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Carboxymethyl high amylose starch as excipient for controlled drug release: Mechanistic study and the influence of degree of substitution

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ABSTRACT

Sodium carboxymethyl high amylose starch, CMHAS(Na), with a DS up to 1.74 were synthesized in non-aqueous medium (with yields as high as 72%) in order to investigate the influence of the degree of substitution (DS) on physical and drug release properties. The CMHAS(Na) was converted to protonated form, CMHAS(H), by acid treatment. The DS and conversion of CMHAS(Na) to CMHAS(H) had a major impact on the physical properties of CMHAS particles and resulting tablets. Dissolution tests performed in simulated gastric fluid (SGF, pH 1.2) and in simulated intestinal fluid (SIF, pH 6.8), with acetaminophen as drug model (20% loading) showed CMHAS as a pH sensitive hydrophilic matrix whereas CMHAS(Na) seems suitable as controlled release matrix. CMHAS(Na) with DS between 0.1 and 0.2 can be preferably used as sustained release excipient since in both dissolution media, the drug release was driven by diffusion over a period up to 12 h. CMHAS(Na) with high DS, between 0.9 and 1.2, can be used as delayed release excipient since drug release in SGF was driven by diffusion over a period up to 20 h and in SIF by fast erosion lowering to less than 3 h the complete release of the drug.

1. Introduction

Starches are widely used as fillers, binders and disintegrants in the pharmaceutical and biopharmaceutical fields as they are cost-effective, renewable materials available in large quantities, non-toxic, biocompatible and biodegradable particularly when administered orally (Pifferi et al., 1999; Demirgoz et al., 2000; Désévaux et al., 2002; Dumoulin et al., 1999). Native starches are not suitable for controlled drug delivery systems due to poor flow and compressibility and, most notably, fast release properties resulting from substantial swelling in aqueous media along with rapid enzymatic degradation by α-amylase in physiological fluids (Kost and Shefer, 1990; Dumoulin et al., 1999). Starch is composed of two distinct polymers: amylose and amylopectin. Amylose is an unramified polymer with glucopyranose units (GU) (≈200–2000) linked through α(1–4) glucosidic bonds. Amylopectin is a highly branched polymer where linear branches (≈20–30 GU linked through α(1–4) glucosidic bonds) are periodically linked through α(1–6) glucosidic bonds (total GU up to 3×10^6) (Wurzburg, 1986).

To overcome these limitations and extend the applications of starch as excipient for sustained drug delivery, the properties of starch can be tailored by physical modification (e.g. retrogradation) (Te Wierik et al., 1997), chemical modification (e.g. cross-linking, esterification) (Lenaerts et al., 1991; Korhonen et al., 2000) or enzymatic hydrolysis (e.g. dextrins) (Stella and Rajewski, 1997). Among these starch derivatives, carboxymethyl high amylose starch (CMHAS, more than 70% amylose), was recently introduced (Mulhbacher et al., 2008) as excipient for oral monolithic tablets able to control the release of active molecules (Ispas-Szabo et al., 2007; Nabais et al., 2007) and of bioactive agents (Calinescu et al., 2005, 2007). As excipient, CMHAS was used as sodium salt, CMHAS(Na), but the acid form (protonated, CMHAS(H)) is also available. It is generally synthesized by etherification of the hydroxyl groups (–OH) of the GU with sodium carboxymethyl groups (CM, CH_2COONa) using monochloroacetic acid (MCA) or its sodium salt (NaMCA) under basic conditions according to the Williamson ether synthesis (Heinze and Koschella, 2005). The aim of starch carboxymethylation in the pharmaceutical industry is to convert native starches to an ionic hydrophilic excipient able to modulate the drug release according to the physiological pH values. In acidic dissolution medium below the pK_a value of CMHAS (about 4.2) hydrogen association, by dimerization of protonated CM groups (–COOH) are expected to enhance the strength of the gel-layer, reducing thus the drug release rate. Otherwise, in a dissolution medium above the pK_a of CMHAS, the deprotonation will reduce the gel-layer strength, by enhancing the matrix hydration and the repulsive forces between dissociated CM groups ($–COO^-$), increasing the drug release rate. The properties of CMHAS(Na),

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including the control of drug release would probably be in functions of the degree of substitution (DS), defined as the average number of CM groups per GU and lies between 0 and 3. Previously, CMHAS(Na) with DS between 0.15 and 0.3 were mainly used since it was shown that these DS were the most suitable for controlled drug delivery (Calinescu et al., 2005; Lemieux et al., 2007). However, drug release from CMHAS(Na) matrix with DS under 0.3 appears to have a low pH sensitivity. The CMHAS(Na) was produced in aqueous reaction medium leading to DS lower than 0.3 (Volkert et al., 2004) with low reaction yields (below 30%). Several studies were carried out to optimize reaction conditions in order to increase DS and reaction efficiency (Tijsen et al., 1999, 2001; Stojanovic et al., 2000; Lazik et al., 2002; Heinze et al., 2004; Volkert et al., 2004; Sangseethong et al., 2005). Essentially, CMHAS(Na) with higher DS was obtained by starch carboxymethylation in organic solvent medium (e.g. methanol, ethanol and isopropyl alcohol) containing only a small fraction of water. The use of organic medium has the advantage of keeping starch in its granular form all along the reaction (Tijsen et al., 1999) and hence simplifying the production process by eliminating additional precipitation steps. In the context of development of a new hydrophilic polymer as excipients for controlled drug delivery, the aim of this study was to investigate the influence of DS on the physical characteristics and drug release properties of CMHAS produced in non-aqueous medium. It was expected that increasing DS will modify the polymer chain interactions according to the environmental pH. Furthermore, an increasing enzymatic resistance of CMHAS to amylolysis by α-amylase was previously reported (Kwon et al., 1997). In order to better understand the polymer chains–chains or chain–medium interactions and their role on the properties of CMHAS, the investigation was also performed with CMHAS(H) in comparison with CMHAS(Na). This study seems to be, at our knowledge, the first contribution to investigate the drug release from CMHAS at high DS (above 0.66). This report was further motivated by the fact that queries still remain about the mechanism of drug release from CMHAS(Na) tablets with low DS. In order to answer these questions, monolithic tablets were deliberately maintained in SGF longer than the physiological gastric residence time, in order to better understand the structural characteristics ensuring tablet shape integrity and release controlling properties.

2. Materials and methods

2.1. Materials

High amylose corn starch (HAS) (Hylon VII) was supplied by National Starch (USA). Pancreatin eight times strength with α-amylase, lipase and proteolytic activities (A&C American Chemicals Ltd., CAN). Acetaminophen (Sigma–Aldrich, USA), sodium monochloroacetate (NaMCA) (Fluka, USA) and other chemicals. were reagent grade and used without further purification.

2.2. Synthesis of CMHAS

An amount of 100 g (dry mass) of granular HAS (15 ± 5 μm) was suspended in 1 kg of 2-propanol/water 90:10 (w/w) at 40 °C in a 2 L jacketed reaction glass vessel (Chemglass, USA) equipped with a servocontrolled speed (N, 400 rpm) and power (P, 18.5 W) monitoring mixer head (Cole-Palmer Instrument model 5000-40, USA) fitted with a Rushton turbine and an anchor agitator to ensure constant and vigorous stirring all along the synthesis. Subsequently, 50 g of NaOH pellets (NaOH/GU molar ratio 2:1) was added to the reaction mixture and stirred for 30 min to induce swelling of the starch granules. The carboxymethylation was started by adding NaMCA and stopped after 4 h by neutralizing the reaction mixture with glacial acetic acid and cooling at room temperature. For this study, CMHAS(Na) with six different DS was synthesized by varying the NaMCA/GU molar ratio (0.15:1, 0.25:1, 0.6:1, 1:1, 1.5:1 and 2:1) in the reaction mixture. CMHAS(Na) was recovered by filtration (Büchner with grade 54 cellulose filter paper, Whatman, USA) and purified by successive resuspension/filtration with a methanol/water (80:20, v/v) solution until the filtrate conductivity was under 25 μS/cm in order to ensure removal of residual salt ions. The purified CMHAS(Na) was then washed with acetone to remove water excess, filtered and dried in oven (Blue M model 200A, USA) for 24 h at 60 °C. The powder was finally screened over a 300 μm sieve prior to use.

