Preparation and properties of core–shell alginate–carboxymethylchitosan hydrogels

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Abstract

BACKGROUND: Hydrogels of alginate (ALG) with partially carboxymethylated chitosan (CMCHI) have been produced for drug delivery, based on the interactions between the negative groups and an ionic crosslinker. In the present work, CMCHI was used to evaluate the influence of amino groups that are positively charged at pH = 4 and 6 on the ALG–CMCHI core–shell hydrogel preparation. An ANOVA statistics tool was used to evaluate the effect of composition, pH and chitosan chemical nature on the morphology and swelling properties of the hydrogels in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

RESULTS: The ALG–CMCHI core–shell hydrogels presented smaller (ca. 2.3 μm) and more homogeneous microparticles than those with unmodified chitosan (ca. 5.5 μm). The ALG–CMCHI hydrogels showed higher thermal stability and lower degree of swelling in SGF (314%) compared to those with chitosan (708%), since in the former hydrogels the protective layers that surround the particles are negatively charged.

CONCLUSION: CMCHI can replace chitosan in the production of core–shell hydrogels with improved properties since the negative charge surrounding the ALG–CMCHI particles favours a lower degree of swelling. The results point out a possible prevention of burst release in SGF, sustaining the swelling ability of the ALG–CMCHI core–shell hydrogels in SIF, promising appropriate drug release.

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Keywords: hydrogels; chitosan; carboxymethylchitosan; alginate; microspheres; degree of swelling

INTRODUCTION

Advances in hydrogel technology have focused on finding biocompatible and non-toxic materials intended for pharmaceutical and biomedical applications.1,2 Alginate (ALG) is a polyanionic copolymer of mannuronic and guluronic sugar residues, while chitosan (CHI) is a polycationic polymer derived from chitin, an abundant polysaccharide present in crustacean shells. Both polymers are ionic, biodegradable, pH sensitive and non-toxic when administered orally, and have been used in the production of hydrogels at the micro- and nanoscale for site-specific delivery.3–11 ALG–CHI hydrogels offer advantages for the oral delivery of peptide and protein drugs, such as mild conditions, preventing denaturation and inactivation of the desired protein.12

CHI is known to be an effective penetration enhancer for peptides in vivo in acid environments, due to its limited solubility in acid media (pH < 6.5). On the other hand, water-soluble CHI derivatives increase the solubility of CHI at neutral pH, enhancing the penetration of drugs in the small intestine.13 Several water-soluble CHI derivatives have been produced by carboxymethylation of CHI and the carboxymethyl groups in the glucosamine units of carboxymethylchitosan (CMCHI) give special characteristics and novel properties to hydrogels.14–21 The aggregation behaviour of CMCHI in dilute aqueous solution with addition of salts19,20 has been investigated, showing that CMCHI forms aggregates at neutral pH and complexes with different sizes as a function of CaCl2 concentration.

CMCHI hydrogels physically crosslinked with calcium21 and co-valently crosslinked with glutaraldehyde22,23 have been produced for drug and protein delivery. Lin et al.21 have produced entangled interpenetrating network hydrogels, based on CMCHI and ALG crosslinked with calcium, whereas Chen et al.24 have developed a semi-interpenetrating network with CMCHI and ALG crosslinked with genipin. Miranda et al.25 have evaluated the effect of N-carboxymethylation of CHI on polymer film properties, and found CMCHI films have higher thermal stability and tension strength with lower elasticity than CHI ones. Colo et al.26 have studied the effect of chitosan hydrochloride and CMCHI, formulated in ophthalmic solutions, on the ocular pharmacokinetics of ofloxacin.

The degree of substitution (SD) of CMCHI is usually above 70 wt% and the ionic crosslinking in hydrogel formation occurs mainly between the carboxyl groups of CMCHI and ALG.21 Few studies have reported the use of CMCHI with lower SD exploring
the possible interaction between the CMCHI residual protonated amino groups and the ALG carboxyl groups.

Hydrogels for drug delivery in the gastrointestinal tract require protection from the harsh acidic environment in the stomach (pH ≈ 1.2) and the slightly alkaline environment in the intestine (pH ≈ 7.4). Thus, hydrogel swelling should be minimal in the stomach and should start in the intestinal tract due to the increasing pH. The mucoadhesive properties of these hydrogels also enhance the residence time in the intestinal media.

