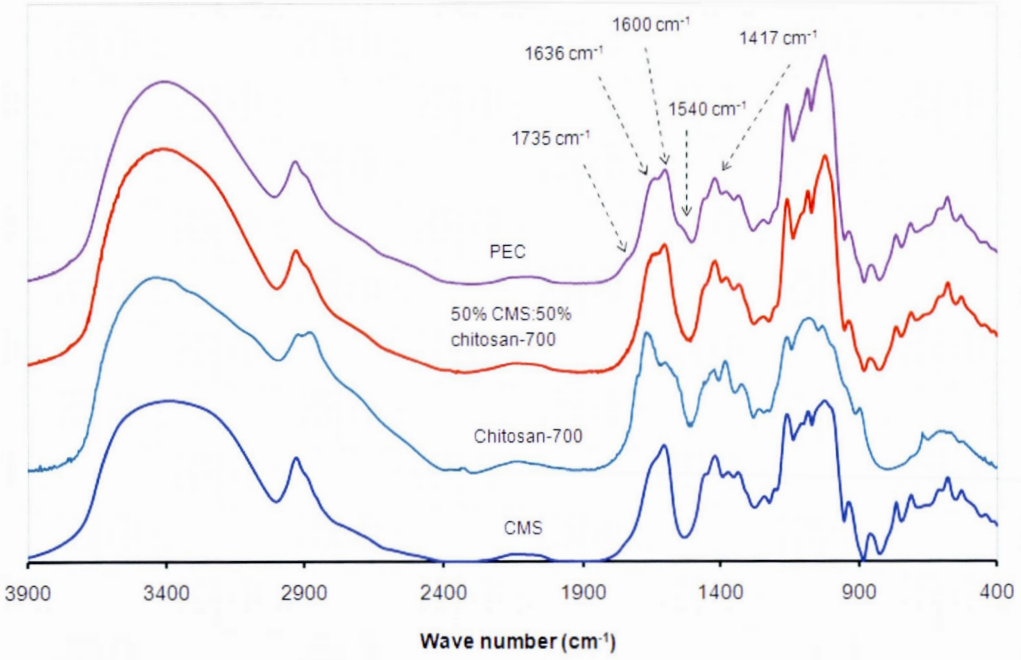

Figure 2: FTIR spectra of CMS, chitosan-700, 50% CMS:50% chitosan-700 and PEC. Pellets (12 mm diameter) were prepared by compression at 3 tonnes of KBr (67 mg) and sample (3 mg) mixtures.

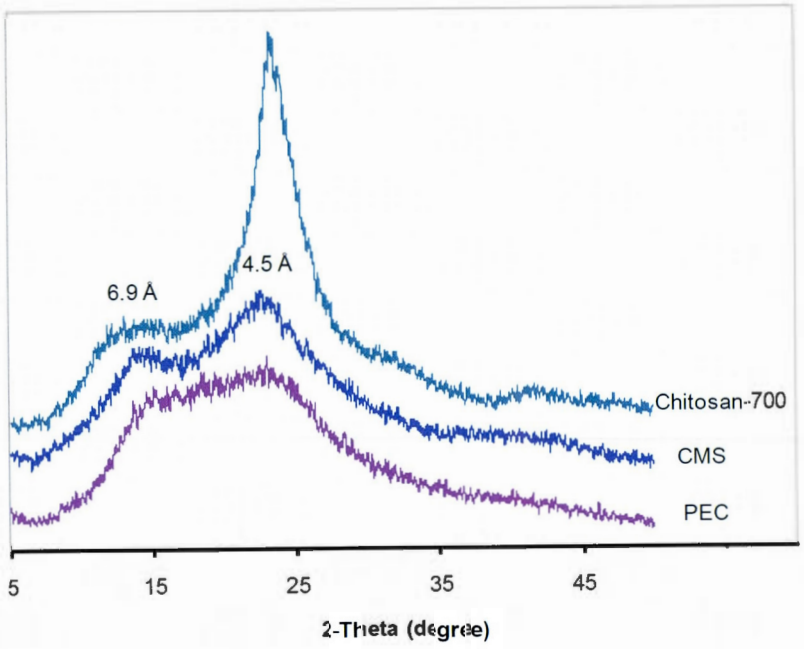

Figure 3: X-ray diffraction patterns of CMS, chitosan-700 and PEC.

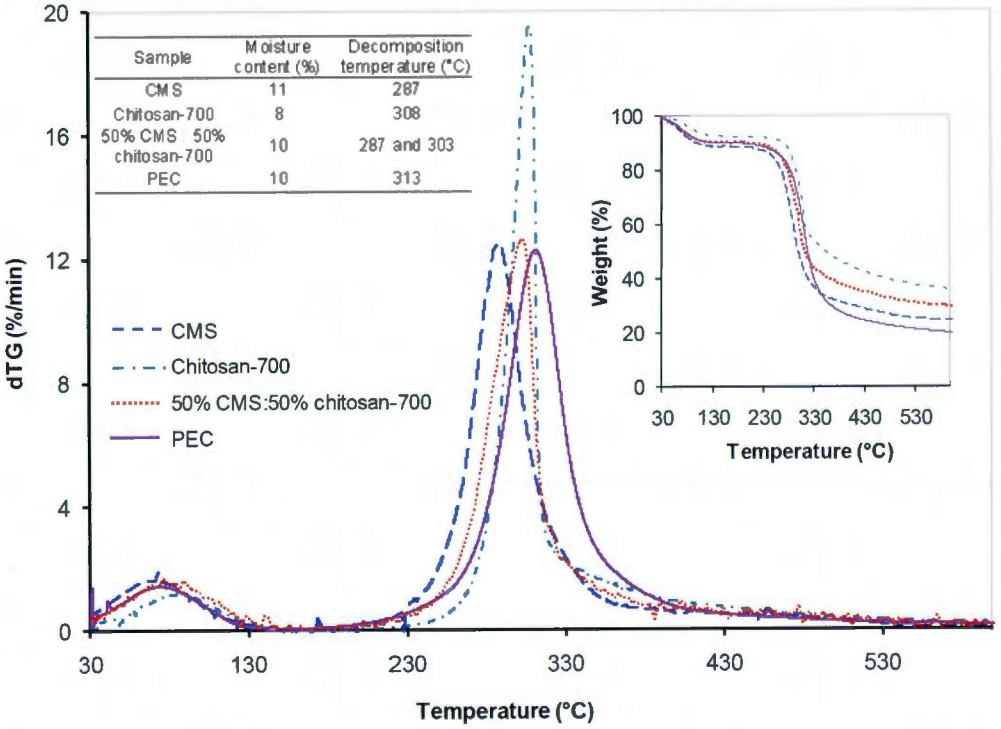

Figure 4: Thermogravimetric patterns of CMS, chitosan-700, 50% CMS:50% chitosan-700, and PEC at heating rate of 10 °C/min between 25 and 600 °C.

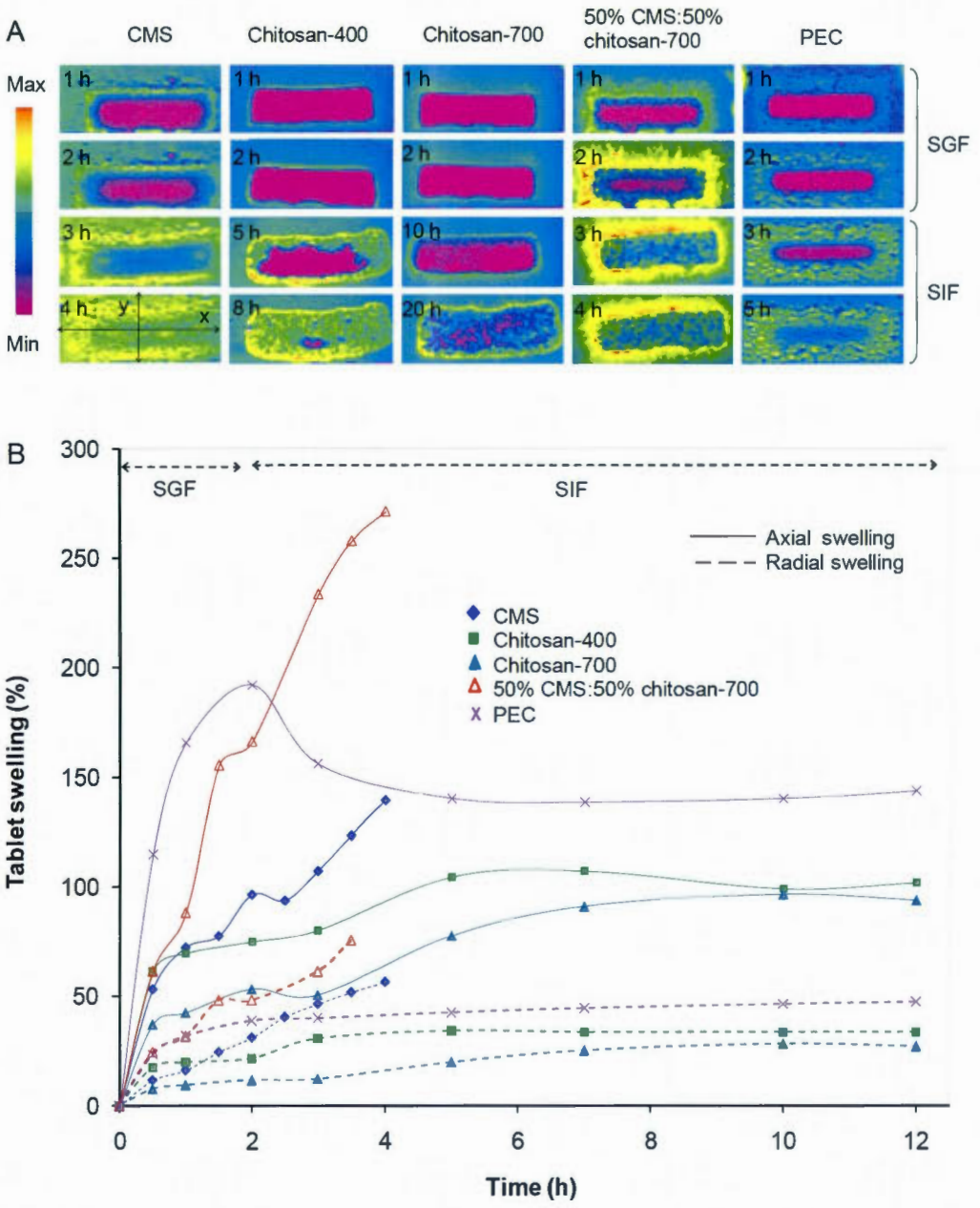

Figure 5: NMR images at various times of unloaded tablets of (CMS, chitosan-400, chitosan-700, 50% CMS:50% chitosan-700 and PEC) incubated for 2 h in SGF and then transferred to SIF: (A) axial side images where x indicates the radial direction and y the axial direction, (B) axial and radial swelling. (For interpretation of the references to color in this figure the reader is referred to the web version of the article.)

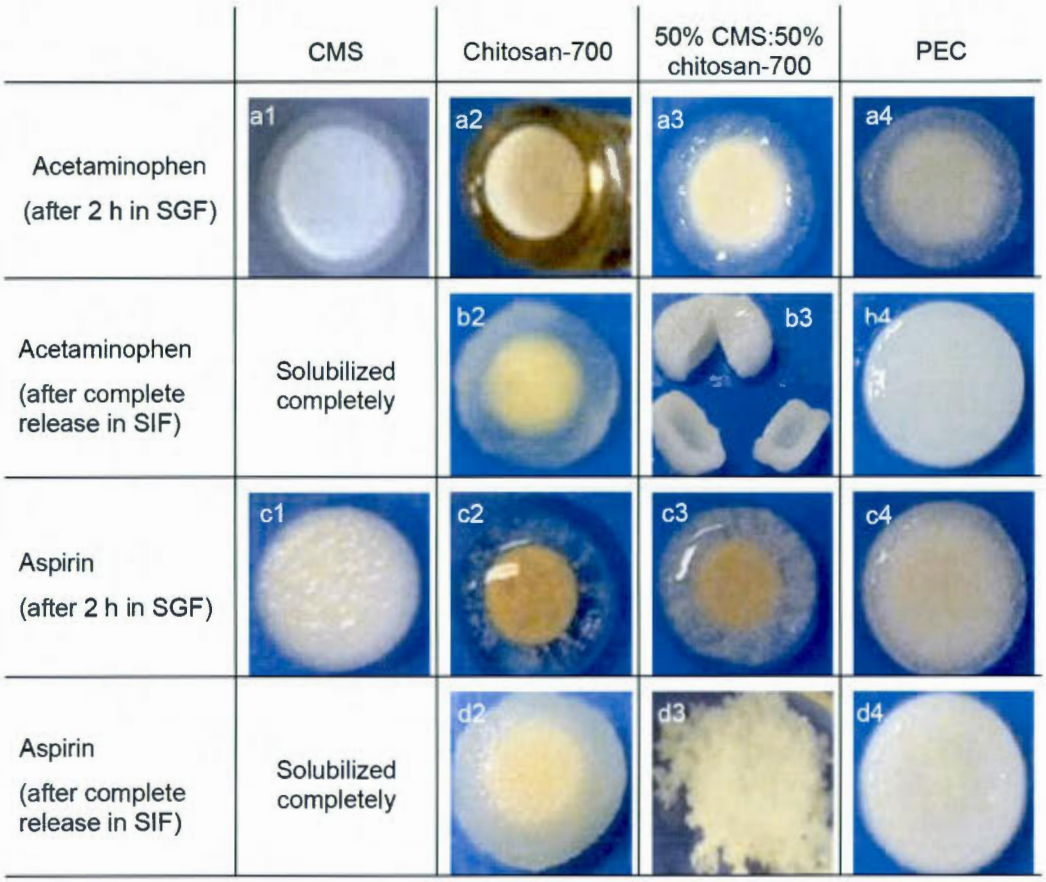

Figure 6: Photographs of CMS, chitosan-700, 50% CMS:50% chitosan-700 and PEC tablets (200 mg, 20% loading) during dissolution tests (1 L, 37 °C, 100 rpm). Photographs were taken for the tablets, first after 2 h of incubation in SGF and then after the complete drug (acetaminophen or aspirin) release in SIF. The sizes of tablets were not normalized.

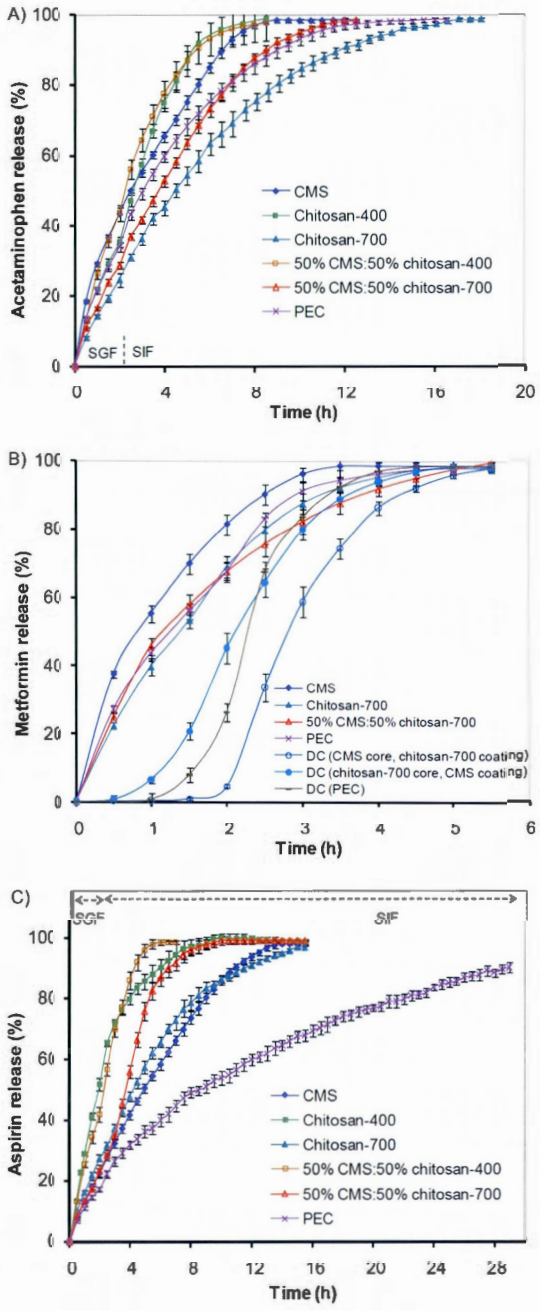

Figure 7: Kinetics of drug dissolution from tablets (200 mg, 20% loading) of CMS, chitosan-400, chitosan-700, 50% CMS:50% chitosan-400, 50% CMS:50% chitosan-700 and PEC. The tablets were incubated (1 L, 37 °C, 100 rpm) for 2 h in SGF and then transferred to SIF. A) acetaminophen; B) metformin, monolithic tablets were incubated only in SGF; C) aspirin.

CHAPITRE VIII

LE COMPLEXE CARBOXYMÉTHYL AMIDON-CHITOSANE COMME EXCIPIENT POUR LE TRANSPORT DES PROTÉINES : ADMINISTRATION DE L'OVALBUMINE PAR VOIE ORALE

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Résumé

Un nouveau complexe polyélectrolyte (CPE) à base de carboxyméthyl amidon (CMA) et de chitosane a été proposé comme excipient pour l'administration orale de l'ovalbumine. Des comprimés monolithiques (200 mg, 2,1x9,6 mm, charge de 50 %) ont été obtenus par compression directe des mélanges de poudres et les profils de dissolution ont été étudiés. Lorsque le CMA a été utilisé comme excipient, plus de 70% de l'ovalbumine formulée a été protégé contre la dégradation par la pepsine dans un fluide gastrique simulé (FGS). La dissolution totale a duré 6 h : une heure dans FGS contenant de la pepsine et 5 h dans un fluide intestinal simulé (FIS) contenant de la pancréatine. Une protection plus élevée (plus de 90 %) et une dissolution prolongée (plus de 13 h) ont été obtenues en utilisant le CPE ou un mélange physique 50 % CMA:50 % chitosane comme excipients. Une plus faible proportion de chitosane (11%) dans le CPE que dans le mélange physique a été nécessaire pour assurer une performance similaire dans la dissolution. La bonne protection contre la dégradation par la pepsine, les différents temps de libération et la mucoadésivité des matrices favorisent le développement des formulations convenables pour la vaccination par voie orale.

Mots clés : carboxyméthyl amidon, chitosane, complexe polyélectrolyte, coexcipient, transport du médicament, ovalbumine.

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Polyelectrolyte complex of carboxymethyl starch and chitosan as protein carrier: Oral administration of ovalbumin

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Abstract

A novel carboxymethyl starch (CMS)-chitosan polyelectrolyte complex (PEC) was proposed as an excipient for oral administration of ovalbumin. The dissolution of ovalbumin from monolithic tablets (200 mg, 2.1x9.6 mm, 50% loading) obtained by direct compression was studied. When CMS was used as an excipient, more than 70% of the loaded ovalbumin remained undegraded after 1 h of incubation in simulated gastric fluid (SGF) containing pepsin. The complete dissolution, after transfer of tablets into simulated intestinal fluid (SIF) containing pancreatin, occurred within total time of about 6 h. Higher protection (more than 90% stability in SGF) and longer dissolution (more than 13 h) were obtained with 50% CMS:50% chitosan physical mixture and with PEC excipients. A lower proportion of chitosan was needed for PEC than for CMS:chitosan mixture to obtain a similar dissolution profile. The high protection against degradation by pepsin, the various release times and the mucoadhesion properties of the matrices favor the development of suitable carriers for oral vaccinations.

Keywords: carboxymethyl starch, chitosan, polyelectrolyte complex, coexcipients, drug delivery, ovalbumin.

Abbreviations: CMS, carboxymethyl starch; PEC, polyelectrolyte complex; SGF, simulated gastric fluid; SGF (Pe), Simulated gastric fluid containing pepsin; SIF, simulated intestinal fluid; SIF (Pan), Simulated intestinal fluid containing pancreatin; DS, degree of substitution (the average number of carboxymethyl groups per glucose unit); DDA, degree of deacetylation of chitosan; 50% CMS:50% chitosan, an excipient containing 50% CMS (w/w) and 50% chitosan (w/w); SDS-PAGE, Sodium dodecylsulfate-polyacrylamide gel electrophoresis; TBST, Tris buffer solution containing 0.2% Tween 20.

1. Introduction

Monolithic tablets based on carboxymethyl starch (CMS) as excipient have been previously shown to protect proteins and microorganisms against gastric acidity and degradation by pepsin [1, 2]. Moreover, it has been shown that the release rate may be reduced and the protection may be enhanced when chitosan powder is added as coexcipient [3]. After incubation of tablets in simulated gastric fluid (SGF), the drug release time in simulated intestinal fluid (SIF) was longer with tablets based on CMS:chitosan than those based on CMS alone [4]. Chitosan can interact with CMS and develop a gel network in SGF, reducing thus the solubility of tablets in SIF.
Recently, a novel CMS-chitosan polyelectrolyte complex (PEC) was suggested as carrier for small molecules [5]. This PEC permitted a longer release than CMS. In addition, analysis by NMR imaging showed that CMS-chitosan PEC tablets have the advantage of a lower swelling than chitosan tablets in SGF and than CMS tablets in SIF [6]. Moreover, the diffusion of SIF into PEC tablets was slower than into CMS tablets.

It is now of interest to evaluate whether monolithic tablets based on PEC can ensure controlled release of proteins. The ovalbumin, a globular phosphoglycoprotein with 385 amino acid residues and with a molecular weight of 45 daltons [7, 8], was chosen as a model protein (tracer) due to its sensitivity to degradation by pepsin. This permits to predict the efficiency of the formulations to afford gastric protection. In addition, ovalbumin is a protein largely used as model antigen in immunological studies [9] and thus can be a good tracer to compare various excipients as carriers for oral vaccination via mucosal immunity.

Several studies [10-12] showed that the degradation of ovalbumin by pepsin depend on pH and on pepsin/protein ratio. Pepsin, which has an optimal activity within pH 1.2 - 3.5 range [13], breaks down ovalbumin into small peptides at pH 2 [11]. This degradaion by pepsin complicates the development of oral vaccines known

to have several advantages over parenteral form of administration [14]. Matrices able to protect ovalbumin against digestion by pepsin would allow higher antigen amounts delivered to intestinal mucosa. In this context, mucoadhesive matrices make the vaccinations more effective. The mucoadhesion could be related to hydrogen bonds, hydrophobic bonds, electrostatic interactions or/and Van der Waals interactions [15, 16]. The mucoadhesion force depends on many factors such as pH of the medium, ratio polymer/mucin, polymer structure and hydrophilicity, ionic character and charge density of polymer, porosity of polymer particles, and molecular dynamics of polyelectrolytes [15-19].

The aims of the present study were: (i) to test the ability of monolithic tablets based on different excipients (CMS, chitosan, CMS:chitosan physical mixture, and CMS-chitosan PEC) to protect ovalbumin against degradation by pepsin in SGF; (ii) to compare the release kinetics of ovalbumin from tablets based on these various excipients in conditions simulating the gastro-intestinal transit.

2. Materials and methods

2.1. Reagents and chemicals

High amylose corn starch (Hylon VII) was from *National Starch* (Bridgewater, NJ, USA) and chitosan from *Marinard Biotech* (Rivière-au-Renard, QC, Canada). Sodium chloroacetate (SCA, 98%), polyoxyethylene sorbitan monolaurate (Tween 20), ovalbumin (albumin from hen egg white, ≥ 90%, grade III) and pepsin (1:10000, from porcine stomach mucosa) were from *Sigma-Aldrich* (St-Louis, MO, USA). Pancreatin, eight times strength, from porcine pancreas was provided by *A&C American Chemicals Ltd.* (Montreal, QC, Canada).

Protein molecular weight markers (broad range) were from *Bio-Rad Laboratories* (Richmond, VA, USA). Chemiluminescent HRP antibody detection reagent and autoradiography films were from *Denville Scientific Inc.* (Metuchen, NJ, USA).
Piglet serum anti-ovalbumin IgA (primary antibody) was produced by *Agriculture and Agri-Food Canada* (Lennoxville, Quebec, Canada) and Anti-pig IgG peroxidase conjugate secondary antibody was from *Sigma-Aldrich.*
The other chemicals were of reagent grade and used without further purification. Simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) were prepared following the USP [20].

2.2. Preparation of CMS, purification of chitosan and preparation of CMS-chitosan PEC

Carboxymethyl starch (CMS) was prepared in aqueous medium from high amylose corn starch as previously described [1, 21], with some modifications. The preparation was done in a jacketed beaker (600 mL) at 55 °C (*Haake* heating circulator bath, D1, Berlin, Germany) as follows. An amount of 40 g of starch was dispersed in 100 mL of distilled water under continuous stirring with a Servodyne mixer system (*Cole- Parmer*, 50000-40, Niles, IL, USA). A volume of 135 mL of 1.5 M NaOH were added for starch gelatinization (30 min). Subsequently, 30 mL of 10 M NaOH and 15 g of sodium chloroacetate were added. After 1 h of reaction, 155 mL of distilled water were added, the mixture was cooled-down and the reaction was stopped by neutralization with acetic acid. The CMS was precipitated with methanol and washed repeatedly with 600 mL of 80% methanol until a conductivity of about 50 µS/cm. CMS was then washed three times with methanol 40%/acetone 60%, air dried at 40 °C for 24 h and finally sieved on a 300 µm screen.

Commercial chitosan was purified as previously described [5], but the precipitation was carried out only with NaOH by increasing the pH. An amount of 20 g of chitosan was solubilized in 350 mL of 0.35 M acetic acid and then the volume

was completed to 2 L with distilled water. The solution was filtered under vacuum on Whatman filter paper to eliminate insoluble particles. Chitosan was then precipitated by adding NaOH solution under continuous stirring until a final pH of 8. The chitosan was washed first with distilled water until conductivity of about 200 µS/cm and then with 100% acetone. Finally, it was air dried at 40 °C for 24 h, ground and sieved on a 300 µm screen.

The CMS-chitosan polyelectrolyte complex (PEC) was prepared by precipitation of CMS and chitosan as previously described [5]. Practically, 1 g of chitosan was solubilized in 44 mL of 0.1 M HCl, then the volume was adjusted to 150 mL with distilled water and the solution was filtered. A solution (1 %) of CMS was prepared by solubilizing 8 g of CMS in 800 mL of distilled water. The precipitation occurred under vigorous mixing by gradually adding the solution of polycation (chitosan) to that of polyanion (CMS) at 1:1 ratio for their respective ionic groups ($-NH_3^+$: $-COO^-$). The pH of the final solution was about 5. The PEC, containing 11% (w/w) of chitosan, was washed first with 70% acetone, and then with pure acetone. Finally, it was dried at 40 °C for 24 h, sieved with a 300 µm screen and stored at room temperature.

2.3. Characterization of excipients

The molecular weight of chitosan and its degree of deacetylation (DDA), as well as the degree of substitution (DS) of CMS were determined as reported elsewhere [5].

The DS of CMS was determined by back-titration as follows. Protonated CMS (n = 3) was solubilized in a precise volume of NaOH (0.05 M) and then the excess of NaOH was titrated with HCl (0.05 M), permitting the calculation of the amount of –COOH groups.

The degree of deacetylation (DDA) of chitosan was determined by acid-base titration. An amount of chitosan solubilized in a precise volume of HCl (0.1 M) was titrated with NaOH (0.1 M). The volumes of NaOH corresponding to the two inflexion points of the titration curve permitted the calculation of DDA.

The molecular weight of chitosan was determined by using viscometric method and reported viscometric constants data [22-24]. Samples with different concentrations (0.07-0.7%) were prepared with a solution containing 0.2 M acetic acid and 0.1 M sodium acetate. The viscosities of chitosan solutions were measured at 30 °C with an electronic viscometer (*Viscosity Monitoring and Control Electronics*, Medford, MA, 180 USA). The data on viscosities and concentrations were used to calculate the reduced viscosities, the intrinsic viscosity and then the average molecular weight of chitosan following the Mark-Houwink-Sakurada's empirical equation $[\eta] = kM\alpha$. The constants k (dL/g) and α (dimensionless) are depend on the solvent-polymer system.

The thermal analysis was carried out by using a differential scanning calorimetry (*Mettler Toledo*, DSC 1). Aluminum pans of 40 µL and 2.8-4 mg of polymer powders were used for analyses. Indium was used for calibration and an empty aluminum pan was used as reference. The heating rate was fixed at 10 °C/min from 25° to 340 °C and the nitrogen flow rate was 50 mL/min.

The solubilities (n = 3) were determined by dispersing 150 mg of polymer powders in 6 mL of SGF or of SIF. The samples were stirred (*Thermolyne, 37600 Mixer*, Dubuque, IA, USA) for one min followed by overnight rotative agitation (*Glas-Col*, USA) and then by centrifugation (20 min, 4000 rpm) at 25 °C. A volume of 1.5 mL of supernatant was evaporated at 65 °C until a constant mass was reached. Controls were prepared similarly with SGF or SIF only (without polymer). The weights of dried samples and of controls were used to calculate the solubilities.

2.4. Characterization of tablets

Preparation of tablets

Monolithic tablets (200 mg, 50% w/w loading) were obtained by direct compression of a homogenous mixture of excipient(s) and ovalbumin powders (flat-faced punches with 9.6 mm diameter, 2.5 tonnes, *Carver* hydraulic press). These tablets were used for tests of crushing strength, disintegration and dissolution. Unloaded (drug-free) tablets were prepared for determination of erosion and fluid uptake, for swelling tests and for adhesion tests. Formulations with CMS:chitosan coexcipients were obtained by simple mixture of powders.

Crushing strength, disintegration, erosion and fluid uptakes

The crushing strength (n = 5) was measured with a tablet hardness tester (*Varian*, VK 200, Cary, NC, USA). The disintegration tests (n = 3) were performed with a disintegration tester (*Electrolab*, ED-2L) at 37 °C according to the method (701) of USP. Disks, 900 mL of SGF and 900 mL of SIF were used.

The erosion and fluid (SGF or SIF) uptake tests (n = 3) were carried out by incubating the unloaded tablets in SGF or in SIF for 1 h (50 mL, rotative agitation). The tablets were then removed from the media, blotted with tissue paper to eliminate surface water excess, and weighed before and after drying. The percentage of erosion and the percentage of fluid uptake by the dry mass of remaining polymer were calculated as previously described [25].

Adhesion

The adhesion tests (n = 3) were performed with a texture analyzer (*Texture Technologies*, TXXT2i, Scarsdale, NY, USA) with a TA-19-kobe probe. Each tablet was fixed on the metallic tablet holder by cyanoacrylate adhesive. The Nitrocellulose Mixed Esters Membranes were dipped in 5% mucin solution (SGF or SIF) for at least 30 min, as described elsewhere [16]. The displacement rate was 1 mm/s, the weight

was 1 g, and the contact time of tablets with mucin on the membrane was 10 min. A Texture Expert Exceed software was used to record the force of detachment.

2.5. Degradation of free ovalbumin by pepsin and by pancreatin

Free (unformulated) ovalbumin (100 mg, n = 3) was solubilized and shaken at 37 °C and 50 rpm in 50 mL of SGF containing pepsin (SGF (Pe)) or in 50 mL of SIF containing pancreatin (SIF (Pan)) by using an incubator shaker (*New Brunswick Scientific*, series 25 D). Samples (250 µL) were taken at 30 min, 1 h and 2 h of incubation in SGF (Pe), and at 1, 2, 3 and 4 h of incubation in SIF (Pan). The pH of each sample was adjusted to 8-9 with 4 M NaOH solution and the total volume was adjusted to 0.5 mL with phosphate buffer saline (PBS, pH 7.4). The control (free ovalbumin) was prepared in the same conditions as the samples, but PBS was used as dissolution medium.

The degradation of ovalbumin by pepsin in SGF and by pancreatin in SIF was evaluated by SDS-PAGE (15%, w/v) followed by Western blot analysis (sections 2.7 and 2.8).

2.6. *In vitro* dissolution of formulated ovalbumin

For dissolution tests, the tablets (200 mg, 50% loading) were first incubated (37 °C, 50 rpm) for 1 h in SGF (Pe) and then transferred into SIF (Pan). After 1 h of incubation in SGF and at every 1 h of incubation in SIF (Pan), tablets (n = 3 for each excipient) were taken and crushed in 50 mL of PBS to extract the ovalbumin remained in the tablets. Samples (250 µL) were taken from these PBS solutions and treated as described before (section 2.5) to adjust the pH to 8-9 and the total volume to 0.5 mL. The control (n = 3) for ovalbumin formulated with each kind of excipients was prepared in the same conditions as for the tablets used for the dissolution tests. The only difference is that for the controls the tablets were agitated for 1 h in PBS and then crushed.

To evaluate the amount of ovalbumin remained within each tablet (not released yet), the samples were analysed by SDS-PAGE followed by Western blot (sections 2.7 and 2.8).

The parameters of the release kinetics in SIF, after 1 h preincubation in SGF, were obtained from the equation described by Peppas $M_t/M_\infty = kt^n$ [26]. Where, M_t/M_∞ is the fraction of drug released at the time t. The kinetic constant (k) incorporates the geometric characteristics of the dosage form and the properties of the matrix and of the drug. The release exponent (n) is characteristic of the drug release mechanism.

2.7. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The experiments were done with a Mini-protean II Electrophoresis (*Bio-Rad Laboratories*) as described elsewhere [2], with some modifications. Each of the 0.5 mL previously described (sections 2.5 and 2.6) was treated (1:1, v/v) with electrophoresis loading buffer (0.5 M Tris-HCl, pH 6.8, 2% SDS, 20% glycerol, 5% β-mercaptoethanol, 0.02% bromophenol blue) and heated at 95 °C for 5 min, and then cooled to room temperature. Volumes of 10 μL of treated samples were applied to SDS-polyacrylamide gels of 15% (w/v). The running time with the electrophoresis buffer (0.015 M Tris Base, pH 8.3, 0.115 M glycine, 0.06% SDS) at room temperature was about 1 h. Molecular weight protein markers (Broad Range) containing electrophoresis buffer were also loaded on each electrophoresis gel.

2.8. Western blot analysis

The gels were separately soaked for 5 min in a transfer buffer solution (0.048 M Tris Base, 0.039 M glycine, 0.04% SDS, 20% methanol). The semidry transfer was done with Trans-Blot SD (*Bio-Rad Laboratories*, Richmond, CA, USA) onto activated polyvinylidene fluoride membranes for 1 h 20 min at 80 mA/gel. These

membranes were blocked for 1 h at 4 °C in a Tris buffer solution (pH 7.7) containing Tween 20 (TBST) and 5% skim milk. They were then washed three times with TBST without milk.

Subsequently, the membranes were dipped overnight in skim milk buffer solution containing piglet serum anti-ovalbumin IgA (1:10000) at 4 °C. After washing four times with TBST, the membranes were incubated for 1 h with anti-pig IgG peroxidase conjugate secondary antibody (1:10000) in the same conditions as above. The chemiluminescence emission was recorded on autoradiography films and the density of bands was quantified by using *Image J* software, considering the density of control as 100%.

2.9. Statistical analysis

The data are presented as means ± SD, with n = 3. The comparison of means was done with *GraphPad InStat* software by using one-way ANOVA followed by Tukey (ns = non-significant with $P > 0.05$, *=$P < 0.05$, **= $P < 0.01$, ***=$P < 0.001$).

3. Results and discussion

Five excipients were tested for their potential as drug carriers: CMS, chitosan, CMS-chitosan polyelectrolyte complex (PEC), physical mixture of 89% CMS:11% chitosan to be compared with PEC that contains the same proportion of chitosan, and finally physical mixture of 50% CMS:50% chitosan.

3.1. Characterization of excipients

The solubilities of the excipients are shown in table 1. As expected, CMS (DS 0.1) was completely soluble in SIF, whereas chitosan (700 kDa, DDA 80%) was completely soluble in SGF and insoluble in SIF. The protonation of CMS in acidic

medium favors inter- and intra-chain attractions between hydroxyl and carboxyl groups, leading to lower solubility of CMS in SGF than in SIF. The solubility of PEC in SGF was higher than that of CMS. This is probably due to the protonation of amino groups, favoring the solubilization of chitosan and reducing interactions between functional groups of CMS. The solubility of PEC in acidic medium could possibly be reduced with a chitosan at a lower DDA. In SIF, the solubility of PEC was lower than that of CMS, suggesting a lesser exposure of the carboxylate groups (–COONa) to the medium due to possible interactions with amino groups of chitosan. The DSC results (Fig. 1) showed an endothermic band for each sample at around 100 °C, which can be assigned to the evaporation of free water. The chitosan decomposition temperature was about 305-309 °C while that of CMS was about 297-301 °C. The physical mixtures of CMS and chitosan showed decomposition temperatures between those of CMS and chitosan. The close proximiting of the decomposition temperatures of CMS and chitosan did not allow for identification of separate peaks in the mixture. The higher the proportion of CMS in the mixture, the more the exothermic decomposition peak shifted towards that of the CMS (lower temperature). The PEC showed a thermal profile different from those of physical mixtures of CMS and chitosan. Thus, a weak transition appeared at about 278 °C probably due to the breakdown of interactions between CMS and chitosan. Moreover, the decomposition temperature of PEC (324-328 °C) was higher than those of CMS and chitosan, and the exothermic transition was weaker. These results indicate that the thermal characteristics of CMS-chitosan complex were different from those of CMS, of chitosan or of the physical mixture of these two polymers.