The reaction yield (Y) is defined as

$$Y\,[\%] = \frac{\mathrm{DS}}{\mathrm{DS_t}} \times 100 \tag{1}$$

for which $\mathrm{DS_t}$ is the maximum theoretical degree of substitution, given by

$$\mathrm{DS_t} = \frac{n_{\mathrm{LR}}}{n_{\mathrm{GU}}} \tag{2}$$

where n_{LR} and n_{GU} are, respectively, the molar amount of NaMCA limiting reactant and glucopyranose units.

In order to further increase DS, CMHAS(Na) was also synthesized by two consecutive reactions steps using a NaMCA/GU molar ratio of 2:1 in each reaction. The synthesis conditions for the second step were identical as previously described but where the ratio of 2-propanol/water was 95:5 (w/w). For two-steps carboxymethylation reaction, the $\mathrm{DS_t}$ is defined as the sum of the individual $\mathrm{DS_t}$ of each reaction ($\sum_{i=1}^{2} \Delta \mathrm{DS}_{\mathrm{t},i}$).

Conversion to the acid form CMHAS(H) was obtained by adding 30 mL of 6 M HCl per 10 g of dry sample in a CMHAS(Na)/acetone suspension (0.1 g/mL) under continuous stirring for 120 min (Stojanovic et al., 2005). To remove the excess of acid, the CMHAS(H) was purified, washed and dried using the same protocol as for CMHAS(Na).

Two non-derivatized controls HAS were prepared under the same reaction conditions as for the synthesis of CMHAS except for: (1) HAS was treated only with 2-propanol/water obtaining $\mathrm{HAS_{IPA}}$ and (2) treated with 2-propanol/water and NaOH, obtaining $\mathrm{HAS_{NaOH}}$. Acid controls were also obtained by acid treatment with HCl and respectively identified as $\mathrm{HAS_{IPA}}$(H) and $\mathrm{HAS_{NaOH}}$(H).

2.3. Determination of degree of substitution (DS)

The DS of CMHAS and HAS controls was determined by back titration (Stojanovic et al., 2005). The CMHAS(H) weighed sample was dissolved in 40 mL of standard 0.1 M NaOH. The solution was then diluted in 100 mL of distilled water. The excess of NaOH contained in 25 mL of the solution was back-titrated with standard 0.05 M HCl using phenolphthalein as indicator. A blank (without HAS) was also titrated. The amount of CM groups is given by

$$n_{\mathrm{COOH}} = 4(V_{\mathrm{b}} - V)C_{\mathrm{HCl}} \tag{3}$$

where V_{b} is the volume of HCl used for the titration of the blank, V is the volume of titration of the sample, C_{HCl} is the concentration of the HCl and 4 is the ratio of the total solution volume over the volume sampled for titration. The DS was calculated from the following equation:

$$\mathrm{DS} = \frac{162 \times n_{\mathrm{COOH}}}{m_{\mathrm{dry}} - 58 \times n_{\mathrm{COOH}}} \tag{4}$$

where 162 g/mol is the molecular weight of a GU, 58 g/mol is the increase in molecular weight accounted for each CM group substitution and m_{dry} is the mass of the dry sample.

2.4. CMHAS particles and powder characterization

2.4.1. Particle size and size distribution

The granulometry of the CMHAS particles was determined using a transmitted light microscopy (Leica model DM2500 M, Germany) interfaced with a color camera (Clemex model L 2.0C CL-13-211, Canada) and an image analysis software (Clemex Vision PE, Canada). Powder samples were randomly spread on a glass slide to obtain a representative sample. Ten thousand particles were observed under a 200× magnification and analysed with a developed algorithm. This algorithm incorporated image processing, gray thresholding and geometric constraint to first differentiate the particles from the background and then to eliminate overlapping particles and particles located at the boundary of the frame. Particles were then characterized by their mean particle size ($\bar{D}$) and their cumulative particle size distribution (D_{10}, D_{50} and D_{90}) where the size of one particle represents the average value of 64 ferets (distance between two parallel tangents on each side of the particle).

2.4.2. Particle morphology

Morphology of the CMHAS particles was examined with a Hitachi S-4300SE/N with variable pressure scanning electron microscope (SEM) (Hitachi High Technologies America, USA), at 15.0 kV and under a magnification of 1000×. Samples were prepared on metallic studs using double-sided conductive tape.

2.4.3. Bulk and tapped densities

The bulk (ρ_b) and the tapped (ρ_t) densities of the powder were determined according to USP method <616> (US Pharmacopeia XXXI, 2008). For each sample, the Carr index (CI, also compressibility index) (Carr, 1965) and the Hausner ratio (HR) (Hausner, 1967) were respectively calculated from Eqs. (5) and (6) interpreted as per USP method <1174> (US Pharmacopeia XXXI, 2008):

$$\mathrm{CI} = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \tag{5}$$

$$\mathrm{HR} = \frac{\rho_t}{\rho_b} \tag{6}$$

2.4.4. Water content

The water content of the powder was measured by Karl-Fisher titration (795 KFT Titrino, Metrohm, USA) according to USP <921>, method 1c (US Pharmacopeia XXXI, 2008).

2.4.5. Relative solubility

The relative solubility of the CMHAS derivatives and the HAS controls was determined following Chen and Jane (1994a) and Volkert et al. (2004) with minor modifications. The method consisted of precisely weighing a dry CMHAS sample (1.0 g) and dissolving it in 10 mL of neutral nanopure water or in acidic medium (0.1 M HCl, pH 1.0) at 25 °C. The solution was vortexed for 1 min and stored at room temperature for 2 h. The solution was then centrifuged for 15 min at 4000 rpm (IEC model HH-II centrifuge, USA), and 5 mL of the supernatant liquid was evaporated in oven (Blue M model OV-12A, USA) at 105 °C until a constant mass was weighed. The mass of dry residue was used to calculate the relative solubility of the sample.

2.5. CMHAS tablet characterization

2.5.1. Tablet preparation

Tablets (500 mg, 12 mm-diameter) of CMHAS derivatives and of HAS controls were formulated with acetaminophen (20%, w/w loading), as drug model, by dry blending and direct compression at 20 kN using flat faced toolings on hydraulic press (Carver, model Mini C, USA). Tablets were finally dedusted over a 600 μm screen.

2.5.2. Physical characterization of tablets

Tablet crushing strength was determined ($n = 6$) with a Vankel tester (model VK200, Varian Inc., USA) according to USP method <1217> tablet breaking force (US Pharmacopeia XXXI, 2008). Tablet friability was determined ($n = 2$) with a Vankel friabilator (Varian Inc., USA) according to USP method <1216> (US Pharmacopeia XXXI, 2008). Finally, thickness of the tablets was measured with a digital calliper.

2.5.3. In vitro drug release properties

The dissolution kinetics of the CMHAS(Na) tablets and controls ($n = 3$) were performed in USP dissolution apparatus II (Distek 5100, USA) at 50 rpm in three different media (900 mL, 37 °C): pepsin-free simulated gastric fluid (SGF, pH 1.2), pancreatin-free simulated intestinal fluid (SIF, pH 6.8) and simulated intestinal fluid containing pancreatin (Pan, pH 6.8) (US Pharmacopeia XXXI, 2008). The dissolution kinetics of the CMHAS(H) tablets were performed in SGF and SIF only for comparison with the CMAHS(Na). Acetaminophen release profiles were measured by UV spectroscopy at 280 nm (Hewlett-Packard spectrophotometer, USA).