Hydrogel behaviour can be controlled by the preparation conditions and functional groups in the polymers. Previously, we investigated several ALG−CHI formulations in order to modulate and control the polyelectrolyte complexation and hydrogel properties. In the study reported here, with the aim of evaluating the influence of amino groups that are positively charged at pH = 4 and 6 on CMCHI−ALG hydrogels, we synthesized a CMCHI with SD of 55 wt% to ensure interactions between CMCHI amino groups and ALG carboxyl groups, in addition to those between the carboxyl groups. A factorial experimental design was used to evaluate the effect of CHI chemical modification, pH and ALG content on hydrogel particle size, thermal stability and degree of swelling in simulated gastric fluid (SGF; pH = 1.2) and simulated intestinal fluid (SIF; pH = 7.4).

MATERIALS AND METHODS

Alginic acid sodium salt (viscosity of ca 250 cP at 25 °C; 64.4 kDa) was purchased from Sigma. Low molecular weight CHI (90 wt% deacetylated; 6.68 kDa) was purchased from Aldrich. Calcium chloride, monochloroacetic acid, isopropyl alcohol and phosphate buffered saline were purchased from Synth. All reagents were of analytical grade and were used as received.

Synthesis of CMCHI

CMCHI was synthesized by reacting CHI and chloroacetic acid in isopropyl alcohol/KOH as previously reported. CHI powder (10 g) was suspended in 200 mL of isopropyl alcohol and stirred in a 500 mL flask at room temperature. The mixture was added gradually into 25 mL of 10 mol L⁻¹ NaOH aqueous solution in a 30 min period, and stirred for another 30 min. Subsequently, monochloroacetic acid (60 g) was gradually added over a 10 min period. The reaction mixture was then heated to 50 °C under stirring for 4 h. Then, the reaction mixture was filtered and the product (CMCHI) was rinsed with methanol and dried in an oven at 60 °C.

Polymer characterization

Fourier transform infrared (FTIR) spectra of CHI and CMCHI were recorded with a Spectrum 1000 spectrometer (Perkin Elmer) using KBr discs. SD of the carboxymethyl groups was determined by potentiometric titration with KOH as described in the literature by dissolving 0.8 g of CMCHI in an HCI solution of pH = 2, and using the following equation adapted from Ge and Luo:

\[
SD = \frac{161(V_{\text{KOH}} \times c_{\text{KOH}})}{m_{\text{CMCHI}} - 58(V_{\text{KOH}} \times c_{\text{KOH}})}
\]

where SD denotes the number of carboxymethyl groups per glucose unit (as a percentage) or the average number of etherified hydroxyl groups in a glucose unit; \(V_{\text{KOH}}\) and \(c_{\text{KOH}}\) are the volume and the molar concentration (mol L⁻¹) of KOH aqueous solution; \(m_{\text{CMCHI}}\) is the weight of CMCHI (g); and 161 and 58 are the CHI repeat unit and carboxymethyl group molecular weight (g mol⁻¹), respectively.

The water solubility of CHI and CMCHI was estimated from solution transmittance measurements made with a Beckman spectrophotometer at 600 nm using a quartz cell with an optical path length of 1 cm. Aqueous solutions of the polymers were prepared using 0.2 mg mL⁻¹ acetic buffer solutions of pH = 4.0, 6.2 and 7.4 for the evaluation of the dependence of solubility on pH. The specific viscosities of CHI and CMCHI were evaluated using 0.2% (w/v) polymer solution in phosphate buffer solution at pH = 6 with a Schott AVS-360 Ubbelohde viscosimeter at 20 ± 0.1 °C by analysing samples in quintuplicate. The viscometric molecular weight (\(M_v\)) of the CHI and CMCHI samples was determined according to the procedure described by Ge and Luo.

Hydrogel preparation

Aqueous solutions of ALG (1% w/v) were prepared and diluted to a final concentration of 0.2% (w/v) with distilled water. CHI and CMCHI solutions (1% w/v) were prepared with pH = 6 acetic buffer solution and further diluted to 0.2% (w/v) with distilled water. CaCl₂ solution (0.002 mol L⁻¹) was prepared with distilled water.