3.2. Characterization of tablets

Crushing strength

The tablets of ovalbumin formulated with the excipients showed crushing strengths between 128 and 183 N, whereas the excipient-free ovalbumin tablets showed lower crushing strength (68 N). This indicates that, in this case, the presence of excipient(s) favors the physical stabilization of tablets following compression. The crushing strength of tablets based on CMS was 173 N while that of tablets based on PEC was 183 N. The presence of CMS basically increased the crushing strength of tablets from 128 N with chitosan only to 176-179 N with CMS:chitosan mixture. The obtained results are in the normal range of crushing strengths that have been reported for common excipients [27].

Disintegration

The higher the amount of chitosan in the excipients based on CMS:chitosan mixture, the shorter was the disintegration time in SGF, but the differences were not significant (Fig. 2). This is due to the solubility of chitosan in SGF (pH 1.2). The tablets based on CMS showed the longest disintegration due to the stabilisation of its chains by carboxyl groups implicated in hydrogen bonds. However, the differences among the disintegration times of the various tablets were weak.

In SIF, the tablets based on CMS, chitosan or CMS:chitosan mixtures showed a disintegration time of about 60-70 min, whereas those based on PEC showed the longest disintegration (135 min). This significant difference ($P < 0.001$) is due to the stabilization of the PEC matrix in neutral medium.

Except for PEC, the disintegration time in SIF was shorter than that in SGF for each excipient. As previously discussed, the protonation of carboxylic groups in acidic medium reduces the solubility of tablets based on CMS [5]. For the tablets based on chitosan, the inability of chitosan to form a hydrogel in neutral medium

leads to a low cohesion between the particles and to a faster disintegration in SIF than in SGF. Differently, the tablets based on PEC showed longer disintegration time in SIF than in SGF due to the lower solubility and the more stability of PEC excipient in neutral medium than in acidic medium.

In both media, the tablets based on ovalbumin alone have the fastest disintegration (less than 30 min), indicating again the role of excipients in the enhancement of tablet stability.

Erosion and fluid uptake

Except for chitosan, the erosion of the unloaded tablets ranged between 2.69 and 7.58% (Table 2). The highest erosion (11.65%) was found with chitosan tablets incubated in SGF due to the solubilization of the outer layer in acidic medium. The chitosan tablets incubated in SIF showed many cracks or were disintegrated without real erosion.

The fluid uptake by tablets depended on the pH of the medium. Thus, in SGF the highest uptake was found with 50% CMS:50% chitosan, whereas in SIF the highest uptake was found with CMS.

As expected, the CMS tablets remained relatively compact in SGF. The formation of a hydrogel layer limited the SGF penetration into the chitosan tablets, whereas the 50% CMS:50% chitosan tablets took up more fluid. These results fit well with NMR imaging data [5], showing faster diffusion of SGF into the 50% CMS:50% chitosan tablets than into CMS and chitosan tablets.

In SIF, the highest fluid uptake by CMS tablets (177%) is due to the hydration of carboxylate groups. This uptake decreased with an increase of chitosan proportion in the CMS:chitosan mixture. Hence, the uptake was decreased from159% to 112% when the proportion of chitosan was increased from 11% to 50%. The PEC tablets showed the lowest uptake (88%). This proves the interactions between functional groups of CMS and of chitosan in PEC, leading to this low hydration.

Significant difference ($P < 0.001$) of erosion and fluid uptake in SGF was found between CMS tablets and the other tablets. By comparison with the erosion of CMS tablets in SIF, the difference was: no significant ($P > 0.05$) with 89% CMS:11% chitosan tablet, significant ($P < 0.01$) with 50% CMS:50% chitosan tablet, significant ($P < 0.001$) with PEC tablet. For fluid uptake in SIF, significant difference was found between CMS tablets and the other tablets: 89% CMS:11% chitosan ($P < 0.01$), 50% CMS:50% chitosan ($P < 0.001$), PEC ($P < 0.001$).

Adhesion

Irrespective of pH, the CMS tablets showed the highest adhesion with significant difference ($P < 0.001$) from the other tablets (Fig. 3). Moreover, significant difference ($P < 0.001$) was found between the adhesion of 89% CMS:11% chitosan tablets and those of the other tablets. Decreasing CMS ratio in the mixture CMS:chitosan reduces the adhesion. It was previously shown by scanning electron microscopy that the chitosan particles displayed a more compact morphology than those of CMS and PEC [5]. This suggests that the porosity of the excipient particles may favor the hydration of the tablets and thus more capillary attraction with the mucin. When the fluid from the space between a dry tablet and a mucosa surface is taken up by the polymer, the capillary attraction forces could be considerable [28].

The PEC tablets showed lesser adhesion than those of CMS and of 89% CMS:11% chitosan ($P < 0.001$), possibly due to interpolymer attractions that reduce attractions with mucin.

The difference between the adhesion forces obtained in SGF and in SIF for each excipient was not significant, possibly because the effects of polymer structures and of particle morphologies prevail over the effect of pH. More investigations, with different parameters and with hydrated tablets, will be needed for a better understanding of adhesion properties of formulations with these excipients.

3.3. Stability of ovalbumin in presence of pepsin and pancreatin

The analysis by Western blot showed two bands at about 42 and 45 kDa (Fig. 4, insert Western blot) for the ovalbumin incubated in phosphate buffer saline (PBS, pH 7.4). The intensity of the bands was reduced to 17% after only 30 min of incubation in SGF (Pe) due to degradation by pepsin (Fig. 4). No intermediate bands appeared, indicating thus an advanced hydrolysis producing peptides of low molecular weights. In fact, at low pH, pepsin is known to attack many peptide bonds in ovalbumin, leading to the formation of small peptides not detectable by SDS-PAGE [29].
In SIF (Pan), the free ovalbumin was gradually proteolyzed. The band at 45 kDa was almost disappeared and the percentage of original bands was reduced to 38% after 1 h of incubation. Intermediate bands appeared in the presence of pancreatin, corresponding to fragments lower than 31 kDa.

3.4. *In vitro* dissolution of ovalbumin

After 1 h of incubation in SGF (Pe), the amount of ovalbumin that still remained within the tablets was between 76% and 94% (Fig. 5), whereas for free ovalbumin the percentage was about 10% (Fig. 4). This indicates that the formulation with the described excipients afforded a high protection of ovalbumin against degradation by pepsin in SGF. Significant difference was found between CMS and PEC ($P < 0.01$).

The release rates of ovalbumin formulated with CMS and with 89% CMS:11% chitosan were almost similar ($P > 0.05$ for each sampling) and higher than those obtained with the other excipients. This is due to the high solubility of CMS in neutral medium (SIF, pH 6.8) compared to those of chitosan and PEC (Table 1). In addition, the degradation of CMS by α-amylase of pancreatin can accelerate the release of active agent [30, 31].

In the case of 89% CMS:11% chitosan, the presence of low proportion of chitosan (11%) can favor the diffusion of acidity (SGF) into the matrix without formation of a thick hydrogel in the outer layer of this matrix. Moreover, the presence of high proportion of CMS (89%) can favor the matrix solubilization in neutral medium (SIF).

Total release times over 13 h were found with chitosan, 50% CMS:50% chitosan and PEC (Fig. 5 C-F). The tablets based on 50% CMS:50% chitosan afforded a longer release than that afforded by the tablets based on 89% CMS:11% chitosan, because the higher proportion of chitosan (50%) contributes to the formation of a thicker hydrogel in SGF and reduces the solubility of the tablets and the degradation by pancreatin (α-amylase) in SIF. The longest release time was found with PEC which is less soluble and then less susceptible to degradation by pancreatin (α-amylase) than CMS. The complexation of CMS with chitosan in the PEC remarkably reduced the hydration and the swelling of the tablets (Fig. 6). Even a low chitosan proportion (11%) could increase the dissolution time from 6 h (for CMS) to more than 13 h (for PEC), whereas the same chitosan proportion in CMS:chitosan physical mixture did not generate an increase of the release time. The longer release time with PEC can be particularly useful for delivery of drug and bioactive agents to other sites than the upper intestine.

Although the chitosan seems able to control the release of ovalbumin, its solubility in SGF and its insolubility in SIF make the release rate mainly dependent on the preincubation time in acidic medium (SGF) when the chitosan tablets form a hydrogel [4]. As the cohesion between chitosan particles is weak in neutral medium, the dissolution profile depends on the cohesion ensured by the bioactive agent after compression with chitosan in monolithic tablets.

The n values obtained from the equation described by Peppas [26] indicate that the release was mostly controlled by diffusion and swelling in the case of chitosan ($n = 0.73$), and by swelling and erosion in the case of CMS ($n = 1.13$), of 89% CMS:11% chitosan ($n = 1.61$), of 50% CMS:50% chitosan ($n = 1.00$) and of PEC

(n = 1.45). The n value between 0.5 and 1 indicates an anomalous diffusion and the n > 1 suggest a super case-II transport.

Taken together, these results show that the formulation with CMS as only excipient can ensure a good protection of ovalbumin against degradation by pepsin and a relatively fast release in SIF. To ensure a longer release time, an adequate amount of chitosan (as a coexcipient) should be added to the formulation. The 50% CMS:50% chitosan mixture and the PEC afforded better protection against degradation by pepsin and longer release times than when CMS alone. It is worth mentioning that no coating was needed for our formulations, because relatively high protection was obtained with uncoated monolithic tablets.

An *in vivo* study was recently carried out on piglets with ovalbumin formulated in monolithic tablets. The CMS was chosen as excipient due to the high protection that it can afford against degradation by pepsin and to the adequate release time of ovalbumin in SIF. Tablets were given to piglets by oral administration with the aim to produce anti ovalbumin IgG. This study will be the subject of another report.

4. Conclusions

Although ovalbumin is highly sensitive to degradation by pepsin, more than 70% of the ovalbumin formulated with CMS in monolithic tablets remained protected after 1 h of incubation in SGF (Pe). The subsequent release in SIF (Pan) occurred over a few hours. The addition of chitosan as coexcipient to CMS at a relatively low percentage (11%) did not modify the release profile found with CMS. In contrast, when chitosan represented 50% of the excipient, the release time was longer than that obtained with CMS. The use of chitosan alone as excipient may have limitations due to chitosan solubility in SGF and to the low cohesion between chitosan particles in neutral medium (SIF).

Both 50% CMS:50% chitosan and PEC seem the most efficient excipients to protect ovalbumin against degradation by pepsin at pH 1.2, with more than 90% of ovalbumin still remaining undegraded after 1 h of incubation in SGF (Pe). They are also the most suitable for extended release. The advantage of PEC lay in the lower proportion of chitosan (11%) needed to obtain a similar dissolution profile to that obtained with CMS:chitosan mixture. The mucoadhesion properties of CMS, of CMS:chitosan physical mixture and of PEC make these excipients more promising as carriers for oral vaccinations.

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Table 1: Solubility of CMS, chitosan and PEC in SGF and in SIF (150 mg/6 mL). The data are presented as means ± SD, (n = 3).

Powder	Solubility in SGF (%)	Solubility in SIF (%)
CMS	56.41 ± 3.19	99.39 ± 5.08
Chitosan	100.00 ± 4.37	1.84 ± 1.52
PEC	80.03 ± 4.12	63.29 ± 3.47

Table 2: Erosion and fluid uptake by tablets (200 mg) of CMS, 89% CMS:11% chitosan, 50% CMS:50% chitosan, chitosan and PEC after 1 h of incubation in 50 mL of SGF or of SIF. The data are presented as means ± SD, (n = 3).

Tablet	Erosion (%)		Fluid uptake (%)	
	SGF	SIF	SGF	SIF
CMS	2.91 ± 0.09	5.26 ± 0.21	80.36 ± 2.24	177.38 ± 5.32
89% CMS:11% chitosan	4.73 ± 0.17	5.78 ± 0.28	99.13 ± 3.08	158.73 ± 4.89
50% CMS:50% chitosan	7.58 ± 0.29	7.53 ± 0.89	162.83 ± 4.72	112.13 ± 3.69
Chitosan	11.65 ± 0.62	Fractured	103.40 ± 4.16	Fractured
PEC	4.46 ± 0.16	2.69 ± 0.16	111.95 ± 3.74	87.51 ± 2.14

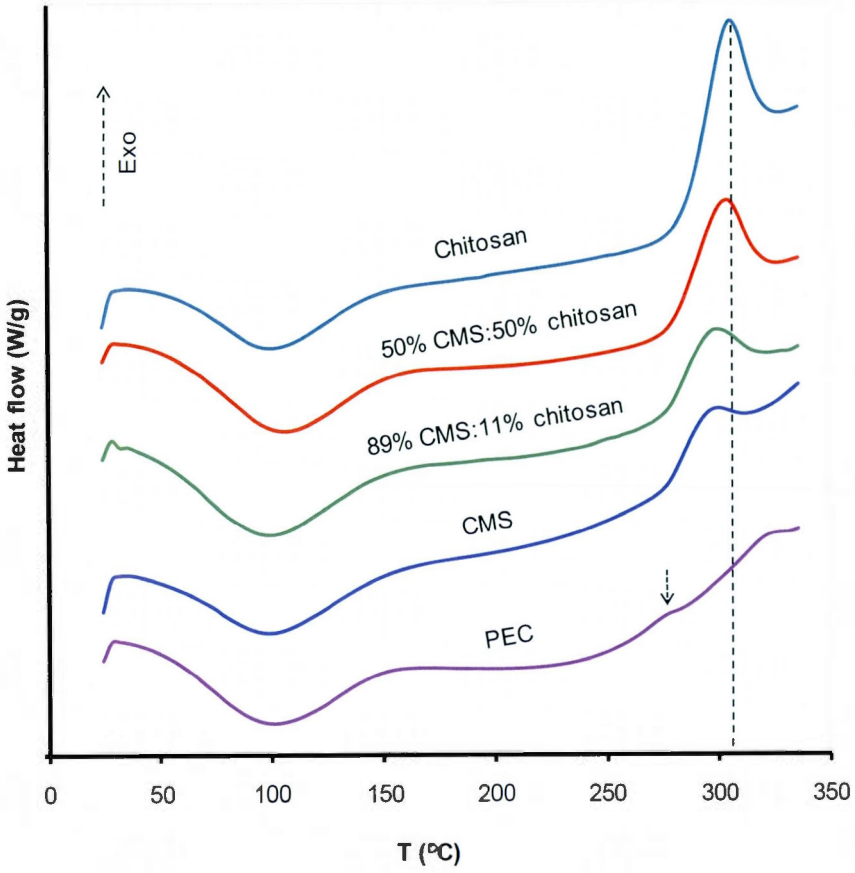

Figure 1: Differential scanning calorimetry analysis of CMS, 89% CMS:11% chitosan, 50% CMS:50% chitosan, chitosan and PEC. Samples (2.8-4 mg) were heated at a rate of 10 °C/min between 25 and 340 °C, (n = 2).

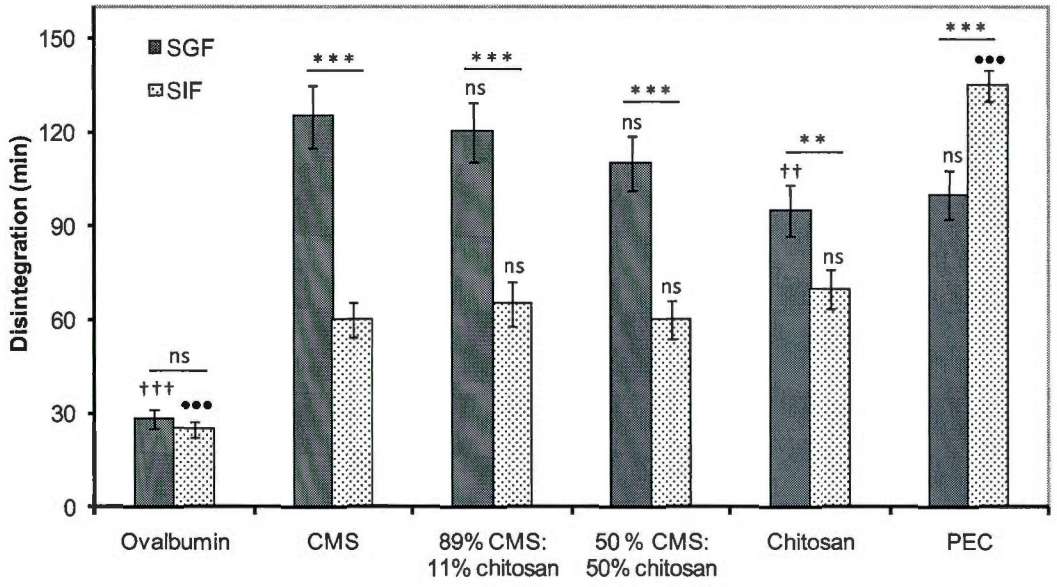

Figure 2: Disintegration of tablets (200 mg) of excipient-free ovalbumin and of ovalbumin formulated with CMS, 89% CMS:11% chitosan, 50% CMS:50% chitosan, chitosan or PEC (900 mL of SGF or SIF at 37 °C). The data are presented as means ± SD, (n = 3). (ns = non-significant, *=P < 0.05, **= P < 0.01, ***=P < 0.001). The symbols used are: (*) to compare the results obtained in SGF and in SIF for each excipient, (†) to compare the results obtained with CMS to those obtained with the other excipients in SGF, (•) to compare the results obtained with CMS to those obtained with the other excipients in SIF.

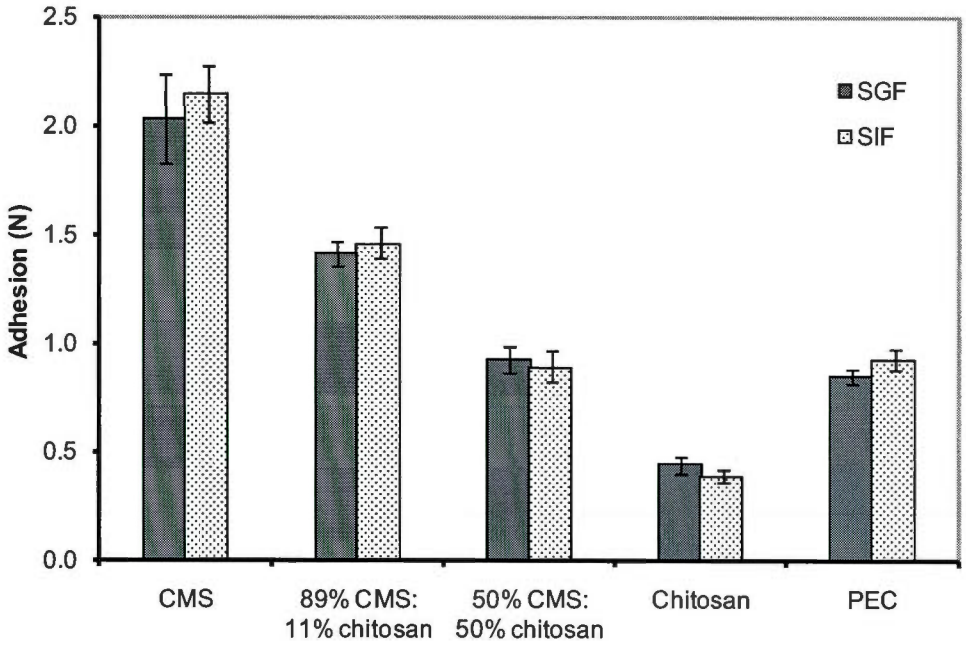

Figure 3: Adhesion on nitrocellulose mixed esters membranes dipped in 5% mucin solution (SGF or SIF) of drug-free tablets (12 mm diameter) of CMS, 89% CMS:11% chitosan, 50% CMS:50% chitosan, chitosan and PEC. The data are presented as means ± SD, (n = 3). Significant difference ($P < 0.001$) between CMS tablet and the other tablets. Significant difference ($P < 0.001$) between 89% CMS:11% chitosan tablet and the other tablets. No significant difference ($P > 0.05$) between results in SGF and in SIF for each excipient.

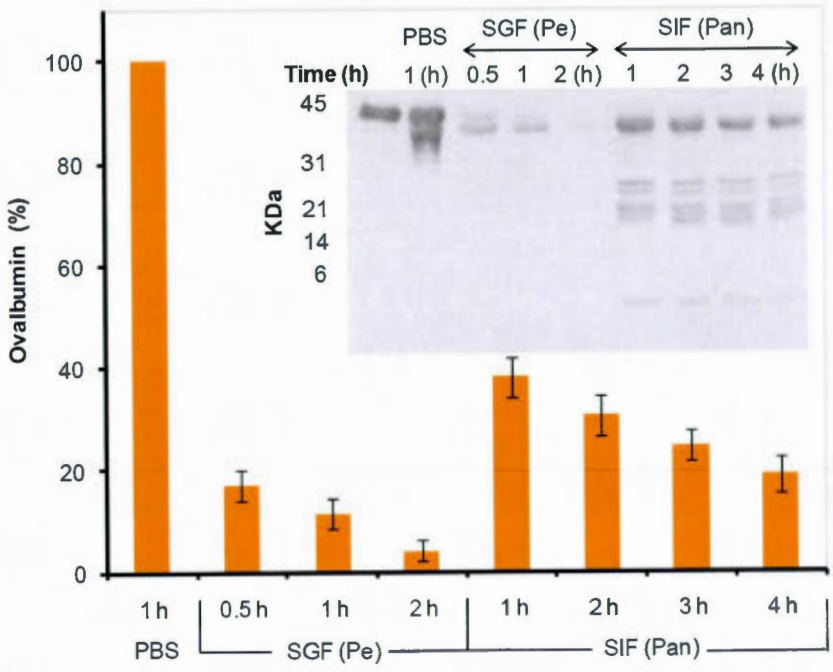

Figure 4: Evaluation of the stability of free (unformulated) ovalbumin in SGF (Pe) and in SIF (Pan) by Western blot and by densitometry analysis. The data are presented as means ± SD, (n = 3).

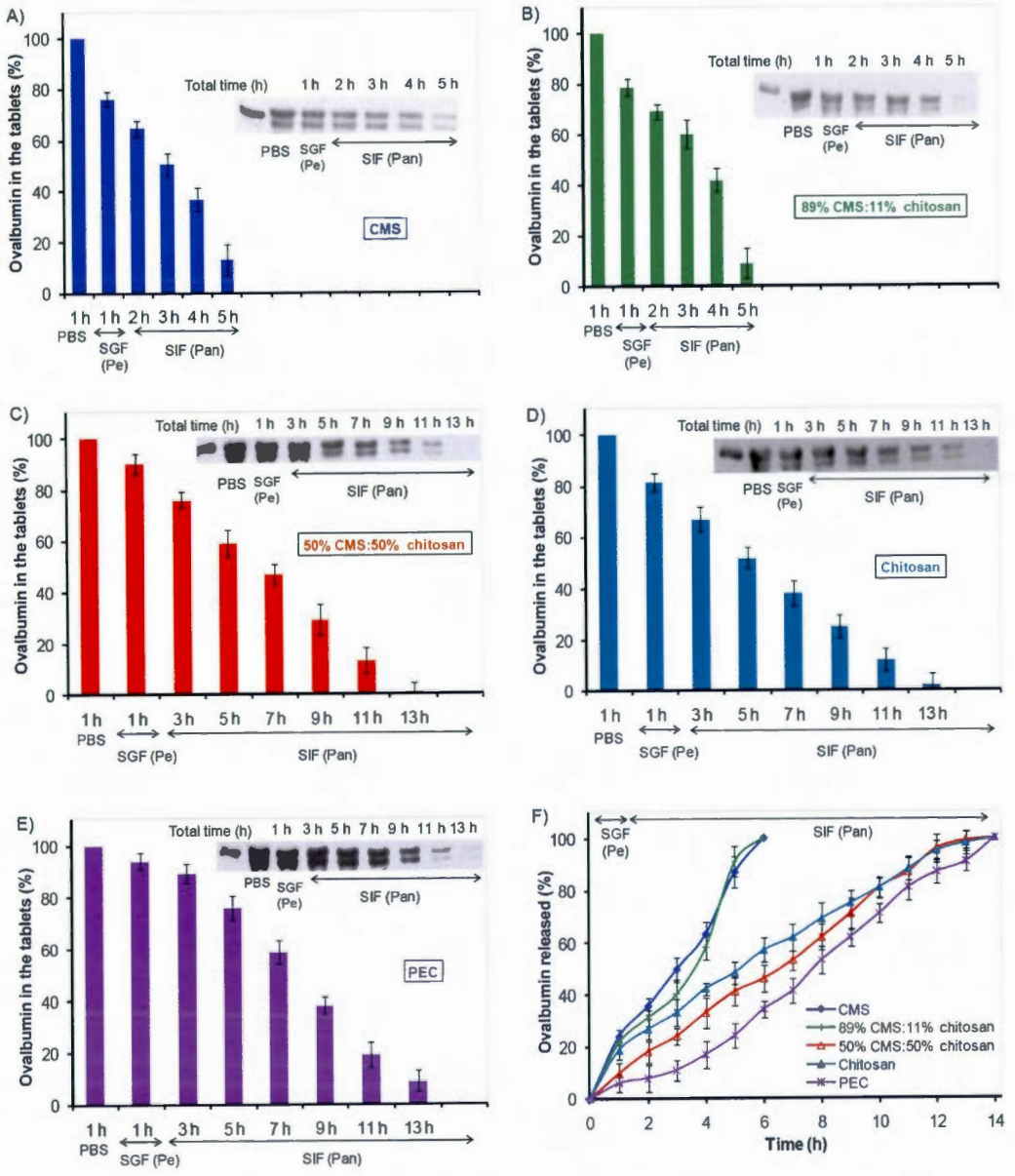

Figure 5: Dissolution of ovalbumin in SIF (Pan) after the incubation of tablets in SGF (Pe) for 1 h. Western blot and densitometry analysis of ovalbumin that still remained within the tablets: CMS (A), 89% CMS:11% chitosan (B), 50% CMS:50% chitosan (C), chitosan (D) and PEC (E).). The kinetics of the ovalbumin release from various formulations (F). The data are presented as means ± SD, (n = 3).

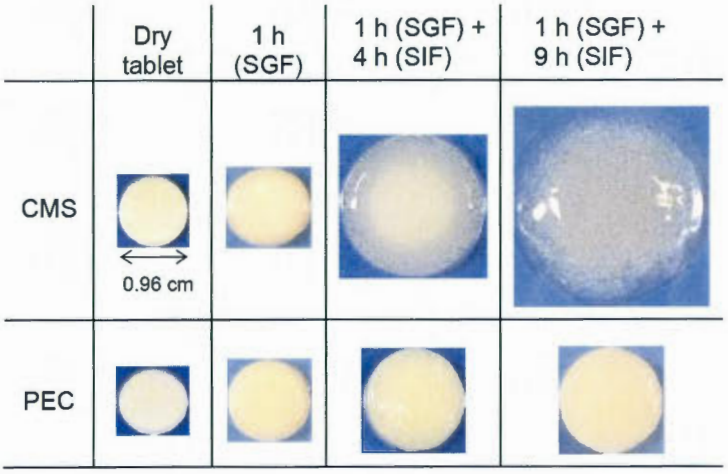

Figure 6: Images showing the swelling of CMS and of PEC tablets (200 mg) in gastric (1 h) and in intestinal (2-9 h) media (50 mL of SGF or SIF, 37 °C, 50 rpm).

CHAPITRE IX

DISCUSSION ET CONCLUSIONS

La forte association des chaînes d'amidon ne permet pas à ce polymère de s'hydrater suffisamment dans un milieu aqueux et de former un hydrogel, d'où la nécessité de le modifier physiquement ou chimiquement afin de l'utiliser dans des formulations pharmaceutiques. L'amidon riche en amylose faiblement réticulé (Contramid®) a été introduit en 1994 comme excipient pour la libération contrôlée avec des matrices hydrophiles (comprimés) (Mateescu, Lenaerts et Dumoulin, 1994). Cet excipient qui ne présente pas des fonctions ioniques stabilise la matrice dans un milieu aqueux par des ponts d'hydrogène via les groupements hydroxyles de l'amidon. Afin de conférer à l'amidon réticulé une certaine sensibilité au pH, ce polymère a été fonctionnalisé avec des groupements carboxyméthyles (Mulbacher *et al.*, 2001). Plus tard, en 2004, le carboxyméthyl amidon non-réticulé (CMA) a été proposé comme nouvel excipient pour la formulation des agents actifs dans des matrices à libération contrôlée (Mulhbacher, Mateescu et Calinescu, 2004). Ces matrices qui sont stabilisées à pH acide par des ponts d'hydrogène via des groupements hydroxyles et carboxyles ont montré qu'elles peuvent limiter la libération dans un fluide gastrique simulé (FGS, pH 1,2) et qu'elles peuvent protéger les agents bioactifs formulés (Calinescu *et al.*, 2005). Afin de prolonger le temps de libération dans un fluide intestinal simulé (FIS, pH 6,8), le chitosane a été utilisé comme coexcipient dans les matrices à base de CMA (Leonida et Mateescu, 2006; Calinescu et Mateescu, 2008). Étant insoluble à pH neutre, le chitosane réduit la solubilité de la matrice dans FIS et, par conséquence, la libération des agents formulés.

Dans le présent projet doctoral, nous avons utilisé l'amidon riche en amylose (Hylon VII) et le chitosane comme produits de départ afin de préparer des excipients et des formulations convenables pour des libérations contrôlées. Les excipients ont été préparés sans avoir recours à l'utilisation d'agents de réticulation. Ce chapitre présente une discussion des principaux résultats obtenus dans le cadre de ce projet qui valorise l'utilisation du CMA dans le domaine de formulation pharmaceutique.

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En tenant compte des variations du pH dans le tractus gastro-intestinal et leur influence sur la libération des substances actives à partir des comprimés, la première étude (Assaad et Mateescu, 2010) a été consacrée à suivre les cinétiques de libération en fonction : (i) du degré de carboxyméthylation du CMA, (ii) du degré de protonation du CMA, (iii) des pH des milieux de dissolution, en effectuant séparément des tests dans un fluide gastrique simulé (FGS, pH 1,2) et dans un fluide intestinal simulé (FIS, pH 6,8), (iv) de la préincubation des comprimés dans FGS avant de les transférer dans FIS.

L'acétaminophène a été utilisé comme substance active modèle (traceur) dans des comprimés de 200 mg à base de CMA présentant différents degrés de substitution (DS : 0,07; 0,09; 0,11; 0,14 et 0,20) et différents ratios de protonation (RP : 10 %, 20 %, 50 %, 70 %, 85 % et 100 %). L'acétaminophène a été choisi principalement en raison de son pK_a de 9,5 qui est supérieur aux pH des milieux de dissolution (FGS, pH 1,2; FIS, pH 6,8). Ceci permet de comparer les cinétiques de libération obtenues dans FGS et dans FIS, en sachant que les changements sont dues aux caractéristiques de l'excipient et non à la différence de solubilité de l'acétaminophène dans les deux milieux. Les DS choisis étaient relativement faibles, car avec des DS élevés le CMA devient très soluble à pH neutre et la libération à partir des matrices devient très rapide dans FIS. Le DS est définie comme étant la moyenne du nombre de groupements carboxyméthyles sur chaque unité de glucose. Le DS théorique maximal

est 3, puisque chaque unité de glucose contient un hydroxyle primaire et deux hydroxyles secondaires.

La fonctionnalisation de l'amidon, la protontion du CMA et la préparation des comprimés ont été effectuées de la même manière pour tous les échantillons. Les deux seuls paramètres qui ont été modifiés sont le DS et le RP, permettant d'analyser les résultats principalement en fonction de ces deux paramètres. Il est important de mentionner que les tailles des particules de tous les échantillons ont été sélectionnées de la même manière (< 300 μm), réduisant ainsi ou même éliminant l'interférence de ce paramètre sur les résultats de dissolution, surtout que le pourcentage de polymère dans les comprimés était relativement élevé (60-80 %). Dans des travaux préliminaires non publiés, nous avons montré que les profils de libération de l'acétaminophène n'ont pas changé en utilisant des particules de CMA de 0-75 μm ou de 75-300 μm.

Les tests de dissolution ont été effectués tout d'abord séparément dans les deux milieux de dissolution (FGS et FIS). Les comprimés préparés avec le témoin qui consiste en un amidon faiblement gélatinisé se sont désintégrés rapidement, puisque ce polymère était presque insoluble (Fig. 5.6) et présentait un polymorphisme de double hélice (type B) assez similaire à celui de l'amidon non modifié (Hylon VII) (Fig. 5.1S). En absence d'une couche d'hydrogel, la diffusion du liquide (FGS ou FIS) dans les comprimés a été très vite, entraînant une libération presque immédiate de l'acétaminophène. En utilisant un amidon plus gélatinisé et donc plus amorphe et plus soluble (environ 30 % dans FGS et 40 % dans FIS), la libération a été contrôlée et lente (environ 11 h), mais les matrices ont présenté des fissures, indiquant une hydratation non suffisante et non homogène (Figs. 5.6, 5.8 et. 5.1S). Pour ces raisons, la carboxyméthylation de l'amidon après la gélatinisation a été nécessaire. La présence des groupements carboxylates/carboxyles permet une meilleure hydratation de la matrice et le développement d'une couche d'hydrogel qui contrôle la libération.

La libération de l'acétaminophène à partir des matrices à base de CMA a été plus lente dans FGS que dans FIS (Fig. 5.2). Pour chaque DS, le temps de libération a augmenté avec l'augmentation du ratio de protonation, puisqu'une protonation plus élevée favorise une association entre les chaînes du CMA par des liaisons d'hydrogène. Les augmentations les plus fortes des temps de libération ont été observées à des RP inférieurs à 50 %, particulièrement dans FIS.

Dans FGS et pour le même ratio de protonation du CMA, l'augmentation du DS a accéléré la libération (Fig. 5.2A). Ceci est probablement dû à l'augmentation de la solubilité avec le nombre croissant de groupements carboxyméthyles. Le CMA(DS 0,07), en raison de sa plus faible hydratation et de sa plus faible solubilité (environ 44 % dans FGS et 68 % dans FIS pour RP 0%), a permis une libération plus lente que celles obtenues avec des DS plus élevés. Les matrices ont gardé leur forme globale dans FGS, même après la libération complète de l'acétaminophène.

Dans le milieu neutre (FIS), les comprimés ont été moins compacts que dans le milieu acide (FGS). Le CMA(DS 0,11) a assuré la libération la plus lente en FIS (Fig. 5.2B), indiquant que le taux d'hydratation était convenable pour développer une couche d'hydrogel de faible solubilité qui peut réduire la diffusion du FIS et la dissolution de l'acétaminophène. La libération la plus rapide dans FIS a été observée avec le DS le plus faible (0,07) et le DS le plus élevé (0,20). La libération accélérée avec CMA(DS 0,07) a été due à l'incapacité de la matrice à développer un hydrogel suffisamment compact pour empêcher une diffusion rapide du FIS dans le comprimé. Dans le cas du CMA(DS 0,20), la libération rapide a été plutôt due à la solubilité élevée de la matrice. Avec CMA(DS 0,11), le temps de libération a été moins affecté par la protonation que dans le cas des autres échantillons de CMA. Ainsi, lorsque le RP a été changé de 0 % à 100 %, le $t_{90\%}$ de CMA(DS 0,07) a augmenté de 2,5 h à 6,3 h (facteur de 2,5) et celui de CMA(DS 0,20) de 2,7 h à 7 h (facteur de 2,6), tandis que le $t_{90\%}$ de CMA(DS 0,11) a augmenté de 5,3 h à 8 h (un facteur de 1,5). Il est logique que l'augmentation du nombre de groupements carboxyles (DS) rende le comportement du polymère plus affecté par la protonation. Toutefois, cette règle n'a

pas été respectée dans le cas du CMA(DS 0,07) et CMA(DS 0,11). Probablement, la protonation du CMA(DS 0,07) a permis la formation d'un hydrogel qui a réduit la porosité de la matrice de façon considérable par rapport à celle de la forme sodium.

Pour chaque DS, l'augmentation du temps de libération avec le ratio de protonation (RP) a été moins prononcée dans FGS que dans FIS, parce que l'acidité du FGS contribue à la protonation du CMA et réduit la différence entre les différents RP.

Malgré le fait que les CMA les plus protonés aient assuré les libérations les plus lentes, une accélération de la libération a été observée en fonction du temps du stockage des échantillons, particulièrement avec les DS élevés. Par exemple, un mois après la protonation totale du CMA, des diminutions de $t_{90\%}$ de 10 % dans FGS et de 21 % dans FIS ont été observées avec CMA(DS 0,11), tandis qu'avec CMA(DS 0,20) les diminutions ont été de 46 % dans FGS et de 71 % dans FIS (Fig. 5.3). De plus, la solubilité des poudres des CMA totalement protonés diminuait progressivement avec le temps (Fig. 5.4). Ces diminutions ont été plus prononcées avec des DS élevés et elles n'ont pas été nécessairement proportionnelles aux diminutions des temps de libération. Ceci suggère une augmentation des interactions attractives inter- et intra-chaîne via les groupements carboxyles du polymère. De plus, les spectres de diffraction de rayons-X de ces échantillons de CMA protonés ont montré qu'il y a eu une modification au niveau du polymorphisme avec le temps (Fig. 5.1S).