To evaluate the drug release mechanism from the CMHAS tablets, the empirical model of Peppas and Sahlin (1989) was used:

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{(2m)} \tag{7}$$

where M_t/M_∞ is the fraction of drug released in time t, k_1 and k_2 are kinetic constants and m is the purely Fickian release exponent. The two terms on the right side of Eq. (7) represent, respectively, the Fickian diffusional contribution (F) and polymer relaxation/erosion (R) contribution to the release profile. The fraction of released drug was fitted, up to $M_t/M_\infty = 0.6$ (60% release), by nonlinear regression to Eq. (7) using Sigmaplot v.11 (Systat software Inc., USA) to determine the values of kinetic constants.

3. Results and discussion

3.1. CMHAS synthesis

The described method of HAS carboxymethylation in non-aqueous medium allowed synthesis of CMHAS of DS as high as 1.23 with a satisfactory yield of 72% for one step synthesis. DS and yield (Y) of the CMHAS synthesis obtained according to the molar ratio of NaMCA/GU (mol/mol) or DS_t are presented in Fig. 1. First of all, DS increased with the increase of the DS_t. On the other hand, the Y of

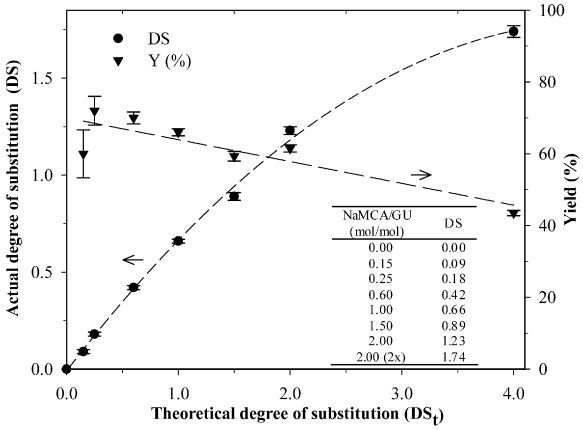

Fig. 1. Actual degree of substitution (DS) and reaction yield (Y) as function of the theoretical DS of CMHAS synthesized in non-aqueous medium ($n = 3$).

Table 1
Particle size and size distribution ($n > 10\,000$ particles), bulk (ρ_B) and tapped (ρ_T) densities, Carr's index (CI) and Hausner ratio (HR) ($n = 3$) of CMHAS(Na) and CMHAS(H) particles.

DS (form)	Granulometry (μm)				Densities and powder flow			
	$\bar{D}$	D_{10}	D_{50}	D_{90}	ρ_B (g/cm^3)[a]	ρ_T (g/cm^3)[a]	CI (%)	HR
HAS_{IPA}								
–	15.31 ± 6.11	7.91	14.38	24.33	0.46	0.75	39 ± 6	1.63 ± 0.11
H	14.26 ± 5.43	7.81	13.45	22.33	0.46	0.75	39 ± 6	1.63 ± 0.11
HAS_{NaOH}								
–	15.42 ± 6.33	7.99	14.40	24.94	0.37	0.63	41 ± 8	1.70 ± 0.15
H	13.35 ± 5.40	7.56	12.08	21.57	0.36	0.64	44 ± 8	1.78 ± 0.15
0.09								
Na	19.55 ± 6.13	8.56	19.40	27.79	0.45	0.64	30 ± 7	1.42 ± 0.11
H	18.76 ± 5.09	8.15	18.22	26.91	0.44	0.64	31 ± 7	1.45 ± 0.11
0.18								
Na	23.93 ± 8.90	12.86	23.18	36.16	0.47	0.67	30 ± 7	1.43 ± 0.10
H	21.28 ± 6.25	11.03	22.05	32.20	0.47	0.69	32 ± 7	1.47 ± 0.11
0.42								
Na	25.79 ± 6.57	13.37	26.41	34.14	0.51	0.75	32 ± 6	1.47 ± 0.10
H	22.66 ± 6.31	13.99	23.13	30.86	0.49	0.78	37 ± 6	1.59 ± 0.11
0.66								
Na	28.07 ± 11.17	15.20	26.51	43.35	0.66	0.87	24 ± 5	1.32 ± 0.07
H	28.70 ± 9.94	15.60	28.26	42.55	0.64	0.87	26 ± 5	1.36 ± 0.07
0.89								
Na	48.00 ± 20.96	22.07	46.15	78.07	0.72	0.96	25 ± 5	1.33 ± 0.07
H	47.03 ± 18.13	23.18	44.83	73.20	0.68	0.98	31 ± 5	1.44 ± 0.07
1.23								
Na	54.71 ± 25.88	26.32	49.08	94.88	0.71	1.04	32 ± 4	1.46 ± 0.08
H	53.88 ± 23.87	25.52	49.76	87.22	0.65	0.93	30 ± 5	1.43 ± 0.07
1.74								
Na	54.90 ± 24.26	26.78	50.58	89.37	0.73	0.98	26 ± 5	1.34 ± 0.07
H	52.32 ± 23.25	25.74	48.16	84.77	0.64	0.92	30 ± 5	1.44 ± 0.08

[a] Standard deviation equal or under 0.02.

reaction decreased with the increase of DS and DS_t probably due to some competitive undesirable side reaction of NaMCA with NaOH that can form sodium glycolate (Heinze et al., 2004; Stojanovic et al., 2000). Indeed the increase of the ratio of NaMCA/NaOH favoured this side reaction (Kooijman et al., 2003). For the synthesis with a low molar ratio of NaMCA/GU (0.15:1), a lower concentration of NaMCA in the reaction medium can reduce the probability to react with starch granules prior to aqueous NaOH which could explain the lower reaction *Y*.

The *Y* of the two-step carboxymethylation showed also notable diminution since the $Y(\Delta DS_{(2-1)}/\Delta DS_{t(2-1)})$ of the second reaction was only 25%. This could be explained by the increased difficulty of NaMCA to react at re-synthesis with the remaining hydroxyl groups with the increase of the DS due to steric hindrance (Sangseethong et al., 2005) or electrostatic repulsion caused by the CM groups (Heinze and Koschella, 2005). Moreover, the diminution of the Y can be explained by the fact that in order to avoid swelling and agglomeration of particles during the non-aqueous synthesis for increase DS (Tijsen et al., 1999; Volkert et al., 2004), the water mass fraction was reduced to 0.05. A reduction of water mass fraction in the reaction decreased the content of NaOH and NaMCA dissolved in the aqueous phase, hindering the diffusion of reagent into the CMHAS granules by reducing their swelling.

3.2. CMHAS particles and powder characterization

Physical properties such as particle size and granulometry, morphology, water content, solubility and resulting flow and compression characteristics are among the factors that can influence the kinetics of drug release from solid dosage forms.

3.2.1. Particle size and size distribution

There is a direct correlation between DS and particle size for both CMHAS(Na) and CMHAS(H), all larger than the controls HAS_{IPA} and HAS_{NaOH} (Table 1). All obtained particles showed a unimodal close to Gaussian particle size distribution. The CMHAS with a DS in the range 0.09–0.66, only led to a slight increase of the $\bar{D}$ from 19 to 28 μm but with a wider particle size distribution. The conformation of CMHAS chains is characterized by single-helical. The increase of DS from 0.66 to 0.89 caused a significant increase of the CMHAS $\bar{D}$ up to 48 μm with narrower size distribution relative to median point. The increase of the DS to 1.23 induced a minor increase up to 54 μm of the $\bar{D}$ with a slightly wider particle size distribution. This size value appears to be the highest and a plateau $\bar{D}$ with no significant differences for further increased of the DS to 1.74 was observed.