The hydrogels were prepared by polyelectrolyte complexation, extruding a previously prepared ALG solution in a 0.45 mm syringe needle at a dripping rate of 1.0 mL min⁻¹ into the CaCl₂ solution obtaining an ALG : CaCl₂ mixture in a molar ratio of 10 : 1. After the ALG : CaCl₂ mixture hardened for 30 min, CMCHI or CHI solution was slowly added dropwise into the solution over 20 min and homogenized for 30 min, producing hydrogel microcapsules. The hydrogels were prepared with ALG/CMCHI (CHI) ratios of 40/60 and 80/20 wt%. The hydrogel microcapsules were centrifuged at 3500 rpm for 20 min and rinsed with distilled water three times and kept in distilled water or dehydrated using air drying. The reaction yield was evaluated by the weight difference of the flask containing the hydrogels before (fresh centrifuged and hydrated) and after drying at 50 °C to constant weight.

Hydrogel characterization

Samples of the hydrogels were dried and sputter coated with gold and analysed with a Jeol JSM 5800 SEM instrument using an acceleration voltage of 5 kV. The surface and point of zero charge of the hydrogel particles at different pH values were determined through zeta potential measurements (ZetaMeter, USA) by analysing samples in quintuplicate. Hydrogel stability as a function of temperature was evaluated with a TGA 2050 TA instrument from 200 to 1000 °C at a heating rate of 20 °C min⁻¹. The hydrogel degree of swelling (%SW) in SIF and SGF media was determined according to a reported method, using the following equation, by analysing samples of the dried hydrogel and SIF-exposed (24 h) and SGF-exposed (5 h) hydrogels using an inverted optical microscope (Carl Zeiss, Axiovert 200):

\[
\%\text{SW} = 100 \left[ \frac{d_{\text{hydr}}}{d_{\text{dry}}} - 1 \right]
\]

where \(d_{\text{hydr}}\) is the hydrated particle size diameter and \(d_{\text{dry}}\) is the dried particle size diameter.

Experimental design and statistical analysis

A two-level factorial experiment design was employed to evaluate the morphology and related properties of the hydrogels,
selecting as dependent variables the hydrogel morphology, particle size and degree of swelling. The independent variables were ALG content, pH and CHI type. The factors related to the independent variables and their high level (HL) and low level (LL) are defined as follows:

- Factor A: ALG content in the hydrogel — HL (+) 80 wt% (A80); LL (−) 40 wt% (A40).
- Factor B: solution pH — HL (+) 6 (P6); LL (−) 4 (P4).
- Factor C: CHI type — HL (+)CMCHI; LL (−) CHI.

Table 1 gives the matrix variables of the designed experiments and particle size and reaction yield of the ALG-based hydrogels produced. A total of 8 runs in triplicate were performed for each independent variable and they were carried out in groups of 4 experiments randomly chosen to nullify the effect of nuisance variables.

The statistical treatment of data was carried out through ANOVA using the Yates algorithm in Excel. The method involves calculating the sum square (SS), degree of freedom (DF) and mean square (MS) for the main effects, interactions and residual part. In this study, a reference F value of 4.0 was used to take into account the variability of the results according to the reaction conditions and the effect of some minor interactions that could be important in the study.

### RESULTS AND DISCUSSION

In our previous paper, it was shown that ALG–CHI hydrogels prepared with low molecular weight (LMW) CHI presented enhanced swelling properties and higher yield compared to those prepared with medium molecular weight CHI, due to the higher binding energy. As a result, LMW CHI was chosen as the precursor of the CMCHI studied in the present work. A mixture of water/isopropyl alcohol (1:8 v/v) was used to favour the carboxymethylation reaction, as pointed out by Chen and Park.