Ces résultats ont montré que la présence des groupements –COOH non liés dans le CMA favorise la stabilité de la matrice et une libération plus lente que celle obtenue avec la forme sodium. Lorsque le DS et le RP du CMA sont suffisamment élevés, la probabilité et la force d'interaction entres les groupements carboxyles via des liaisons d'hydrogène augmentent. Ces CMA, présentant des fortes interactions inter- et intra-chaîne, ne s'hydratent pas suffisamment au sein de la matrice et ne développent pas une couche d'hydrogel. Ceci explique la libération rapide à partir de ces matrices ou même leur désintegration immédiate lorsqu'elles entrent en contact avec les milieux de dissolution.

Des comprimés de CMA(DS 0,11) chargés avec 20 % ou 40 % en acétaminophène ont été préincubés durant 2 h dans FGS, puis incubés dans FIS jusqu'à la dissolution complète. Les vitesses de libération étaient similaires, indépendamment du ratio de protonation (0 % et 50 %) et de la charge en acétaminophène (20 % et 40 %) (Fig. 5.9A). En comparant ces résultats à ceux obtenus avec une incubation dans FGS seul ou dans FIS seul, nous avons conclu qu'une protonation partielle du CMA(DS 0,11) rend les cinétiques de libération presque indépendantes de la préincubation dans FGS. De plus, il semble que l'augmentation de la charge en acétaminophène n'a pas empêché le polymère de former une couche d'hydrogel homogène et cohérent et d'assurer les mêmes profils de libération.

L'étude menée sur le degré de substitution et sur la protonation a montré que la libération la plus lente dans des conditions simulant le transit gastro-intestinal est obtenue avec un CMA de DS 0,11. Une protonation modérée du CMA rend la vitesse de libération moins dépendante de la préincubation dans FGS et favorise une libération plus lente que celle obtenue avec la forme sodium.
Avec le CMA comme seul excipient dans les comprimés, il était possible d'obtenir des formulations permettant une libération immédiate (CMA totalement protoné) ou une libération contrôlée.

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Le CMA a été ensuite comparé à l'hydroxypropylméthyl cellulose (HPMC) qui est un des excipients les plus utilisés dans les comprimés à administration orale. Le HPMC est un excipient hydrophile non ionique, tandis que le CMA est anionique dont le comportement dépend du pH du milieu de dissolution. Des comprimés à base de CMA et de HPMC ont été préparés en utilisant l'acétaminophène et des bactéries *Lactobacillus rhamnosus* comme modèles de petites molécules et de probiotiques,

respectivement. La sensibilité des bactéries *Lactobacillus rhamnosus* à l'acidité permet d'évaluer l'efficacité des matrices à assurer la protection de ces bactéries contre l'acidité gastrique. Les *Lactobacillus*, utilisés comme probiotiques, font partie de la flore gastro-intestinale (Avlami *et al.*, 2001). Les probiotiques sont considérés bénéfiques pour l'équilibre de la flore bactérienne intestinale et pour réduire l'effet des maladies inflammatoires intestinales (Mach, 2006).

L'utilisation d'un indicateur de pH (vert de bromocrésol) dans des comprimés monolithiques de 200 mg incubés durant 2 h dans un fluide gastrique simulé (FGS, pH 1,2) a montré que le CMA peut limiter la pénétration de l'acidité à l'intérieur des comprimés (Fig. 6.2). Dans les mêmes conditions, les comprimés à base de HPMC ont gardé des cœurs solides et secs, montrant que la couche d'hydrogel formée est suffisamment consistante pour empêcher l'entrée du FGS à l'intérieur des comprimés.

Avec des comprimés de 500 mg préincubés durant 2 h dans FGS avant d'être transférés dans un fluide intestinal simulé (FIS, pH 6,8), les matrices à base de CMA ou de HPMC ont montré une capacité de charge en acétaminophène relativement élevée (60 %). Les cinétiques de libération de l'acétaminophène ont été indépendantes des charges (20 à 60 %), avec un temps total de libération de 12 h pour CMA et de 34 h pour HPMC (Figs. 6.4 et 6.5). Tel qu'expliqué au chapitre I (section 4.5. *Les facteurs influençant la libération à partir des matrices hydrophiles*), au-delà d'un certain pourcentage de polymère dans le comprimé, l'hydrogel formé devient complètement homogène et cohérent. Ce pourcentage représente un point critique au-delà duquel le polymère peut assurer des profils de libération assez similaires pour des différentes charges en substances actives solubles (Maderuelo, Zarzuelo et Lanao, 2011). De plus, il semble que les molécules d'acétaminophène n'interagissent pas entre elles ou avec l'excipient de façon à affecter les cinétiques de libération. Dans l'intervalle de charge en acétaminophène de 20 % à 60 %, la vitesse de libération a été apparemment constante avec CMA, tandis qu'avec HPMC la libération s'est

ralentie après les premières heures d'incubation des comprimés. Avec les matrices à base de HPMC, il y a eu premièrement une libération de l'acétaminophène se trouvant dans partie superficielle des comprimés, puis après la formation de l'hydrogel, la libération a été plus limitée et plus contrôlée par un mécanisme de diffusion.

La présence de la pancréatine dans FIS a accéléré la libération à partir des comprimés à base de CMA, à cause de l'hydrolyse des chaînes d'amidon par l'α-amylase. Ce phénomène n'a pas été observé avec les matrices à base de HPMC, puisque la cellulose n'est pas sensible à l'action de l'α-amylase.

Les comprimés à double noyau chargés de 20 % en acétaminophène ont permis une libération retardée de 4 h avec CMA comme excipient et de 6 h avec HPMC comme excipient. L'utilité de ce type de comprimé est qu'il empêche la libération de la substance active au niveau de l'estomac, favorisant ainsi sa disponibilité en plus grande quantité au niveau de l'intestin où elle sera absorbée. Même en présence de la pancréatine, un retardement de la libération a été toujours observé avec des comprimés à double noyau.

La libération de *Lactobacillus rhamnosus* à partir des comprimés monolithiques (200 mg) à base de CMA a eu lieu en 7 h au total (Fig. 6.3). Par contre, les bactéries formulées avec du HPMC, malgré qu'elles ont été bien protégées dans le milieu gastrique (85 % de bactéries viables), n'ont pas été capables de diffuser dans FIS, même après 15 h d'incubation (2 h dans FGS et 13 h dans FIS). Ceci est dû à la formation d'une épaisse couche d'hydrogel qui empêche l'entrée des milieux de dissolution à l'intérieure des matrices, l'hydratation des poudres des bactéries lyophilisées et, par conséquence, la libération des bactéries qui ont relativement une grande taille. Ceci montre que le CMA utilisé comme seul excipient dans la formulation pourrait assurer une libération du probiotique au niveau de l'intestin, ce qui ne semble pas le cas du HPMC.

La diffusion, le gonflement et la solubilisation sont les trois mécanismes qui contribuent apparement à la libération à partir des comprimés à base de CMA ou de HPMC.

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Après avoir étudié les propriétés du CMA et son efficacité dans la libération contrôlée des substances actives, il s'est avéré intéressant de concevoir un excipient permettant une libération prolongée (libération soutenue ou « sustained release ») à partir des matrices tout en utilisant le CMA comme principal produit. Ceci peut être réalisé en réduisant la solubilité du CMA en milieu neutre et, par conséquence, en diminuant la sensibilité du polymère à l'action de l'α-amylase. C'est dans cette optique qu'un complexe du CMA et du chitosane a été préparé, en sachant que la littérature ne rapporte pas d'informations sur un tel complexe polyélectrolyte et ses applications dans le domaine pharmaceutique (Assaad *et al.*, 2011b). Dans notre projet, le produit résultant de l'interaction entre le chitosane et le CMA a été nommé « complexe polyélectrolyte », puisque les deux polymères impliqués portent des charges opposées. Dans certaines études, le complexe formé entre le chitosane et un polymère anionique a été nommé « complexe interpolymère » (De la Torre, Torrado et Torrado, 2003; Chen et Fan, 2007).

Le complexe polyélectrolyte (CPE) de CMA et de chitosane a été obtenu par une simple précipitation des deux polymères après avoir mélangé du CMA (DS 0,11-0,14) solubilisé dans l'eau avec du chitosane (masse moléculaire moyenne approximative de 700 kDa, degré de désacétylation de 80 %) solubilisé dans l'acide chlorhydrique. La précipitation immédiate peut être expliquée par une interaction effective entre les groupements fonctionnels du CMA et ceux du chitosane. Le CPE obtenu à pH 5 contenait environ 11-14 % de chitosane (m/m).

Les caractérisations par microscopie électronique à balayage (Fig. 7.1), par diffraction de rayons-X (Fig. 7.3) et par analyses thermiques (Figs. 7.4 et 8.1) ont montré que le complexe forme une nouvelle entité dont les caractéristiques sont différentes de celles du CMA et du chitosane, ainsi que de celles du mélange physique de poudres du CMA et du chitosane.

La solubilité du CPE dans FGS a été plus élevée que celle du CMA (tableau 7.1), probablement à cause de la protonation des groupements amines en milieu acide. Au contraire, la solubilité du CPE dans FIS a été plus faible que celle du CMA, suggérant une plus faible exposition des groupements carboxylates (–COONa) au milieu en raison de leurs interactions avec les groupements amines au sein du complexe.

L'analyse par imagerie RMN a montré que les comprimés monolithiques (200 mg) de CMA n'ont pas été hydratés de l'intérieur après 2 h d'incubation dans FGS (Fig. 7.5A). Lorsque ces comprimés ont été transférés dans FIS, la diffusion du liquide s'est accélérée pour avoir une hydratation complète des comprimés après seulement 2 h d'incubation dans ce milieu de pH neutre. La transformation des groupements –COOH en –COONa dans FIS a favorisé l'hydratation de la matrice pour atteindre un gonflement axial de 150 % et un gonflement radial de 60 % après un temps total d'incubation de 4 h (Fig. 7.5B). Dans les mêmes conditions d'incubation, l'hydratation des comprimés de CPE a été plus lente que ceux de CMA et de 50 % CMA:50 % chitosane. Ceci montre que l'association du CMA et du chitosane au niveau moléculaire dans le complexe favorise plus les interactions entre ces deux polymères que dans le cas du mélange physique.

Les analyses menées par Wang *et al.* (2011) sur des comprimés incubés directement dans l'eau ou dans FIS, sans préincubation dans FGS, ont montré que la vitesse de diffusion du liquide dans les comprimés de CPE et le taux de gonflement de ces comprimés ont été plus faibles que dans le cas des comprimés de CMA (Figs. A1, A3 et AS2). Il semble que les interactions CMA-chitosane au sein du complexe réduisent l'hydratation des groupements carboxylates. Ceci pourrait favoriser une libération

prolongée des substances actives formulées avec des matrices à base de CPE. La matrice de CPE reste sensible au pH et à la force ionique du milieu de dissolution, même si elle est moins sensible que la matrice de CMA. La matrice du CPE gonfle plus à pH acide (FGS) qu'à pH neutre (H_2O et FIS) à cause de la protonation des groupements amines du chitosane (tableau A2 et Figs. A3 et A4). Aussi, elle gonfle plus dans FIS que dans l'eau à cause de l'hydratation facilitée par la présence des cations K^+ dans FIS. Il est utile de mentionner que le CPE obtenu à pH 5 contient une certaine proportion de groupements carboxyles (Fig. 7.2) qui pourraient se transformer en carboxylates dans FIS (pH 6,8).

Avec des comprimés de 200 mg et une charge de 20 % en acétaminophène, le CPE et le 50 % CMA:50 % chitosane ont permis une libération plus lente (11 h) que celle obtenue avec CMA (8 h) (Fig. 7.7A). Cependant, le profil de libération à partir des matrices à base de mélange physique CMA:chitosane qui contient la même proportion de chitosane que celle du complexe a été similaire à celui obtenu avec CMA comme seul excipient.

La libération du chlorhydrate de metformine a été relativement rapide, indépendamment des excipients utilisés (CMA, mélange physique CMA : chitosane ou CPE), à cause de la solubilité élevée de cette molécule (Fig. 7.7B).

Le CMA a montré une libération prolongée de l'aspirine (13 h) (Fig. 7.7C). Il semble que des interactions entre l'excipient et la substance active via des groupements carboxyles favorisent une libération lente. Cette libération a été accélérée lorsque le chitosane a été utilisé comme coexcipient dans les comprimés. Probablement, cette accélération est due à la réduction de la quantité de CMA et à des plus faibles interactions CMA-aspirine. Une libération remarquablement prolongée (30 h) a été obtenue avec le CPE, indiquant des possibles interactions entre aspirine, CMA et chitosane sans que les chaînes du CMA et du chitosane au sein du complexe soient dissociées. Un facteur important à ne pas négliger est l'interaction attractive entre les molécules d'aspirine par des ponts d'hydrogène via les groupements carboxyles.

Cette association des molécules d'aspirine explique l'augmentation considérable du temps de libération (environ de 15 h à 30 h) lorsque la charge en aspirine a été augmentée de 20 % à 60 % dans des comprimés de 500 mg à base de CMA (Assaad, Azzouz et Mateescu, 2008). Ce même phénomène a été observé dans un tampon de pH 7,2 avec des matrices à base de carboxyméthyl amidon réticulé présentant un DS proche de ce qui était utilisé dans notre étude (Mulhbacher *et al.*, 2001).

Les tests de dissolution effectués avec l'acétaminophène, l'aspirine et le chlorhydrate de metformine ont permis de comparer le comportement de chacun des excipients vis-à-vis des trois molécules présentant différentes propriétés acide/base et différentes solubilités. Toutefois, l'acétaminophène reste le plus convenable pour comparer les excipients entre eux, puisque ses molécules ne présentent pas une charge ionique et ne s'associent pas entre elles ou avec l'excipient. En conclusion, le CPE semble plus avantageux que le CMA pour la libération prolongée de médicaments.

Finalement, le CMA et son complexe avec le chitosane ont été testés pour leur capacité de protéger l'ovalbumine dans les matrices et de la libérer de façon contrôlée (Assaad *et al.*, 2011a). L'ovalbumine a été choisie comme protéine modèle en raison de sa grande sensibilité à l'action de la pepsine et de son utilisation courante comme antigène dans des études immunologiques. Ceci permet d'évaluer l'efficacité de la formulation à protéger l'ovalbumine contre la dégradation et d'obtenir des éléments pour une vaccination par voie orale.

Avec CMA comme excipient et après 1 h d'incubation des comprimés (200 mg) dans 50 mL de FGS en présence de la pepsine, plus de 70 % de l'ovalbumine formulée (charge de 50 %) ont été protégés contre la dégradation (Fig. 8.5A). La dissolution complète, après le transfert des comprimés dans 50 mL de FIS contenant de la pancréatine, a eu lieu dans une durée totale de 6 h. Une protection plus élevée (plus de 90 %) et une dissolution plus lente (plus de 13 h) ont été obtenues en utilisant un mélange physique 50 % CMA:50 % chitosane ou le CPE comme excipients (Fig. 8.5C, 5E). La présence du chitosane dans la formulation a réduit la solubilité de

la matrice en milieu neutre et, par conséquence, a diminué sa sensibilité à l'érosion par l'α-amylase. Une plus faible proportion de chitosane dans le CPE que dans le mélange physique a été suffisante pour assurer une performance similaire dans la dissolution. Il est important de mentionner que des profils de libération similaires ont été obtenus en utilisant comme excipients le CMA seul ou un mélange physique CMA : chitosane contenant la même proportion de chitosane que le CPE (Fig. 8.5B). Les comprimés de CMA, de mélange physique CMA : chitosane et de CPE ont montré une bonne capacité d'adhésion sur des membranes préalablement imprégnées dans des solutions de mucine (Fig. 8.3). Ceci rend ces excipients plus promettant dans un cadre de développement des formes galéniques solides pour des vaccinations par voie orale.

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L'ensemble des travaux du projet doctoral a permis une meilleure compréhension des propriétés du CMA utilisé comme excipient pour le transport des agents actifs par des matrices administrées par voie orale. Il a permis aussi de montrer qu'il est possible d'assurer un transport des agents actifs aux différentes parties du tractus gastro-intestinal en modifiant le degré de carboxyméthylation ou le ratio de protonation du CMA ou même en complexant le CMA avec du chitosane. Ceci représente un grand intérêt, puisque le CMA et le chitosane sont des produits d'origine naturelle provenant des sources renouvelables largement accessibles au Canada. De plus, le CMA est un produit économique et le chitosane utilisé n'a pas été modifié chimiquement. Ajoutons aussi que les comprimés monolithiques obtenus par compression directe de poudres sont les plus faciles à préparer au niveau industriel.

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Enfin, il sera important d'étudier dans de futurs projets les propriétés du complexe CMA-chitosane en fonction de certains paramètres tels que le degré de

substitution du CMA, la masse moléculaire du chitosane, le degré de désacétylation du chitosane, les ratios CMA/chitosane, etc. Ces changements de paramètres pourraient aboutir à l'obtention des complexes présentant de différentes propriétés et de différents comportements vis-à-vis du pH du milieu et des enzymes gastro-intestinales. De plus, il sera d'une grande importance de profiter des propriétés du CMA pour préparer des complexes avec des dérivés du chitosane, en sachant que jusqu'à présent il n'y a pas d'études sur ce sujet. Il sera aussi intéressant d'utiliser le CMA dans des formes galéniques autres que les comprimés obtenus par compression directe. Dans ce sens, la préparation des microcapsules, des microparticules ou des nanoparticules pourrait être envisagée. Ces formes qui ont attiré beaucoup d'attention durant la dernière décennie peuvent modifier les propriétés pharmacocinétiques et pharmacodynamiques de la substance active par une approche physique.

ANNEXES

Imagerie RMN pour des comprimés de carboxyméthyl amidon et de chitosane : gonflement et hydratation du complexe polyélectrolyte

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Résumé

Les effets du pH et de la force ionique sur l'hydratation et le gonflement des comprimés de chitosane, de carboxyméthyl amidon et de complexe polyélectrolyte de ces deux polysaccharides ont été étudiés par imagerie RMN. Les comprimés ont été incubés dans de l'eau ou dans des fluides physiologiques simulés. Le pH du miieu d'incubation avait plus d'influence que sa force ionique sur le gonflement des comprimés. De plus, ces comprimés ont été comparés à ceux préparés avec de l'amidon réticulé. Les matrices à base de complexe CMA-chitosane ont montré plus d'integrité et moins de gonflement à pH neutre que ceux à base de CMA. Les vitesses de libération des substances actives à partir des matrices dans les milieux physiologiques simulés ont été discutées. Une libération prolongée de l'aspisine formulé avec le complexe a été observé. Ce phénomène est probablement dû aux interactions entre l'aspirine et l'excipient.

Mots clés : Imagerie RMN, gonflement, carboxyméthyl amidon, chitosane, complexe polyélectrolyte, transport du médicament.

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NMR imaging of chitosan and carboxymethyl starch tablets: Swelling and hydration of the polyelectrolyte complex

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Abstract

The hydration and swelling properties of the tablets made of chitosan, carboxymethyl starch, and a polyelectrolyte complex of these two polysaccharides have been studied by NMR imaging. We studied the effect of pH and ionic strength on the swelling of the tablets and on the diffusion of fluid into the tablets in water and simulated physiological fluids. The pH value of the fluids exerts a more significant effect than their ionic strengths on the swelling of the tablets. The tablets are compared also with those made of crosslinked high amylose starch. The formation of complex helps to keep the integrity of the tablets in various media and render a slow and restricted swelling similar to that of the tablets of the cross-linked high amylase starch, which is significantly lower than the swelling of chitosan and of carboxymethyl starch. The capacities to modulate the release rate of drugs in different media are discussed by comparing the matrices and evaluating the preparation process of the complex. A sustained release of less soluble drugs such as aspirin in gastrointestinal fluids can be provided by the complex, due to the ionic interaction and hydrogen bonding between the drug and the biopolymer complex.

Keywords: NMR imaging, swelling, carboxymethyl starch, chitosan, polyelectrolyte complex, drug delivery.

1. Introduction

Polysaccharides are among the most abundant macromolecules in nature and present several advantageous characteristics for pharmaceutical applications. They are highly stable, non-toxic, hydrophilic, biodegradable and some of them bio-adhesive. The presence of hydroxyl groups and of amine groups in certain polysaccharides allows for chemical derivatization and crosslinking. Improved physicochemical properties can be achieved by chemical modifications. For these reasons, drug delivery with solid oral dosage forms has often used polysaccharides, such as starch, cellulose, chitosan, collagen and pectin. For example, hydroxypropyl methylcellulose (HPMC) is one of the most widely used polymer carriers in the pharmaceutical industry. The ratio of hydroxypropyl groups to methoxyl groups can be modified to produce HPMC products of different hydrophilicities (Jain, 2008).

Cross-linked high amylose starch (CHAS) is an excellent excipient for controlled drug release because the cross-linking and hydrogen bonding keep the polymeric network from erosion and retrogradation and, more importantly, restrains swelling of the matrix. The sustained release was optimized for a CHAS matrix with a cross-linking degree of 6% (often defined as the weight ratio of the cross-linking agent to the starch) (Dumoulin et al., 1998). The pK_a of natural starch is about 12–14 (Lammers et al., 1993; Wong, 1989). Therefore, pH has no effect on the swelling of the CHAS tablets at pH 2–8 in the gastrointestinal tract. Carboxymethyl starch (CMS), on the other hand, is an anionic polymer with a pK_a of about 4.8 (Assaad and Mateescu, 2010) due to the presence of carboxyl groups (Mulhbacher et al., 2004; Sen and Pal, 2009). Its swelling is thus suppressed in gastric fluid. The resultant oral dosage forms could decrease irritation to the stomach caused by soluble drugs and increase their bioavailability. In intestine surroundings, CMS is deprotonated and the polymer chains swell, leading to the release of the drug entrapped in the matrix. CMS was proposed as a pharmaceutical excipient for oral dosage forms of bioactive agents

such as peptides (Calinescu et al., 2007), enzymes (Rathbone, 2008) and probiotics (Calinescu and Mateescu, 2008). In contrast to starch, chitosan is a non-ramified cationic polysaccharide due to the presence of amine groups. Its pK_a value is about 6.3 (Kumar et al., 2004). In acidic media, unmodified chitosan dissolves due to protonation of its amine groups. The control of drug release depends on the dissolution rate and this can be modulated by the excipients used in formulations.

The pH-dependent drug release can cause *in vivo* variability, and thus it is difficult to correlate *in vitro* release with *in vivo* drug availability. Complexation of ion pairs represents an effective way to modulate the pH-sensitive swelling of polyelectrolytes. A complex can be formed (Bhattarai et al., 2010) in the presence of chitosan and a polyanionic polymer (such as polysaccharides (Kaur et al., 2010), synthetic polymers (Park et al., 2008), proteins (Zhang et al., 2009) and DNA (Mao et al., 2001)). The complexation occurs without cross-linking agents, catalysts or organic solvents, which alleviates the concerns on their safety in the body (Berger et al., 2004; Bhattarai et al., 2010). In this study, the complex of CMS and chitosan obtained from direct precipitation has been investigated.

In general, the water uptake properties of anionic and cationic polymeric excipients are provided by the ionization of the functional groups, which depends on the pH and on the ionic strength of the external medium (Swarbrick, 2006). Although the effect of pH on the swelling of the above-mentioned four excipients is qualitatively predictable, a quantitative study will generate useful information on their capabilities to control the drug release. The hydration data will also serve to the modulation of the excipient preparation for better reproducibility.

Nuclear magnetic resonance imaging (NMRI) is one of the ideal methods to record *in situ* the swelling behavior of solid oral dosage forms, thanks to its noninvasive and nondestructive nature. Magnetic field gradients are used to encode the spatial distribution of the spin density (Richardson et al., 2005). Researchers can

use NMRI to evaluate the polymer concentration profile in the tablets (Baumgartner et al., 2005; Djemai and Sinka, 2006), quantify dimensional properties (thickness, area and volume) during the swelling (Baille et al., 2002; Malveau et al., 2002; Thérien-Aubin et al., 2005, 2008; Wang et al., 2010), and define the diffusion front by a sharp gradient in signal intensity (Baille et al., 2002; Malveau et al., 2002). Furthermore, the NMRI studies can provide the diffusion coefficient of a liquid component (Thérien-Aubin et al., 2008; Wang et al., 2010), and the spin-lattice and spin–spin relaxation times which relate to the environment of the penetrant and its physical bonding to the polymer system (Fyfe and Blazek, 1997).

We have used NMRI in the study of the CHAS tablets with and without loaded drugs (Baille et al., 2002; Malveau et al., 2002; Thérien-Aubin et al., 2005, 2008; Thérien-Aubin and Zhu, 2009; Wang et al., 2010), and investigated the effect of temperature, tablet size and the drug loading on the swelling and water uptake of the CHAS tablets. In this study, we would like to compare the characteristics of the tablets of the four matrices in three different media, i.e., simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and water. The tablets were made to be homogenous and do not contain any drugs in an effort to compare their swelling properties, as the dissolution studies of the drug-containing tablets have been done previously (Assaad et al., 2011). This present work should provide a better understanding of the polysaccharides used in drug delivery systems. CHAS, a well-characterized polymer matrix for sustained release of drugs, served as a basis of comparison in the study of the other three matrices.

2. Materials and methods

2.1. Preparation of matrices and tablets

High amylose starch (corn starch Hylon VII) was cross-linked at 6% with epichlorohydrin as reported (Dumoulin et al., 1998). The CMS was prepared by the alkali-catalyzed reaction of the high amylose corn starch with chloroacetic acid as previously described (Assaad et al., 2011; Mulhbacher et al., 2001). The degree of substitution of CMS determined by back-titration method is about 0.14 (Assaad et al., 2011). Chitosan (Marinard Biotech, Rivière-au-Renard, QC, Canada) was purified by solubilization in acetic acid followed by filtration (Assaad et al., 2011). The degree of deacetylation of the chitosan was about 80% according to acid–base titration and its approximate molecular weight determined by Mark–Houwink–Sakurada method was about 700 kDa (Assaad et al., 2011). A stoichiometric CMS–chitosan complex was prepared by coagulation of CMS and chitosan in an aqueous medium. The complex contains 14 wt% of chitosan. The unloaded tablets of 200 mg were obtained by direct compression (2.5 tons) of the excipient powder. Flat-faced punches and a Carver hydraulic press were used to obtain tablets of 9.6 mm × 2.1 mm.

2.2. Preparation of media

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared following the USP methods (2009), without pepsin nor pancreatin being added. SGF (pH 1.2) was prepared by dissolving 2.0 g of NaCl in 1 L of solution containing 7 mL of concentrated HCl (35 wt%). SIF (pH 6.8) was prepared by dissolving 6.8 g of KH_2PO_4 in 250 mL of distilled H_2O, and adding 77 mL of 0.2 M NaOH solution followed by dilution to 1 L with distilled water.

2.3. NMR imaging

All NMR imaging experiments were carried out at 37.0 °C on a Bruker Avance-400 NMR spectrometer operating at a frequency of 400.27 MHz for protons and equipped with a microimaging probe with a 20 mm inner diameter. A standard spin-echo pulse sequence was used to obtain spin density images of each tablet in a 20 mm o.d. NMR tube containing 20 mL of the media (distilled water, SGF, or SIF). A slice of 0.5 mm in thickness was selected either perpendicular or parallel to the main magnetic field using a sinc-shaped pulse. Eight scans were accumulated to obtain 128 × 128 pixel images for a field of view of 2.0 cm, leading to an in-plane resolution of 156 μm. An echo time of 3 ms and a repetition time of 1 s were fixed, leading to an acquisition time of about 17 min for each image.

In addition to observing the tablet swelling in the same medium until equilibrium, some tablets were studied by changing the media: first observing the tablets in SGF for 2 h and then in SIF until swelling equilibrium was reached. This was used to simulate the situation of a tablet's transit through the gastrointestinal tract, which is denoted as SGF–SIF hereafter.

3. Results and Discussion

3.1. NMR imaging and proton density profiles

NMR imaging provides a visual representation of the spatial distribution of water by acquiring signals directly from the protons. Fig. 1 presents the NMR images of tablets based on the CMS–chitosan complex in media of different pH values. The images of the tablets made of other excipients are shown in Figs. S1–S3 in the Supporting Information. The image cross-sections clearly demonstrate the time-dependent ingress of water into the polymer matrix. Moreover, the swelling of a tablet in the fluid can be visualized from the NMR images. ^{1}H tuning and matching

strongly depend on the ionic strength of the sample. A buffer solution, such as SGF, may cause de-tuning of the probe and some artifacts in the NMR images of the highly ionic samples (Bock et al., 2001, 2002), as observed in some images in Fig. 1. The higher intensity of the water inside the tablets is attributed to the different longitudinal relaxation time (T1), 5 s for the bulk water vs. 800 ms for the water inside the tablet. The repetition time was fixed at 1 s, so that all the images obtained are T1-weighted. The magnetization of the bulk water does not have enough time to return to equilibrium, contrary to the magnetization of water inside the tablet, leading to a higher proton signal intensity than that of the bulk water initially at the interface of the tablet and aqueous media.

The proton density profiles shown in Fig. 2 are taken from the NMR images of the samples and offer a clearer picture of magnitude of the water signal as a function of time. The profiles were plotted along the radial direction going through the center of the tablets. As mentioned earlier, all the images are T1-weighted due to the short repetition time. Water concentration in the gel layer is not strictly proportional to the proton density but the trend is correct inside a tablet.

3.2. Comparison of polymer matrices

The NMR images and the proton density profiles show that the CHAS tablets swell at the slowest rate among the four matrices (Figs. S1 and S4). The dry core of a CHAS tablet disappears after immersion in a medium for more than 7 h. The hydrated tablet continues to swell very slowly until reaching an equilibrium at more than 24 h. As expected, the swelling of the CHAS tablet shows little difference in the different external media used since the matrix is stabilized mainly by hydrogen bonding. Basket-shaped spindensity profiles with flat bottoms present the slow process of water penetration in a CHAS tablet. Low water signals have already been

detected in the core after 2 h, long before the polymer concentration becomes homogeneous in 10 h. The signal intensity of the core gradually increases with time.

The NMRI studies provide a direct comparison on the diffusion of water in the tablets made of different polymer matrices. The slow diffusion of water as shown by NMRI explains in part the prolonged drug delivery by the PEC tablets in comparison to the tablets made of CMA previously (Assaad et al., 2011). In contrast, water moves very fast in CMS and the water fronts meet inside a CMS tablet after immersion for only 3 h. It appears (Fig. S2) that the CMS tablets lack a well-defined edge owing to the similar proton density of the loose gel at the periphery. Since SGF has a pH lower than the pK_a (4.8) of CMS, the carboxyl groups are protonated and the resultant hydrogen bonding in the polymer network restricts the movement or relaxation of the gel and keeps the integrity of the tablets. The swelling ratio in SGF is the lowest for the CMS tablets. These results fit well with previous observation of the shape and behavior of the CMS tablets (Assaad and Mateescu, 2010; Calinescu et al., 2005; Lemieux et al., 2010). In SIF and water with pH values higher than the pK_a of CMS, the carboxyl groups are ionized. Hydrogen bonds involving carboxylic groups were disrupted and an electrostatic repulsion occurred among polymer chains, allowing water to readily diffuse into the hydrogels. A higher swelling ratio was observed. A bump-shaped change in the proton density was observed in the hydrated outer layer when the gel is very loose, which is common for the CMS tablets in SIF and water (Fig. S5). The water front moves the most slowly in the case of SGF. A sharp "peak" well defines the border of a tablet at 0.5 h. The slow rate to reach equilibrium for a CMS tablet in SIF and water shows that the anionic matrix may offer limited sustained drug release.

Chitosan dissolved in acidic SGF and a thick layer of transparent gel was formed very quickly around the hard core. The size of the core decreases gradually with time while the matrix dissolves in the medium. Within 2 h the hydrogel already

filled the NMR tube with a diameter of 18.2 mm. No proton signal was detected in the dry core during the process, as shown by the clear-cut feature of the proton density profile (Figs. S3 and S6). The chitosan tablets rapidly disintegrated in both SIF and H_2O, and no NMR image could be acquired to permit any measurement of the swelling ratio.

A tablet made of CMS–chitosan complex presents swelling characteristics similar to those of CHAS, but with slightly faster swelling. The features are very different from those of the individual components, CMS and chitosan. The interaction between CMS and chitosan retards the diffusion of water. The tablets of the complex swell much more slowly than those of CMS and chitosan. A gel layer was formed more rapidly in SGF than in other media and kept expanding until reaching the tube wall in 7 h (Fig. 1). The gel is mostly formed by chitosan which dissolves in SGF. At the same time, the dry core diminishes faster than in the other media. At the 5th hour, the proton density profile shows that the proton signal inside the tablets became uniform, while the tablets in the other media needed a few more hours to complete the process. When the fluid was changed from SGF to SIF, the volume of the gel layer either leveled off or decreased due to the still protonated chitosan.

The preparation procedure may help to explain the compact shape of the complex tablets in neutral media (H_2O and SIF) and their pronounced swelling in SGF. The chitosan solution (pH 3.6) was added to the CMS solution (pH 6.8) to prepare the complex. During the mixing process, the pH value (5.2–6.8) became close to the pK_a of chitosan. The formation of the complex was due to the electrostatic interaction between NH_3^+ and COO^- and the hydrogen bonds between the NH_2, OH and COOH groups. Since at pH 5–7 the majority of the amino groups of the chitosan were non-ionized while the carboxyl acid groups were ionized (deprotonated) when the precipitation took place, the complex formed by these two polymers may contain idle NH_2 groups (not involved in the interaction). In SGF (pH 1.2), most of the

carboxylic acid groups and the amino groups were protonated, leading to the dissociation of the NH_3^+ and COO^- groups. The chitosan (non-associated) in the complex exhibited significant swelling. A similar chitosan-based complex with pectin (Bigucci et al., 2008) was prepared at pH 5.0 at mixing molar ratios (pectin:chitosan) of 9:1, 7:3, and 1:1. The swelling of the complex of chitosan (pK_a 6.3) and pectin (pK_a 4.0) showed significant pH dependence when the molar ratio is high. The pH effect was substantially reduced when the molar ratio decreased (Bigucci et al., 2008).

3.3. Swelling characteristics of the tablets

The radial and axial swelling data extracted from the images at different immersion times (Fig. 3) provide quantitative information of the change in shape and dimension of the tablets. In the case of SGF–SIF treatment, when the tablets are switched to SIF at 2 h, a sudden change occurs and the swelling curve is approaching the curve of SIF. This behavior is most obvious in the case of the complex tablets (Figs. 3E–F).

The percentage of the swelling of the tablets is defined by

$$S = \frac{d - d_0}{d_0} \times 100\% \quad (1)$$

where S is the percentage of swelling and d and d_0 are the dimensions (thickness or diameter) of the tablet at immersion time t and at the beginning, respectively. The swelling data can be fitted to

$$S = S_{max}(1 - e^{-k_s t}) \quad (2)$$

where S_{max} is the swelling at equilibrium, and k_s the rate constant of the swelling process.

For all the tablets, the swelling in the axial dimension was higher than that in the radial direction, and the rate constant along axial direction is an order of magnitude higher (Table 1). As expected, the swelling of the CHAS tablets did not show any pH effect, while ionic strength influenced the swelling. The dimensions of the swollen tablets in both SGF and SIF are smaller than those in distilled water (65% vs. 90% radially and 100% vs. 130% axially). An increase in the ionic strength causes a decrease in the osmotic pressures (due to the hydration of ionic species), leading to a reduced swelling. For the other three matrices, i.e., CMS, chitosan and the complex, the effect of pH was more significant than that of ionic strength (Figs. 3C–F). The swelling of the CMS tablets was lower in SGF than in neutral media due to the protonation of the COOH groups. In SGF–SIF, the tablets swelled to a similar extent as in SGF. In contrast, the swelling of the complex tablets in SGF was almost twice of those in other media (Table 1). The tablets in distilled water had the solid-like appearance due to a very slow swelling process. According to the swelling characteristics, drug could be released in a controlled way by the complex after an initial burst dissolution of the drug located near the surface of the tablet.

The swelling process of the tablets in the different media can be directly observed, even though the content of water and water penetration fronts cannot be visualized in this case. Table 2 shows the photographs of the PEC tablets in 4 different media at 2, 5 and 10 h. Several observations can be made. In pure water, the tablet swells to a less extent than in the other media. SIF is also a neutral medium, but the presence of ionic species such as K^+ and Na^+ facilitates the hydration of the tablet. It is in SGF that the tablet swells the most and the fastest. Apparently in an acidic environment such SGF, the protonation of functional groups in the polymer matrix favors the swelling of the tablet, leading to the partial dissolution of the tablets and the formation of a hydrogel. The swelling remained continuous even after a long incubation, while in other media the swelling reaches a stable plateau at shorter time intervals. When the tablet is transferred to SIF (a neutral medium) after 2 h in SGF,

the swelling of tablet is slowed down, with similar dimensional change at the end as the tablet in SIF alone. The radial swelling properties of the tablets observed in the four different media are plotted as a function of time in Fig. 4. These results are in good agreement with those obtained by NMRI analyses (Fig. 3E). It is to be noted that NMRI provides additional information on the water diffusion in the tablet.

