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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).

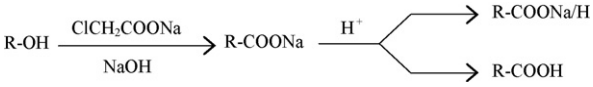
2.2. Synthesis of sodium CMS

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).

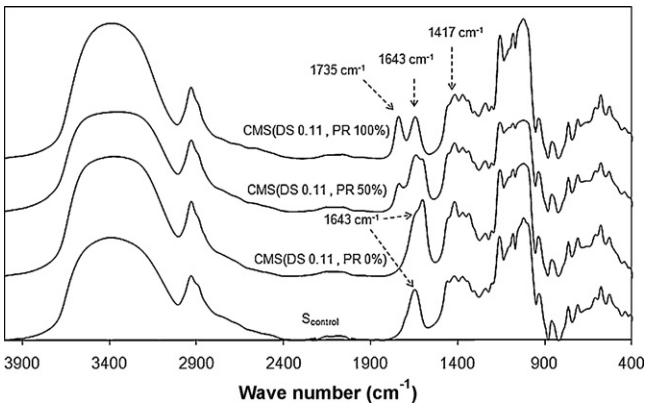


Fig. 1. FTIR spectra of $S_{control}$ and of CMS(DS 0.11) at various protonation ratios (0%, 50% and 100%). Pellets (12 mm diameter) of KBr (67 mg) and samples (3 mg) mixture obtained by compression at 3 tonnes.

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

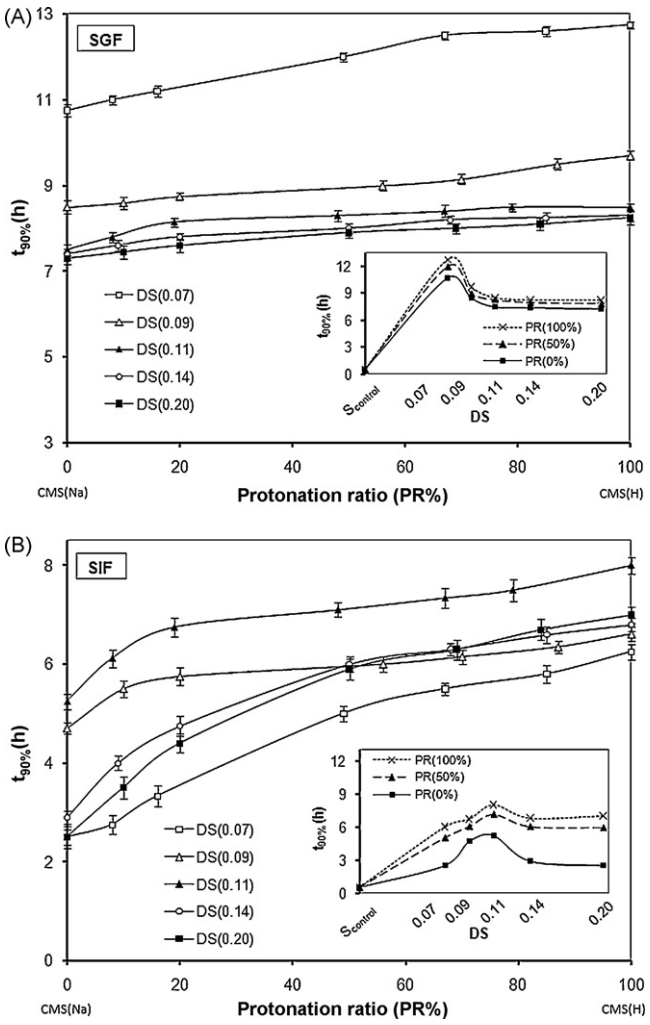


Fig. 2. Time of acetaminophen release ($t_{90\%}$) from CMS at various DS in (A) SGF and (B) SIF. Tablets of 200 mg (20% loading) were used for the dissolution tests (900 mL, 37 °C, 100 rpm).

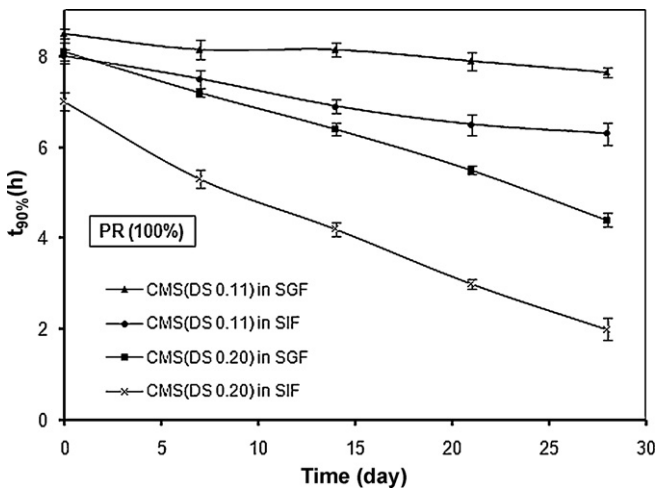


Fig. 3. Time of acetaminophen release ($t_{90\%}$) from totally protonated CMS(DS 0.11 and 0.20) over storage times. Tablets of 200 mg (20% loading) were used for the dissolution tests (900 mL, 37 °C, 100 rpm) in SGF and in SIF.