![FTIR spectra of CHI and CMCHI.](image)

Figure 1 compares the FTIR spectra of LMW CHI and its derivative (CMCHI). The degree of deacetylation of the CHI sample is 90% and the FTIR spectrum shows characteristic peaks at 1654 cm$^{-1}$, assigned as amide I, and at 1596 cm$^{-1}$, assigned as symmetric and asymmetric bending of amine and amide II, in accordance with similar result reported by Lawrie et al. However, comparing both spectra, the amide I peak for CMCHI shifts to 1624 cm$^{-1}$ ($\delta = 20 $ cm$^{-1}$) indicating intermolecular hydrogen bonding between the amide and carboxyl groups (O═C═N$\cdot\cdot\cdot$O═COH) in the CHI derivative sample. As reported, this shift occurs because the hydrogen bonding in CMCHI is stronger than that in CHI, since the resultant dipole moment of N—H bonds is smaller than that of O–H bonds. The CMCHI spectrum also shows absorption peaks at 1744 cm$^{-1}$ assigned to carboxymethyl interaction or dimer formation (O═COH$\cdot\cdot\cdot$O═COH). The carboxymethyl groups present in CHI can also be estimated by the peak intensities ratio $A_{1029}/A_{1070}$, which has a relationship to the C–O stretching of secondary (1029 cm$^{-1}$) and primary (1070 cm$^{-1}$) hydroxyl groups in cyclic alcohols. The calculated values of the $A_{1029}/A_{1070}$ ratio for both polymers decrease from 0.97 to 0.90 showing that carboxymethylation has occurred in the CHI hydroxyl groups in accordance with the report of Chen and Park about the temperature effect on the substitution of OH sites by carboxymethyl groups.

Figure 2 shows the titration curve for CMCHI at 25°C which correlates the pH variation as a function of KOH volume. The two inflection points in the titration curve reveal different acid groups in the medium. The first corresponds to the neutralization of the free hydrogen chloride of the HCl solution and the attainment of CMCHI isoelectric point at pH = 4.4 (ca 30 mL) and the second corresponds to the whole titration of the carboxyl group at pH = 8.6 (ca 42 mL). The difference in volume between the two inflection points represents the volume of the KOH solution ($V_{\text{KOH}}$) consumed in the carboxymethyl titration, which corresponds to SD of 56 wt%, calculated according to Eq. (1).

The solubility of both CHI and CMCHI, evaluated from the transmittance of their solutions at different pH, the specific viscosity ($η_{sp}$) and viscosimetric molecular weight ($M_v$) are given in Table 2. The higher the polymer solubility, the better the transmittance, and thus CMCHI is more soluble than CHI independent of the pH of the solution. It is known that CHI is only soluble in acidic media because the amino groups are protonated (NH$_3^+$). An increase in the solution pH reduces the charge density of the amino groups, thus decreasing the
solution pH changes the nature and extension of the interactions among amino groups of CHI or CMCHI depends on the medium acidity. The amino groups of the polymers affect ionic complex formation between the anionic acid and basic hydrogel particles, since variation in the diffusion coefficients can increase the solution’s resistance to flux and gel formation. If intermolecular positive and negative charges coexist in the structure, which is in agreement with the SD value. Furthermore, if intermolecular positive and negative charges coexist in the molecules, ion–molecule interactions become stronger, thereby increasing the solution’s resistance to flux and gel formation. Increasing the polymer viscosity can affect the morphology of the hydrogel particles, since variation in the diffusion coefficients can affect ionic complex formation between the anionic acid and basic groups of the polymers.  

Table 2. Specific viscosity ($\eta_{sp}$), viscosimetric molecular weight ($M_w$) and solution transmittance of CHI and CMCHI.  

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH = 4</th>
<th>pH = 6.3</th>
<th>pH = 7.4</th>
<th>$\eta_{sp}$ (g dL$^{-1}$)</th>
<th>$M_w$ (g mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI</td>
<td>40</td>
<td>36</td>
<td>23</td>
<td>0.62</td>
<td>39 000</td>
</tr>
<tr>
<td>CMCHI</td>
<td>85</td>
<td>82</td>
<td>79</td>
<td>0.97</td>
<td>50 000</td>
</tr>
</tbody>
</table>