3.4. Comparison to the dissolution characteristics of the tablets

The *in vitro* dissolution experiments of monolithic tablets made of the complex loaded with 20 wt% aspirin have been carried out in a previous study (Assaad et al., 2011). It showed a sustained release up to 30 h, longer than expected. In contrast, 90% of the loaded aspirin was released in about 11 h when CMS or chitosan alone was used as the excipient (Assaad et al., 2011). Therefore, the water diffusion rate as visualized by NMRI is related to the drug release rates obtained. Slower water diffusion leads to prolonged drug release. Aspirin is slightly soluble in water and its pK_a is 3.5 (Dewick, 2006; Schrör, 2009). The relatively long sustained drug release of aspirin from the complex tablets can be a result of a binding interaction with either CMS or chitosan in acidic and neutral media, respectively. During the first 2 h in SGF, the carboxyl acid groups of aspirin form hydrogen bonds with CMS, which slowed down the drug release. In SIF, the ionic interaction between aspirin and CMS may become more predominant than the hydrogen bonding in the system, reducing the diffusion rate of aspirin toward the outside of the matrix. In the case of acetaminophen (Assaad et al., 2011), a drug with higher water solubility and with higher pK_a (9.5) (Dewick, 2006), the longer release was afforded by the chitosan excipient alone due to the formation of an outer layer hydrogel in SGF. The CHAS tablets showed a similar release profile of acetaminophen to those of the complex (Assaad et al., 2011; Wang et al., 2010).

4. Conclusion

Tablets made of both CMS and chitosan have demonstrated pH-dependent swelling capabilities. The comparison of the tablets revealed that the tablets made of the CMS–chitosan complex presented a combined benefit of a lower swelling in acidic media than the chitosan tablets and a slower water uptake than the CMS tablets in neutral media. A large decrease in swelling (60% radially and 150% axially) in neutral media is observed by NMRI for the complex tablets in comparison with those of CMS and of chitosan. Similar to CHAS, the complex tablets can keep their integrity even after their swelling reaches an equilibrium. The interaction between the carboxyl groups of CMS and amine groups of chitosan showed a stabilizing effect (in terms of limited swelling) similar to that of hydrogen bonding in the case of covalently cross-linked starch. The *in vitro* dissolution experiments presented a particularly slow release of aspirin from the monolithic tablets with 20 wt% drug loading (Assaad et al., 2011). The results showed that the CMS–chitosan complex is a promising polymer excipient for sustained drug release, by partially retaining the gastro-protective effect of CMS and by modulating its solubility in neutral media through ionic association with chitosan. This behavior makes the novel complex a good excipient to colon delivery. Its swelling properties may be modulated by pH and ionic strength of the processing medium and by the molar ratio of the two components.

Supporting Information:

Figures providing the NMR images, the proton density profiles, and the comparison of the radial and axial swelling of the CHAS, CMS, and CMS–chitosan complex tablets.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.08.008.

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Table 1: The swelling behaviors of the tablets made of CHAS, CMS and CMS-chitosan complex. Parameters obtained by fitting to Eq. 2.

Media	radial dimension			axial dimension		
	S_{max} (%)	k_s ($10^{-5}s^{-1}$)	R^2	S_{max} (%)	k_s ($10^{-4}s^{-1}$)	R^2
CHAS						
H_2O	86.8±1.1	6.1±0.2	0.992	109±3	3.6±0.5	0.877
SGF	62.2±0.7	7.5±0.3	0.989	91.8±2.1	2.2±0.2	0.894
SIF	62.2±1.2	8.2±0.4	0.985	96.9±3.1	2.0±0.3	0.886
SGF-SIF	63.0±0.7	7.4±0.3	0.990	92.7±2.3	2.1±0.3	0.878
CMS						
H_2O	57.4±7.9	30±9	0.903	139±7	6.8±1.5	0.932
SGF	66.6±3.8	8.8±0.8	0.991	109±6	3.0±0.6	0.841
SIF	103±7	14.2±1.8	0.982	159±4	4.5±0.5	0.963
SGF-SIF	128±24	4.1±1.0	0.996	135±14	1.8±0.5	0.937
CMS-chitosan complex						
H_2O	37.1±1.2	9.2±1.1	0.907	92.6±2.1	2.8±0.4	0.890
SGF	91.4±2.8	9.6±0.8	0.976	229±4	4.6±0.4	0.959
SIF	61.4±1.1	5.0±0.2	0.987	117±3	2.0±0.2	0.911
SGF-SIF	45.1±0.7	34.9±2.6	0.966	151±4	9.4±2.0	0.883

* The measurements were carried out on triplicates; the reported values are the averages and the uncertainties correspond to the standard deviations.

Table 2: Photographs showing the swelling of the PEC tablets in water, SGF, SIF and SGF–SIF (SGF for 2 h followed by replacing SGF by SIF) and at three different time intervals.

Dry Tablet	Time (h)	Medium			
		H_2O	SGF	SIF	SGF-SIF
 0.96 cm	2				
	5				
	10				

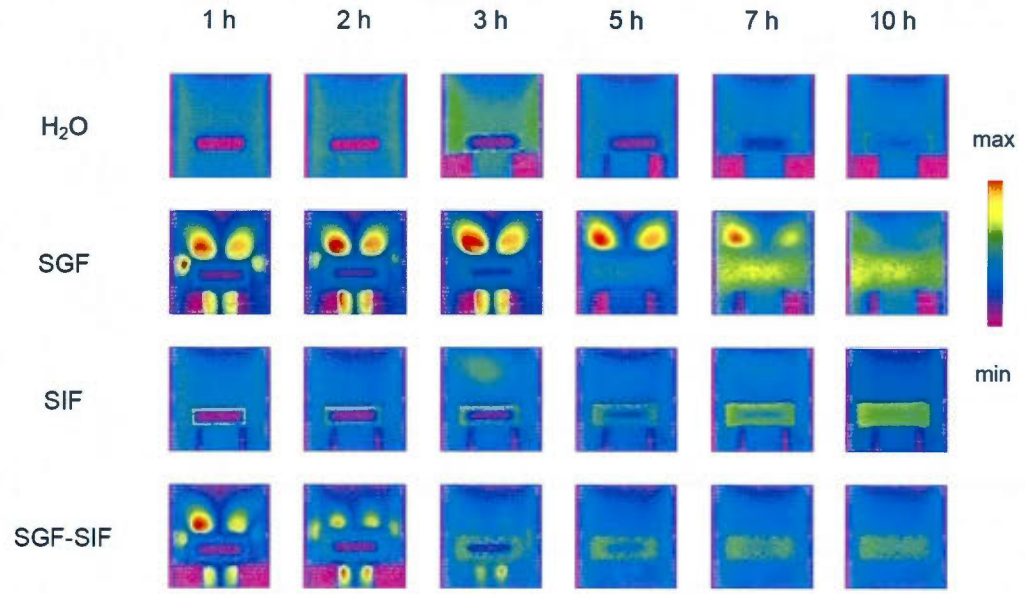

Figure 1: The NMR images of the CMS-chitosan complex tablets immersed in various media at 37 °C for 1, 2, 3, 5, 7 and 10 h. The bright spots above the tablets resulted from an abrupt change of magnetic susceptibility at the interface between the gel layer and the liquid. SGF-SIF indicates that the tablets were transferred from SGF to SIF after immersion for 2 h in SGF.

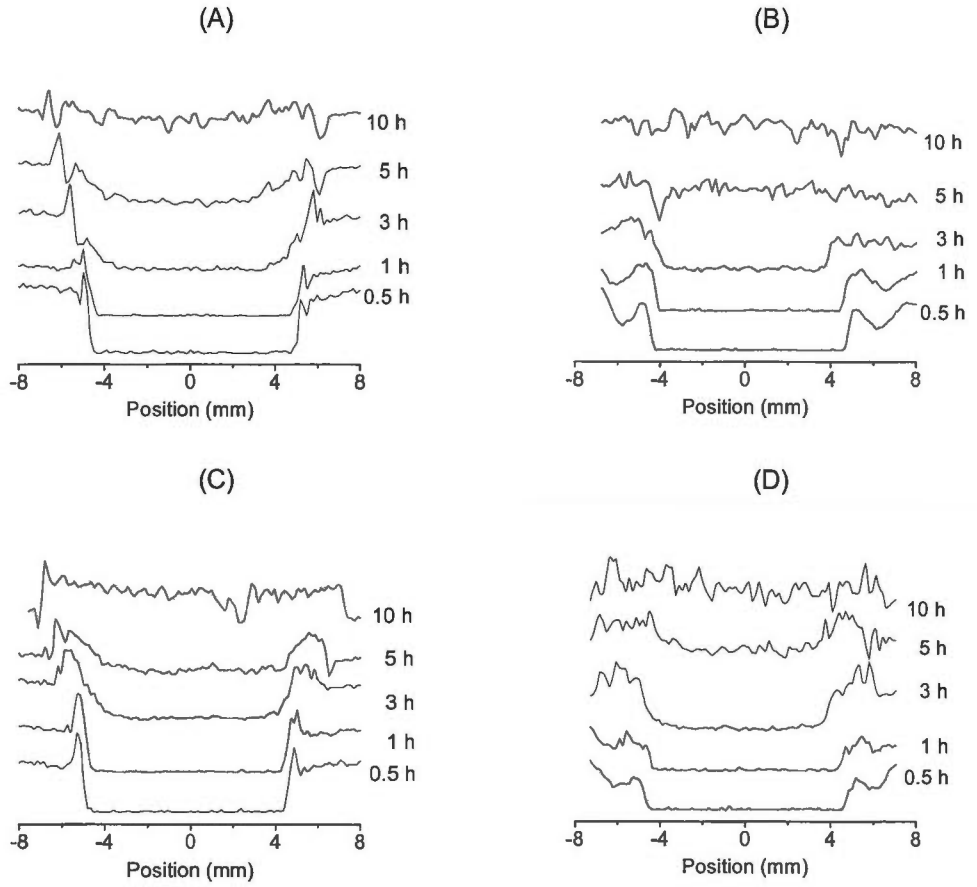

Figure 2: The change of the proton density profiles of CMS-chitosan complex tablets immersed in (A) H_2O, (B) SGF, (C) SIF, (D) SGF-SIF at 37 °C at different immersion times.

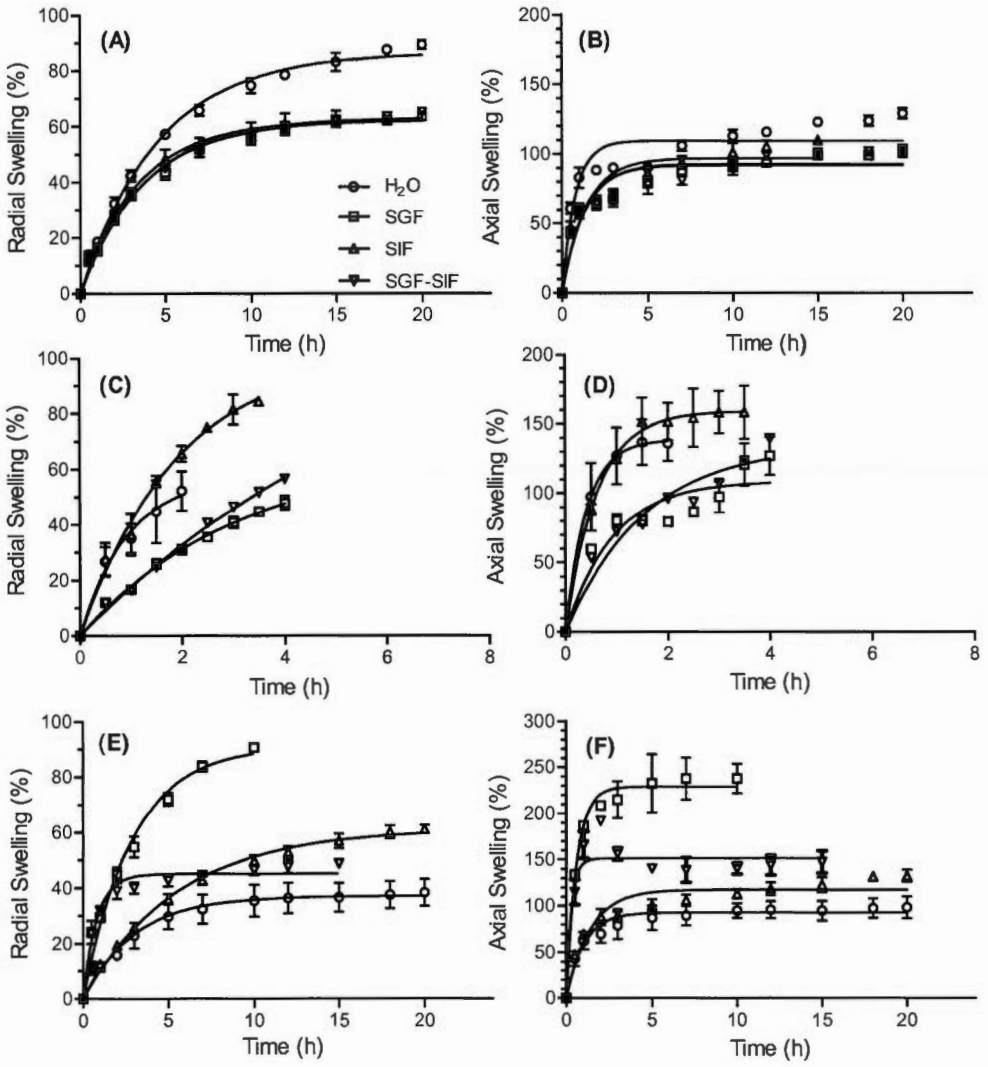

Figure 3: The radial and axial swelling of the tablets in H2O (circles), SGF (squares), SIF (up triangles), and SGF–SIF (down triangles). CHAS tablets (A and B), CMS tablets (C and D), and CMS–chitosan complex tablets (E and F). Lines are best fits to Eq. (2). For the CMS tablets, the swelling reached equilibrium quickly within ca. 4 h.

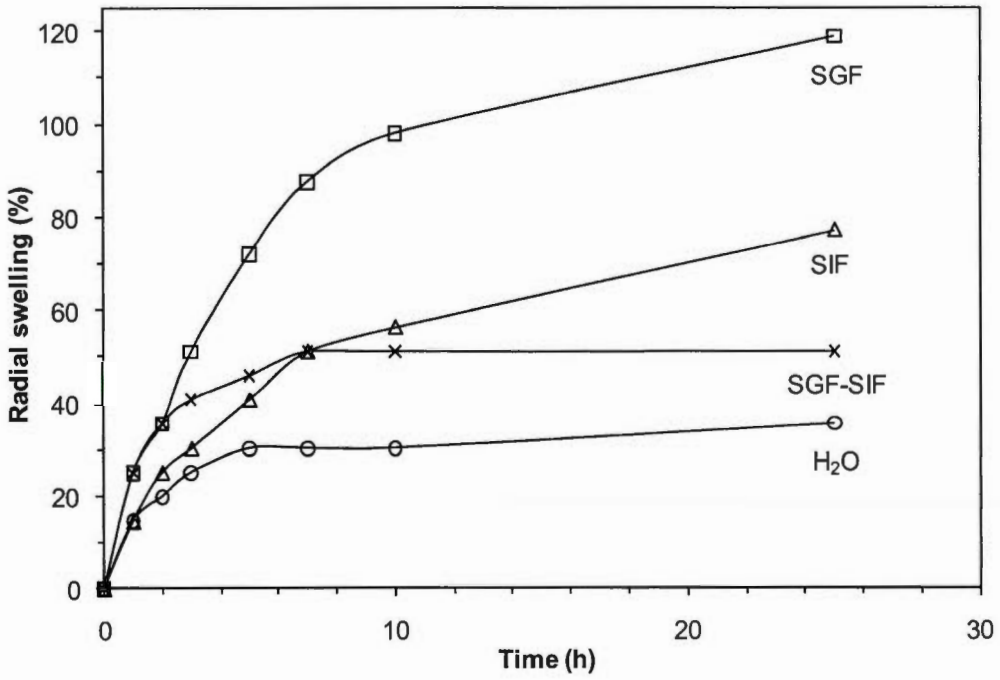

Figure 4: The apparent radial swelling of the PEC tablets observed visually as a function of time in the four different media tested.

Supporting Information

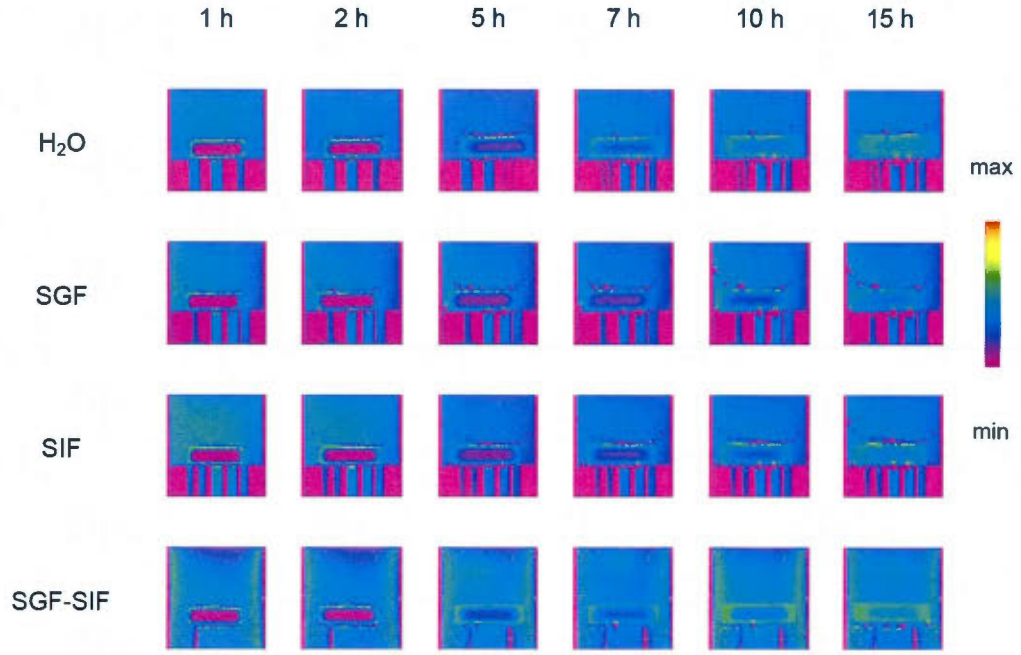

Figure S1: The NMR images of the CHAS tablets immersed in various media at 37 °C for 1, 2, 5, 7, 10 and 15 h. The purple part at the bottom of the images is the Teflon support. The dark spots around the tablets are air bubbles.

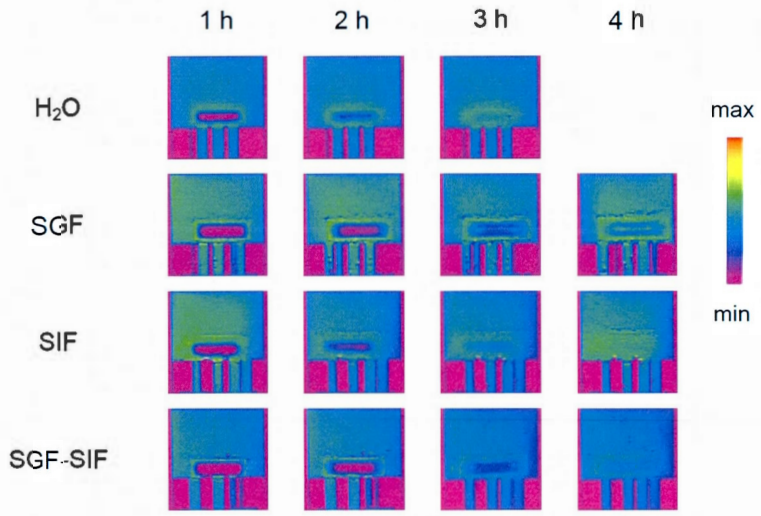

Figure S2: The NMR images of the CMS tablets immersed in various media at 37 °C for 1, 2, 3 and 4 h.

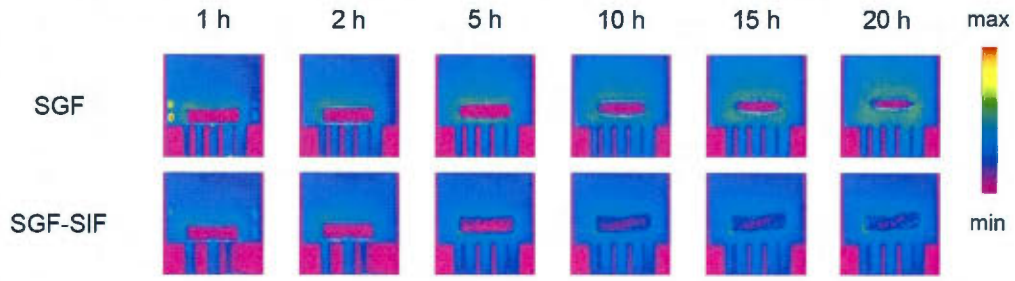

Figure S3: The NMR images of the chitosan tablets immersed in various media at 37 °C for 1, 2, 5, 10, 15 and 20 h.

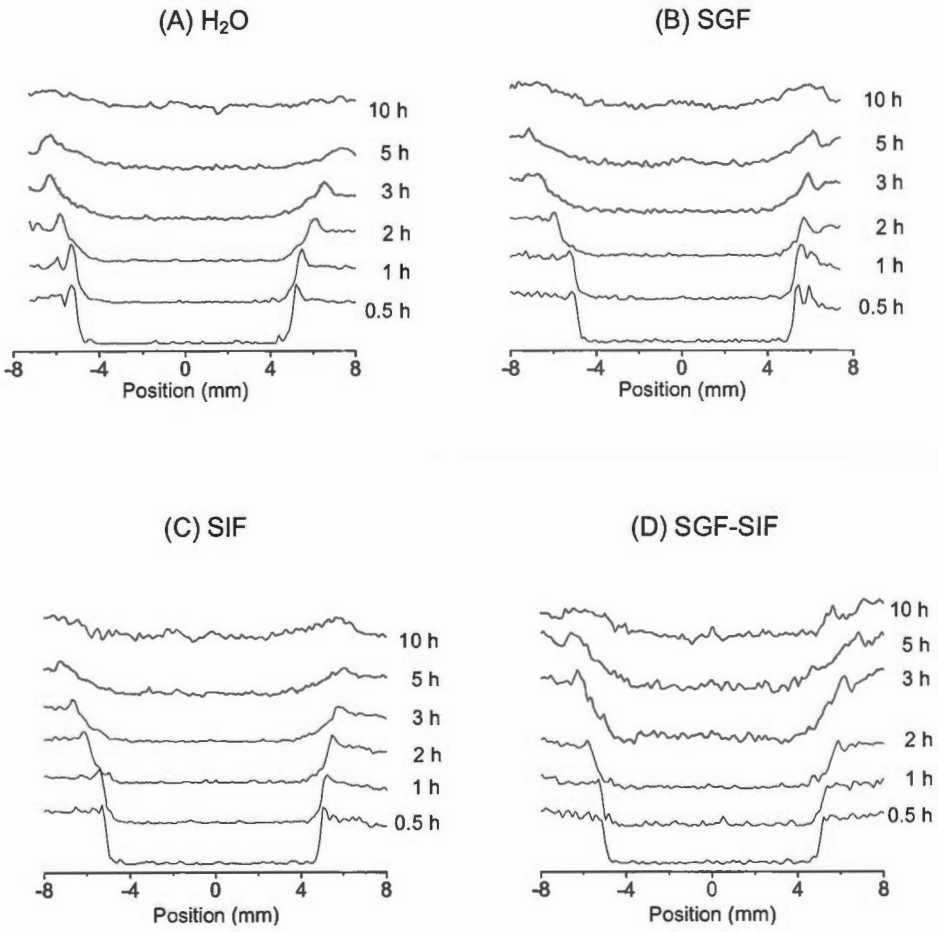

Figure S4: The change of the proton density profile of the CHAS tablets immersed in varoius media at 37 °C.

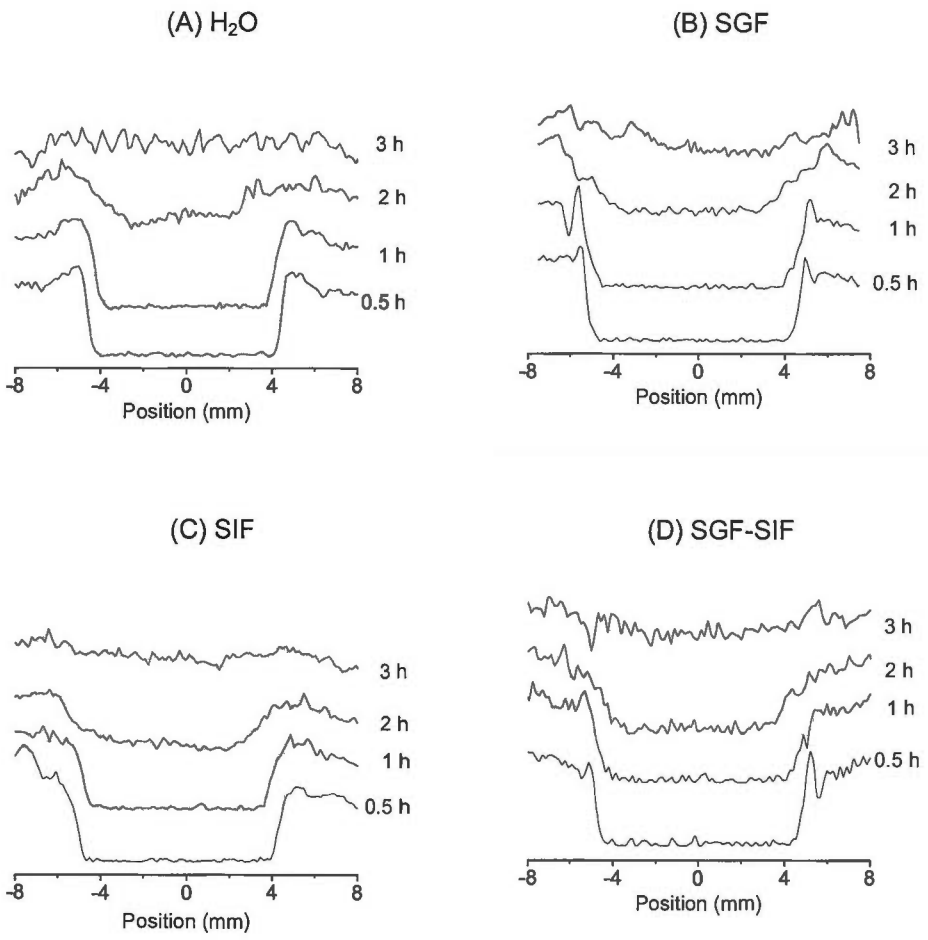

Figure S5: The change of the proton density profile of the CMS tablets immersed in various media at 37 °C.

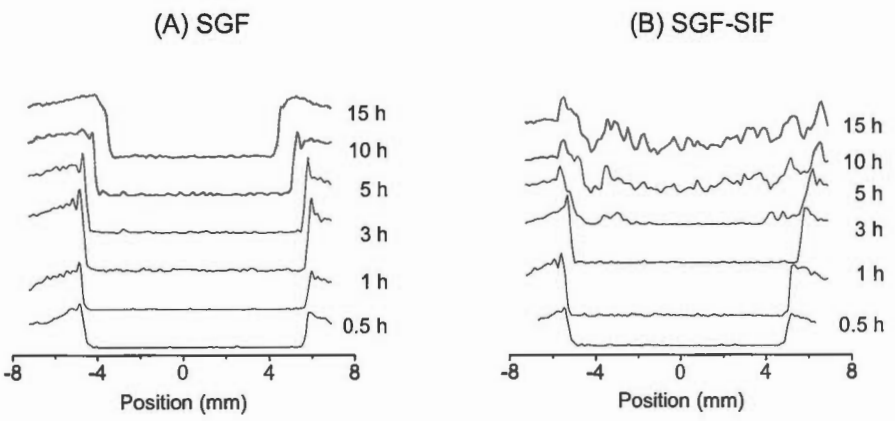

Figure S6: The change of the proton density profile of the chitosan tablets immersed in various media at 37 °C. These tablets disintegrated quickly in H_2O and SIF.

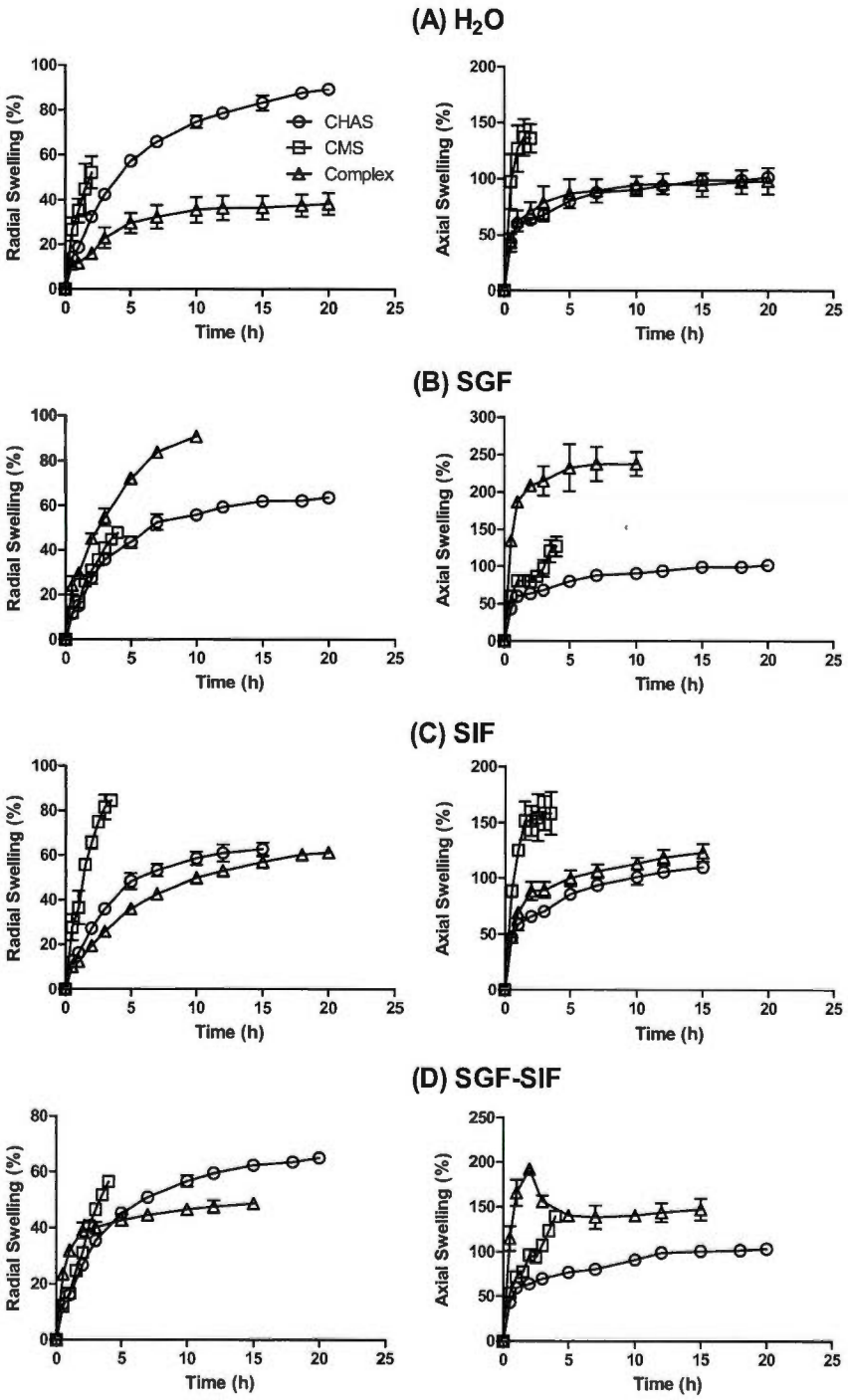

Figure S7: The radial and axial swelling of the CHAS, CMS and CMS-chitosan complex tablets in various media at 37 °C.

Les participations aux colloques et aux conférences

35th Annual Meeting & Exposition of Controlled Release Society (CRS), 2008: Influence of bentonite on drug release by sodium carboxymethyl starch excipients. Présentation par affiche.

36th Annual Meeting & Exposition of Controlled Release Society (CRS), 2009: Influence of carboxylation degree and of protonation ratio on drug delivery from carboxymethyl starch excipients. Présentation par affiche.

92e Congrès et Exposition Canadiens de Chimie, 2009 : Drug release from chitosan and carboxymethyl starch excipients. Présentation par affiche.

2e Colloque de Pharmaqam, 2010 : Formulations à base de carboxyméthyl amidon et de chitosane pour administration de médicaments par voie orale. Présentation par affiche.

78e Congrès de l'Association Francophone pour le Savoir (Acfas), 2010 : Effet du ratio de protonation et du degré de substitution sur les propriétés du carboxyméthyl amidon utilisé comme excipient. Présentation orale.

79e Congrès de l'Association Francophone pour le Savoir (Acfas), 2011 : Carboxyméthyl amidon et chitosane comme excipients pour le transport d'agents bioactifs par des comprimés administrés par voie orale. Présentation orale.

3e Colloque de Pharmaqam, 2011. Complexe carboxyméthyl amidon-chitosane comme nouvel excipient pour le transport des substances actives : Analyse par imagerie RMN. Présentation par affiche.

1ère journée scientifique du Réseau Québécois de Recherche sur les Médicament (RQRM), 2011 : Complexe carboxyméthyl amidon-chitosane comme nouvel excipient : Cinétiques d'hydratation analysées par imagerie RMN. Présentation par affiche.

Influence of Bentonite on Drug Release by Sodium Carboxymethyl Starch Excipients

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Abstract

Sodium carboxymethyl starch (Na-CMS) in monolithic tablets can afford a partial gastric protection of drug and an intestinal controlled release. The addition of an adequate amount of sodium-bentonite (Na-Ben) to the formulation leads to a longer drug release, without affecting the physicochemical properties of tablets.

Introduction

The crosslinked CMS form was described as an efficient excipient for the formulation of small molecules in monolithic tablets[1]. The uncrosslinked CMS was previously proposed as excipient for the formulation of probiotic bacteria (*Lactobacillus rhamnosus*)[2].

The present study describes a model formulation for the controlled release of small molecules. The excipients are based on Na-CMS or on a mixture of Na-CMS and Na-form bentonite (Na-Ben). When associated, both components of the excipients are able to generate a hydrogel structure that can control the release of drug. Tablets with Na-CMS excipient are relatively compact in Simulated Gastric Fluid (SGF) and becomes more hydrated and swelled in Simulated Intestinal Fluid (SIF). The Na-Ben can interact with the hydroxyl and carboxymethyl groups of Na-CMS and also with the polar functions of drugs via silanol and aluminol groups. The electrostatic interactions with the permanent charge of Na-Ben and the possible hydrogen bonds between Na-CMS, Na-Ben and drug seem involved in the drug release mechanisms.

Experimental methods

Synthesis of Na-CMS

Na-CMS was prepared as previously described[2]. High amylose starch (Hylon VII, *National Starch*, USA) and monochloroacetic acid were allowed to react in alkaline medium for 1 h. Na-CMS was characterized by FTIR, XRD, SEM and the degree of substitution was determinated via potentiometric titration.

Modification of bentonite

Natural bentonite (*Aldrich*, USA) was purified by repeated settlings, and then ion-exchanged by sodium cations. The obtained Na-Ben was characterized by FTIR, SEM and XRD.

Physicochemical and *in vitro* tests

Monolithic tablets (500 mg) based on Na-CMS or on a mixture of Na-CMS and Na-Ben were obtained by direct compression (2.5 T/cm^2, *Carver* hydraulic press). Four drugs were used as tracers: acetaminophen, aspirin, metformin hydrochloride and chlorpheniramine maleate.

Tablets were characterized by hardness, friability and disintegration tests. The *in vitro* dissolution tests were performed using a *Distek* dissolution 2100A paddle system (100 rpm) coupled with an UV *Hewlett Packard* spectrophotometer. The tablets were maintained for 2 h in 1 L of pepsin-free SGF and then in 1 L of pancreatine-free SIF. These tests were carried-out following the USP 24 method.

Results and discussion

The hardness of tablets depends on the formulated drug. In fact, aspirin induced higher hardness value than acetaminophen. This feature decreased with the drug amount in the tablets. The friability also depends on the loading (weight loss increases with loading). For the same drug loading (20%), the disintegration test showed similar results for chlorpheniramine maleate and metformin hydrochloride. At similar high tablet loading (60%), aspirin displayed longer disintegration time than acetaminophen.

The presence of low percentage (2% and 5%) of bentonite in the excipient did not influence significantly the hardness, friability and disintegration of tablets (Table 1).