3.2.2. Particle morphology

The study of the morphology of the particles (Fig. 2) allowed to explain the mechanism that controlled the increase of the $\bar{D}$ with the increase of the DS. Firstly, the comparison of the HAS_{IPA} particles with the native Hylon VII particles (Fig. 2a and j), showed that the alcohol/heat treatment did not induce any visible modification of the particle morphology. Under these conditions, the swelling of the starch granules by the absorption of water is low (<10% increase in diameter) and reversible suggesting that HAS_{IPA} and native HAS have equivalent properties. Secondly, Fig. 2b–f shows that HAS_{NaOH} and CMHAS with a DS of 0.09–0.66 had similar particles morphology characterized by a deformed sphere shape with an indented surface when compared to the sphere like shapes with a smooth surface of the HAS_{IPA} particles. Deformed surfaces were probably created under alkaline conditions, where the structure

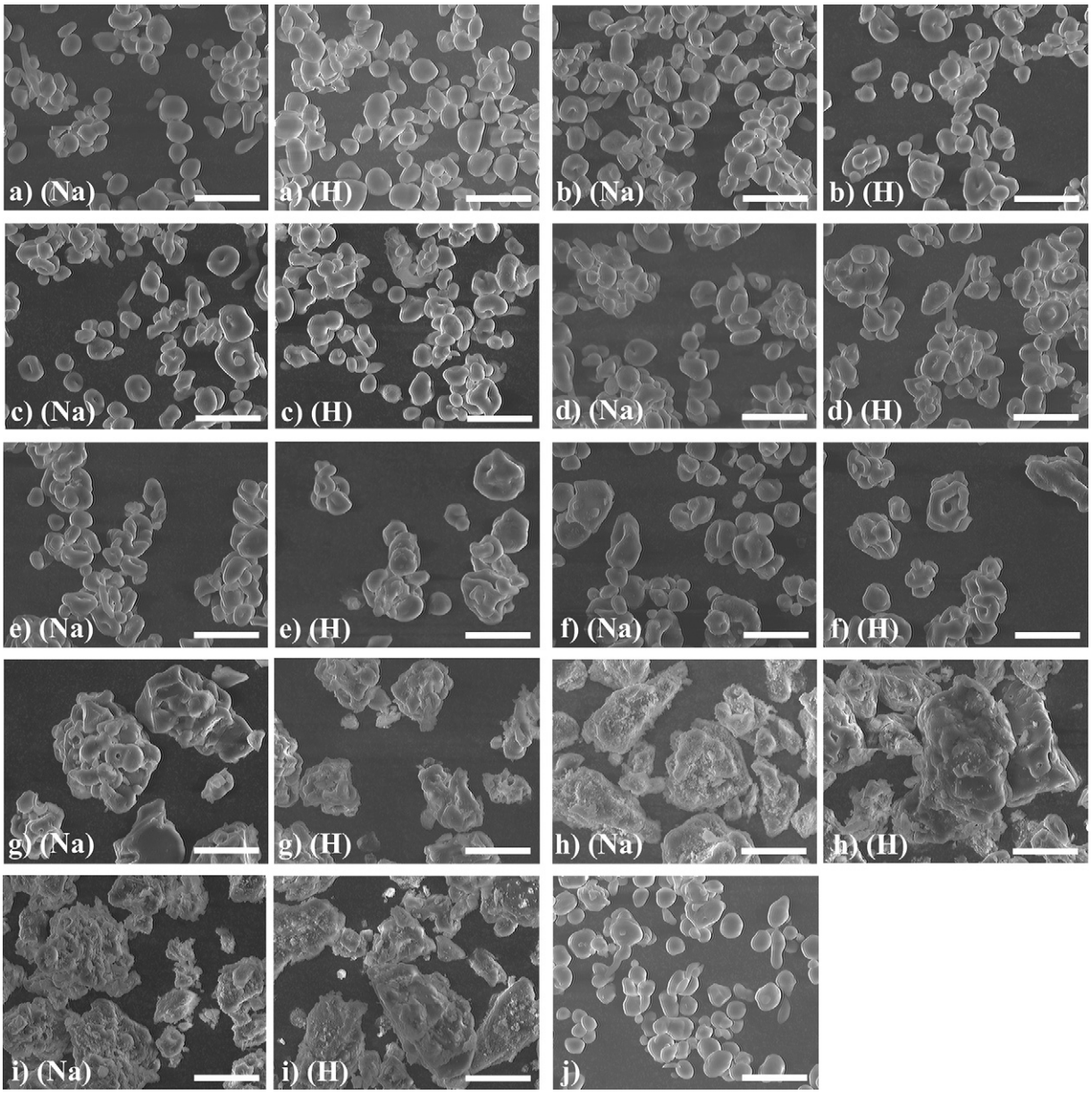

Fig. 2. Scanning electron micrograph of sodium CMHAS(NA) and protonated CMHAS(H) particles of: (a) HAS_{IPA}, (b) HAS_{NaOH}, DS of (c) 0.09, (d) 0.18, (e) 0.42, (f) 0.66, (g) 0.89, (h) 1.23, (i) 1.74 and (j) native HAS (SEM at a voltage of 15.0 kV and a magnification of 1000×; scale bar 25 μm).

of the swollen particles was consequently weakened and easily altered by the vigorous stirring in the reactor, as well as by removal of the alcohol and dehydration process during the final washing and drying phase. Moreover, Fig. 2 suggests that the reaction occurred not only at the surface but also inside the starch granules. Thirdly, at a DS of 0.89 (Fig. 2g), CMHAS particles are agglomerated but individual particles could still be distinguished at the surface of the agglomerates. To avoid this phenomenon, the water mass fraction should be reduced, but with the consequence of reducing the reaction yield as previously discussed. Finally, with DS of 1.23 and 1.74 (Fig. 2h and i), the CMHAS particles were fully agglomerated and showed irregular prismatic morphology.

Table 1 and Fig. 2 show that no significant difference prevailed between the CMHAS(Na) and CMHAS(H) in terms of $\bar{D}$, granulometry and particle morphology as a function of DS, suggesting that mainly protonation occurred during the acid treatment conversion.

3.2.3. Bulk and tapped densities

Density values and resulting flowability are presented in Table 1. The increase from 0.5 to 0.7 of the ρ_B and from 0.75 to 1.0 of the ρ_T, with the increase of the DS for both CMHAS(Na) and (H) may be, in a certain extent, ascribed to the increase of the average molecular mass of the CMHAS chains by the addition of the CM groups to the GU. The increase of ρ_B and the ρ_T can also be correlated to the increase of the CMHAS particles $\bar{D}$. Moreover, the increase of ρ_B and ρ_T were not found to be directly proportional to the DS, to alteration of the particle morphology and to variations in water content into the CMHAS particles. Probably the substitution with highly polar carboxylic groups and water retention increased the flowability via density. According to Carr index (CI) and Hausner ratio (HR) values of Table 1, flowability of the CMHAS lies between passable and very poor (CI = 24–32%, HR = 1.32–1.59), which represents an improvement when compared to controls HAS_{IPA} and the HAS_{NaOH} both classified as very, very poor (CI > 39, HR > 1.60) following US Pharmacopeia XXXI (2008). Nevertheless, all obtained

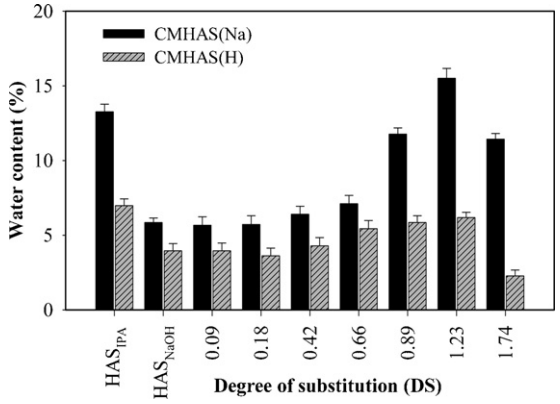

Fig. 3. Water content (%) of CMHAS(Na) and of CMHAS(H) particles.