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

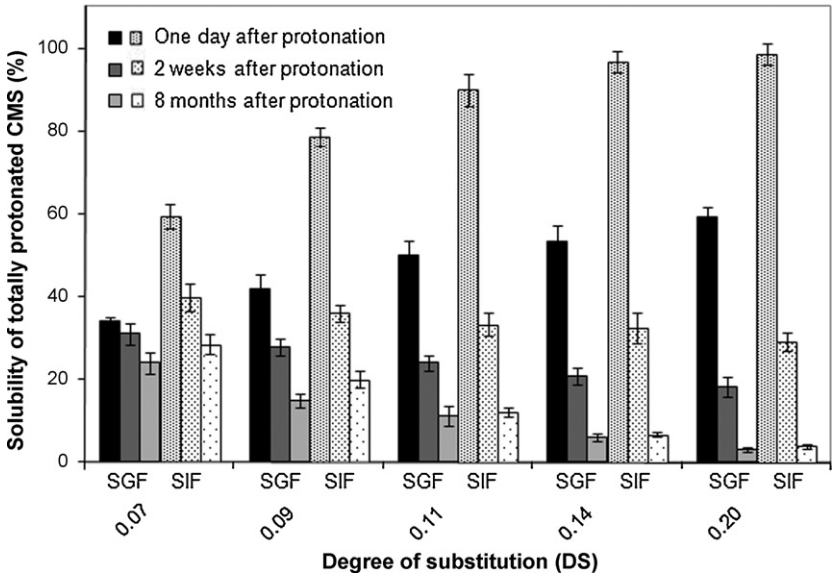


Fig. 4. Solubility in SGF and in SIF of totally protonated CMS(PR 100%, different DS, various storage times).

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.

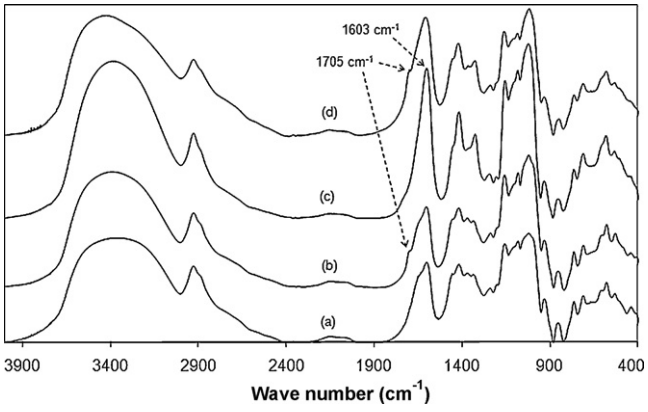


Fig. 5. FTIR spectra of CMS obtained by conversion of totally protonated form (PR 100%) at various storage times to sodium salt form: CMS(DS 0.11) deprotonated at day 1 (a) or at 8 months after protonation (b), and CMS(DS 0.20) deprotonated at day 1 (c) or at 8 months after protonation (d). Pellets (12 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-

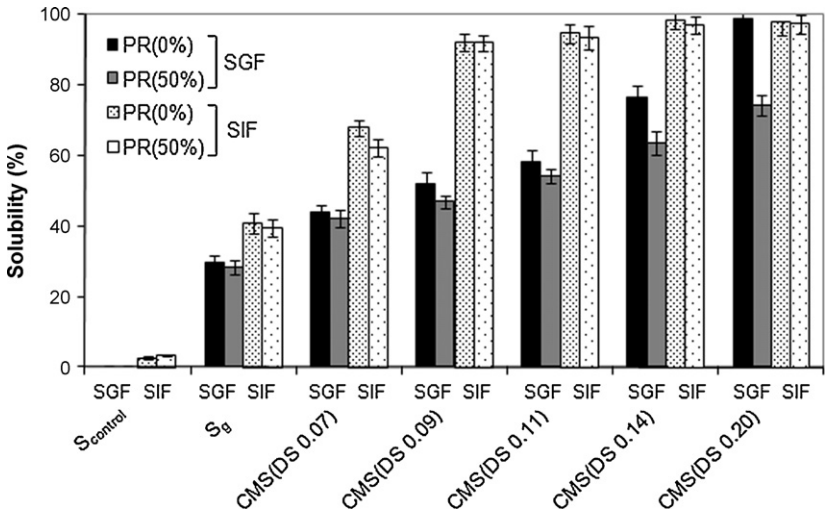


Fig. 6. Solubility in SGF and in SIF of powders of $S_{control}$, S_g, sodium CMS, and CMS with 50% protonation ratio.