Dissoluton kinetics are strongly depending on the drug solubility on SGF. Indeed, metformin hydrochloride and chlorpheniramine maleate were released relatively fast in SGF, while their dissolution was much slower in SIF (Fig.1 a and b). This indicates that formulation of these two drugs in double-coated Na-CMS based tablet would ensure a good protection in SGF and a controlled release in SIF. The release kinetics for acetaminophen and aspirin were almost linear (Fig.1 c and d). Therefore, Na-CMS appears as an interesting excipient for oral drug release from monolithic tablet obtained by direct compression of powders.

Small amounts of bentonite (2% and 5% in the excipient) can prolong the drug release. This is presumably due to electrostatic interactions and hydrogen bonding between bentonite and polar groups of drug and Na-CMS.

However, relatively high ratio of Na-Ben seems to accelerate the drug release (data not shown) due to the high swelling properties of bentonite[3], which may affect the tablet compaction.

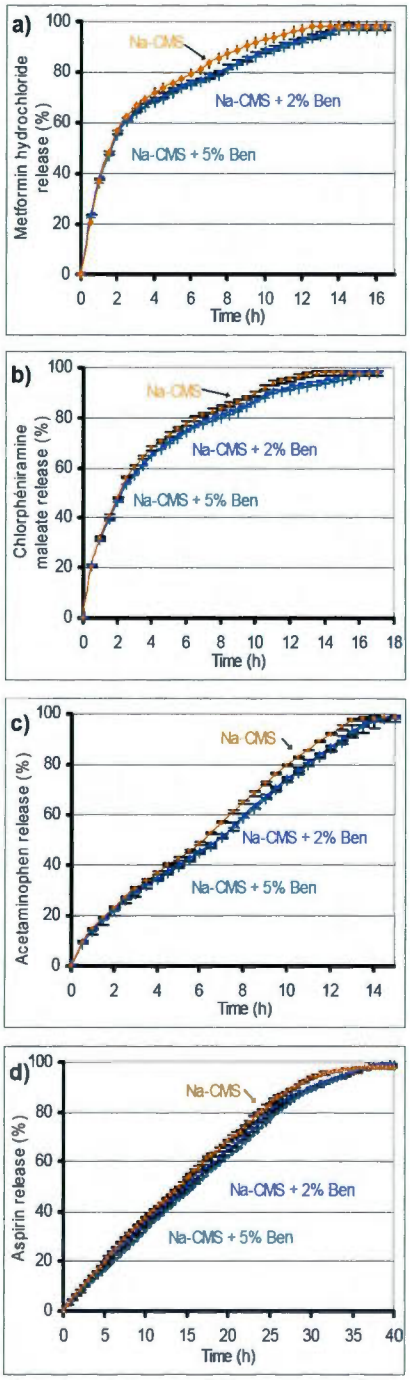

Figure 1: Drug release from monolithic tablets a) metformin hydrochloride, b) chlorpheniramine maleate, c) acetaminophen and d) aspirin.

Table 1: Hardness, friability and disintegration of tablets with different tracers and loading

Hardness (kp mode)	Loading	Bentonite %		
		0	2	5
Acetaminophen	60%	19.6	19.5	19.3
Aspirin	60%	24.5	23.1	22.2
Metformin hydrochloride	20%	>35	>35	>35
Chlorpheniramine maleate	20%	>35	>35	>35

Friability	Loading	Bentonite %		
		0	2	5
Acetaminophen	60%	0.54	0.54	0.55
Aspirin	60%	0.37	0.39	0.36
Metformin hydrochloride	20%	0.04	0.04	0.05
Chlorpheniramine maleate	20%	0.04	0.05	0.05

Disintegration (min)	Loading	Bentonite %		
		0	2	5
Acetaminophen	60%	121	117	115
Aspirin	60%	202	219	214
Metformin hydrochloride	20%	319	314	316
Chlorpheniramine maleate	20%	328	321	315

Conclusions

The ability of sodium carboxymethyl starch to protect a drug in SGF depends on the solubility of this drug in acidic medium. The results with acetaminophen, aspirin, metformin hydrochloride and chlorpheniramine maleate indicate that Na-CMS can afford a good protection of drugs with medium or low solubility in SGF and a controlled release in SIF. Addition of optimal amounts of bentonite in the tablet formulation can prolong the drug release.

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1. J. Mulhbacher, P. Ispas-Szabo, V. Lenaerts and M. A. Mateescu. *J.Control.Release*. 2001, 76, 51-58.
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INFLUENCE OF MONTMORILLONITE ON DRUG RELEASE BY SODIUM CARBOXYMETHYL STARCH EXCIPIENTS

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ABSTRACT

Sodium carboxymethyl starch (Na-CMS) was proposed as excipient for the controlled release of some tracer drugs, such as metformine hydrochloride, chlorpheniramine maleate, aspirin and acetaminophen. The effects of the addition of a Na-form montmorillinite (NaMt) upon drug release and physicochemical properties of monolithic tablets were studied.
The dissolution kinetics strongly depends on the drug solubility on simulated gastric fluid (SGF). Relatively quick release of metformine hydrochloride and chlorpheniramine maleate takes place in SGF, despite their dissolution was much slower in simulated intestinal fluid (SIF). The tablets mechanical features depend on the incorporated drug, since aspirin induced higher hardness and lower friability than acetaminophen. Na-CMS in monolithic tablets can afford a muted release in SGF and a controlled release in SIF for acetaminophen and for aspirin. Addition of adequate amounts of Na-Montmorillonite leads to a delay of drug release, without affecting the tablet physicochemical properties.

OBJECTIVES

- **Preparation of Na-CMS / NaMt composites for pharmaceutical formulations**
- **Formulation of various bioactive molecules with Na-CMS / NaMt composites**
- **Assessment of the effect of NaMt upon drug release and physicochemical properties of tablets**

MATERIALS & METHODS

- Na-CMS was prepared from high amylose starch (Hylon VII) and the powder was obtained by precipitation with acetone or by spray dryer (Büchi Mini Spray Dryer, B-290)
- Natural bentonite from *Aldrich* was purified by repeated settlings and then ion-exchanged by sodium cations. The powder was obtained by spray dryer (NaMt) or by lyophilization (L-NaMt)
- Na-CMS/NaMt composites were obtained by mixing aqueous suspensions of both products and further by precipitation in acetone (Na-CMS-NaMt (A)) or by drying with spray dryer (Na-CMS-NaMt (SD))
- Monolithic tablets (500 mg) based on Na-CMS or a mixture of Na-CMS and NaMt were obtained by direct compression (2.5 T, Carver hydraulic press, 13 mm cylinder outfits)
- Each dry-coated (DC) tablet of 500 mg with 20 % loading has a core (100 mg of drug + 50 mg of excipient) compressed in 9 mm cylinder outfit. This core was double dry-coated by 350 mg of excipient and compressed in 13 mm cylinder outfit
- The *in vitro* dissolution tests were performed, according to the USP 24 method, using a dissolution system (Distek, premiere 5100) coupled with an UV *HP* spectrophotometer. Each tablet was maintained for 2 h in 1 L of pepsin-free SGF and then in 1 L of SIF, with 100 rpm agitation
- SEM (HITACHI S-4300SE/N (VP-SEM)), BET (QuantaChrome Autosorb-1 instrument) and other techniques were used for characterization

EXPERIMENTAL & RESULTS

Sodium carboxymethyl starch (Na-CMS)

Na-CMS can be prepared by gelatinization of starch in alkaline medium and by reaction with sodium monochloroacetate or monochloroacetic acid

Figure 1 synthesis of carboxymethyl starch

Montmorillonite

- **Lamellar structure clay minerals with permanent negative charge**
- **Interesting features: swelling, adsorption and ion-exchange capacities**
- **Dispersion of the clay sheets yield colloidal suspension in aqueous media**

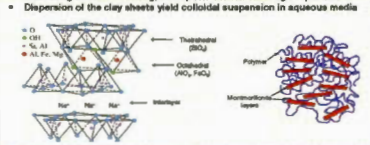


Figure 2 structure of montmorillonite layers Figure 3 montmorillonite polymer nanocomposite

1. Products caracterisation

- BET specific surface area: **NaMt : 34 m²/g** **L-NaMt: 60 m²/g**
- Degree of substitution (DS) of Na-CMS: **0.14**

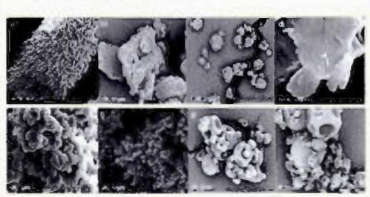


Figure 4 Scanning Microscopies of (a) gelatinized starch (b) natural bentonite (c) spray dried montmorillonite (d) lyophilized montmorillonite (e) Na-CMS precipitated with acetone (f) Na-CMS-NaMt (A) composite (g) spray dried Na-CMS (SD) (h) Na-CMS-NaMt(2%)-(SD) composite

Gelatinized starch shows a very porous structure; Na-CMS and Na-CMS-NaMt (A) are more compact; Na-CMS (SD) and Na-CMS-NaMt (SD) are of small particles size with spherical forms presenting cavities; lyophilized NaMt is more exfoliated than spray dried NaMt sample

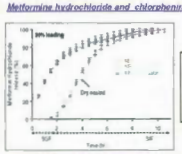
2. Physicochemical characterizsition of the tablets

Table 1 hardness friability and disintegration results for monolithic tablets

		Hardness (Kp mode)			Friability			Disintegration (min)		
		NaMt %			NaMt %			NaMt %		
Drug loaner	Loading	0 (%)	2 (%)	5 (%)	0 (%)	2 (%)	5 (%)	0 (%)	2 (%)	5 (%)
Acetaminophen	60%	19.6	19.5	19.3	0.54	0.54	0.56	121	117	115
Aspirin	60%	24.6	23.1	22.2	0.37	0.38	0.36	212	219	214
Metformin hydrochloride	20%	>35	>35	>35	0.04	0.04	0.05	319	314	306
Chlorpheniramine maleate	20%	>35	>35	>35	0.04	0.08	0.06	389	331	319

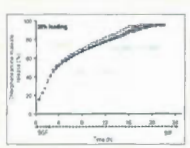
3. Dissolution tests

Metformine hydrochloride and chlorpheniramine maleate (20 % loading)



- Fast metformine release in SGF due to high solubility in acidic media
- Dry-coating protect metformine in SGF and retard the release
- Addition of 2% and 5% of NaMt delay slightly the metformine release

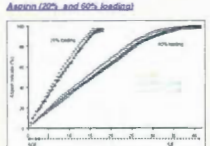
Figure 5 metformine hydrochloride and dry-coated tablets containing NaMt



- Relatively fast release of chlorpheniramine maleate in SGF due to high solubility in acidic media
- Addition of 2% and 5% of NaMt delay slightly the release of chlorpheniramine maleate

Figure 6 chlorpheniramine maleate tablets containing NaMt

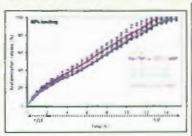
Aspirin (20% and 60% loading)



- Dissolution kinetics of aspirin is almost linear
- The release for 60% loading is slower than that for 20%, indicating an interaction between the aspirin molecules and stabilization of the formulations
- Addition of 2% and 5% of NaMt in the tablet formulation can delay slightly the release of aspirin

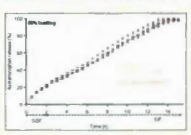
Figure 7 aspirin release by monolithic tablets containing NaMt

Acetaminophen (60% loading; 20% loading with pancreatine)



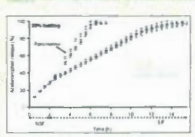
- Kinetic dissolution is almost linear with relatively small release in SGF
- Release can be delayed by addition of 2% and 5% of montmorillonite; no difference was seen between the effect of spray dried and lyophilized NaMt on kinetic release
- 20% spray dried NaMt accelarated release (swelling of particles); 20% lyophilized NaMt have almost no effect on release (exfoliated layers, reduced swelling)

Figure 8 acetaminophen release by monolithic tablets containing L-NaMt or NaMt



- Two percent of montmorillonite were introduced in the excipients by three different ways: physical mixture of powders, composite dried with spray dryer and composite precipitated with acetone
- The introducing way of the 2% of montmorillonite has no effect on kinetic release of acetaminophen (60% loading)

Figure 9 acetaminophen release by monolithic tablets with physical mixture or composites excipient



- There is a slight influence of 2% and 5% montmorillonite on the kinetic release for 20% acetaminophen loading
- Pancreatine in SIF can accelerate significantly the release of acetaminophen, because Na-CMS still a substrate for alpha-amylase

Figure 10 acetaminophen release by monolithic tablets containing NaMt without pancreatine

CONCLUSION

- **The ability of sodium carboxymethyl starch for drug protection in SGF depends on the solubility of the given drug in acidic media**
- **Na-CMS can afford a good protection of drugs with medium or low solubility in SGF and a controlled release in SIF**
- **Addition of optimal amounts of montmorillonite in tablet formulation can delay the release of drug: This is presumably due to ternary electrostatic interactions and hydrogen bonding between the montmorillonite surface, polar groups of drugs and Na-CMS**

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1. J. Muhlbacher, P. Ispas-Szabo, V. Lenaerts and M. A. Mateescu, *J.Control.Release*, 2001, 76, 51-58.
2. C. Calinescu, J. Muhlbacher, E. Itadeau, JM Fortunither and M. A. Mateescu. *Eur J Pharm Biopharm.* 2005, 60, 53-60.
3. F. Bergaya, B.K.G. Theng and G. Lagaly. 2006. *Handbook of Clay Science*. London, Elsevier. 1224 p.

Influence of Carboxylation Degree and of Protonation Ratio on Drug Delivery from Carboxymethyl Starch Excipients

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Abstract

Carboxymethyl starch (CMS) is a pH sensitive matrix. In monolithic tablets based on CMS excipient, the drug release is influenced by both the pH of the media and the percentage of the protonated carboxymethyl groups. This study shows the influence of the degree of substitution (DS) and of the protonation ratio (PR)* on the properties of the CMS matrix. Optimum DS and optimum PR were found for CMS matrix; they can provide a good protection in gastric medium and a controlled drug release in intestinal medium. At these optimums DS and PR, CMS presents an amelioration compared to sodium CMS form (CMS(Na)), in terms of drug controlled release.

*PR is the percentage of protonated groups (COOH) compared to the total carboxyl groups (COOH + COONa)

Introduction

Sodium carboxymethyl starch in association with chitosan, was previously found as an interesting matrix to formulate probiotic bacteria (*Lactobacillus rhamnosus*)[1]. The present study is focused on the understanding of the mechanisms that control the release of small molecules by CMS. Several variants of CMS with different DS and different PR were prepared and studied with the aim to evaluate whether the DS value and the protonation of (–COONa) groups can affect the properties of CMS matrix and the control of drug release by CMS excipient. Dissolution tests in acid and neutral media proved the influence of pH on the acetaminophen release by CMS and the influence of DS value and protonation ratio on the properties of CMS. The matrix becomes more or less swollen or soluble depending on the DS, on the protonation ratio and on the pH of the medium.

Experimental methods

Synthesis of CMS(Na)

The CMS(Na) (PR 0%) were prepared in aqueous media from high amylose starch (Hylon VII, *National Starch*, USA) as previously described[1], with some modification. Sodium chloroacetate was used to add (–CH_2–COONa) groups on glucosidic units. Methanol (100%) was used to precipitate the sodium CMS and 80% methanol was used to wash the powders. Different amounts of sodium chloroacetate were used to obtain various degrees of substitution (DS 0.07; 0.09; 0.11 and 0.24).

Modification of protonation ratio

Each sodium CMS (with a specific DS) was divided into several samples that were dispersed in 80% methanol and then the pH of samples were adjusted with hydrochloric acid. Thus, variants protonation ratios (from 0% to 100%) were obtained for each degree of substitution. The samples were then washed with 80% methanol and after that with (methanol 40% / acetone 60%), finally they were dried at 40C°.

Samples were characterized by FTIR, and by back titration to determine the degrees of substitution and the protonation ratios.

Dissolution tests

Monolithic tablets (200 mg) of CMS loaded with 20% of acetaminophen were obtained by direct compression (2.5 T, *Carver* hydraulic press). The *in vitro* dissolution tests were done using a *Distek premiere 5100* dissolution paddle system coupled with an UV *Hewlett Packard* spectrophotometer. The tablets were incubated, up to complete release, in 900 mL of pepsin-free simulated gastric fluid (SGF), or in 900 mL of pancreatin-free simulated intestinal fluid (SIF) following the USP 24 method and the paddle speed was adjusted to 100 rpm.

Results and discussion

Dissolution tests in acid medium and neutral medium provided interesting information on the properties of CMS matrix and on the influence of DS and of PR on drug release.

In SGF, the lower DS (0.07) provides the slower release of acetaminophen (Fig. 1). The release becomes faster when the DS increases and slower with PR increase. It is worth to note that the percentage of acetaminophen released after the first 2h in SGF is relatively similar for all CMS. The longer dissolutions in SGF were done in order to better understanding the influence of DS and PR on the CMS matrix.

In SIF, the middle DS (0.11) provides the slower release of acetaminophen (Fig.2). Like in SGF, higher the protonation ratio is, slower the release of acetaminophen. The time corresponding to the release of 90% of acetaminophen in SIF is highly influenced by DS and protonation ratio.

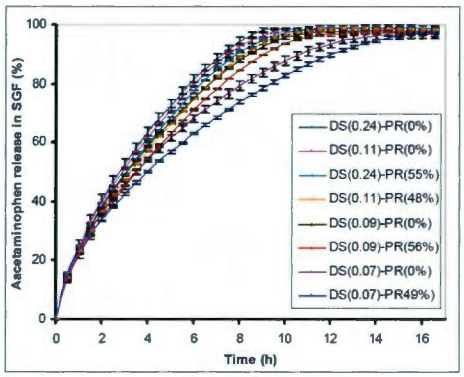

Figure 1: Acetaminophen release from monolithic tablets of CMS in SGF.

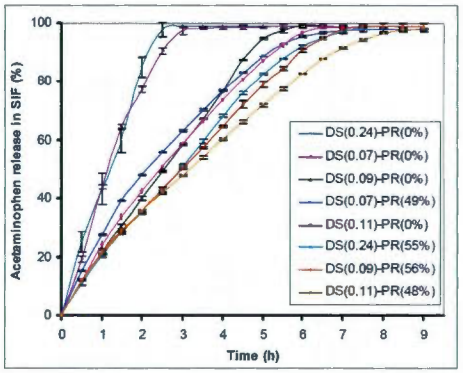

Figure 2: Acetaminophen release from monolithic tablets of CMS in SIF.

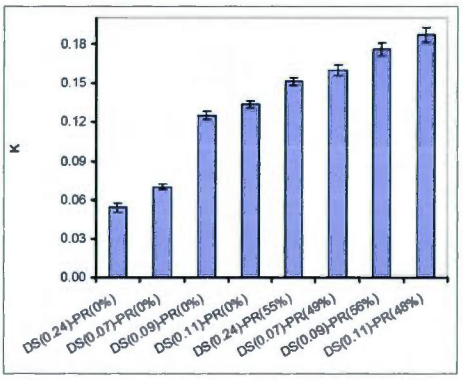

Figure 3: Constant K (time for 90% release in SIF (h) / percentage of release after 2h in GSF (%)) for acetaminophen release from monolithic tablets of CMS.

In SIF the tablets are less compact than in SGF and the dissolution kinetics are almost linear. The faster release was with CMS(Na) that have the lower and the higher DS (0.07 and 0.24). This fast release of acetaminophen by CMS (DS 0.24) is due to its high solubility compared to the others CMS. When protonated, its solubility is reduced and the release is much slower in SIF. In the case of CMS with lower DS (0.07), the fast release is almost due to its inability to generate a hydrogel structure that can control the release of drug. The slower release was with partially protonated CMS that have the medium DS (0.11). A very good release profile and a good stability of the matrix were found with a protonation ratio not more than 50%. The constant K* shows clearly the differences between the matrices (Fig. 3). Partially protonated CMS (DS 0.11) has the higher K value, while the CMS(Na) (DS 0.07 and DS 0.24) have the lowest values. It seems that the release in acid medium is controlled more by diffusion, while the release in neutral medium is controlled more by the solubility of CMS.

The highly protonated CMS provides the best protection in SGF and the slower drug release in SIF. However, we have to mention that the stability of these matrices is not very long and studies are now in course to improve this aspect. The moderately protonated CMS provide a good protection in SGF and a better controlled release than CMS(Na) in SIF. Other DS will be also studied to choose the best CMS matrix for controlled drug release.

In a previous study[2], it was shown that dissolution kinetics are strongly depending on the drug solubility, we are now showing that these dissolutions depend also on the CMS matrix solubility.

* K is the ratio (time for 90% release in SIF (h) / percentage of drug release after 2h in SGF (%))

Conclusions

The degree of substitution and the protonation ratio have an important influence on the properties of the CMS matrix, particularly in the control of the drug release kinetics. An optimum DS can provide a good protection of drug in SGF and a prolonged controlled release in SIF. An optimum protonation ratio can provide a slower drug release compared to the sodium CMS form and can afford a good stability of the matrix with the time.

References

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2. E. Assaad, A Azzouz and M. A. Mateescu, *Transaction of 35th Annual Meeting & Exposition of CRS*, New York, 2008, # 512.

OPTIMAL DEGREE OF SUBSTITUTION AND PROTONATION RATIO FOR CONTROLLED DRUG RELEASE BY CARBOXYMETHYL STARCH SYNTHETISED IN AQUEOUS MEDIUM

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UQÀM Pharmaqam

CRS Canada Local Chapter

ABSTRACT

Carboxymethyl starch (CMS) is a pH sensitive matrix. In monolithic tablets based on CMS excipient, the drug release is influenced by both the pH of the media and the percentage of the protonated carboxymethyl groups. This study shows the influence of the degree of substitution (DS) and of the protonation ratio (PR)* on the properties of the CMS matrix. An optimal DS and an optimal PR were determined for CMS excipient. They can provide a relatively low release in gastric medium and a controlled drug release in intestinal medium. At these optimal DS and PR, CMS presents an amelioration compared to sodium CMS form (CMS(Na)), in terms of drug controlled release. It is concluded in this study that a relatively low DS (0.09-0.14) and a protonation ratio up to 50% are necessary for controlled drug release by CMS excipients.

*PR is the percentage of protonated CMS (H) with -COOH groups compared to the total CMS(H,Na) with -COOH and -COONa groups

OBJECTIVES

1- Evaluate the role of degree of substitution (DS) of carboxymethyl starch (CMS) excipient on drug release rate.

2- Establish the effect of protonation ratio (PR) of CMS on drug dissolution.

3- Determine the effect of dissolution medium (pH) on drug release rate by CMS.

4- Establish the best parameters (DS and PR) of CMS excipient for a low drug release in gastric fluid and a controlled release in intestinal fluid.

MATERIALS & METHODS

Synthesis of CMS(Na) (PR 0%): was carried out in aqueous medium from high amylose starch (Hylon VII, National Starch, USA) as previously described[1], with some modifications: use of sodium chloroacetate (SCA) for substitution and methanol for precipitation and washing of the CMS(Na). Different amounts of (SCA) were used to obtain various degrees of substitution: DS (0.07; 0.09; 0.11; 0.14 and 0.24) or mequ (0.42; 0.55; 0.68; 0.83 and 1.35). Gelatinized starch (GS) was obtained by the same method without adding (SCA).

Modification of protonation ratio: Variants protonation ratios (from 0% to 100%) were obtained for each CMS sample by dispersing samples in 80% methanol and by adjusting the pH with hydrochloric acid. Samples were characterized by FTIR, and by back titration to determine the degrees of substitution and the protonation ratios.

Monolithic tablets preparation: Tablets (200 mg) of CMS loaded with 20% of acetaminophen were obtained by direct compression (2.5 T, *Carver* hydraulic press, 9 mm cylinder outfits).

Dissolution tests: The *in vitro* dissolution tests were done using a *Distek premiere 5100* dissolution paddle system coupled with an UV *Hewlett Packard* spectrophotometer. The tablets were incubated, up to complete release, in 900 mL of pepsin-free simulated gastric fluid (SGF), or in 900 mL of pancreatin-free simulated intestinal fluid (SIF) following the USP 24 method (100 rpm).

Water uptake: Tablets of 200 mg of CMS were incubated 2h in SGF or in SIF in the same conditions as for dissolution tests. The tablets were then measured and weighed before (wet tablets) and after freeze-drying (dried tablets).

EXPERIMENTAL & RESULTS

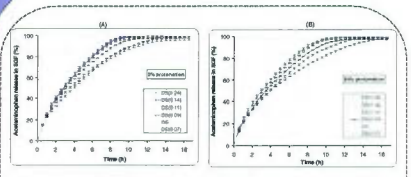

Figure 1: Acetaminophen (20% loading) release in simulated gastric fluid from monolithic tablets of GS and CMS with different DS: (A) sodium form and (B) 50% protonated form

- In SGF, the lower DS(0.07) provides the slower release of acetaminophen. The release becomes faster when the DS increases.
- The acetaminophen release after the first 2h in SGF is almost similar for all matrices.
- The 50% protonated CMS showed slower release than sodium CMS (0% protonation)
- The gelatinized starch (GS) affords a sustained release, but the tablets present capping and cracks (see Fig.5)

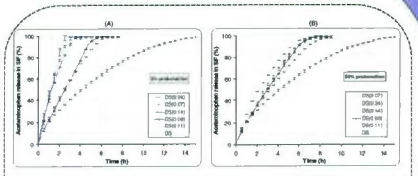

Figure 2: Acetaminophen (20% loading) release in simulated intestinal fluid from monolithic tablets of GS and CMS with different DS: (A) sodium form and (B) 50% protonated form

- In SIF, the tablets were less compact than in SGF (see Fig. 5) and CMS with DS(0.11) provides the slowest release from CMS matrices.
- The highest release rate was from CMS(Na) with the lowest and highest DS (0.07 and 0.24). In the later case, the fast release was due to the highest solubility of excipient. The fast release from CMS with (DS 0.07) seems due to its inability to generate a consistent hydrogel structure that can control the drug release.
- GS presents almost the same release rate in SIF as in SGF
- Compared to CMS(Na), the 50% protonated CMS presented a slower and controlled drug release for all excipients, irrespective of DS.

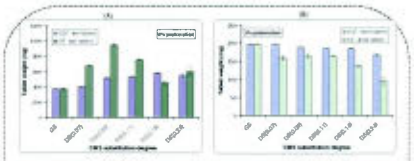

Figure 3: Mass after 2h of monolithic tablets of GS and CMS(Na): (A) wet tablets (B) dried tablets

- Water uptake by CMS tablets is higher than by GS tablets, due to the presence of -COONa/H groups
- The weight of GS tablets is almost the same in SGF and SIF and no mass was lost.
- In SIF, the wet weight increases with DS up to DS(0.09), due to the presence of more -COONa groups. Tablets with DS(0.09) and DS(0.11) have the same dried weight, however those with DS(0.09) have a higher wet weight, probably because the hydrogel generated by DS(0.11) is consistent enough to reduce the water diffusion into tablets. From DS(0.09) to DS(0.24) the weight decreases due to the increased solubility of CMS. These results can explain why the drug release from CMS (DS 0.11) was slower than from other CMS.
- In SGF, increasing DS generates higher water uptake. The weight of dried tablets in SGF decreases of increasing DS in a slower rate than in SIF, due to the low solubility of CMS in acidic medium.

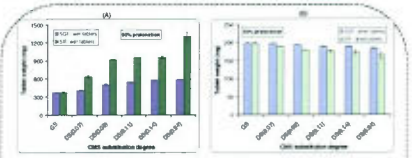

Figure 4: Mass after 2h in SGF or SIF, of monolithic tablets of GS and 50% protonated CMS: (A) wet tablets (B) dried tablets

- Increasing DS generates higher water uptake by 50% protonated CMS, probably due to the higher hydration of -COONa/H groups compared to hydroxyl groups and to the lower solubility of 50% protonated excipients.
- The mass of dried tablets compared to their initial weight (200 mg) suggests a relatively low mass loss (lower than in the case of not protonated excipients).
- Increasing DS leads to higher loss of dried mass in SGF and in SIF.

ASPECT OF MATRICES

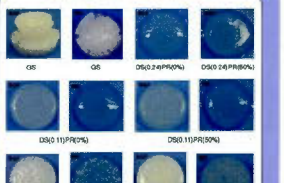

Figure 5: Tablets based on GS and CMS excipients at the end of dissolution tests

CONCLUSIONS

- The degree of substitution and the protonation ratio have an important influence on the properties of the CMS excipient.
- An optimal DS(0.11) found for CMS can reduce drug release in SGF and ensure a controlled release in SIF.
- Moderately protonated CMS excipient can provide a slower drug release compared to the sodium CMS and afford a good storage stability of the matrix with the time.
- A relatively low DS (0.09-0.14) and a protonation ratio up to 50% favor a controlled drug release by CMS excipient. The number of substituted groups must be enough to ensure a formation of gel network, but not too high, in order to maintain a limited solubility of excipient.
- Highly protonated CMS excipients are not stable in time, probably due to the dimerization of carboxyl groups. When protonated at 100%, the CMS excipients allow the best controlled sustained release, but after several weeks of storage, tablets based on 100% protonated CMS disintegrate rapidly in dissolution media.

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Drug Release from Chitosan and Carboxymethyl Starch Matrices

92th Canadian Chemical Conference and Exhibition
92e Congrès et Exposition Canadiens de Chimie

Elias Assaad and Mircea-Alexandru Mateescu

Carboxymethyl Starch (CMS) was already shown that it can be used as excipient for controlled drug release. However, the partial protection afforded by CMS for some drugs can be enhanced when an adequate amount of chitosan is added to the excipient. Chitosans with three different molecular weights, and three tracer drugs (acetaminophen, aspirin and metformin) were studied. When associated in monolithic tablets with CMS, the low molecular weight chitosan accelerates the drug release from matrix, whereas the high molecular weight chitosan (HMWCh) can enhance the acetaminophen protection against acidity. The mixture of an adequate amount of HMWCh with CMS can decrease the release of acetaminophen in gastric medium and make the release in intestinal medium slower, without affecting the linearity of dissolution curve. For aspirin, CMS matrix affords the best protection against acidity and the slowest release in intestinal medium. In this case, addition of chitosan leads to an acceleration of aspirin release. For metformin, which is highly soluble in acidic medium, neither CMS nor chitosan can afford protection against acidity. In conclusion, for drugs with moderate solubility, addition of HMWCh to CMS matrix can be advantageous for protection against acidity and for prolonged release in neutral medium.

DRUG RELEASE FROM CHITOSAN AND CARBOXYMETHYL STARCH MATRICES

UQÀM

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Pharmaqam

ABSTRACT

Carboxymethyl Starch (CMS) was already introduced as excipient for controlled drug release. However, the release of some drugs in simulated gastric medium (SGF) by CMS can be reduced when an adequate amount of chitosan is added to the excipient. Chitosans with three different molecular weights, and three tracer drugs (acetaminophen, aspirin and metformin) were studied. When associated in monolithic tablets with CMS, the low molecular weight chitosan (LMWC) accelerates the drug release from matrices, whereas an adequate amount of the high molecular weight chitosan (HMWC) can decrease the release of acetaminophen in SGF and makes the release in intestinal medium slower, without affecting the linearity of dissolution curve. The presence of enzymes (pepsin and pancreatin) in dissolution media accelerates the release of acetaminophen. For aspirin, CMS matrix affords the lowest release in SGF and the slowest release in simulated intestinal medium (SIF). In this case, addition of chitosan leads to an acceleration of aspirin release. For metformin, highly soluble in acidic medium, neither CMS nor chitosan can afford a limit release in SGF. In conclusion, for drugs with moderate solubility, addition of HMWC to CMS matrix can be advantageous to reduce the release in acidic medium and for sustained release in neutral medium.

OBJECTIVES

1- Reducing the drug release in gastric medium

2- Providing a controlled drug release in intestinal medium

3- Proving the efficiency of excipients based on a mixture of CMS and chitosan

4- Evaluating the influence of chitosan molecular weight on drug release

MATERIALS & METHODS

- CMS at a degree of substitution (DS) 0.16 was prepared from high amylose starch (Hylon VII) and the powder was obtained by precipitation with acetone.
- Natural chitosans (degree of deacethylation 80%) with three different molecular weights (approximately 400 kDa, 600 kDa and 700 kDa) from *Marinard Biotech* were purified by solubilization in acetic acid and filtration. The powders were obtained by precipitation with acetone in acidic medium.
- Excipients based on CMS:chitosan were obtained by homogenising physical mixtures of CMS powder and chitosan powder (0.25, 0.5 and 0.75 mean respectively 25%, 50 % and 75% of excipient w/w) .
- Monolithic tablets (200 mg, 20% loading) were obtained by direct compression (2.5 T, Carver hydraulic press, 9 mm cylinder outfits).
- Dry-coated (DC) tablets of 200 mg with 20 % loading were formulated with a core (40 mg of drug + 40 mg of excipient compressed in 7 mm cylinder outfit) which was then double dry-coated by 120 mg of excipient and compressed in 9 mm cylinder outfit.
- The *in vitro* dissolution tests were performed, according to the USP 24 method, by using a dissolution system (Distek, premiere 5100) coupled with an UV *HP* spectrophotometer. Each tablet was maintained for 2 h in 1 L of simulated gastric fluid (SGF) and then in 1 L of simulated intestinal fluid (SIF) with or without enzymes. The agitation was fixed to 100 rpm.

DISSOLUTION TESTS

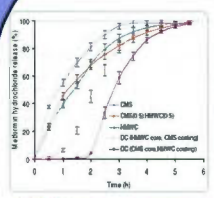

Figure 1. Metformin release from monolithic tablets in SGF and from dry-coated (DC) tablets in SGF (2h) and SIF.

- Neither CMS nor chitosan can limit the release of metformin in SGF.
- Metformin release was very fast in SGF and in SIF due to its high solubility.
- Dry coated tablets reduce metformin release in SGF. However the release in SIF is still fast.

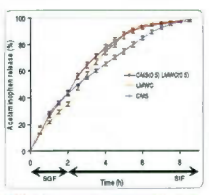

Figure 2. Acetaminophen release from tablets based on CMS and LMWC

- CMS tablets afford a controlled release for acetaminophen and a linear dissolution kinetics.
- LMWC and CMS(0.5):LMWC(0.5) release acetaminophen faster than CMS.
- In acidic medium chitosan make a hydrogel that can reduce the diffusion of acidity into the tablets (see Fig.7a)
- In intestinal medium, LMWC looses the external hydrogel and the release is accelerated.

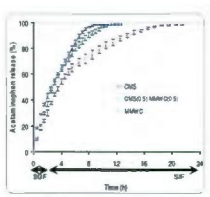

Figure 3. Acetaminophen release from monolithic tablets based on CMS and MMWC

- Release of acetaminophen in SGF is slower with MMWC than that with CMS.
- In SIF the release from MMWC tablets is reduced due to the conversion of hydrogel into a semi-solid form that limits the diffusion of the medium into the tablets.
- Addition of MMWC to CMS reduce the release of acetaminophen in SGF.
- CMS(0.5):MMWC(0.5) shows faster release in SIF than MMWC (CMS accelerates the diffusion of SIF into the tablets).

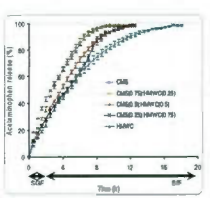

Figure 4. Acetaminophen release from tablets based on CMS and HMWC

- HMWC shows the lowest release in SGF and a sustained release in SIF, but the release becomes very slow after 80% of acetaminophen release.
- CMS(0.5):HMS(0.5) shows a limited release in SGF and a controlled drug release in SIF. HMWC enhances a limited release in SGF and CMS moderately enhances the controlled release in intestinal medium. At the end of the dissolution tests the tablets still contain a mixture of CMS and HMWC (see Fig. 9).

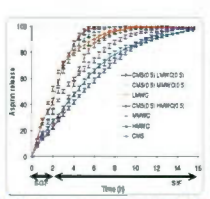

Figure 5. Aspirin release from tablets based on CMS, LMWC, MMWC and HMWC

- CMS and HMWC afford the lowest release in SGF and the best sustained release in SIF for aspirin.
- Higher the molecular weight of chitosan, slower is the aspirin release.
- Adding chitosan to CMS accelerates the release of aspirin. Chitosan seems to reduce the interactions between aspirin and CMS (which accelerates the diffusion of medium into the tablets).

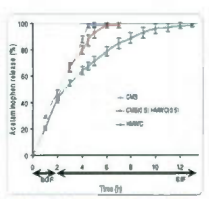

Figure 6. Acetaminophen release from tablets based on CMS and HMWC of different particle sizes

- Pancreatin accelerates the release of acetaminophen from CMS tablets.
- Gastric acidity, pepsin and pancreatin accelerate the release of acetaminophen from HMWC tablets due to the chitosanolysis properties of these enzymes.
- Adding CMS to chitosan accelerates the release in SIF

Abbreviations and symbols: CMS (carboxymethyl starch); LMWC, MMWC, HMWC (respectively low, medium and high molecular weight chitosan); SGF (simulated gastric fluid); SIF (simulated intestinal fluid); DD (degree of deacethylation); DS (degree of substitution); DC (double-coated); CMS(0.5):HMWC(0.5) means that the excipient contain 50% of CMS and 50% of HMWC.