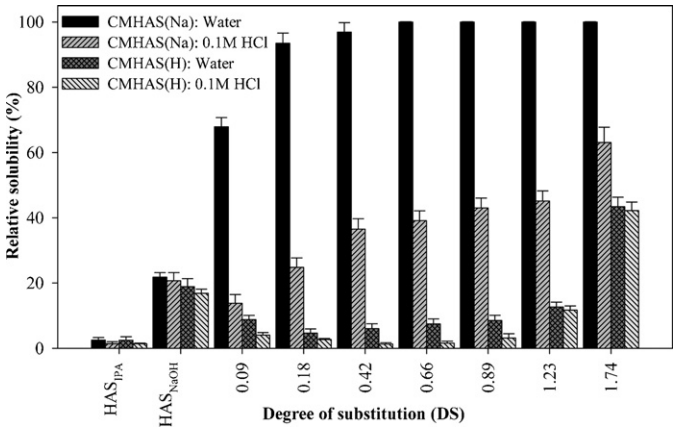

Fig. 4. Relative solubility (%) of CMHAS(Na) and of CMHAS(H) dry powders in nanopure water (pH 7.0) and in HCl 0.1M (pH 1.0).

CMHAS presented fairly high and typical density values compared to standard common excipients (Rowe et al., 2006).

3.2.4. Water content

Fig. 3 shows that moisture of increased from 5.8% (the base value of HAS_{NaOH}) to 15.5% for CMHAS(Na) with the increase of the DS up to 1.23. This trend is probably due to the hydrophilic nature of the CM(Na) groups. The water content at the base value of 5.8% is required by the polymer to maintain its physical integrity. Water content value went down from 15.5 to 12% for the high DS of 1.74, obtained by the two-steps reaction. This decrease of moisture was probably due to intensive washing of the powder with organic solvents after carboxymethylation reactions as well as to the reduction of the 2-propanol/water ratio in the reaction medium during the second carboxymethylation step. The higher water content of HAS_{IPA} of 13.3% versus HAS_{NaOH} controls showed that physical modifications (swelling, deformation) of the particles under alkaline treatment allowed more water to be removed during the washing process. The acid treatment for the conversion of the CMHAS(Na) to CMHAS(H) appears responsible for the decreased moisture for control and CMHAS. A slight increase of moisture was noticed with increased DS up to 1.23 followed by a decrease for DS of 1.74, more important than for the CMHAS(Na). Lesser moisture can be explained by the lower hydrophilicity of the CM(H) groups compared to the CM(Na) groups.

3.2.5. Relative solubility

Addition of highly hydrophilic CM(Na) groups of to the starch generates an increase of the relative solubility in cold water. This CMHAS(Na) presents a good water solubility (Fig. 4) at DS above 0.18 (with relative solubility of about 93.5%). Even at very low DS (0.09), the 65% relative solubility of CMHAS(Na) was considerably higher when compared to the insoluble HAS_{IPA} (relative solubility lesser than 2.5%) and to the poorly soluble HAS_{NaOH} (relative solubility slightly less than 20%). The low solubility of the HAS_{IPA} further confirmed that its properties were similar to those of the native HAS which is insoluble in cold water. Furthermore, the higher solubility of the HAS_{NaOH} compared to that of HAS_{IPA} showed that the crystalline starch structure was altered during the synthesis of CMHAS(Na). Indeed, under alcohol-alkaline conditions, the hydroxyl groups of the starch chains (–OH) are probably converted into alkoxide groups ($–O^-$) disrupting the hydrogen bound between the GU causing extensive swelling of the starch granules (Chen and Jane, 1994a,b) leading to the breakup of crystalline structure by uncoiling and dissociation of the double-helical chains. After neutralization, the starch chains were reported to formed single-helical complexes with alcohol (V-type pattern). The significant decrease of the relative solubility of CMHAS(Na) when placed into 0.1 M HCl seems related to the protonation. The solubility of the acid form of CMHAS(H) was markedly low in both water and 0.1 M HCl media. This behavior fits well with characteristics of non-dissociated carboxylic acid (Heinze and Koschella, 2005). Solubility of CMHAS(H) in both solutions increased with the increase of the DS as for (but less evident) the CMHAS(Na). For CMHAS(H), the noticeable increase in relative solubility at highest DS of 1.74 was probably due to the high amount of CM groups that can increase the hydration of the CMHAS chains through hydrogen association between carboxylic group and water leading to higher hydration and solubilisation.

3.3. CMHAS tablets characterisation

3.3.1. Physical characterization of tablets

Direct compression properties of CMHAS are mainly related to the plastic deformation of the particles under strength leading to formation of bonds between the surface of the deformed particles by the action of distance forces (van der Waals, hydrogen bonds), solid bridges and mechanical interlocking (Adolfsson et al., 1997; Nystrom and Karehill, 1996). For a specific compression force, a decrease in $\bar{D}$, an increase of the particle roughness, and optimal water content will result in a higher bonding surface area between the deformed particles and, subsequently, in a higher tablet strength (Korhonen et al., 2002). Particles deformation and association were assessed by the thickness of the tablets. Fig. 5a and b revealed that the thickness of the CMHAS tablets cannot be correlated to their crushing strength. Indeed, the thickness of CMHAS tablets decreased from a DS of 0.09–0.18 and remained similar for a DS of 0.18–0.66 while the crushing strength decreased, suggesting additional contributions of elastic deformation at these DS. The thickness of the CMHAS tablets continued to decrease with increasing DS to reach the minimum crushing strength value at a DS of 0.89, followed by a considerable increase at a DS of 1.23. Further increase of the DS (1.74), produced thinner tablets with higher crushing strength. These results suggested that CM groups or the modification of the CMHAS chain structure according to the DS, may play a role in the compaction properties of CMHAS. No significant difference was observed between the CMHAS(Na) and the CMHAS(H) tablets thickness and crushing strength indicating that only limited physical modifications occurred during the acid treatment for a DS up to 0.66. At DS higher than 0.89 increased crushing strength of the CMHAS(Na) versus CMHAS(H) tablets may be due to the difference of the water content (Fig. 3). In fact, water have participated at stabilization by hydrogen bonding, and can act like a plasticizer during the compression of plastic deforming polymer (Amidon and Houghton, 1995; Gupta et al., 2005; Sun, 2008). The friability of

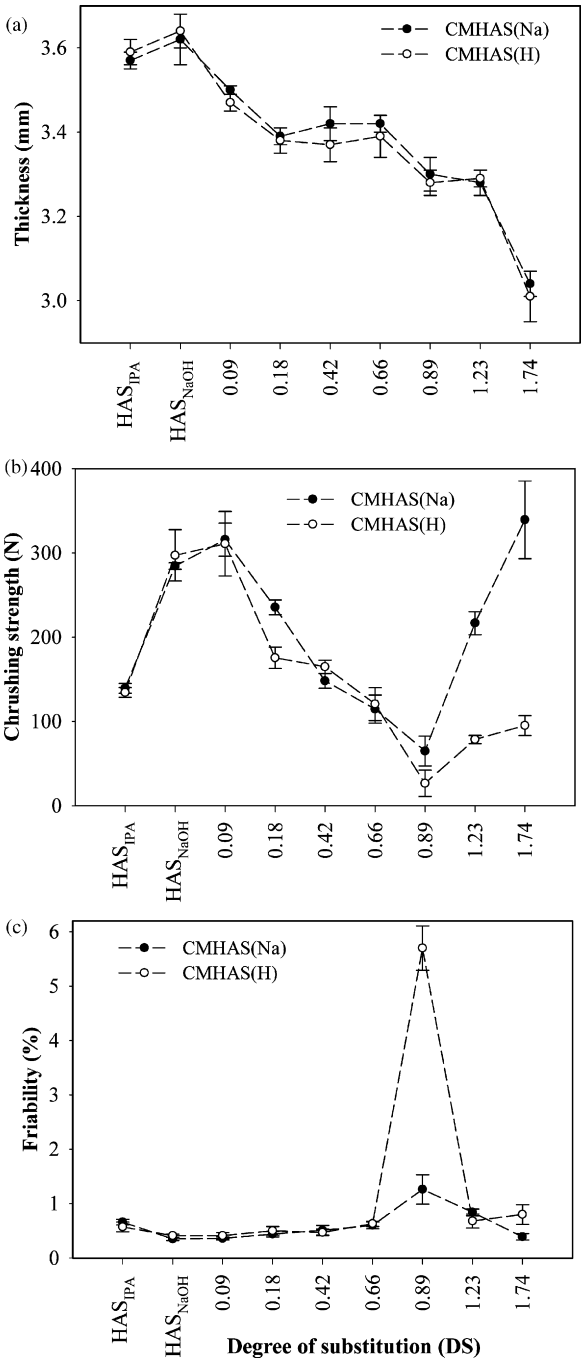

Fig. 5. (a) Thickness ($n = 6$), (b) crushing strength ($n = 6$) and (c) friability ($n = 2$) of CMHAS(Na) and CMHAS(H) monolithic tablets (500 mg, diameter 12 mm) loaded with acetaminophen (20%, w/w).