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.

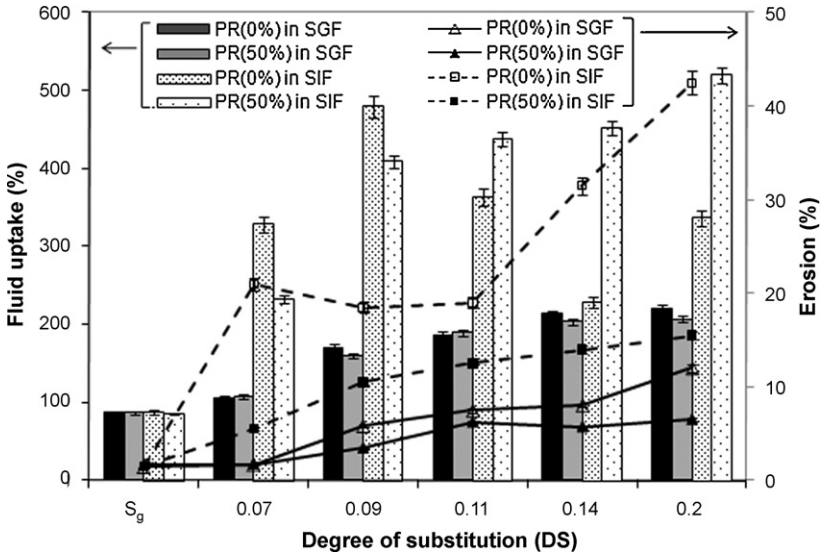


Fig. 7. Erosion and fluid uptake by tablets (200 mg) of CMS(PR 0% and 50%) after 2 h in SGF or 2 h in SIF (900 mL, 37 °C, 100 rpm).

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.