ASPECT OF MATRICES

Figure 7. HMWC after 2h in SGF (a) and at the end of dissolution test in SIF (b)

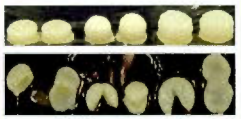

Figure 8. Tablets of chitosan based on CMS(0.5) chitosan(0.5) at the end of dissolution test (top: whole shape, bottom: cross-sections). From left to right: LMWC, MMWC, HMWC

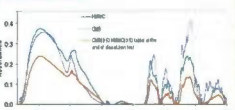

Figure 9. FTIR spectra of CMS and HMWC powders and for CMS:HMWC(0.5) tablet at the end of dissolution test

CONCLUSIONS

- CMS can afford sustained release for acetaminophen and aspirin.
- Release from LMWC tablets is fast: higher the molecular weight of chitosan, slower is the release.
- With acetaminophen: addition of HMWC to CMS matrix can be advantageous to sustained release.
- With aspirin: addition of chitosan to CMS matrix accelerates the release.
- The presence of enzymes (pepsin and pancreatin) accelerates the release in all cases (tablets based on CMS and chitosan)

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2[e] colloque annuel de Pharmaqam, 2010

Formulations à base de carboxyméthyl amidon et de chitosane pour administration de médicaments par voie orale

Le carboxymétyl amidon (CMA) a été utilisé, individuellement ou en association avec des chitosanes de différentes masses moléculaires, comme excipient dans la préparation des comprimés monolithiques. Des agents actifs de différentes charges et solubilités (acétaminophène, metformine et aspirine) ont été formulés. Afin d'assurer une libération prolongée dans un milieu intestinal, l'ajout de chitosane dans la formulation à base de CMA pourrait être bénéfique. Ainsi, l'interaction entre un polymère polyanionique (CMA) et un polymère polycationique (chitosane) favorise la stabilisation de la matrice. De plus, le chitosane (insoluble dans un milieu neutre) peut réduire la solubilité de la matrice dans un milieu intestinal et peut favoriser le transport d'un agent actif jusqu'au côlon. L'ajout de chitosane de faible masse moléculaire dans des formulations à base de CMA a entraîné une accélération de la libération du médicament. Par contre, l'ajout d'une quantité adéquate de chitosane de masse moléculaire appropriée ($\geq$ 700 kDa) a entraîné une diminution de la vitesse de libération de l'acétaminophène. Pour l'aspirine, le CMA a montré une libération prolongée qui a été accélérée lorsque du chitosane a été ajouté à la formulation. Cette accélération est probablement due à la réduction des interactions CMA-aspirine. La solubilité des agents actifs avait un rôle majeur dans la vitesse de libération. Ainsi, la libération était plus vite pour la metformine que pour l'acétaminophène et pour l'aspirine. Des analyses (diffraction de rayon-X, imagerie RMN etc.) ont été effectuées, afin d'étudier les propriétés des excipients, le mécanisme de diffusion de l'eau dans les comprimés et le taux de gonflement des comprimés.

CARBOXYMETHYL STARCH AND CHITOSAN AS COEXCIPIENTS FOR ORAL DRUG DELIVERY

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ABSTRACT

The blends of CMS and chitosan (dry powder mixture) were proposed to improve the properties of each of these two polymeric excipients for controlled drug release by oral solid dosage forms. The dissolution of three drug tracers (acetaminophen, metformin and aspirin) under conditions simulating the gastrointestinal transit showed release rates highly dependent on drug solubility in dissolution medium. Higher the molecular weight of chitosan (Ch1: 400 kDa, Ch2: 700 kDa), slower is the drug release. The low hydration of chitosan and its insolubility in neutral medium can be a limitation for drug delivery by this matrix. Adding chitosan of proper molecular weight and in adequate amount to the formulation based on CMS favored a lower release rate of acetaminophen. The release of metformin was fast irrespective of the excipient used in the formulations. The sustained release of aspirin provided by CMS matrix was accelerated when chitosan was added as a coexcipient.

OBJECTIVES

- Reducing the drug release in gastric medium and providing a controlled drug release in intestinal medium.
- Compare the dissolution profiles of drugs with different charges and solubilities.
- Comparing the drug dissolution from tablets based on anionic water-soluble excipient (CMS), on cationic water-insoluble excipient (chitosan), or on physical mixture powder of these two excipients.
- Evaluating the influence of chitosan molecular weight on drug release rate.

MATERIALS & METHODS

- CMS at a degree of substitution (DS) 0.14 was prepared from high amylose corn starch (Hylon VII) in aqueous medium.
- Natural chitosans (degree of deacethylation 80%) with two different molecular weights (Ch1, 400 kDa and Ch2, 700 kDa) from *Marinard Biotech* were purified by solubilization, filtration and precipitation
- The Excipients 50% CMS : 50% chitosan were obtained by homogenising physical mixtures of CMS powder and of chitosan powder (50%/50%, w/w)
- Monolithic tablets (200 mg, 20% loading) were obtained by direct compression (2.5 T, Carver hydraulic press, 9 mm cylinder outfits)
- Dry-coated (DC) tablets of 200 mg with 20 % loading were formulated with a core (40 mg of drug + 40 mg of excipient compressed in 7 mm cylinder outfit) which was then double dry-coated by 120 mg of excipient and compressed in 9 mm cylinder outfit.
- The *in vitro* dissolution tests were carried out at 100 rpm and 37°C in an USP dissolution apparatus II (Distek 5100) coupled with an UV spectrophotometer (Hewlett Packard 8452A). The tablets (n = 3) were incubated in SGF (1 L) for 2 h and then in SIF (1 L) up to complete release.
- SEM (Hitachi S-4300SE/N (VP-SEM)), FTIR (*Nicolet* 4700, Madison, WI, USA), NMR spectrometer (Avance-400, Germany) and other techniques were used for characterization.

MORPHOLOGY OF THE SAMPLES

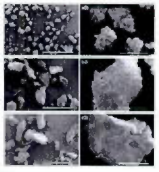
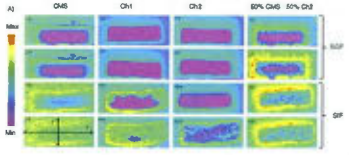
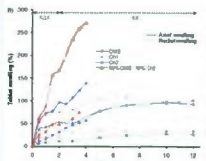


Fig. 1 Scanning electron microscopy micrographs of (a) CMS, (b) Ch1 and (c) Ch2. Magnification of 100x and 500x and voltage of 15 kV

- CMS: porous particles
- Chitosans: compact particles

NMR IMAGING TESTS





- After 2 h in SGF, the CMS tablets still showed a dry core (purple).
- The diffusion of (SGF or SIF) for chitosan tablets was slower than for CMS tablets.
- In SIF, the size of chitosan tablets was stabilized due to chitosan insolubility in neutral medium.
- For Ch1, the core was almost completely hydrated after 8 h of incubation, whereas for Ch2 with higher molecular weight the core still showed dry regions even after 20 h.
- Axial swelling was higher than radial swelling due to the formation of flat particles in tablet after axial compression
- Tablets of 50% CMS : 50% Ch2 showed the fastest fluid diffusion and the highest swelling.

Abbreviations. CMS, carboxymethyl starch; Ch, chitosan; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; DDA, degree of deacetylation; DS, degree of substitution; DC, double-coated, 50% CMS : 50% Ch, an excipient containing 50% (w/w) of CMS and 50% (w/w) of Ch; t90%, time (h) for the release of 90% of drug.

DISSOLUTION TESTS

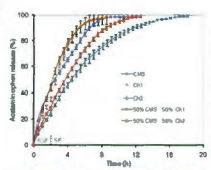


Fig. 3 Kinetics of acetaminophen dissolution from tablets 200 mg 20% loading of CMS, Ch1, Ch2, 50% CMS-Ch1, and 50% CMS-Ch2

Acetaminophen release

- Ch1 and 50% CMS : 50% Ch1 matrices showed higher rate release than CMS, while Ch2 and 50% CMS : 50% Ch2 matrices showed lower rate.
- The higher the molecular weight of chitosan, the lower was the release rate.
- A molecular weight higher than 400 kDa and an adequate ratio in dry blends are required for chitosan to favor a longer drug release time.
- CMS favors the tablet hydration and accelerates the diffusion of SIF into the CMS : chitosan tablets.

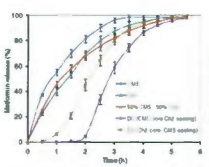


Fig. 4 Kinetics of metformin dissolution from tablets 200 mg, 20% loading of CMS, Ch2, 50% CMS-Ch2, and DC (CMS core-Ch2 coating)

Metformin release

- Neither CMS nor chitosans, separately or in association, were able to control the release of metformin in monolithic dosage form due to its high solubility.
- Dry coated (DC) tablets can delay release in SGF, especially when the outer part of tablet is based on Ch2.

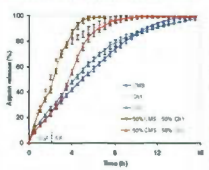


Fig. 5 Kinetics of aspirin dissolution from tablets 200 mg, 20% loading of CMS, Ch1, Ch2, 50% CMS-Ch1, and 50% CMS-Ch2

Aspirin release

- CMS and Ch2 provided low release of aspirin in SGF and long sustained release in SIF due to the interactions with carboxyl groups of aspirin.
- The dissolution of aspirin from Ch2 matrix after 80% of release was remarkably reduced.
- CMS : chitosan tablets showed an accelerated release of aspirin compared to those based on CMS or chitosan only. Maybe this is due to reduction of interactions between these two polymers.

Acknowledgements for financial support

MITACS Accelerate FQRNT

ASPECT OF MATRICES

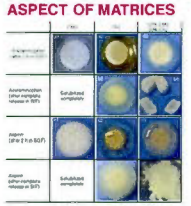
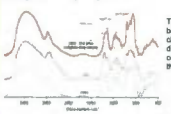


Fig. 6 Photographs of CMS, Ch2 and 50% CMS : 50% Ch2 tablets (200 mg, 20% loading) during dissolution tests (1 L, 37 C, 100 rpm)



The crust of tablets based on CMS : chitosan after complete drug release still containing a mixture of these two polymers

Fig. 7 FTIR spectra of (a) CMS, Ch2 and 50% CMS : 50% Ch2 Pellets prepared with polymer powder and KBr

CONCLUSIONS

- CMS and Ch2 matrices showed a fast release of metformin, a controlled release of acetaminophen and a sustained release of aspirin.
- Drug solubility has a major influence on release rate irrespective of the charge of drug and of excipient.
- The low hydration and the insolubility in neutral medium can be a limitation for drug delivery by chitosan alone.
- With acetaminophen: using Ch2 as coexcipient with CMS can be advantageous to sustained release.
- With aspirin: using Ch2 as coexcipient with CMS accelerates the release.
- Physical or chemical interactions can occur between CMS and chitosan during the dissolution.

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78e Congrès de L'Association Francophone pour le Savoir (Acfas)

Effet du ratio de protonation et du degré de substitution sur les propriétés du carboxyméthyl amidon utilisé comme excipient

Le carboxyméthyl amidon (CMA), sous sa forme sodique, est considéré comme un excipient sensible au pH du milieu. La cinétique de libération des médicaments à partir des formulations à base de CMA pourrait donc être dépendante du pH du tractus gastro-intestinal. La présente étude montre que cette sensibilité au pH pourrait être modulée en fonction du degré de substitution (DS: 0,07-0,20) et du ratio de protonation (RP: 0-100 %) où RP est le rapport (%) du nombre de groupements carboxyles protonés (-COOH) sur le nombre total de groupements carboxyliques. Les propriétés de l'excipient synthétisé en milieu aqueux, et le mécanisme de libération de l'acétaminophène à partir des comprimés monolithiques dans un milieu gastrique simulé (MGS, pH 1,2) et dans un milieu intestinal simulé (MIS, pH 6,8) ont été investigués. Il a été trouvé que la libération du médicament devient plus lente avec l'augmentation du RP. Cependant, en conditions de conservation prolongée la forme totalement protonée a montré un certain réarrangement structural et une réduction de solubilité. Le CMA (DS 0.11) avec une protonation partielle a montré une libération prolongée légèrement dépendante du pH et de la charge en acétaminophène (20 % et 40 %). Différemment, le CMA (DS 0.20) a montré une grande sensibilité au pH du milieu de dissolution. Dans un MGS, un mécanisme de libération par diffusion a été suggéré, tandis que dans un MIS, la libération a été plutôt contrôlée par le gonflement et par l'érosion des comprimés.

Acfas 2011

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79e Congrès de L'Association Francophone pour le Savoir (Acfas)

Carboxyméthyl amidon et chitosane comme excipients pour le transport d'agents bioactifs par des comprimés administrés par voie orale

Le carboxyméthyl amidon (CMA) et le chitosane ont été testés comme des vecteurs des petites molécules (acétaminophène, aspirine, metformine) et d'une protéine (ovalbumine). Les cinétiques de dissolution dans des conditions simulant le passage gastro-intestinal des comprimés ont montré une dépendance des propriétés du polymère utilisé (CMA, polymère anionique ; chitosane, polymère cationique) et du pH du milieu de dissolution. Une libération limitée et une bonne protection contre l'acidité et la digestion enzymatique ont été observées dans un fluide gastrique simulé (pH 1,2) lorsque chacun des polymères a été utilisé individuellement pour formuler l'agent actif. La vitesse de libération dans un fluide intestinal simulé (pH 6,8) a été modulée par la solubilité de l'agent actif et de l'excipient, et par la sensibilité de l'excipient à la digestion par les enzymes pancréatiques. L'ajout d'une quantité adéquate de chitosane (coexcipient) de masse moléculaire moyenne à la formulation à base de CMA a entrainé une libération plus prolongée avec l'acétaminophène et avec l'ovalbumine, et une accélération de la libération avec l'aspirine. La metformine a montré une libération accélérée à partir des comprimés monolithiques, mais une libération retardée a été observée avec des comprimés à double noyau. Les excipients et les comprimés ont été caractérisés (diffraction RX, imagerie RMN, microscopie, etc.), et les propriétés des formulations et les mécanismes de libération ont été étudiés.

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3e colloque annuel de Pharmaqam, 2011

Complexe carboxyméthyl amidon-chitosane comme nouvel excipient pour le transport des substances actives : Analyse par imagerie RMN

Un nouveau complexe polyélectrolyte (CPE) de carboxyméthyl amidon (CMA) et de chitosane a été préparé, caractérisé et testé *in vitro* comme matrice pour le transport des substances actives par voie orale. Ce complexe a été obtenu par un simple mélange des solutions aqueuses du carboxyméthyl amidon (polymère polyanionique) et du chitosane (polymère polycationique). Les interactions ioniques entre les deux polymères et la précipitation du complexe ont été immédiates. Les spectres de rayons-X ont montré que le CPE était plus amorphe que le CMA et le chitosane. Le CPE contenait 10-15% de chitosane et il était moins soluble dans le fluide intestinal simulé que le CMA. De plus, l'analyse par imagerie RMN a montré que la diffusion du fluide intestinal simulé dans les comprimés du CPE a été plus lente que dans les comprimés du CMA. Ceci a permis une libération plus prolongée des substances actives (acétaminophène, aspirine) avec les matrices de CPE qu'avec les matrices de CMA. Cette libération prolongée permet le transport des substances actives jusqu'au côlon. Le potentiel du complexe CMA-chitosane est que la méthode de préparation est facile, ne nécessitant pas l'utilisation des réactifs chimiques. De plus, le chitosane a été utilisé sous sa forme native.

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Complexe carboxyméthyl amidon-chitosane comme nouvel excipient : Cinétiques d'hydratation analysées par imagerie RMN

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Hypothèse(s) : Les interactions ioniques ou par ponts d'hydrogène entre un polymère anionique, carboxyméthyl amidon (CMA), et un polymère cationique, chitosane, favorisent la stabilisation de la matrice. Ceci pourrait réduire la solubilité de la matrice et permettrait une libération prolongée des substances actives.

Méthode(s) : Le CMA a été obtenu par la réaction de l'amidon avec du monochloroacétate de sodium en milieu basique. Le complexe polyélectrolyte CMA-chitosane a été préparé par précipitation du CMA avec le chitosane. Les comprimés monolithiques ont été obtenus par compression directe des poudres. La dissolution des substances actives dans des milieux physiologiques simulés a été suivie avec un appareil de dissolution (USP, apparatus II, paddle). La diffusion des milieux physiologiques simulés dans des comprimés non chargés et les taux de gonflement des comprimés ont été analysés par imagerie RMN.

Résultat(s) : La diffraction de rayon-X a montré que le complexe a une structure plus amorphe que celles du CMA et du chitosane. Les tests physicochimiques ont montré que le complexe présente des propriétés différentes de celles des produits du départ. L'analyse par imagerie RMN a montré que la diffusion du fluide gastrique simulé (FGS, pH 1,2) et du fluide intestinal simulé (FIS, pH 6,8) dans les comprimés dépend du pH du milieu, du caractère ionique du polymère, ainsi que de l'interaction entre le CMA et le chitosane. La libération de l'acétaminophène et de l'aspirine à partir des comprimés a été plus prolongée avec le complexe qu'avec le CMA.

Conclusion(s) : Le CMA utilisé comme excipient dans des comprimés peut assurer une libération contrôlée des substances actives. L'interaction du CMA avec le chitosane amène à la formation du complexe qui a des propriétés différentes de ceux des produits du départ. Le complexe CMA-chitosane semble favorable pour assurer une libération prolongée convenable pour le transport des substances actives jusqu'au côlon.

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The influence of protonation ratio on properties of carboxymethyl starch excipient at various substitution degrees: Structural insights and drug release kinetics

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ABSTRACT

Carboxymethyl starch (CMS) with pH sensitivity modulated by the protonation ratio (PR 0–100%) and the degree of substitution (DS 0.07–0.20) was synthesized in aqueous medium. The properties of CMS excipient and the mechanism of acetaminophen release from monolithic tablets in simulated gastric fluid (SGF, pH 1.2) and in simulated intestinal fluid (SIF, pH 6.8) were investigated. Compared to sodium CMS, the protonated CMS provided a longer release time which increases with the increase of PR. Over storage time, the highly protonated CMS showed a decrease in solubility and a progressive structural alteration due to hydrogen bonded carboxyl groups. Simultaneously, an acceleration of release rate of formulated drug was observed. The CMS(DS 0.11) with PR up to 50% showed relatively low sensitivity to dissolution medium pH and sustained release pattern almost independent of tablet preincubation in SGF and of drug loading (20% and 40%). The CMS(DS 0.20) was more sensitive to pH and showed an accelerated release rate in SIF. For the CMS formulations, a diffusion mechanism was suggested in SGF, whereas in SIF the release was mostly controlled by swelling and erosion.

1. Introduction

Despite the advent of synthetic biodegradable polymers, the natural biodegradable polymers continue to be a predilected area in the field of excipients for oral drug delivery. One of the most available, renewable and versatile natural polymers is starch which is composed of two polysaccharides: amylose (unbranched-chain structure with glucose units joined by α-1,4 linkages) and amylopectin (branched-chain structure with glucose sequences joined at branching points by α-1,6 linkages). Starch is susceptible to physical, enzymatic and chemical modifications that can modulate its properties according to intended applications. For instance, chemical modifications of starch involve esterification, etherification or oxidation of the hydroxyl groups of glucose units in aqueous or organic medium. Although the latter procedure allows a higher substitution, salts and modifying reagent by-products can still remain in the final material (Chiu and Solarek, 2009).

Carboxymethyl high amylose starch (CM-HAS) was proposed (Mulhbacher et al., 2004b) as a novel pH sensitive excipient for bioactive agents delivery (Calinescu et al., 2005; Brouillet et al., 2008). Carboxymethyl starch is not a new product. Indeed, it was known in reticulated form and mostly used as disintegrant (e.g. Primojel®, Explotab®) (Edge et al., 2002). In acid (gastric) fluid the carboxyl groups seem to enhance the stability of tablet by dimerization and by hydrogen bonds. When tablet is transferred in neutral (intestinal) fluid, the matrix becomes more hydrated due to the exchange of protons with cations. This hydration facilitates the swelling of the matrix and the release of bioactive agent (Mulhbacher et al., 2004a; Calinescu et al., 2007). Increasing degree of substitution (DS 0.03–0.25) of CM-HAS synthesized in aqueous medium generates a longer drug release time (Lemieux et al., 2007).

Three main processes were identified in tablets based on swellable polymers: swelling, diffusion and erosion. These processes correspond respectively to fronts generated at the interface between the dry central core and the hydrated gel region, at the locus where the drug concentration forms a maximal gradient, and at the contact region between tablet and dissolution medium (Colombo et al., 1999; Barba et al., 2009).

It was shown that a protonated form of carboxymethyl starch presented a better gastro-resistance than its sodium salt form (Ispas-Szabo et al., 2007). However, no investigations were carried out on the influence of percentage of protonation at different degrees of substitution. Recently, it was reported that the sodium

Abbreviations: CMS, carboxymethyl starch; SGF, simulated gastric fluid (pH 1.2); SIF, simulated intestinal fluid (pH 6.8); DS, degree of substitution (the average number of carboxymethyl groups per glucose unit); PR, protonation ratio as percentage of carboxyl groups (–COOH) in the sum of carboxylate (–COONa) and carboxyl groups; $S_{control}$, starch treated with NaOH to obtain the control of CMS samples; S_g, gelatinized starch; $t_{90\%}$, time (h) for the release of 90% of drug.

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salt form of CM-HAS synthesized in non-aqueous medium is preferred for controlled release, whereas the protonated form presented a fast release (Lemieux et al., 2009).

Considering the effect of carboxyl/carboxylate groups on matrix stabilization, on water uptake, on swelling and gel forming, and on tablets erosion, it is now of interest to elucidate the role of protonation/deprotonation of carboxymethyl starch (CMS) in drug delivery. Hence, the aims of the present study on CMS synthesized in aqueous medium are (i) to evaluate the effect of substitution and of protonation degrees on CMS properties, (ii) to investigate the effect of pH of dissolution medium on the mechanism and the rate of drug release by CMS, and (iii) to establish the advantageous parameters, protonation ratio (PR) and degree of substitution, for the desired rate or mechanism of drug release from CMS formulations.

To our knowledge, this is the first investigation on the effect of protonation degree on the properties of CMS excipient and the first detailed study on the effect of DS on kinetic drug release by CMS synthesized in aqueous medium. The comprehension of the role of PR and DS will facilitate the obtention of formulations permitting better drug delivery at various sites of intestinal tract.

2. Materials and methods

2.1. Reagents and chemicals

High Amylose Corn Starch (Hylon VII) was provided by National Starch (Bridgewater, NJ, USA). Sodium chloroacetate (SCA, 98%) and acetaminophen were from Sigma–Aldrich (St-Louis, MO, USA). The other chemicals were of reagent grade and used without further purification. Pepsin-free simulated gastric fluid (SGF, pH 1.2) and pancreatin-free simulated intestinal fluid (SIF, pH 6.8) were prepared following the USP (US Pharmacopeia, XXIV, 2000).

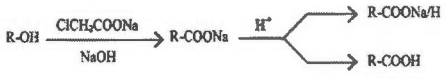
2.2. Synthesis of sodium CMS

Sodium carboxymethyl starch [CMS(Na) or CMS(PR 0%)] was prepared in aqueous medium from high amylose corn starch. A jacketed beaker (2 L) and a heating circulator bath (*HAAKE*, D1, Berlin, Germany) were used to ensure a constant temperature (55 °C) during the synthesis. An amount of 140 g of starch was dispersed in 340 mL of distilled water under continuous vertical stirring (*Servodyne* Mixer, 50000-40, IL, USA). Then, 470 mL of 1.5 M NaOH were added and the stirring was maintained (30 min) for gelatinization. The carboxymethylation (nucleophilic substitution) occurred by adding sodium chloroacetate to the alkaline (0.9 M NaOH) mixture. After 1 h of reaction, a volume of 540 mL of distilled water was added, the mixture was cooled-down, and the reaction was stopped by neutralization with acetic acid. The synthesized CMS(Na) was precipitated with methanol and washed repeatedly with 2 L of 80% methanol until a conductivity of about 50 μS/cm was reached. CMS(Na) was then washed with methanol 40%/acetone 60%, dried at 40 °C for 24 h, and sieved on a 300 μm screen. Different amounts (70, 78, 84, 112 and 208 g) of SCA were used in similar conditions to obtain CMS(Na) at various degrees of substitution. Except CMS with DS(0.20), the CMS presented small granulometry and no grinding was necessary.

The control ($S_{control}$) was prepared following the same procedure as for CMS preparation, but without adding SCA. To prepare gelatinized starch (S_g), 10 M NaOH was added until a final concentration of about 2 M in the mixture.

2.3. Preparation of CMS with different protonation ratios

Each sample of CMS(Na) with a specific DS was dispersed in 80% methanol and the pH was then adjusted with acid solutions in order to obtain derivatives with various protonation ratios (up to 100%), as follows:



(1)

The PR(100%) or CMS(H) was obtained by maintaining the pH at 1.5 for 1 h using 10% HCl, whereas the other PR were obtained by adjusting the pH at 5.8, 5.2, 4.1, 3.9 and 3.6 with (4% acetic acid + 8% HCl) solution maintaining overnight agitation. The samples were filtered and the wet slurries were each washed with 400 mL of 80% methanol and then with (methanol 40%/acetone 60%) as mentioned so far. Finally, samples were air-dried overnight at 40 °C and sieved to retain particles smaller than 300 μm. The $S_{control}$ and the S_g were treated in the same way as CMS(Na) in order to obtain $S_{control}$ (PR 50%), $S_{control}$ (PR 100%), S_g (PR 50%) and S_g (PR 100%). All the samples used in this study were kept under the same conditions in screw top containers stored at room temperature.

Conversion of CMS(PR 100%) to sodium salt form was done by solubilization of 40 mg of polymer in 2 mL of 0.1 M NaOH under overnight agitation. Then 20 mg of NaCl were added and solubilized by mixing for 2 min. Finally, the CMS was precipitated and washed as described so far, then dried at room temperature for 2 days.

2.4. Characterization of unmodified and modified starch samples

2.4.1. FTIR analysis

The carboxymethylation of starch and the protonation status of CMS were assessed by Fourier Transform Infrared (FTIR) spectroscopy (*Nicolet* 4700, Madison, WI, USA). The spectra were recorded from 4000 to 400 cm^{-1} at 2 cm^{-1} resolution and with a total of 32 scans. The pellets were made with a homogenous mixture of dried KBr (67 mg) and polymer sample (3 mg). The compression at 3 tonnes was done in flat-faced punches with 12 mm diameter by using a hydraulic *Carver* press (Wabash, IN, USA).

2.4.2. DS, PR and pK_a

Pure CMS(PR 100%), washed until a conductivity of less than 15 μS/cm, was used to determine the DS by back-titration. An amount of 300 mg of CMS(PR 100%) was solubilized in 20 mL of 0.05 M NaOH ($n = 3$). Subsequently, the excess of NaOH was determined by titration with 0.05 M HCl using phenolphthalein as indicator. Blank ($n = 3$) consisting of 20 mL of 0.05 M NaOH was also titrated. The amount of –COOH groups and the DS were calculated as described by Stojanovic et al. (2005) using the following equations:

$$n_{COOH} = (V_b - V) \times C_{HCl} \quad (2)$$

$$DS = \frac{162 \times n_{COOH}}{m - 58 \times n_{COOH}} \quad (3)$$

where V_b (mL) is the volume of HCl used for the titration of the blank; V (mL) is the volume of HCl used for titration of the sample; C_{HCl} (mol/L) is the concentration of HCl; 162 (g/mol) is the molar mass of glucose unit; 58 (g/mol) is the increase in the mass of glucose unit by substitution with one carboxymethyl group, and m (g) is the mass of dry sample.

The protonation ratios (PRs) were determined by titration of samples by the same method as for DS. The pK_a were determined by direct titration ($n = 2$) as follows: an amount of 300 mg of CMS(PR 100%) was dispersed in 150 mL of distilled water, and titrated until pH 11 with 0.1 M NaOH. Finally, pH was plotted against the volume of added NaOH to obtain the pK_a value at half-equivalence point.

2.4.3. Tapped density, solubility and polymorphism of powders

The tapped densities of the samples were measured according to the USP ⟨616⟩ method by using *Varian*'s Vankel tapped density tester (NC, USA). The solubilities were determined by dispersing 150 mg of polymer powders in 6 mL of SGF or of SIF and stirring (*Thermolyne*, 37600 Mixer, Dubuque, IA, USA) at full speed for 3 min and 3 times to ensure that all the soluble fractions were solubilized. The obtained mixture was centrifuged (20 min, 4000 rpm) at room temperature, and then 1.5 mL of supernatant were evaporated at 65 °C until a constant mass was reached. Controls were prepared similarly with SGF or SIF only (without polymer). The weights of dried samples and of controls were used to calculate the solubilities.

The polymorphism of samples was evaluated by X-ray diffractometer (XRD, *Siemens* D5000, Munich, Germany) at 1.789 Å wavelength. The original XRD spectra, recorded between 5° and 50° (2-theta), were treated using Excel software (regression type: moving average, period 10).

2.5. Tablet preparation and crushing strength measurement

Monolithic tablets [200 mg, 20% or 40% (w/w) loading] were obtained by direct compression of a homogenous mixture of excipient and acetaminophen powders (flat-faced punches with 9.6 mm diameter, 2.5 tonnes, *Carver* hydraulic press). Unloaded tablets of 200 mg were prepared with excipient only and without acetaminophen. The monolithic tablets of 500 mg with 20% and 40% acetaminophen loading were prepared by the same way using punches of 12 mm diameter. The crushing strength ($n=6$) was measured with a Tablet Hardness Tester (*Varian*, VK 200, Cary, NC, USA).

2.6. In vitro dissolution tests

The *in vitro* dissolution tests were carried out at 100 rpm and 37 °C using an USP dissolution apparatus II (*Distek* 5100, North Brunswick, NJ, USA) coupled with an UV spectrophotometer (*Hewlett Packard* 8452A). The acetaminophen release from tablets ($n=3$) in 900 mL of enzymes-free dissolution media was measured at 244 nm. The dissolution was followed: (i) in SGF until complete drug release, (ii) in SIF until complete drug release, and (iii) in SGF for 2 h and then in SIF up to complete release.

The drug release kinetics was evaluated following the equation described by Peppas (1985):

$$\frac{M_t}{M_\infty} = kt^n \tag{4}$$

where M_t/M_∞ is the fraction of drug released at the time t and k is a kinetic constant incorporating the properties of the matrix, the properties of the drug, and the geometric characteristics of the dosage form. The release exponent (n) is characteristic of the drug release mechanism, where $n=0.45$ suggests a diffusion-controlled release (Fickian diffusion), $n=0.89$ a swelling-controlled release, and n between 0.45 and 0.89 indicates an anomalous diffusion (Wei et al., 2009). The $\log(M_t/M_\infty)$ was plotted against $\log(t)$ up to 90% of release to obtain the values of n, k and the correlation coefficient (R^2).

2.7. Erosion and fluid uptake

Erosion and fluid (SGF or SIF) uptake by the unloaded tablets were evaluated in the same conditions (100 rpm, 37 °C) as for dissolution tests. The tablets (200 mg) were incubated for 2 h in SGF or in SIF. They were then removed from the media, blotted with tissue paper to eliminate the water excess on surface, and weighed before (wet tablets) and after freeze-drying (dried tablets).

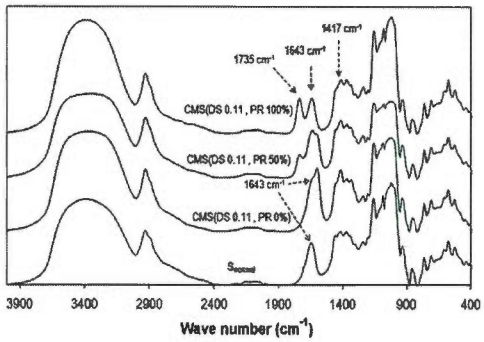


Fig. 1. FTIR spectra of $S_{control}$ and of CMS(DS 0.11) at various protonation ratios (0%, 50% and 100%). Pellets (12 mm diameter) of KBr (67 mg) and samples (3 mg) mixture obtained by compression at 3 tonnes.

The percentage of erosion and the percentage of fluid uptake by unit of remaining polymer were calculated as described per Kavanagh and Corrigan (2004) and Calinescu et al. (2007):

$$\%\text{Erosion} = \frac{(W_i - W_d)}{W_i} \times 100 \tag{5}$$

$$\%\text{Fluid uptake} = \frac{(W_w - W_d)}{W_d} \times 100 \tag{6}$$

where W_i is the initial weight of the tablet, W_w is the weight of wet tablet and W_d is the weight of dried tablet.

3. Results and discussion

The properties of the obtained CMS materials were studied and their efficiencies as excipients for acetaminophen controlled release from monolithic tablets were evaluated. Unless other indications are present, the tablets are of 200 mg with 20% loading.

3.1. Characterization of CMS (carboxymethylation, DS, PR, pK_a)

The FTIR analysis revealed no difference between the spectrum of native starch (Hylon VII) and those of starch after gelatinization ($S_{control}$ and S_g), whereas carboxymethylation and protonation of CMS generated some characteristic bands. The patterns of $S_{control}$ as an example of unsubstituted starch, and that of CMS(DS 0.11) as an example of carboxymethylated starch are presented in Fig. 1. The CMS(PR 0%) presents two new characteristic bands, one at 1417 cm^{-1} and one at 1603 cm^{-1} which overlaps that at 1643 cm^{-1}. For totally protonated form (PR 100%), no band was seen at 1603 cm^{-1}, while a new band appeared at 1735 cm^{-1}. The bands at 1417 and 1603 cm^{-1} were attributed to symmetrical and asymmetrical stretching vibration of $-COO^-$ groups, whereas those at 1643, and 1735 cm^{-1} were assigned to –OH groups and –COOH groups, respectively (Yang, 1991; Silverstein et al., 2005; Zoldakova et al., 2005). The CMS(PR 50%) presented the bands corresponding to $-COO^-$ groups and to –COOH groups, but with lesser intensity compared to those of CMS(PR 0%) and CMS(PR 100%), respectively (Fig. 1). The FTIR patterns confirmed that CMS(PR 0%) was under carboxylate form and that CMS(PR 100%) presented no more carboxylate form (as proof of total protonation), whereas CMS(PR 50%) presented both groups.

The DS of CMS samples determined by back-titration were 0.07, 0.09, 0.11, 0.14 and 0.20. These values correspond respectively to 0.42, 0.55, 0.68, 0.83 and 1.15 milliequivalents of functional groups per gram of polymeric powder (meq./g). The determination of pK_a (4.8–5.0) was done in order to facilitate the choice of pH interval for

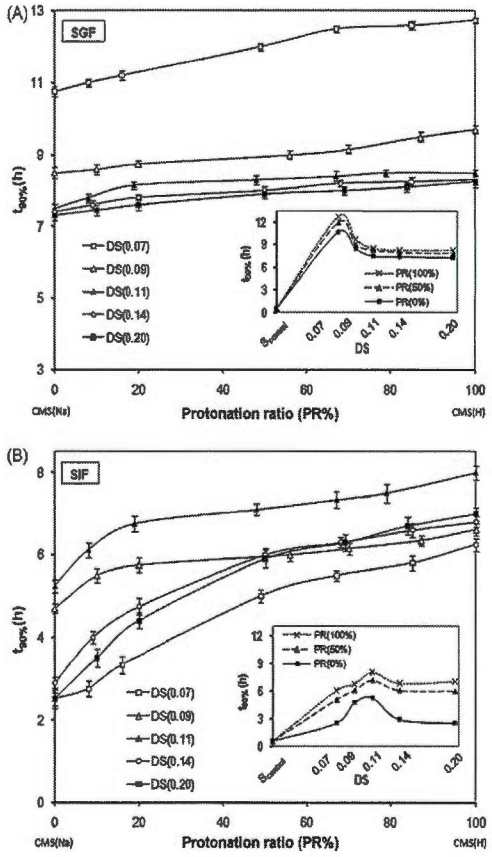


Fig. 2. Time of acetaminophen release ($t_{90\%}$) from CMS at various DS in (A) SGF and (B) SIF. Tablets of 200 mg (20% loading) were used for the dissolution tests (900 mL, 37 °C, 100 rpm).

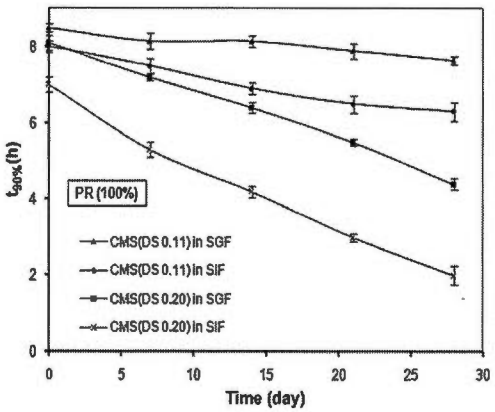


Fig. 3. Time of acetaminophen release ($t_{90\%}$) from totally protonated CMS(DS 0.11 and 0.20) over storage times. Tablets of 200 mg (20% loading) were used for the dissolution tests (900 mL, 37 °C, 100 rpm) in SGF and in SIF.

protonation. Thus, the pH values were adjusted at approximately 5.8, 5.2, 4.1, 3.9, 3.6 and 1.5 to obtain PR of about 10%, 20%, 50%, 70%, 85% and 100%, respectively.