CMHAS tablets decreased with increasing tablet crushing strength (Fig. 5b and c). All CMHAS tablets showed low friability (<1%), except for 0.89 DS, where low crushing strength resulted in high friability. These results show that both CMHAS(Na) and CMHAS(H) with low DS (<0.18) and CMHAS(Na) with high DS (>1.23), have good compression properties, significantly better than the HAS_{IPA} control.

3.3.2. *In vitro drug release properties*

Figs. 6 and 8 present the dissolution profiles in SGF and SIF media obtained for CMHAS(Na) and CMHAS(H), respectively. Fig. 9 presents the dissolution profiles obtained for CMHAS(Na) in SIF

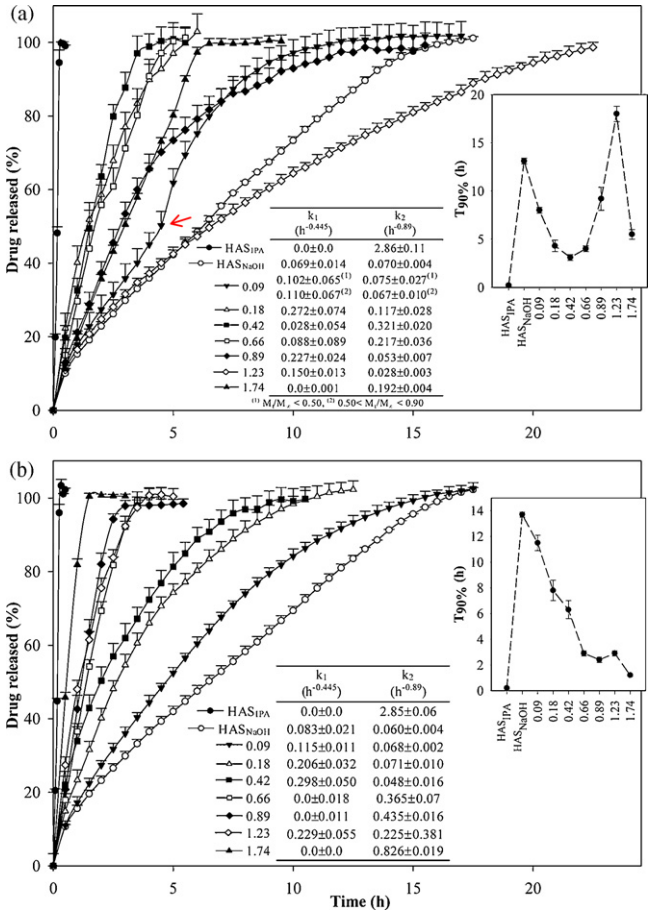

Fig. 6. Dissolution profiles of acetaminophen from CMHAS(Na) tablets (500 mg, 20%, w/w loading) in: (a) simulated gastric fluid (SGF, pH 1.2) and (b) pancreatin-free simulated intestinal fluid (SIF, pH 6.8).

with pancreatin (containing α-amylase) preparation (Pan). In each of these figures, an insert presents the 90% drug release time ($T_{90\%}$) and a table insert presents the kinetic constants, k_1 and k_2 (Eq. (7)) obtained by non linear regression ($R^2 > 0.95$ and p-value < 0.01). The Fickian release exponent (m) used in this study (0.445) was an average value obtained from the correlation of Peppas and Sahlin (1989) with the minimal and maximal CMHAS tablet thickness (Fig. 5a). These statistic assumptions were not used for profiles obtained from the CMHAS(H) with a DS of 1.23 tablets in SIF (Fig. 8b) where a high burst effect induced a lack of fitting to the model ($R^2 < 0.4$). Furthermore, for fast dissolution release profiles (90% release in less than 15 min), kinetic constants were not determined since insufficient data were available.

The mechanism and the rate of drug release from a hydrophilic polymer matrix can be a function of water permeation, polymer swelling, drug dissolution and diffusion, and matrix erosion (Colombo et al., 2000). These factors can affect the gel network structure and strength due to physical properties of the polymer itself, the polymer chain–chain interaction and polymer chain–solvent interactions. Each dissolution test with HAS_{IPA} as tablet matrix resulted in rapid drug release due to a fast erosion (about 10 min) of the tablet. On the other hand, HAS_{NaOH} showed pH independent sustained release over 15 h probably related to the formation of a strong gel-layer by hydrogen bonding between the hydroxyl groups of the soluble gelled starch chains (Fig. 4). For HAS_{NaOH}(H) faster drug release and higher constants were obtained compared to those of the HAS_{NaOH}(Na) due to a prominent burst effect. However, after full matrix hydration (7 h), differences in dissolution profiles between the two HAS_{NaOH} (Na, H) disappeared.