CHI solubility, as was observed in this work by the variation in the transmittance from 40 to 20%. However, the transmittance of the CMCHI solution at pH = 7.4 is higher and increases even more with a decrease of pH, which could be related to a larger number of acetylated chain segments. The higher intrinsic viscosity of the CMCHI solution (0.97 g dL$^{-1}$) compared to that of the CHI solution (0.62 g dL$^{-1}$), evaluated at pH = 6.0, indicated a higher $M_w$ caused by carboxymethyl groups inserted in the CHI structure, which is in agreement with the SD value. Furthermore, if intermolecular positive and negative charges coexist in the molecules, ion–molecule interactions become stronger, thereby increasing the solution’s resistance to flux and gel formation. Increasing the polymer viscosity can affect the morphology of the hydrogel particles, since variation in the diffusion coefficients can affect ionic complex formation between the anionic acid and basic groups of the polymers. 

Hydrogel morphology

Polyelectrolyte complexation was used to produce ALG–CHI and ALG–CMCHI hydrogels with core–shell structure, as shown schematically in Fig. 3. First, the ALG and CaCl$_2$ solutions were prior mixed and the ALG molecules were crosslinked by calcium ions producing ALG–Ca$_{2+}$ complexes (A in Fig. 3). As a second step, the CHI or CMCHI solution was added into the mixture and the CHI (B) or CMCHI molecules (C) covered the ALG–Ca$_{2+}$ complex, producing an outer shell around the ALG–Ca$_{2+}$ core template (D and E). Control of pH in the solution during hydrogel preparation (pH = 4 or 6) seems to be crucial, since the protonation of the amino groups of CHI or CMCHI depends on the medium acidity. The solution pH changes the nature and extension of the interactions between the charged amino groups and the ALG–Ca$_{2+}$ core. The ALG–Ca$_{2+}$ complexes when surrounded by CHI molecules constitute positively charged core–shell particles. On the contrary, if partially carboxymethylated CMCHI molecules (with negative and positive charges) are used a strong ion interaction is formed between the positive amino groups with the ALG–Ca$_{2+}$ core and the carboxymethylated negative charges surround the hydrogel particles.

The hydrogel morphology or structure and resultant charge by using CHI or CMCHI was evaluated following the hydrogel behaviour regarding the average particle size, degree of swelling, thermal stability and zeta potential. The experimental design was used to identify the most significant factors affecting the particle size and degree of swelling of the hydrogels. Two levels of each factor were evaluated applying ANOVA analysis to point out the calculated effects. A reference $F$ value of 4.0 was used for a statistical reliability of 93%.

The ALG-based hydrogels show particles with spherical or spheroid shape and size dependence on the solution pH and CHI chemical nature. Table 1 gives the average size and standard variation of the dehydrated hydrogels obtained in all reaction conditions. ALG–CHI core–shell hydrogels are larger than those produced with CMCHI and present a variation in size depending on the reaction conditions. The significance of the ALG content (A) in the hydrogel, solution pH (B) and CHI chemical nature (C), and the interaction between these variables were evaluated using the particles average size and standard variation. Table 3 gives the ANOVA statistics results for the hydrogel particle size, the sum of squares (SS), degree of freedom (DF), mean square (MS) and $F$-test values for all variables and their interactions. The significant effects on the particle size are C, B and BC, i.e. CHI chemical nature, solution pH and the interaction between these. CHI chemical nature is the most important factor, with an $F$ value equal to 22.5. This effect provides statistical confirmation that hydrogels produced with CMCHI have on average smaller diameters than those produced with CHI, as seen in the average size values (2.2 ± 0.5 and 5.5 ± 1.5 µm, respectively, for CMCHI and CHI) in Table 2. The small size could be a consequence of fewer amino groups in the partially carboxymethylated CHI which limits the ionic network expansion at the tested pH. Furthermore, the ALG–CMCHI hydrogels are more homogeneous in size having lower deviation. The pH effect (B) on the average size is also significant with an $F$ value of 4.8, which indicates that hydrogels produced at pH = 4 are larger than those produced at pH = 6. However, the combination of this factor and the CHI chemical nature presents a synergistic effect producing the interaction effect BC, with an $F$ value of 4.2.