3.2. Effect of DS and of PR on drug release time

The times (h) for the release of 90% of drug ($t_{90\%}$) were obtained from dissolution kinetics of acetaminophen release in SGF or in SIF (Fig. 2). For all CMS variants, the release was longer in SGF than in SIF. Also, for each CMS with a specific DS, the $t_{90\%}$ increased with protonation ratio in both SGF and SIF and the major increase occurred at PR lesser than 50%. In addition, fast release and disintegration (less than 30 min) were seen in all cases for $S_{control}$ (Fig. 2 – insert). The explanation of this disintegration may be that the temperature (40 °C) and the concentration of NaOH (0.09 M) used to prepare $S_{control}$ were not high enough to produce a soluble gelatinized starch.

In SGF, for the same protonation ratio, an increase of DS makes the release faster (Fig. 2A), probably due to a higher solubility at increasing number of carboxymethyl groups. An important output is that a low substitution (DS 0.07) generated the longest release time (10.8–12.8 h), whereas unsubstituted ($S_{control}$) starch was rapidly disintegrated. This suggests that a low DS generates major difference in release behavior. This phenomenon is relatively comparable to that of crosslinked starch when low crosslinking drastically enhances the release time (Ispas-Szabo et al., 2000).

In SIF, the CMS(DS 0.11) showed the longest $t_{90\%}$ and a release rate less affected by protonation than those obtained with DS(0.07) and DS(0.20) (Fig. 2B). Thus, when PR changed from 0% to 100%, the $t_{90\%}$ of CMS(DS 0.07) increased from 2.5 to 6.3 h (a factor of 2.5-fold) and that of CMS(DS 0.20) from 2.7 to 7 h (2.6-fold), while the $t_{90\%}$ of CMS(DS 0.11) increased from 5.3 to 8 h (only 1.5-fold).

For each CMS, the increase of $t_{90\%}$ with protonation ratio is less pronounced in SGF than in SIF, because the acidity of SGF contributes to the protonation of –COONa groups reducing thus the differences between different preprepared PR.

The preparation of CMS from low gelatinized starch ($S_{control}$) permits a better examination of the influence of carboxymethyl groups on the properties of CMS excipients. When CMS was prepared from S_g, even a very low DS(0.03) was enough to achieve controlled release of acetaminophen, but a higher substitution (about 0.07) still required to prevent tablet cracks (data not shown).

3.3. Effect of storage time on the properties of totally protonated CMS

Although the highly protonated CMS presented the lowest release rates, acceleration of the release was observed in function of samples storage time, especially for high DS. This acceleration was examined over 4 weeks for CMS(DS 0.11, PR 100%) and CMS(DS 0.20, PR 100%) as examples of totally protonated CMS. Thus, after 28 days of storage, a decrease of $t_{90\%}$ by 10% in SGF and by 21% in SIF was found for CMS(DS 0.11), whereas for CMS(DS 0.20) the decrease was about 46% in SGF and 71% in SIF (Fig. 3).

In an attempt to elucidate the origin of this change of $t_{90\%}$ over storage time, the solubility of CMS(PR 100%) was evaluated (Fig. 4) and FTIR spectra were recorded after conversion of CMS(PR 100%) to sodium salt form (Fig. 5). Storage duration and samples chosen for analyses in this study are considered as non-exclusive examples to show the effects of protonation on CMS properties.

From day 1 at 2 weeks and at 8 months after total protonation of CMS, the solubility in SGF and in SIF decreased over time (Fig. 4). This decrease was more pronounced at high DS and was not necessarily proportional to the decrease in $t_{90\%}$. For instance, after 2 weeks of storage, the solubility of CMS(DS 0.11) was decreased by 52% in SGF and by 63% in SIF, whereas the $t_{90\%}$ was reduced only by 4% and 14%, respectively. For CMS(DS 0.20) the solubility

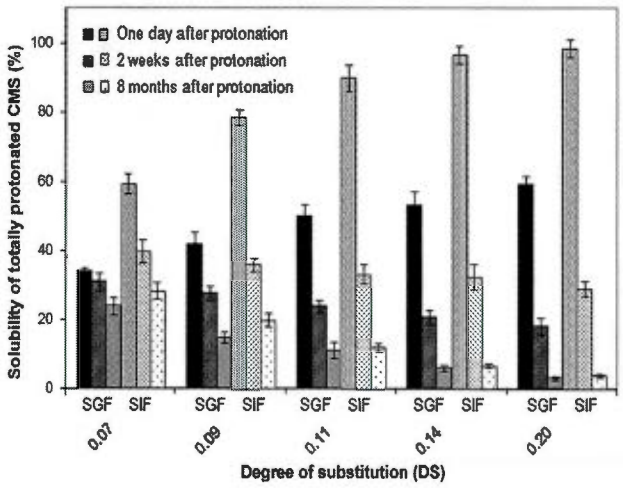


Fig. 4. Solubility in SGF and in SIF of totally protonated CMS(PR 100%, different DS, various storage times).

was decreased by 68% in SGF and by 71% in SIF, while the $t_{90\%}$ was reduced by 21% and 40%, respectively (Figs. 3 and 4).

It is worth to mention that at day 1 after protonation the solubility of CMS increased with DS, whereas after 2 weeks and 8 months the CMS at high DS became less soluble than CMS at low DS. Thus, after 8 months of storage, a certain solubility is still retained for the CMS at low DS, while the solubility of CMS at high DS was drastically reduced (Fig. 4). The high solubility in SIF of CMS(PR 100%) freshly prepared suggests that the carboxyl groups are still non-associated and that no crosslinking occurred via ester link after protonation and drying processing of samples. The subsequent decrease of solubility over storage time of totally protonated CMS suggests an augmentation of intra- and inter-chain interactions via carboxyl groups. These results on solubility fit well with data of Heinze and Koschella (2005) on carboxymethyl cellulose, where the treatment with mineral acids leads to water insoluble polymer.

The conversion of totally protonated CMS to sodium salt form at day 1 after protonation or at 8 months after protonation was done in order to investigate by FTIR whether there are any modifications on carboxyl group vibrations. The FTIR patterns of CMS(DS 0.11) and of CMS(DS 0.20) are presented in Fig. 5 as examples of CMS samples. After conversion to sodium salt form the band at 1735 cm^{-1} ascribed to non-associated carboxyl groups disappeared and that at 1603 cm^{-1} corresponding to carboxylate groups reappeared (Figs. 1 and 5). The patterns of samples deprotonated at day 1 after protonation were similar to those of initially unprotonated form (PR 0%), whereas the patterns of those deprotonated at 8 months after protonation showed a new shoulder at around 1705 cm^{-1} (Figs. 1 and 5). This shoulder indicates the presence of hydrogen bonded carboxyl groups which absorb at a lower (1705 cm^{-1}) frequency than non-bonded groups (1735 cm^{-1}) as reported elsewhere (Harada et al., 2004; Silverstein et al., 2005). In addition, a higher relative intensity of peak at 1603 cm^{-1} can be correlated with the increase of DS (Fig. 5a and c). Furthermore, the intensity of this peak was lower for samples deprotonated after 8 months than those converted at day 1 (Fig. 5).

Overall, the decrease of release time (Fig. 3), the reduction of solubility (Fig. 4) and the FTIR data (Fig. 5) suggest an increase of carboxyl–carboxyl and carboxyl–hydroxyl interactions over storage time of CMS(PR 100%) samples. These interactions concern only the protonated form of CMS and not sodium form.

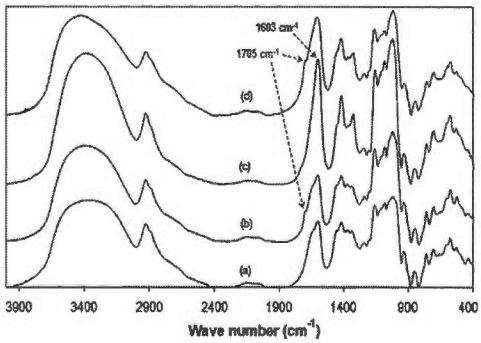


Fig. 5. FTIR spectra of CMS obtained by conversion of totally protonated form (PR 100%) at various storage times to sodium salt form: CMS(DS 0.11) deprotonated at day 1 (a) or at 8 months after protonation (b), and CMS(DS 0.20) deprotonated at day 1 (c) or at 8 months after protonation (d). Pellets (12 mm diameter) of KBr (67 mg) and samples (3 mg) mixture obtained by compression at 3 tonnes.

3.4. Crystalline type of unmodified and modified starch samples

The patterns of native starch (Hylon VII) present prominently a B-type crystalline structure with X-ray diffraction (XRD) peaks at 3.7, 4.0, 4.5, 5.2 (strongest peak), 5.9, 7.1 and 14.5 Å. The $S_{control}$, treated with 0.9 M NaOH, retained a structure almost similar to that of untreated native starch. Differently, the S_g, treated with more concentrated NaOH (2 M), presented a clear reduction of the peak at 5.2 Å and an increase of peaks at 4.5 and 6.9 Å (Supplementary Fig. 1). These XRD patterns show the effect of NaOH concentration during gelatinization of starch on the subsequent structural organization of chains in dried powders. Thus, in the presence of alkaline solution, the starch becomes negatively charged (alkoxide) with dissociation of the protons of –OH groups. Further negative charges generate extensive swelling and may lead to dissociation of double-helical regions and to break-up of crystalline structure (Chen and Jane, 1994).

After carboxymethylation of $S_{control}$, the initial B-type order was lost and a new V-type organization appeared with the two charac-

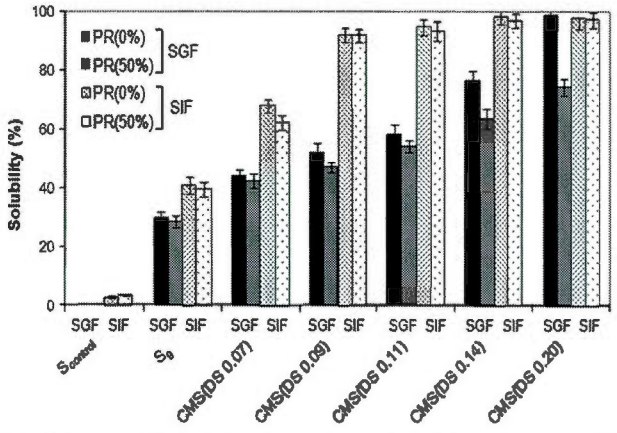


Fig. 6. Solubility in SGF and in SIF of powders of $S_{control}$, S_g, sodium CMS, and CMS with 50% protonation ratio.

teristic peaks at 4.5 and 6.9 Å (Supplementary Fig. 1). The change of crystalline structure from double to single helices after carboxymethylation, suggests that carboxymethyl groups enhance the starch granules swelling.

To clarify whether the CMS(PR 100%) undergo any crystalline modification over storage time, XRD patterns were recorded for these excipients at day 1 and after 8 months. Freshly protonated CMS(PR 100%) presented the same XRD patterns as CMS(PR 0%), but a decrease in intensity of the peak at 6.9 Å can be observed for CMS(PR 100%) over storage time (8 months) (Supplementary Fig. 1). Furthermore, this decrease, indicating lesser order, is particularly evident for CMS with high DS.

Taken together, the results on CMS excipients indicate that the presence of –COOH groups favors progressive intra- and inter-chain interactions via hydrogen bonds. When the DS and the PR are high enough, the carboxyl–carboxyl and carboxyl–hydroxyl interactions can even lead to a kind of structural alteration or rearrangement (Supplementary Fig. 1). A further stability study, including accelerated stability, will allow a better understanding of the structural rearrangement of CMS(PR 100%).

3.5. Properties of CMS(PR 0%) and of CMS(PR 50%)

To investigate the influence of partial protonation on the properties of CMS, only sodium CMS and freshly prepared CMS(PR 50%), unless other indications are present, were retained for the subsequent experiments. We supposed that CMS(PR 50%) can be a model for partially protonated samples and then similar interpretations of acquired data can be applicable to PR less than 50%.

3.5.1. Solubility and density of powders and crushing strength of tablets

As shown in Fig. 6, the control ($S_{control}$) is almost insoluble, whereas the solubility of S_g is about 30% in SGF and 40% in SIF. This higher solubility of S_g is probably related to the higher concentration of NaOH (2 M) used in treatment for gelatinization liberating more polysaccharide chains from starch granules and inducing crystalline disorder (Supplementary Fig. 1). Since polyhydroxylic S_g is non-ionized in used dissolution media, its higher solubility in SIF may be related to solvation power of the medium.

For the CMS samples, the solubility of powder increases with the DS and it was higher in SIF than in SGF. Similar solubility was found for CMS(DS 0.07, PR 0%) and CMS(DS 0.07, PR 50%). At higher DS, the solubility of CMS(PR 0%) in SGF was higher than that of CMS(PR 50%). It appears that the partial protonation of –COONa groups (PR 50%) reduces solubility in SGF, due to the augmentation of intra- and inter-chain interactions and to a lesser hydration of polymer chains when the sodium is replaced by proton. In SIF, no differences were found between the solubility of CMS(PR 0%) and CMS(PR 50%), probably because the –COOH groups turn all to unprotonated form in neutral medium.

As an overall behavior of samples, high tapped density was associated with low crushing strength, irrespective to DS and to PR (Supplementary Fig. 2). The $S_{control}$ and CMS(DS 0.20) presented the lowest crushing strength (177–188 N) with the highest tapped density (0.38–0.41 g/cm^3), whereas for the other samples the crushing strength was in the range 236–278 N with 0.26–0.34 g/cm^3 tapped density. The relatively high tapped density and low crushing strength of $S_{control}$ are probably due to the low hydration and swelling of starch particles when treated with 0.09 M NaOH. In the case of CMS(DS 0.20), high density can be explained by stronger interactions between particles and agglomeration during the precipitation. The obtained values of tapped density and crushing strength are in the normal range compared to common excipients (Rowe et al., 2006).

3.5.2. Erosion of tablets and fluid uptake

Erosion and fluid (SGF or SIF) uptake by the unloaded tablets were examined in the same conditions as for the drug dissolution tests: The tablets were incubated 2 h in SGF to simulate the approximate retention time in stomach, or 2 h in SIF to compare the effect of acid medium to that of neutral medium on tablet erosion and fluid uptake. The $S_{control}$ were not considered for these experiments due to the rapid tablets disintegration.

The S_g showed very low and similar erosion percentages, irrespective of dissolution medium (SGF or SIF) (Fig. 7). For all CMS samples, the erosion in SGF was lower than in SIF (Fig. 7), fitting well with the results of powders solubility (Fig. 6). For the same DS, the erosion of CMS(PR 50%) was lower than that of CMS(PR 0%), due to the network stabilization by hydrogen bonds between carboxyl–carboxyl groups and carboxyl–hydroxyl groups.

The erosion increases with DS for CMS(PR 0%) and CMS(PR 50%) incubated in SGF, and for CMS(PR 50%) incubated in SIF. Differently, the CMS(PR 0%) at DS(0.07, 0.09 and 0.11) incubated in SIF showed almost the same erosion which increased at DS(0.14) and DS(0.20) due to the higher hydration and solubilization of tablets.

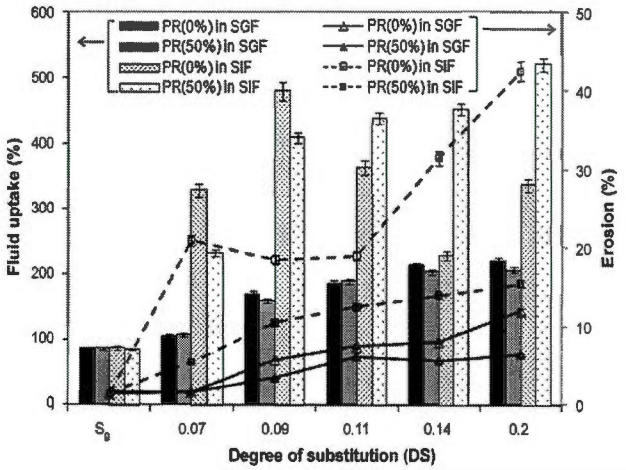


Fig. 7. Erosion and fluid uptake by tablets (200 mg) of CMS(PR 0% and 50%) after 2 h in SGF or 2 h in SIF (900 mL, 37 °C, 100 rpm).

The fluid uptake was the lowest (87%) for S_g in both SGF and SIF media (Fig. 7). It increased with CMS due to the presence of –COONa/H groups, and it was lower in SGF than in SIF. In SGF, the fluid uptake by CMS(PR 50%) was similar to that by CMS(PR 0%), because the SGF acidity contributes to the protonation of carboxylate groups and thus the differences between initially non-protonated and protonated CMS diminished.

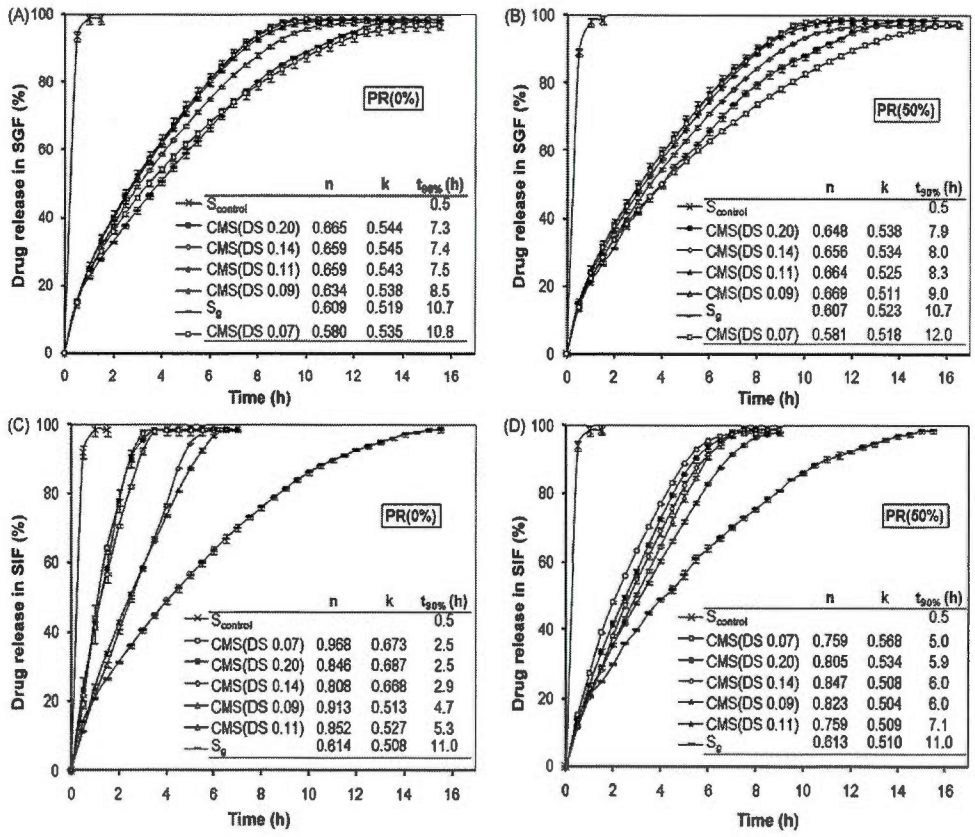


Fig. 8. Kinetics of acetaminophen dissolution (900 mL, 37 °C, 100 rpm) from tablets (200 mg, 20% loading) of $S_{control}$ and of CMS. (A) Sodium form in SGF, (B) 50% protonated form in SGF, (C) sodium form in SIF, (D) 50% protonated form in SIF.

For all CMS samples in SGF and for CMS(PR 50%) in SIF, increasing DS generated higher fluid uptake, probably due to the higher hydration of –COONa/H groups compared to hydroxyl groups and to the low erosion ensured by the stabilization of the network by hydrogen bonds.

For CMS(PR 0%) in SIF, the CMS(DS 0.09) and CMS(DS 0.11) have the same erosion with lower fluid uptake for DS(0.11), indicating that the gel layer generated by CMS(DS 0.11) is consistent enough to reduce the medium diffusion into tablets (Fig. 7). From DS(0.09) to DS(0.14) the fluid uptake decreases in spite of the increase of the number of –COONa groups due to the increasing of tablet erosion (solubilization). At DS(0.20) the fluid uptake by CMS increases due to the difficulty to wipe well this fragile tablet before weighing.

3.5.3. In vitro dissolution tests

Dissolution tests in SGF (pH 1.2 lower than CMS pK_a) and in SIF (pH 6.8 higher than CMS pK_a) (Fig. 8) provided useful information on the properties of the CMS matrix and on the influence of DS and of PR on the kinetics drug release.

The correlation coefficients (R^2) obtained by plotting $\log(M_t/M_\infty)$ against $\log(t)$ were higher than 0.996 indicating that Peppas's equation is applicable to the present devices. In both SGF and SIF media, the control ($S_{control}$) showed a fast release of acetaminophen and a rapid disintegration of tablets (less than 30 min) due to its low solubility and its inability to develop hydrogel (Figs. 6 and 8).

In SGF, longer dissolution assays were conducted in order to better understand the influence of acidity on the properties of CMS matrix. It was found that the percentage of acetaminophen released after the first 2 h in SGF was almost similar for all CMS (Fig. 8A and B), that all CMS tablets preserved their shape even after the complete drug release, and that the S_g tablets presented cracks after approximately 2 h. It was also found that S_g and CMS(DS 0.07), due to the low fluid uptake, low erosion and low solubility, afford longer sustained release than CMS at higher DS (Figs. 6, 7, and 8A and B). For the same DS, the PR(50%) showed slightly slower release than the sodium form (PR 0%), since a higher protonation enhances inter-chain associations via hydrogen bonding. The exponent (n) values (0.580–0.669) suggested an anomalous diffusion mechanism in acid medium.

In neutral medium (SIF), the tablets are less compact and the $t_{90\%}$ is lower than in acid medium (SGF) (Fig. 8C and D). The S_g, which is not pH sensitive, presented almost the same release rate in neutral and in acid media. Like in SGF, the CMS(PR 50%) provided a lower release rate than CMS(PR 0%), in agreement with the lower erosion of CMS(PR 50%) tablets (Figs. 7, and 8C and D). The fastest release was found with CMS at lowest DS(0.07) and at highest DS(0.20), irrespective of the protonation ratio (0% and 50%). The fast release of acetaminophen from CMS(DS 0.07) is almost due to its inability to generate a hydrogel structure compact enough to prevent fast fluid diffusion within the tablet and thus to control the drug release. In the case of CMS(DS 0.20), the fast release is due to the high solubility (Fig. 6) and the high erosion (Fig. 7) of this matrix compared to the others CMS. When protonated (PR 50%), the solubility of CMS(DS 0.20) was reduced and the release became slower. The slowest release of acetaminophen was provided by the middle DS(0.11), allowing hydration just enough to generate a low soluble hydrogel layer which can reduce the fluid diffusion and drug dissolution. The exponent (n) values (0.759–0.968) suggest a mechanism controlled mostly by swelling of CMS matrices in neutral medium (Fig. 8C and D).

3.5.4. Effect of drug loading and of SGF acidity on drug release rate in SIF

Tablets of CMS(DS 0.11 and 0.20) loaded with acetaminophen (20% and 40%) were first incubated for 2 h in SGF to mimic the gastric residence and then in SIF until complete dissolution. The CMS(DS 0.11) was chosen for its ability to ensure sustained release and the CMS(DS 0.20) was chosen for its high sensitivity to the pH of the dissolution medium.

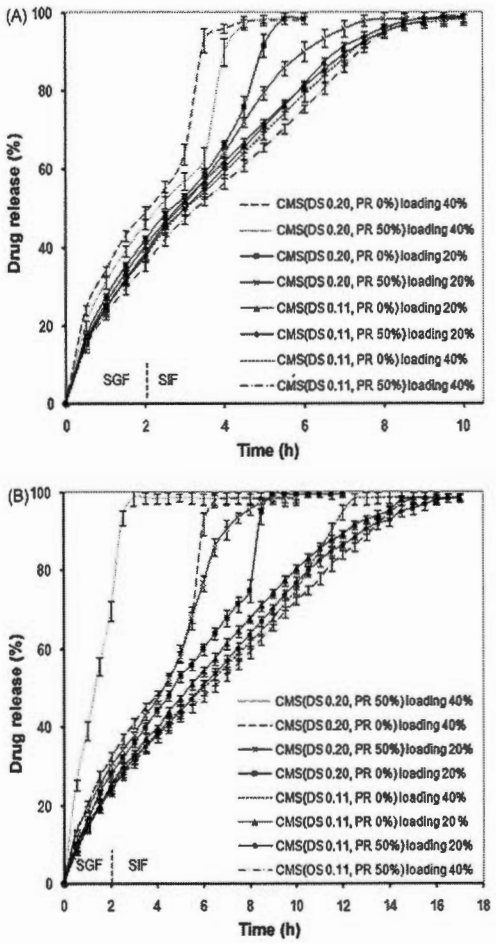


Fig. 9. Kinetics of acetaminophen dissolution (900 mL, 37 °C, 100 rpm) from tablets of CMS(DS 0.11 and 0.20, PR 0% and 50%). Tablets of (A) 200 mg or (B) 500 mg (20% or 40% loading) incubated 2 h in SGF and then transferred in SIF until complete dissolution.

The drug release from CMS(DS 0.11) showed almost similar rates irrespective of protonation ratio (0% and 50%) and of loading (20% and 40%) (Fig. 9A). In these cases, $t_{90\%}$ (7–7.5 h) are close to that (7.1 h) obtained with CMS(DS 0.11, PR 50%) and higher than that (5.3 h) obtained with CMS(DS(0.11, PR 0%) in SIF only (Figs. 8C, D and 9A). These results indicate that a partial protonation of DS(0.11) makes dissolution kinetics almost independent of time retention in SGF.

For CMS(DS 0.20, PR 50%) at 20% loading, the $t_{90\%}$ (6 h) is close to that in SIF only (Figs. 8D and 9A). For the same loading (20%), the CMS(DS 0.20, PR 0%) showed higher $t_{90\%}$ (5 h) than in SIF only (2.5 h), but with final acceleration of release (late burst) (Figs. 8C and 9A). The CMS(DS 0.20) at 40% loading presented similar dissolution profiles to that with CMS(DS 0.20, PR 0%) at 20% loading, but the release rate was higher (Fig. 9A). Therefore, the longer dissolution observed with DS(0.20, PR0%) is almost due to the preincubation of tablets in

acid medium (SGF) which generates a kind of preconditioning for further intestinal release.

Contrarily to CMS(DS 0.20), the CMS(DS 0.11) did not show a final acceleration of release due to its low erosion in SIF (Fig. 7). Thus, at high DS(0.20), the progressive conversion of carboxyl to salt form in SIF induces a high water absorption by the tablet until the inside pressure becomes enough to disrupt the weak erodible hydrogel and to liberate the drug.

In order to evaluate the effect of tablet size and of storage time on drug release rate, the same experiments as before were done with tablets of 200 mg and 500 mg after 1 year of CMS storage (Fig. 9B).

For tablets of 200 mg, same results were obtained as with freshly prepared samples except for CMS(DS 0.20, PR 50%) where the dissolution becomes faster upon samples storage (not shown). These results indicate that for high DS a protonation ratio less than 50% is necessary to limit the intra- and inter-chains interactions and to ensure maintaining of excipient properties at storage. Interestingly, the CMS(DS 0.11) with protonation ratio up to 50% did not show any modification even after 1 year of storage. Maybe, the DS and the PR are not high enough and thus the number of –COOH groups is not sufficient to permit high interactions between carboxyl and hydroxyl groups. Moreover, the presence of –COONa groups may reduce the intra- and inter-chains interactions.

For tablets of 500 mg, the CMS(DS 0.11) allowed almost the same sustained release irrespective of protonation ratio (0% and 50%) and of loading (20% and 40%) (Fig. 9B). The CMS(DS 0.20) showed final acceleration of release as with tablets of 200 mg. Also, the release with CMS(DS 0.20, PR 50%) was faster than with CMS(DS 0.20, PR 0%). The effect of DS and of PR on release profile for 500 mg tablets was similar to that for 200 mg tablets, but the time release was longer with larger tablets.

Except for $S_{control}$ and CMS(DS 0.20), the crushing strength of tablets are close. This suggests that the differences of drug release rate from various formulations are not due to crushing strength effect (Supplementary Fig. 2 and Fig. 8).

It was recently shown (Brouillet et al., 2008) that adding of NaCl (loading 27.5%) to CMS tablet formulation was necessary to maintain the integrity of matrices used for sustained release. In our conditions, formulations with CMS(DS 0.11) can keep integrity and ensure sustained release without adding any electrolyte to the formulation.

Taken together, these results indicate that oral solid dosage forms based on CMS(DS 0.20) are suitable for duodenum and upper intestinal delivery, whereas those based on CMS(DS 0.11) are useful for sustained drug release.

4. Conclusions

This first detailed study on the effect of protonation ratio and of degree of substitution on the properties of carboxymethyl starch synthesized in aqueous medium, provides useful information about the delivery mechanisms with this excipient type. The control ($S_{control}$) disintegrated rapidly in the media, whereas the CMS showed fast or slow release of acetaminophen depending on the PR and the DS. To ensure a gel network formation and to maintain a limited solubility of the matrix, the amount of carboxyl groups (DS) must be enough but not too high. Protonation of CMS excipients made the drug release rate lower than that provided by sodium CMS. High protonation, especially at high DS(0.20), lead to time-dependant reduction of solubility and alteration of crystalline structure of CMS. The CMS(DS 0.20) was remarkably sensitive to the pH of dissolution medium and showed high release rate in SIF. Longer release time was observed after tablet preincubation in SGF due to the protonation acquired in acid medium. The CMS(DS 0.09–0.11) with PR up to 50% appears as the most suitable excipients for drug sustained release. When partially protonated, CMS(DS 0.11) was slightly sensitive to pH of dissolution medium and showed almost similar sustained release rate with 20% and 40% loading.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2010.04.037.

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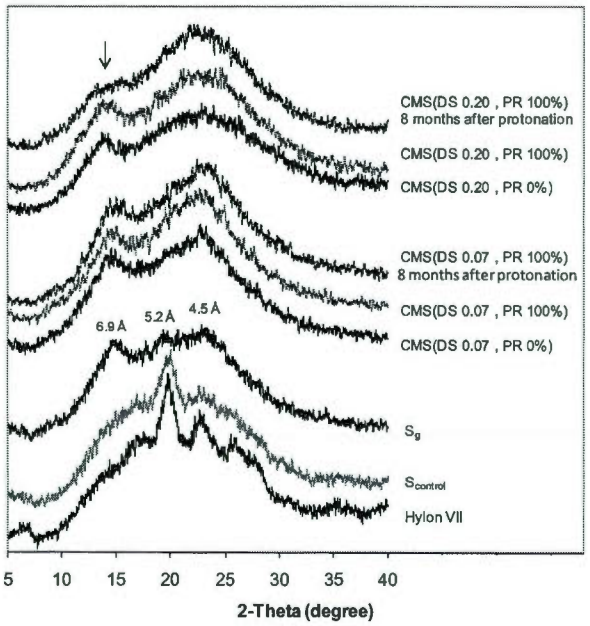
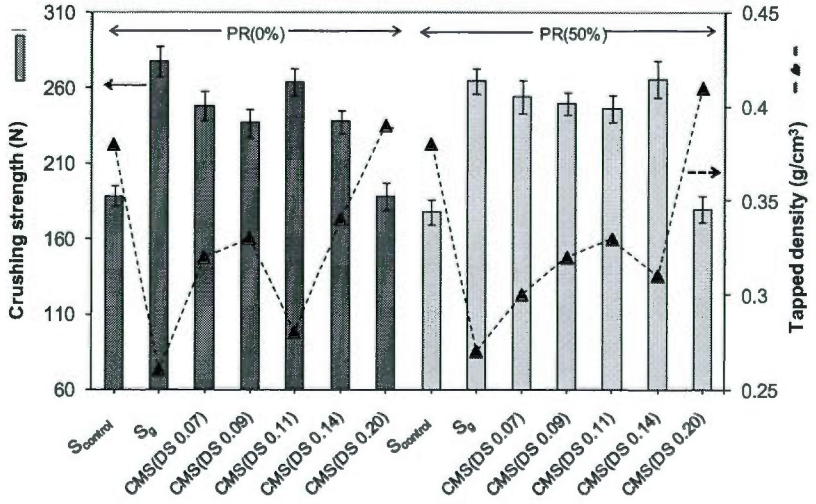
Appendix A. Supplementary data



Supplementary Fig. 1. X-ray diffraction patterns of native starch (Hylon VII), $S_{control}$, S_g, sodium CMS, and of totally protonated CMS (freshly protonated or after 8 months of storage).



Supplementary Fig. 2. Crushing strength of tablets and tapped density of powders of $S_{control}$, S_g, sodium CMS, and CMS with 50% protonation ratio.

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Polyelectrolyte complex of carboxymethyl starch and chitosan as drug carrier for oral administration

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ABSTRACT

A novel polyelectrolyte complex (PEC) of carboxymethyl starch (CMS) and chitosan was prepared, characterized and tested *in vitro* as a carrier for oral drug delivery. This PEC, containing 14% (w/w) of chitosan, showed a polymorphism with a lower order degree than those of CMS and of chitosan. Under conditions simulating the gastrointestinal transit, NMR imaging analysis showed slower fluid diffusion inside PEC monolithic tablets than inside CMS tablets. The PEC seems to be a more suitable drug carrier for colon targeting than CMS, since it can prolong acetaminophen release time from 8 h to 11 h and aspirin release time from 13 h to 30 h. In contrast, chitosan used as a coexcipient accelerated aspirin release from matrices based on a CMS:chitosan physical mixture.

1. Introduction

Since carboxymethyl starch (CMS) was proposed (Mulhbacher, Mateescu, & Calinescu, 2004) as an excipient for controlled drug release from oral solid dosage forms (tablet), several studies have been undertaken in order to investigate the properties and the efficiency of this excipient. The influence of the degree of substitution (DS), of the degree of protonation, and of the formulated drug type and loading on release kinetics of small molecules from CMS matrices has been recently studied (Assaad & Mateescu, 2010; Assaad, Azzouz, & Mateescu, 2008; Ispas-Szabo, De Koninck, Calinescu, & Mateescu, 2007; Lemieux, Gosselin, & Mateescu, 2009). Moreover, the effects of certain formulation parameters, such as compression force and NaCl electrolyte particle size, on drug release rate have been investigated (Brouillet, Bataille, & Cartilier, 2008; Nabais et al., 2007). CMS has also been suggested for the formulation of large size bioactive agents, such as pancreatic enzymes (α-amylase, lipase and trypsin) (Massicotte, Baille, & Mateescu, 2008), *Escherichia coli*, filamentous surface proteins of *Escherichia coli* (F4 *fimbriae*) and *Lactobacillus rhamnosus* probiotic (Calinescu & Mateescu, 2008; Calinescu, Mulhbacher, Nadeau, Fairbrother, & Mateescu, 2005; Calinescu, Nadeau, Mulhbacher, Fairbrother, & Mateescu, 2007). These studies have shown that CMS can reduce the damaging effect of the acidity of gastric medium on bioactive agents and affords a controlled drug release in intestinal medium. In simulated gastric fluid (SGF, pH 1.2), the CMS in the outer layer of tablet is protonated, making the matrix compact. At higher pH (simulated intestinal fluid, SIF, pH 6.8), the carboxyl groups are deprotonated and ionized, thus favoring hydration, swelling and finally solubilisation of tablet. The solubility of CMS in neutral medium (SIF) and its digestion by pancreatic α-amylase can be limiting factors to effect a sustained drug release (Assaad & Mateescu, 2010; Calinescu & Mateescu, 2008). With the aim to ensure a longer time of drug release and targeting to the colon, chitosan dry powder has been used as a coexcipient in such formulations (Calinescu & Mateescu, 2008; Leonida & Mateescu, 2006). Chitosan has been shown to interact with unmodified starch via intermolecular hydrogen bonds, leading to the formation of chitosan–starch complex (Xu, Kim, Hanna, & Nag, 2005).

There is a recent growing interest for polyelectrolyte complexes of chitosan due to its cationic character and biocompatibility (Chen & Fan, 2007): some of them have been proposed for delivery of bioactive agents, such as chitosan–xanthan complexes (Chitoxan TM) for controlled drug delivery (Chellat et al., 2000); chitosan–carboxymethyl konjac glucomannan and

Abbreviations: CMS, carboxymethyl starch; PEC, polyelectrolyte complex; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; DS, degree of substitution; DDA, degree of deacetylation; DC, dry-coated; 50% CMS:50% chitosan, an excipient containing 50% (w/w) of CMS and 50% (w/w) of chitosan; $t_{90\%}$, time (h) for the release of 90% of drug.