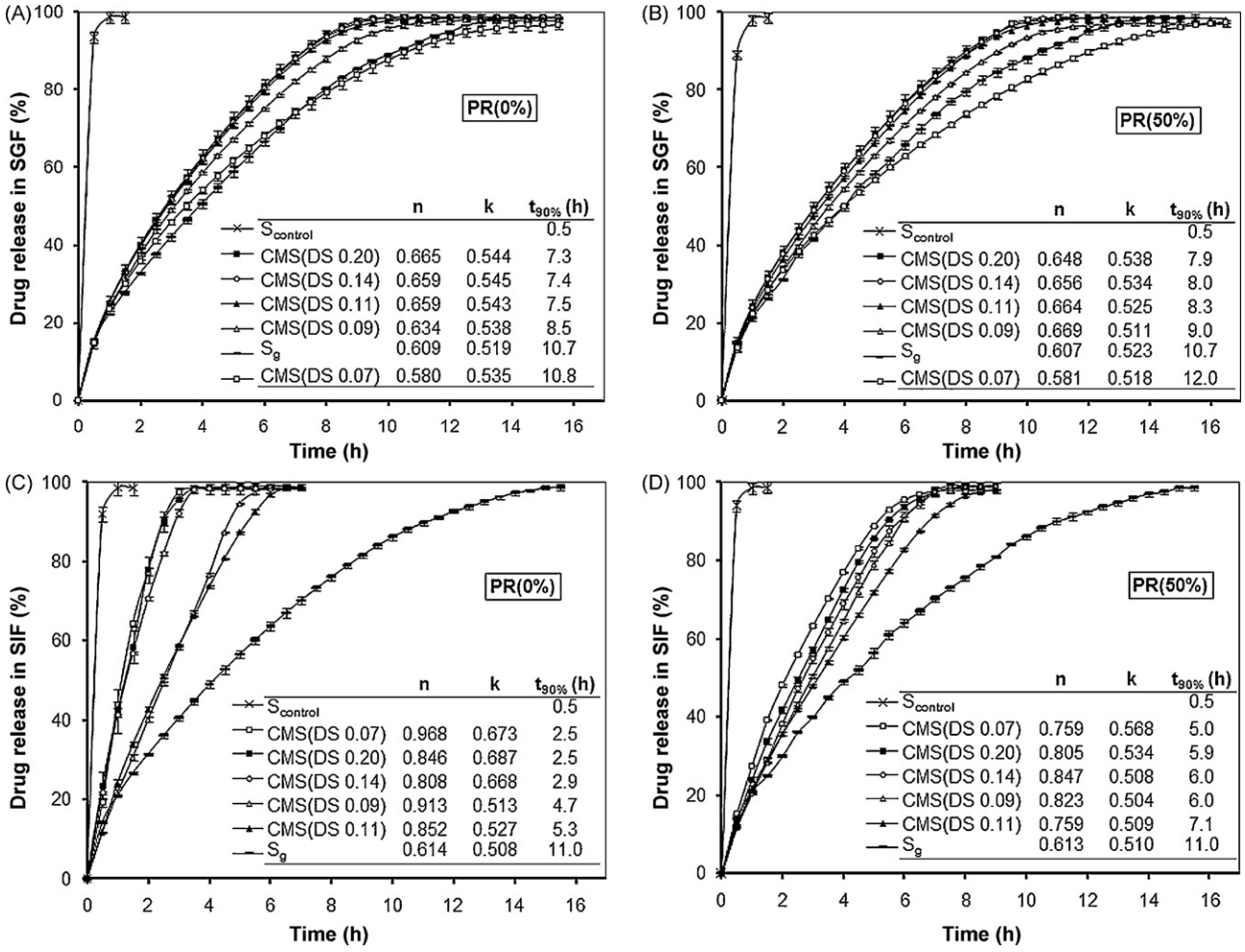


Fig. 8. Kinetics of acetaminophen dissolution (900 mL, 37 °C, 100 rpm) from tablets (200 mg, 20% loading) of $S_{control}$ and of CMS. (A) Sodium form in SGF, (B) 50% protonated form in SGF, (C) sodium form in SIF, (D) 50% protonated form in SIF.

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.

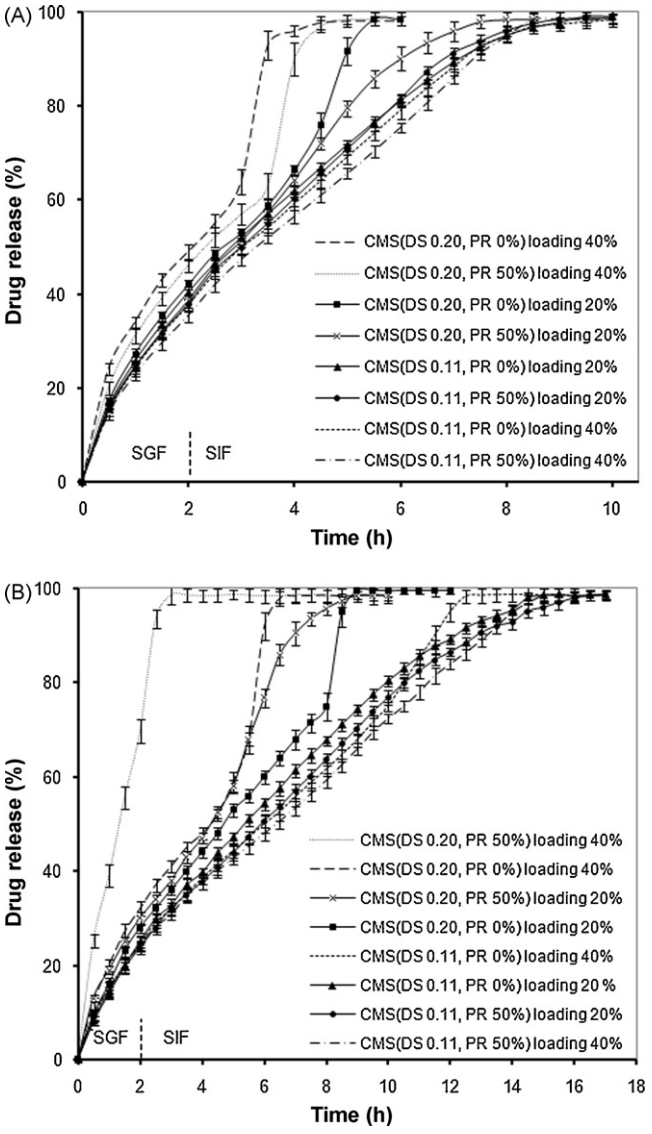


Fig. 9. Kinetics of acetaminophen dissolution (900 mL, 37 °C, 100 rpm) from tablets of CMS(DS 0.11 and 0.20, PR 0% and 50%). Tablets of (A) 200 mg or (B) 500 mg (20% or 40% loading) incubated 2 h in SGF and then transferred in SIF until complete dissolution.

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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