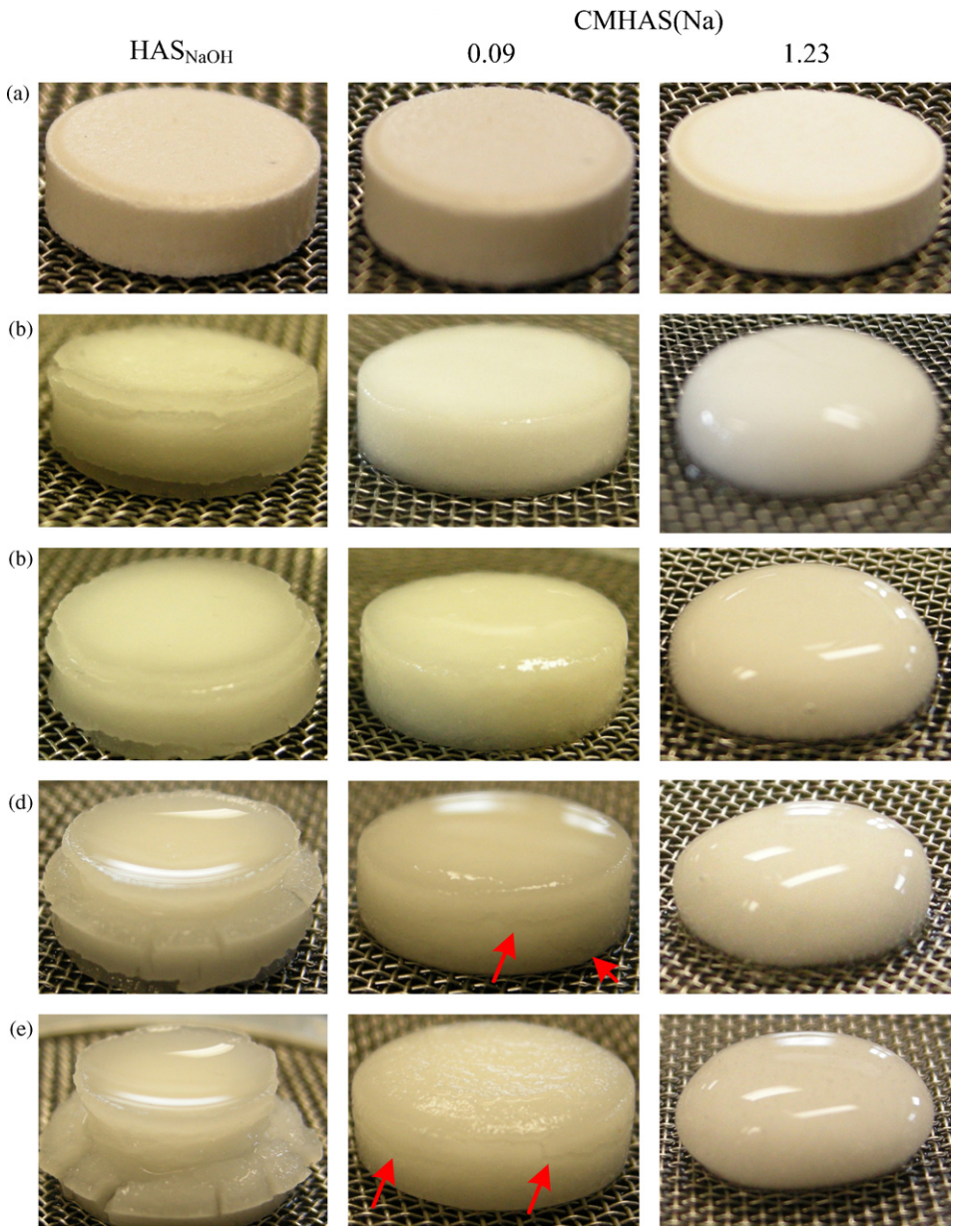

Fig. 7. Physical appearance of typical tablets of HAS_{NaOH} and CMHAS(Na) with a DS of 0.09 and 1.23 incubated in SGF after: (a) initial (0 h), (b) 3 h, (c) 5 h, (d) 7 h and (e) 9 h.

Fig. 6a shows a non-monotonous relationship of the $T_{90\%}$ release from CMHAS(Na) with the DS in SGF. Such non-monotonous dependency release rate versus DS of CMHAS(Na) was not found in SIF (Fig. 6b). At low DS in SGF, the formation of the gel-layer seems favoured by hydrogen bonds between hydroxyl groups, hydroxyl group with CM groups and between protonated CM groups (increasing with the DS). Increasing drug release rate in SGF for DS (0.09–0.42) can be due to higher water content in the outer gel-layer and thus enhancing water permeation inside the matrix which increases the drug diffusion through the gel-layer and the swelling of the matrix as shown by the increase of the k_1 and k_2 constants (Fig. 6a – table insert). For moderately low DS, the higher hydrogen association between the polymer chains by dimerization of protonated CM groups (COOH) was not sufficient to counterbalance the swelling/erosion forces with the penetration in the gel-layer of the dissolution medium, due to higher solubility of CMHAS(Na) in SGF (Fig. 4). The formation of a gel-layer appeared stronger with higher DS up to 1.23, and was associated to a decrease of both k_1 and k_2 constants. These results also showed that tablet strength appeared to have only a limited influence on the drug release properties for CMHAS(Na) with high DS. Indeed, despite the fact that CMHAS(Na) tablets with a DS of 0.89 presented the lowest crushing strength (Fig. 5b), they did not show a faster dissolution rate in SGF. For CMHAS(Na) tablets with a DS of 1.74, with the highest tablet crushing strength, the reduction of the $T_{90\%}$ was probably due to continuous dissolution of the gel-layer induced by a marked increase of the chain solubility in SGF at this DS (Fig. 4).

The dissolution profile of the CMHAS(Na) tablet with a DS of 0.09 presented a significant discontinuity (Fig. 6a) in the drug release rate after 4.75 h in the SGF. A higher drug dissolution rate in the gel-layer can enhance the drug concentration and more water penetration inside the gel-layer inducing a higher swelling of the polymer chains (Colombo et al., 1999). In our case, if the swelling kinetics of the polymer chain is low and the strength of the gel-layer is high, as shown by the slow drug release before 4.75 h for the CMHAS(Na) at DS of 0.09, the increased amount of dissolved drug

in the gel-layer and dissolution medium can increase the osmotic pressure inside the gel-layer, thus, at a certain time, accelerating the release rate. This phenomenon seems to be specific to this DS since no other dissolution profile had shown such discontinuity. This discontinuity in SGF was not the result of or did not induce the so-called "cracking" phenomenon as reported with CMHAS with low DS in SIF, since tablet and gel-layer integrity were maintained intact before and after the discontinuity (Fig. 7c and d). In fact a rapid and significant loss of gel-layer integrity was observed only for the HAS_{NaOH} tablets in all dissolution media. For the DS 0.09 CMHAS(Na) tablet, a low surface cracking appears only after 7 h in the SGF (Fig. 7d) and also in SIF (not shown) probably resulted from the low amount CM groups which can induce non-uniform polymer chain swelling. The presence of CM groups can alter the hydrogen bonding between the hydroxyl groups involved of the V-type structure (Sarko and Wu, 1978) by higher hydration of the CM groups causing higher swelling of the regions closer to CM groups compared to unsubstituted regions. For the HAS_{NaOH} tablet, this cracking probably resulted from differences between axial and radial swelling of the polymer chain due to non-uniform tablet hydration. At higher DS (≥0.18), no cracking was observed since the gel-layer of these tablets slowly dissolved in SIF or eroded with the hydration front advancing inside the matrix due to the higher hydration of the gel-layer associated with the increase of the DS. As explanation, a typical shape of CMHAS(Na) tablet with a DS of 1.23 is presented in Fig. 7. However, this tablet cracking did not induce modification of the drug release mechanism, shown by the continuous dissolution profile of HAS_{NaOH} tablets and the almost identical kinetic constants before and after the discontinuity of DS 0.09 CMHAS(Na) (Fig. 6a) since the drug was released from the gel-layer before the cracking occurred. Nevertheless, for other drugs with different release mechanisms this eventual cracking at low DS can induce uncontrolled and unpredictable drug release rates through the gastrointestinal tract, undesirable for medical treatment. Therefore, a minimal DS, affording enough hydration of the tablet and uniform swelling is necessary for the use CMHAS(Na) synthesized in non-aqueous media as controlled delivery excipient. This minimum DS can be equal to or slightly higher than 0.1 since at a DS of 0.09 the cracking of the gel-layer occurred late during the dissolution tests and was relatively low compared to the cracking of the HAS_{NaOH}.

The increase of drug release rates in SIF (pH 6.8) from CMHAS(Na) tablets with increased DS (Fig. 6b) is probably due to higher hydration of the gel-layer and increased repulsive forces between dissociate CM groups (–COO^-). Indeed, from a DS of 0.09–0.42 the decrease of the $T_{90\%}$ was associated with lower gel-layer strength due to increasing hydration in the gel-layer and therefore increased diffusion rate of the drug (k_1). At higher DS (0.66 and more), the drug release was accelerated by fast erosion of the gel-layer probably enhanced by higher repulsive forces between CM groups (–COO^-) as shown by the marked increase value of k_2 and the close to zero value of the constant k_1 (Fig. 6b – table insert). This DS of 0.66 seems allowing the dimerization of the CM groups in SGF (Fig. 6a) sufficient to counterbalance the swelling/erosion force in the gel-layer of the tablets."

Overall, CMHAS with high DS (≥0.66) showed slower release in SGF than in SIF. In SGF higher attractive interchain hydrogen bonding from the dimerized protonated CM groups and the lower swellability of the polymer caused reduced water penetration and slower drug release rate, whereas the repulsive forces between ionized CM groups (–COO^-) and the Na^+ with its hydration sphere enhance the release rate in SIF. However, CMHAS with lower DS (≤0.42), where repulsive interactions between CM groups or hydration are low, showed faster release in SGF than in SIF. The small difference in solubility of acetaminophen (Takahashi et al., 2005) in SGF (21.3 mg/mL) and in SIF (17.8 mg/mL), was not sufficient to explain dissolution results for CMHAS(Na) at low DS.