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chitosan–heparin for delivery of albumin (Du et al., 2005; Liu, Jiao, Liu, & Zhang, 2007); chitosan–dextran sulfate and chitosan–alginate for oral delivery of insulin (Sarmento et al., 2006); chitosan–polyaspartate for delivery of 5-fluorouracil (Zheng et al., 2007). A number of polyelectrolyte complexes of chitosan and polyuronans have been prepared and spray-dried as microspheres (Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004).

The objectives of the present study are (i) to prepare CMS–chitosan polyelectrolyte complex (PEC) and to investigate its performance in drug delivery; (ii) to evaluate the influence of chitosan molecular weight on drug release rate; (iii) to compare the drug dissolution from tablets based on anionic water-soluble excipient (CMS) alone, on cationic water-insoluble excipient (chitosan) alone, on physical mixture powder of these two excipients, or on PEC; and (iv) to compare the dissolution profiles of drugs with different charges and solubilities.

2. Materials and methods

2.1. Materials

High amylose corn starch (Hylon VII) was obtained from *National Starch* (Bridgewater, NJ, USA) and crab shell chitosans were from *Marinard Biotech* (Rivière-au-Renard, QC, Canada). Acetaminophen was from *Sigma-Aldrich* (St-Louis, MO, USA). Metformin (1,1-dimethylbiguanide hydrochloride) was from *MP Biomedicals* (Solon, OH, USA). Aspirin (acetylsalicylic acid) and monochloroacetic acid were from *Fisher Scientific* (Fair Lawn, NJ, USA). The other chemicals were of reagent grade and used without further purification. Pepsin-free simulated gastric fluid (SGF, pH 1.2) and pancreatin-free simulated intestinal fluid (SIF, pH 6.8) were prepared following the USP methods (US Pharmacopeia, XXIV, 2000).

2.2. Preparation of CMS and purification of chitosans

Sodium carboxymethyl starch (CMS) was prepared in aqueous medium from high amylose corn starch as previously described (Calinescu et al., 2005; Mulhbacher, Ispas-Szabo, Lenaerts, & Mateescu, 2001), with minor modifications. Briefly, an amount of 70 g of Hylon VII was suspended in 170 mL of distilled water in a Hobart mixer (Vulcan, Canada) at 55 °C. Then, 235 mL of 1.5 M NaOH were added for gelatinization under continuous mixing for 30 min. Subsequently, 55 mL of 10 M NaOH and a freshly prepared solution of monochloroacetic acid (45.5 g in 40 mL of distilled water) were added. After 1 h of reaction, a volume of 130 mL of distilled water was added and the slurry was cooled-down to room temperature and neutralized with acetic acid. The CMS was then precipitated from the slurry by gradually adding 600 mL of acetone. After that, the CMS was washed by repeated dispersion in volumes of 1 L of 70% acetone and filtrations until a final conductivity of filtrate decreased at about 50 μS/cm. The CMS mass was again washed three times with acetone, and then dried at 40 °C for 24 h. The obtained powder of sodium form CMS was sieved with a 300 μm screen and stored at room temperature.

Two chitosans of different molecular weights were each purified by solubilization in acetic acid and by filtration as follows: an amount of 20 g of chitosan was solubilized in 350 mL of 0.35 M acetic acid and the volume was adjusted to 2 L with distilled water. The acidic solution was filtered under vacuum through Whatman filter papers (medium 40). Subsequently, the chitosan was precipitated with 0.1 M NaOH under continuous stirring. The mass was washed with distilled water, then with nanopure water (volumes of 2 L) until conductivity of about 200 μS/cm and finally with acetone. The chitosan was dried at 40 °C for 24 h, ground and sieved on a 300 μm screen.

2.3. Preparation of CMS–chitosan PEC

A CMS–chitosan polyelectrolyte complex (PEC) was prepared by coagulation of CMS and chitosan-700 in aqueous medium at room temperature. Essentially, 1 g of chitosan-700 was solubilized in 44 mL of 0.1 M HCl, and the volume was adjusted to 150 mL with distilled water. A 1% solution of CMS was prepared by solubilizing 6 g of CMS in 600 mL of distilled water. The precipitation occurred under vigorous mixing by adding the solution of polycation (chitosan-700) to that of polyanion (CMS) at 1:1 ratio ($-NH_3^+$:$-COO^-$), with a final pH of about 5. The PEC, containing 14% (w/w) of chitosan-700, was washed and dried with acetone by the same procedure as for CMS.

2.4. Physical and chemical characterizations of excipients

2.4.1. The degree of substitution

The degree of substitution (DS) of CMS was determined by back-titration as previously described (Assaad & Mateescu, 2010). Briefly, 300 mg of protonated CMS ($n = 3$) were solubilized in 20 mL of 0.05 M NaOH and then the excess of NaOH was titrated with 0.05 M HCl using phenolphthalein as indicator. The blank (20 mL of NaOH) was also titrated by the same method. The amount of –COOH groups and the DS were calculated by using the following equations (Stojanovic, Jeremic, Jovanovic, & Lechner, 2005):

$$n_{COOH} = (V_b - V) \times C_{HCl} \quad (1)$$

$$DS = \frac{162 \times n_{COOH}}{m - 58 \times n_{COOH}} \quad (2)$$

where V_b (mL) is the volume of HCl used for the titration of the blank; V (mL) is the volume of HCl used for the titration of the sample; C_{HCl} (mol/L) is the concentration of HCl; 162 (g/mol) is the molar mass of glucose unit; 58 (g/mol) is the increase in the mass of glucose unit by substitution with one carboxymethyl group, and m (g) is the mass of dry sample.

2.4.2. The degree of deacetylation

The degree of deacetylation (DDA) of each chitosan was determined by acid-base titration. An amount of 150 mg of chitosan was solubilized in 20 mL of 0.1 M HCl and the volume was completed to 200 mL with distilled water. A titration was done with 0.1 M NaOH and the pH and the conductivity were recorded. The DDA was calculated following the method and the equation given by Broussignac (1968) and Muzzarelli (1977):

$$DDA(\%) = \frac{203 \times (v_2 - v_1) \times M \times 100}{m + 42 \times (v_2 - v_1) \times M} \quad (3)$$

where V_1 and V_2 are the volumes of NaOH solutions corresponding to the two inflexion points of the curve obtained by titration; M is the concentration of NaOH (mol/L); m is the weight of chitosan (g); 203 (g/mol) is the molar mass of acetylated unit, and 42 (g/mol) is the difference between molar mass of acetylated unit and that of deacetylated unit.

2.4.3. The molecular weights

The molecular weights of chitosans were determined by viscometric method, using experimental reported viscometric constants data (Kasaai, 2007; Knaul, Kasaai, Bui, & Creber, 1998). Samples were dissolved in a solution containing 0.1 M acetic acid and 0.2 M sodium chloride for chitosan-400 and in a solution containing 0.2 M acetic acid and 0.1 M sodium acetate for chitosan-700. The viscosities of chitosan solutions with different concentrations (0.07–0.7%)

were measured by using an electronic viscometer (*Viscosity Monitoring and Control Electronics*, Medford, MA, USA). The temperature was adjusted at 25 °C for chitosan-400 and at 30 °C for chitosan-700 as reported elsewhere (Roberts & Domszy, 1982; Wang, Bo, Li, & Qin, 1991).

The data on viscosities and concentrations were used to calculate the reduced viscosities. Plotting reduced viscosities against chitosan concentrations gives the intrinsic viscosity ($[\eta]$) by extrapolation of the straight line obtained by linear regression to zero concentration. The average molecular weight (M) of chitosan was calculated from the intrinsic viscosity by Mark–Houwink–Sakurada's empirical equation:

$$[\eta] = kM^{\alpha} \quad (4)$$

where k (dL/g) and α (dimensionless) are constants that depend on the solvent-polymer system.

2.4.4. The Fourier transform infrared spectra

The Fourier transform infrared spectra (FTIR) of the samples were recorded from 4000 to 400 cm^{-1} at 2 cm^{-1} resolution with a total of 32 scans by using a *Nicolet* 4700 spectroscopy (Madison, WI, USA). To prepare the pellets, homogenous mixtures of dried KBr (67 mg) and of polymer powders (3 mg) were compressed at 3 tonnes (*Carver*, Wabash, IN, USA) in flat-faced punches with 12 mm diameter.

2.4.5. The polymorphism

The polymorphism of samples was evaluated by X-ray diffractometer (XRD, *Siemens* D5000, Munich, Germany) at 1.789 Å wavelength. The original XRD spectra, recorded between 5° and 50° (2θ), were treated using Excel software (regression type: moving average, period 10).

2.4.6. The thermogravimetric analyses

The thermogravimetric analyses were carried out in platinum crucible at a heating rate of 10 °C/min between 25 and 900 °C under nitrogen atmosphere (flow rate 100 mL/min). A *Seiko* TG/DTA 6200 (Japan) instrument was used and the alumina was taken as reference material.

2.4.7. The morphology

The morphology of the sample particles was examined by a *Hitachi* (S-4300SE/N) scanning electron microscopy with variable pressure (Hitachi High Technologies America, Pleasanton, CA, USA) at voltage of 15 kV and magnifications of 100× and 500×. Samples were mounted on metal stubs and sputter-coated with gold.

2.4.8. The density

The density of the polymer powders was determined according to the (616) USP method, using a Vankel tapped density tester (*Varian*, NC, USA).

2.5. Preparation of tablets

Monolithic tablets (200 mg, 20% (w/w) loading) were obtained by direct compression (2.5 tonnes) of a homogenous mixture of excipient and drug (acetaminophen, metformin or aspirin) powders. The unloaded (drug-free) tablets of 200 mg were prepared with excipient only. Flat-faced punches with 9.6 mm diameter and a *Carver* hydraulic press were used.

Dry-coated (DC) tablets (200 mg, 20% (w/w) loading) were prepared with a core consisting in a homogenous mixture of drug (40 mg) and excipient (40 mg) and compressed in a 7 mm cylinder outfit. This core was then dry coated with 120 mg of excipient, giving tablet of about 9.6 mm diameter and 2.1 mm thickness after compression.

2.6. Nuclear magnetic resonance (NMR) imaging analysis

NMR imaging analyses were carried out at 37 °C with a *Bruker* Avance-400 NMR spectrometry (Germany) as previously reported (Baille, Malveau, Zhu, & Marchessault, 2002; Malveau, Baille, Zhu, & Marchessault, 2002; Thérien-Aubin & Zhu, 2006, 2009; Thérien-Aubin, Baille, Zhu, & Marchessault, 2005; Thérien-Aubin, Zhu, Ravenelle, & Marchessault, 2008; Wang, Ravenelle, & Zhu, 2010). A standard spin-echo pulse sequence (90-τ-180-τ-Acquisition) was used to obtain spin density images of the unloaded tablets (n = 3) in a NMR tube (20 mm diameter) containing 20 mL of dissolution media (SGF or SIF). A slice of 0.5 mm in thickness was selected either perpendicular or parallel to the main magnetic field (axial axis). Eight scans were accumulated with a field of view of 2 cm and an in-plane resolution of 156 μm. An echo time of 3 ms and a repetition time of 1 s were fixed, leading to an acquisition time of about 17 min for each image. Each tablet was first incubated for 2 h in SGF and then in SIF until the end of the test. The percentage of axial and radial swelling was calculated by comparison to the initial dimension of the tablet.

2.7. In vitro dissolution tests

The *in vitro* dissolution tests were carried out at 100 rpm and 37 °C in an USP dissolution apparatus II (*Distek* 5100, North Brunswick, NJ, USA) coupled with an UV spectrophotometer (*Hewlett Packard* 8452A, USA). The tablets (n = 3) were incubated in SGF (1 L) for 2 h and then in SIF (1 L) up to complete release. The drug release from tablets was evaluated by measuring the absorbance at the appropriate wavelength (acetaminophen at 244 nm, metformin at 218 nm, and aspirin at 246 nm).

3. Results and discussion

3.1. Characterization of the excipients

The degree of substitution of carboxymethyl starch (CMS) determined by the back-titration method was about 0.14, representing the average number of carboxymethyl groups per glucose unit. The degrees of deacetylation of chitosans determined by acid-base titration were about 80% and the approximate molecular weights determined by Mark–Houwink–Sakurada method were about 400 kDa for chitosan-400 and 700 kDa for chitosan-700.

The scanning electron microscopy micrographs showed that chitosan particles were compact, whereas those of CMS and PEC were porous (Fig. 1). The morphology of the polyelectrolyte complex (Fig. 1, d1 and d2) appeared homogenous, indicating a uniform distribution and a good compatibility between CMS and chitosan.

Chitosan-400 and chitosan-700 showed the highest tapped densities (0.61 and 0.64 g/mL, respectively) due to their compact morphology, whereas PEC showed the lowest density (0.20 mg/mL) due to its higher granulometry and porosity (Fig. 1). Intermediate density (0.36 mg/L) was found for CMS.

3.2. CMS–chitosan interactions and preparation of PEC

When the chitosan-700 solution was added to the CMS solution, immediate coagulation and precipitation occurred. This suggests effective interactions between functional groups of CMS and of chitosan-700 with possible partial charges neutralization, leading to the formation of a polyelectrolyte complex. To verify this

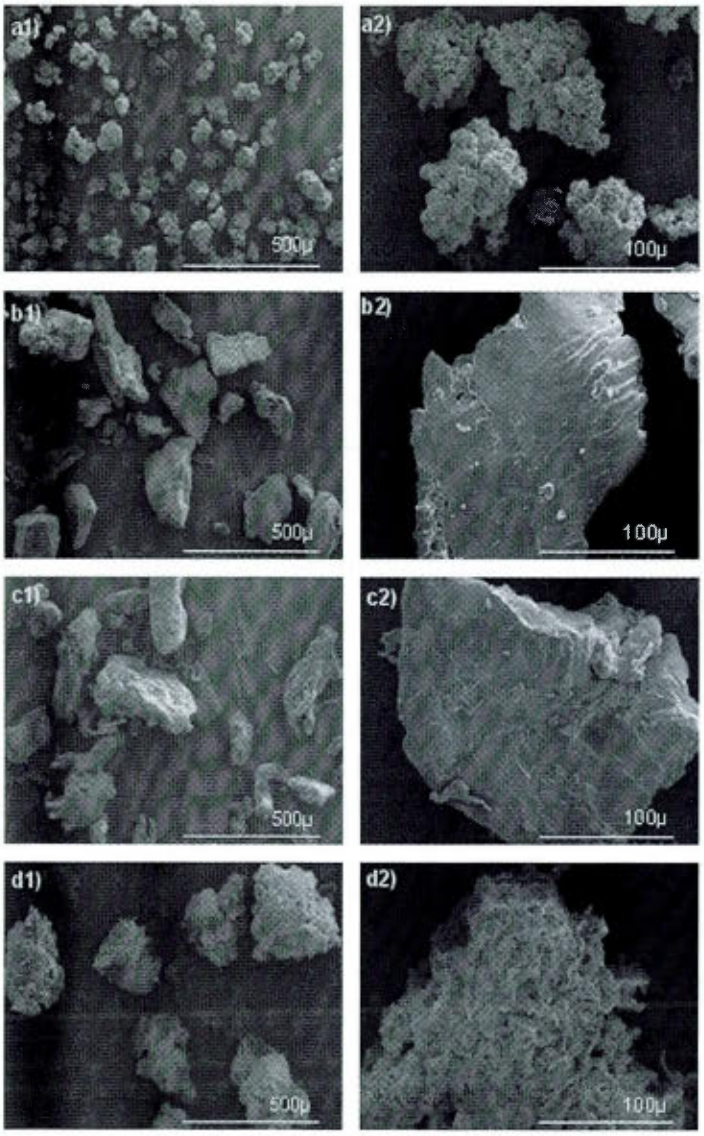

Fig. 1. Scanning electron microscopy micrographs of (a) CMS, (b) chitosan-400, (c) chitosan-700, and (d) PEC at magnifications of 100× and 500× and voltage of 15 kV.

hypothesis, the products were characterized by FTIR spectroscopy, by X-ray diffractometry (XRD) and by thermogravimetry (TGA) (Figs. 2–4).

The FTIR spectrum of CMS (Fig. 2) presents two characteristic bands at 1603 and 1417 cm^{-1}. They were attributed respectively to asymmetrical and symmetrical stretching vibration of $-COO^-$ groups (Silverstein, Webster, & Kiemle, 2005; Zoldakova, Srokova, Sasinkova, Hirsch, & Ebringerova, 2005). The bands at 2930 and 1643 cm^{-1} are assigned respectively to C–H stretching and to O–H groups.

The spectrum of chitosan-700 shows characteristic absorption bands of chitosan at 1653 and 1597 cm^{-1} ascribed to $-CONH_2$ stretching vibrations, and two bands at 2922 and 2876 cm^{-1} due to C–H stretching. The bands at 1417 and 1376 cm^{-1} were assigned to the C–H symmetrical deformation mode as per Mathew and Abraham (2008).

The polyelectrolyte complex (PEC) shows a spectrum similar to that of 50% CMS:50% chitosan-700, with bands at about 2923–2880, 1636, 1600, 1417 and 1376 cm^{-1}. This indicates the presence of both CMS and chitosan in the PEC. Roughly similar spectra of dry blend polymer powders and polyelectrolyte complexes were reported in other studies, as for chitosan and carboxymethyl cellulose polymers (Fukuda, 1980). The weak shoulders at around 1735 and 1540 cm^{-1} for PEC obtained at pH 5 could be assigned respectively to –COOH and $-NH_3^+$ groups. These shoulders suggest that interactions between CMS and chitosan in the PEC may occur via hydrogen bonds (–OH, –COOH) or ionic interactions ($-COO^-$, NH_3^+).

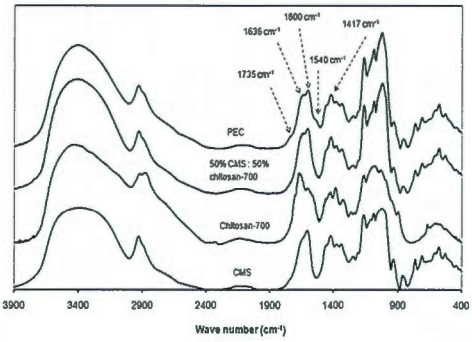

Fig. 2. FTIR spectra of CMS, chitosan-700, 50% CMS:50% chitosan-700, and PEC. Pellets (12 mm diameter) were prepared by compression at 3 tonnes of KBr (67 mg) and sample (3 mg) mixtures.

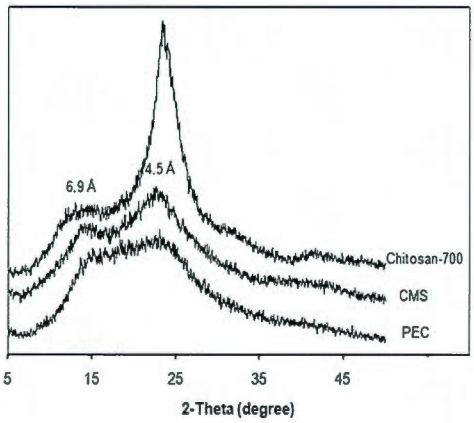

Fig. 3. X-ray diffraction patterns of CMS, chitosan-700, and PEC.

The XRD pattern (Fig. 3) of CMS shows the two characteristic peaks at 6.9 and 4.5 Å, indicating a V-type single helix structure as previously reported (Assaad & Mateescu, 2010). The pattern of chitosan-700 shows characteristic crystalline peaks at around 6.9 and 4.4 Å (major one), fitting well with the typical XRD pattern of chitosan (Choi, Kim, Pak, Yoo, & Chung, 2007; Wang et al., 2005).

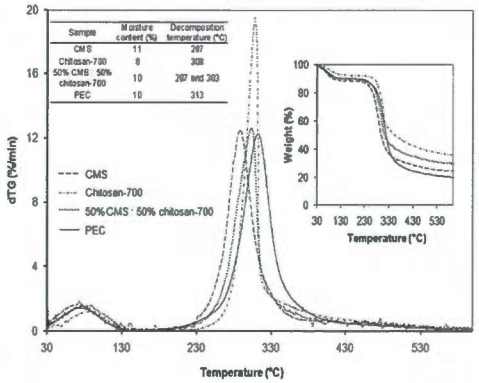

Fig. 4. Thermogravimetric patterns of CMS, chitosan-700, 50% CMS:50% chitosan-700, and PEC at heating rate of 10 °C/min between 25 and 600 °C.

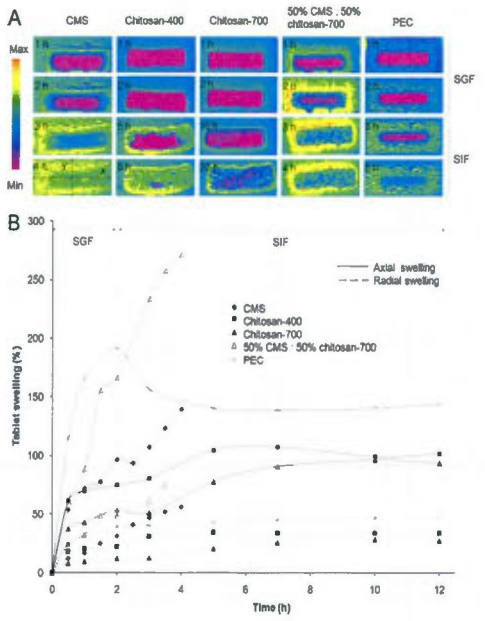

Fig. 5. NMR images at various times of unloaded tablets of (CMS, chitosan-400, chitosan-700, 50% CMS:50% chitosan-700 and PEC) incubated for 2 h in SGF and then transferred to SIF: (A) axial side images where *x* indicates the radial direction and *y* the axial direction, (B) axial and radial swelling. (For interpretation of the references to color in this figure the reader is referred to the web version of the article.)

The order degree of the PEC is definitely lower to those of CMS and chitosan-700. The suppression of crystalline peak of chitosan-700 at 4.4 Å and the broad amorphous pattern of the PEC indicate a good compatibility and strong interactions between CMS and chitosan with a complete dispersion of chitosan chains. These intermolecular interactions could prevent macromolecules to crystallize individually as reported for some interpolymer complexes (Mathew & Abraham, 2008; Sakurai, Maegawa, & Takahashi, 2000; Xu et al., 2005; Yin, Yao, Cheng, & Ma, 1999).

The TGA results (Fig. 4) show relatively lower moisture content for chitosan-700 than for CMS and PEC, maybe due to higher hydrogen association of chitosan chains. The 50% CMS:50% chitosan-700 shows a nonsymmetrical dTG peak with a weak shoulder at around 287 °C and a maximum at 303 °C, indicating the presence of two components. The difference of decomposition temperatures between CMS (287 °C) and chitosan-700 (308 °C) seems not enough to identify two separate peaks for the dry powder mixture of these two polymers. Differing from 50% CMS:50% chitosan-700, the PEC presented a symmetrical dTG peak and the highest decomposition temperature (313 °C).

Overall, these results suggest a good compatibility between CMS and chitosan, a strong interaction between the chains of these two polymers, and the formation of a homogenous polyelectrolyte complex.

3.3. Examination of tablets hydration and swelling by NMR

Water penetration into unloaded tablets (Fig. 5A) and the axial and radial swelling (Fig. 5B) were followed by NMR imaging in SGF for 2 h and then in SIF to simulate the gastrointestinal tran-

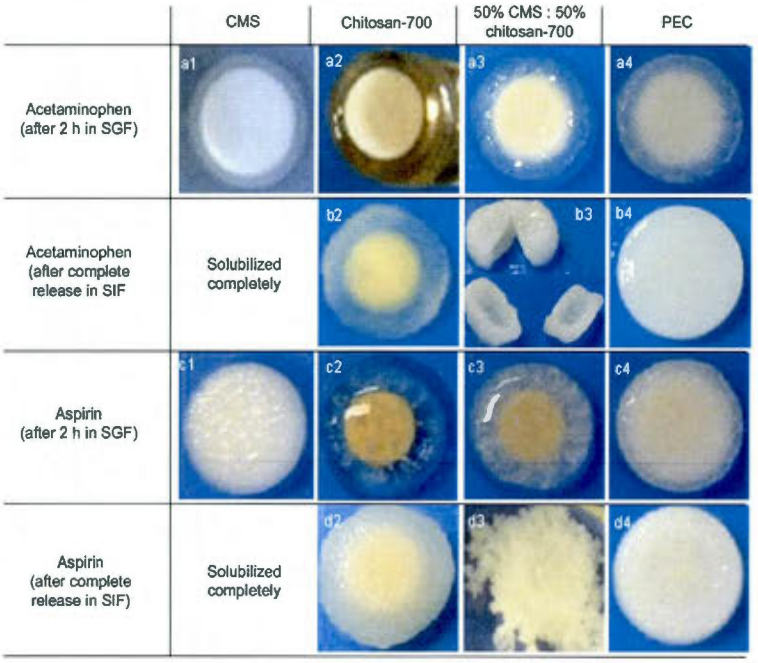

Fig. 6. Photographs of CMS, chitosan-700, 50% CMS:50% chitosan-700 and PEC tablets (200 mg, 20% loading) during dissolution tests (1 L, 37 °C, 100 rpm). Photographs were taken for the tablets, first after 2 h of incubation in SGF and then after the complete drug (acetaminophen or aspirin) release in SIF. The sizes of tablets were not normalized.

sit. The location where the water concentration matches 1/6 of the maximal concentration corresponding to free fluid (SGF or SIF) was considered as the front of fluid diffusion inside the tablets (Baille et al., 2002; Malveau et al., 2002).

For all tablets, the axial swelling was higher than radial swelling (Fig. 5B). This may be explained by the formation of flat oriented particles in tablet after axial compression of polymer powders. Upon tablet hydration, the stress resulting from compression is released, leading to a higher swelling in the direction where compression force was applied (Le Bail, Morin, & Marchessault, 1999; Malveau et al., 2002; Thérien-Aubin & Zhu, 2006, 2009; Thérien-Aubin et al., 2005, 2008; Wang et al., 2010).

After 2 h in SGF, the CMS tablet still showed a dry core (purple) with a partial penetration of SGF and formation of a gel network in the outer layer (blue and green) (Fig. 5A). In acidic medium (SGF, pH 1.2), the carboxylate groups (–COONa) of the outer layer are converted to carboxyl groups (–COOH), thus reducing the solubility of the CMS excipient and limiting the gastric fluid penetration into the tablets. When tablets were transferred to SIF (pH 6.8), the fluid advanced rapidly to the core which became hydrated within 2 h in this neutral medium. The protonation acquired in SGF is lost and the –COOH groups turn into their salt form (–COOK), increasing thus the solubility of the excipient and accelerating intestinal fluid advancement to the core of the tablet. The axial and radial swelling of CMS tablet increase relatively fast, reaching 150% and 60%, respectively, after 4 h of incubation (Fig. 5B).

The diffusion of fluid (SGF or SIF) into the chitosan (chitosan-400 and chitosan-700) tablets was slower than into the CMS tablets (Fig. 5A). A gel network was developed by chitosan in SGF due to the protonation of amino groups exposed to the acid medium. In SIF, the tablet size was stabilized (Fig. 5B) due to chitosan insolubility in neutral medium while an anisotropic fluid diffusion was observed (Fig. 5A). For chitosan-400 the core was almost completely hydrated after 8 h of incubation, whereas for chitosan-700 the core still showed dry regions even after 20 h. Thus, chitosan-700 with a higher molecular weight seems to provide a thicker (more substantial) outer layer gel than chitosan-400.

The tablets of 50% CMS:50% chitosan-700 mixture showed the fastest fluid diffusion (Fig. 5A) and the highest swelling (Fig. 5B). The gel network formed in SGF was less substantial than that formed with chitosan tablets due to close neighboring of CMS in the mixture. In SIF, the chitosan would be deprotonated, whereas the CMS would be converted to the salt form triggering a higher in situ hydration of tablet previously swollen in SGF.

The tablets of PEC presented slower fluid diffusion than CMS and 50% CMS:50% chitosan-700 tablets, particularly in SIF (Fig. 5A). This suggests that association of CMS and chitosan at molecular level as PEC favors more interactions between these two compounds than in physical mixture of powders. A more extensive swelling occurred in the first two hours of incubation in SGF due to the protonation and the hydration of chitosan within the PEC. When SGF was changed to SIF, the size of PEC tablets was reduced due to the deprotonation and dehydration of chitosan chains in neutral medium, indicating a stronger interaction between chitosan and CMS than that between CMS and water. The shape of tablets was after that as stable as those of the chitosans, despite the low ratio (14%) of chitosan in PEC. This is an important aspect and can be related to the insolubility of chitosan in neutral medium and to a lower tendency of CMS to swell when intimately complexed with chitosan.

3.4. *In vitro dissolution tests*

All dissolution tests, except for metformin formulated in monolithic tablets, were followed first in SGF for 2 h and then in SIF until complete drug release, simulating thus the gastrointestinal transit.

The shape of tablets and the dissolution profiles of acetaminophen, metformin and aspirin are presented in Figs. 6 and 7. Unless otherwise specified, the tablets (200 mg) used for dissolution were monolithic.

The release rates of acetaminophen from chitosan-400 and 50% CMS:50% chitosan-400 matrices were higher than from CMS matrix, whereas the release rates from chitosan-700 and 50% CMS:50% chitosan-700 matrices were lower than from CMS matrix (Fig. 7A). That is why the chitosan-700 was chosen to prepare the PEC. In addition, the 75% CMS:25% chitosan-700 matrix showed almost the same release rate as CMS (not shown). It seems that a molecular weight of 700 kDa rather than 400 kDa and an adequate ratio in dry blends are required for chitosan to favor a longer drug release time.

Although the chitosan-700 matrix showed the lowest release rate, chitosan alone does not seem suitable for controlled drug release, because the transformation of the gel developed in SGF (Fig. 6, a2) to a semi-solid form (Fig. 6, b2) that limits the diffusion of SIF into the tablet makes the release slow (Fig. 7A). It is worthwhile to note that the solid core of tablet was still compact and insoluble even after the complete acetaminophen release (Fig. 6, b2).

The faster release from tablets based on CMS:chitosan-700 powder mixture compared to that from those with chitosan-700 as only excipient, indicates that the CMS favors the tablet hydration and accelerates the diffusion of SIF into the tablets. These results are in agreement with those obtained by NMR imaging (Fig. 5A). At the end of the dissolution tests, the tablets based on a mixture of CMS and chitosan powders appeared as a water-insoluble empty shell (Fig. 6, b3) with a crust still containing a mixture of these two polymers as confirmed by FTIR analysis (not shown). This indicates that although the tablets are based on dry blend of polymer powders, physical or chemical interactions can occur between CMS and chitosan during the dissolution.

The release rate of acetaminophen from PEC matrix was lower than that from 50% CMS:50% chitosan-700 matrix (Fig. 7A). This is an interesting advantage for PEC which contains only 14% (w/w) of chitosan-700, considering the higher cost of chitosan compared to that of CMS.

Metformin is a freely soluble drug (US Pharmacopeia, 2000) and its release from hydrophilic excipients is usually fast. Neither CMS nor chitosans, separately or in association, were able to control the release of metformin in monolithic dosage form (Fig. 7B). Dry coated (DC) tablets can delay this release in SGF, especially when the outer part of tablet is based on chitosan-700. However, when the fluid reached the inner core of the tablets the release was accelerated. The dissolution profiles of metformin from tablets based on chitosan-400 were similar to those based on chitosan-700, but with higher release rate (not shown). The dry coating formulation can be of interest, considering the very high hydrosolubility of metformin and the fact that the release is undesired in stomach.

For aspirin, which is slightly soluble in water (US Pharmacopeia, 2000), the higher the molecular weight of chitosan, the lower was the release rate (Fig. 7C). CMS and chitosan-700 provided a low release of aspirin in SGF and a long sustained release in SIF. Probably, this is due to the interactions of carboxyl groups of aspirin with carboxyl groups and hydroxyl groups of CMS, and to the formation of consistent outer layer gel network in tablets based on chitosan (Fig. 6, c2). As with acetaminophen, the dissolution of aspirin from chitosan-700 matrix after 80% of release was reduced probably due to the formation of chitosan insoluble outer layer in SIF (Fig. 6, d2). The matrices based on CMS:chitosan mixture showed an accelerated release of aspirin compared to those based on individual excipient (CMS or chitosan). It seems that the aspirin–CMS and aspirin–chitosan interactions compete and reduce those between these two polymers, favoring a faster

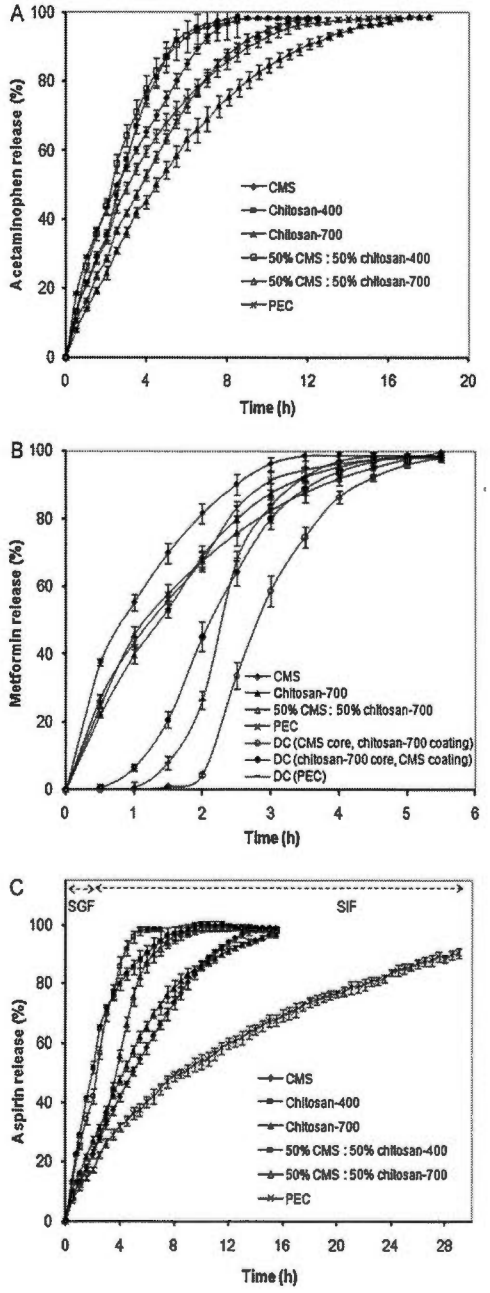

Fig. 7. Kinetics of drug dissolution from tablets (200 mg, 20% loading) of CMS, chitosan-400, chitosan-700, 50% CMS:50% chitosan-400, 50% CMS:50% chitosan-700 and PEC. The tablets were incubated (1 L, 37 °C, 100 rpm) for 2 h in SGF and then transferred to SIF. (A) Acetaminophen; (B) metformin, monolithic tablets were incubated only in SGF; (C) aspirin.

tablet erosion and aspirin release. Similar to what was observed with acetaminophen, a water-insoluble excipient residue was still present after complete release of aspirin (Fig. 6, d3), indicating the presence of CMS–chitosan attractions. A sustained release of aspirin, over more than 30 h, was observed with PEC (Fig. 7C). This time release is markedly longer than $t_{90\%}$ obtained with 50% CMS:50% chitosan-700 (6.5 h), CMS (11 h) or chitosan-700 (11.5 h). This difference is ascribed to the interactions between aspirin, CMS and chitosan within the PEC matrix. These interactions reduce the diffusion of both aqueous fluid and the drug, leading thus to the lower release rates. The remained tablet based on the PEC after the complete release of drug (Fig. 6, d4) further supports the existence of the interpolymer attractions. In this case, aspirin interacts with the hydroxyl, carboxyl and amino groups of the PEC without dissociation of interactions between the chains of CMS and chitosan.

Taken together, these results showed the advantage of PEC for monolithic formulations. The PEC excipients, containing only 14% of chitosan-700, can afford controlled release of acetaminophen and aspirin. Moreover, the tablets of the PEC were homogenous and less swellable than those of 50% CMS:50% chitosan-700 (Figs. 5B and 6).

4. Conclusion

The CMS–chitosan polyelectrolyte complex (PEC) showed a polymorphism with a lower order degree than those of carboxymethyl starch (CMS) and of chitosan-700. The fluid (SGF or SIF) diffusion and the swelling were lower with PEC tablets than with those based on CMS:chitosan-700 powder mixture. The PEC provided a controlled release of acetaminophen and a markedly slower sustained release of aspirin than that provided by CMS or chitosan-700, making this excipient favorable to colon targeting.

Chitosan at molecular weight of about 400 kDa (chitosan-400) did not afford a long release time for any of the three tracer drugs (metformin, acetaminophen and aspirin). CMS and chitosan-700 matrices showed a fast release of metformin, a controlled release of acetaminophen and a sustained release of aspirin. This indicates that the drug solubility has a major influence on release rate irrespective of the charge of drug and of excipient. The low hydration of chitosan and its insolubility in neutral medium can be a limitation for drug delivery with this excipient alone, but it can be an advantage in the case of PEC. Adding an adequate amount of chitosan with an appropriate molecular weight to the formulations based on CMS can prolong the release time of acetaminophen. Contrarily, the aspirin release from CMS matrix was accelerated when chitosan was added as coexcipient.

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