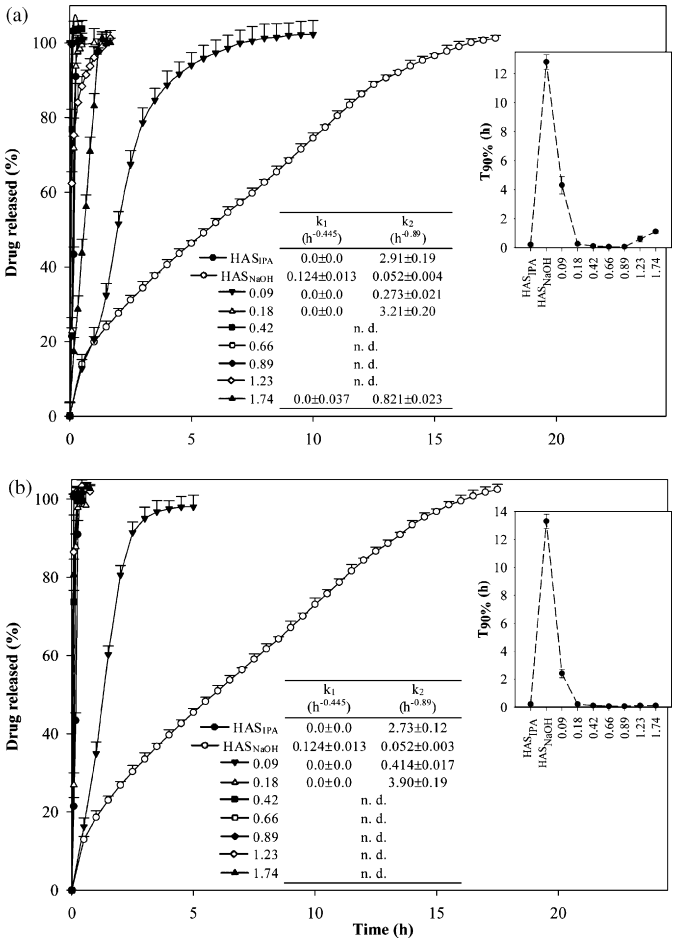

Fig. 8. Dissolution profiles of acetaminophen from CMHAS(H) tablets (500 mg, 20%, w/w loading) in: (a) simulated gastric fluid (SGF, pH 1.2) and (b) pancreatin-free simulated intestinal fluid (SIF, pH 6.8).

The drug release from CMHAS(H) tablets with DS of 0.18 and higher (Fig. 8) was very fast, resulted from the rapid disintegration of the tablet in both dissolution media. The low solubility of CMHAS(H) chains in both media (Fig. 4), the low water content (Fig. 3) and reduced tablet strength at high DS (>0.89) (Fig. 5b) allow fast water penetration inside the matrix, annihilating the cohesive forces between the particles and inducing a faster swelling without formation of a gel network controlling the drug release. The only exception was for DS of 0.09 in both dissolution media and for 1.74 DS in SGF. For DS 0.09, the slower swelling rate allowed the formation of a weak gel-layer mainly from hydrogen bonding between hydroxyl groups, as for the HAS_{NaOH}(H). For DS 1.74, a significant increase of the solubility (Fig. 4) of CMHAS(H) can explain the lower dissolution rate in SGF since the solubilisation of the chains will allow the formation of hydrogen association between protonated CM groups and thus the formation of a gel-layer. Differently to CMHAS(Na) with a DS of 0.09 and a DS of 1.74, the corresponding CMHAS(H) presents a lower drug release rate in SGF than in SIF. Increasing the solubility of CMHAS(Na) chains allows the formation of the drug release controlling gel-layer. The presence of the Na^+ ion induced stabilization of the gel-layer in SIF. At low DS (under 0.42), stabilization of the gel-layer was probably due to a reduction of the osmotic pressure inside the gel, explained by the Donnan equilibrium (Khare and Peppas, 1995). Indeed, in a gel network with weak immobile charge (–COO^-), the presence of solubilized Na^+

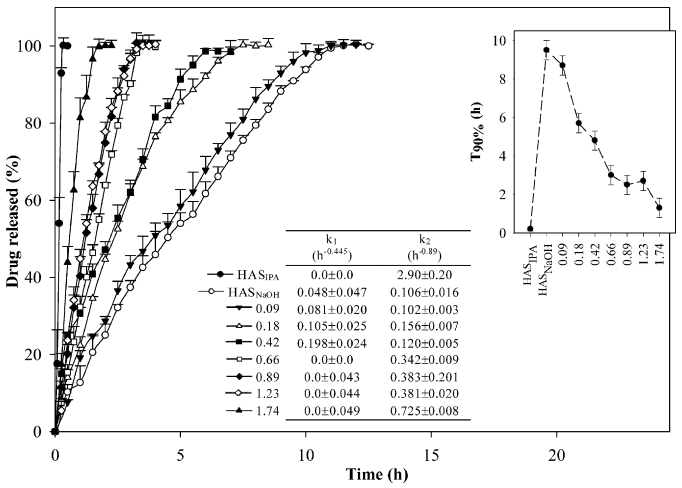

Fig. 9. Dissolution profiles of acetaminophen from CMHAS(Na) tablets (500 mg, 20%, w/w loading) in simulated intestinal fluid with pancreatin (Pan, pH 6.8).

mobile counterions inside the gel network of the CMHAS(Na) tablet resulting from the dissociation of the CM groups in SIF, reduced the osmotic pressure and thus the water penetration inside the gel-layer owing to slower drug release rate compared to the drug release rate from CMHAS(Na) tablet in SGF. At high DS (above 0.66), stabilization of the gel-layer was probably due to the stabilization of the forces between dissociated CM groups through ionic interaction and/or the Na^+ ion (Mulhbacher et al., 2004; Nabais et al., 2007).

Finally, in order to evaluate the influence of α-amylase on CMHAS, the drug release kinetics from the CMHAS(Na) tablets were evaluated in SIF medium containing Pancreatin (Pan) (Fig. 9). The hydrolytic action of α-amylase is expected to be proportionally reduced with the increase of DS due to steric hindrance of CMHAS substrate. The access to α-amylase active site is supposed limited by the presence of the CM groups (Kwon et al., 1997). The comparison of Figs. 6b and 9 showed that the $T_{90\%}$ was reduced by 31% for the HAS_{NaOH}, 24% for DS 0.09, 27% for DS 0.18 and 20% for DS 0.42 under the bioerosion action of α-amylase. This faster release caused by α-amylase was not directly proportional to DS since its action mechanism appears also as function of its diffusion through the hydrated gel-layer (Dumoulin et al., 1999). At higher DS (>0.66), no significant differences were observed between the dissolution profiles of the CMHAS(Na) tablets in the two SIF media (with and without Pan) since their drug release properties in SIF were already driven by a faster dissolution mechanism.

4. Conclusion

The aim of this study was to investigate the influence of the DS on the physical and drug release properties of CMHAS produced in non-aqueous medium. This synthesis allows carboxymethylation at high DS up to 1.23 with yields as high as 72%. Further increase of the DS to 1.74 was also obtained with two consecutive reactions steps but with significantly lower yields (43.5%). The DS and conversion of CMHAS(Na) to CMHAS(H) had a major impact on the physical properties of particles and of resulting tablets, CMHAS appearing as a versatile excipient for direct compression. Dissolution tests performed in SGF, SIF and Pan with acetaminophen as drug model showed CMHAS to be a pH sensitive hydrophilic matrix. Only the sodium salt form CMHAS(Na) seems suitable for oral solid dosage forms for controlled release since the CM(Na) groups showed adequate properties and capacity to form gel-layer in SGF and gel network in SIF. The CMHAS(Na) at low DS (between 0.1 and 0.2) can be used as excipient for sustained release formulations. The advantage of CMHAS(Na) consisted in possibility to adjust the release profiles in function of DS and to ensure an increased resistance of tablets to bioerosion from α-amylase. The CMHAS(Na) with high DS (between 0.9 and 1.2) can be an excipient of choice for delayed release since the drug dissolution rate in SGF was significantly lower than in SIF. The faster dissolution in SIF appears mostly controlled by pH rather than by α-amylase since drug release mechanism is already driven by faster swelling and erosion mechanism.

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