Figure 4 shows SEM micrographs of the ALG–CHI and ALG–CMCHI core–shell hydrogels with 80 wt% of ALG at pH = 4 and 6. The hydrogels prepared at pH = 4 (Figs 4(a) and (c)) present a larger particles size than those produced at pH = 6 (Figs 4(b) and (d)), corroborating the BC effect, in which at pH = 4, hydrogel particles prepared with CHI were larger than those prepared with CMCHI. In general, for drug delivery purposes, hydrogels with smaller particles and size deviation are preferred in order to achieve more homogeneous encapsulation and controlled release. The best reaction conditions are low ALG content (40 wt%) and pH = 6 for hydrogel production with average particle size of 3.2 ± 1.5 µm for CHI and 2.2 ± 0.5 µm when CMCHI is used.

Hydrogel degree of swelling

The average size of the hydrated hydrogels in SGF and SIF was used for the determination of the degree of swelling (SW). SW in SGF was calculated from Eqn (2) and evaluated statistically using

Figure 2. Alkalimetric titration curve for CMCHI sample at 25 °C.
Table 3. ANOVA statistics data of ALG-based hydrogel particle size: independent variables and their interactions ($F_{ref} = 4.0$)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Sum of squares (SS)</th>
<th>Degree of freedom (DF)</th>
<th>Mean square (MS)</th>
<th>$F$-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.5</td>
<td>0.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>9.0</td>
<td>1.00</td>
<td>9.0</td>
<td>4.8</td>
</tr>
<tr>
<td>AB</td>
<td>0.6</td>
<td>0.6</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>42.3</td>
<td>1.00</td>
<td>42.3</td>
<td>22.5</td>
</tr>
<tr>
<td>AC</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>BC</td>
<td>7.8</td>
<td>1.00</td>
<td>7.8</td>
<td>4.2</td>
</tr>
<tr>
<td>ABC</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>22.6</td>
<td>12.00</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>84.4</td>
<td>15.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA. Table 4 shows the sum of squares (SS), degree of freedom (DF), mean square (MS) and $F$-test values for all variables and their interactions. The significant effects on SW are C, i.e. CHI chemical nature, and the interaction AB, i.e. the interaction between ALG content and pH, both with $F$ values of 4.1.

Figure 5 shows the degree of swelling of the ALG-based hydrogels in SGF according to their preparation. Those prepared with CHI present higher SW than those prepared with CMCHI (Fig. 5(a)), since there is a higher concentration of protonated amino groups at acidic pH which are responsible for a higher water diffusion into the hydrogel network, causing a greater expansion in acid medium. On the other hand, CMCHI has approximately 50 wt% of carboxyl groups and lower protonated amino group concentration. In this case the interaction of CMCHI with ALG may occur via two different processes: first, some of the CMCHI carboxyl groups could crosslink with the ALG carboxyl groups by some remaining calcium ions in the solution, which is the same type of interaction described in Lin et al.; second, the remaining amino groups of CMCHI that are protonated during the preparation at pH = 6 could complex with the carboxyl groups of ALG. Thus, the amino group content in CMCHI contributes to the formation of an entangled system of CMCHI and ALG polyelectrolytes, ensuring microsphere stability and a significant swelling reduction in SGF compared to the ALG–CHI hydrogels.

Figure 5(a) also shows that ALG–CHI or ALG–CMCHI hydrogels produced at a solution pH of 4 with 40 wt% of ALG have lower SW than those prepared with 80 wt%, regardless of the solution pH in which they were produced. The higher SW of the hydrogel with 80 wt% of ALG may be a consequence of low amino group concentration leading to a porous hydrogel which does not
Table 4. ANOVA statistics data of ALG-based hydrogel degree of swelling in SGF: independent variables and their interactions ($F_{ref} = 4.0$)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Sum of squares (SS)</th>
<th>Degree of freedom (DF)</th>
<th>Mean square (MS)</th>
<th>$F$-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>861 402</td>
<td>1.0</td>
<td>861 402</td>
<td>3.1</td>
</tr>
<tr>
<td>B</td>
<td>196 169</td>
<td></td>
<td>196 169</td>
<td>0.7</td>
</tr>
<tr>
<td>AB</td>
<td>1 154 293</td>
<td>1.0</td>
<td>1 154 293</td>
<td>4.1</td>
</tr>
<tr>
<td>C</td>
<td>1 152 293</td>
<td>1.0</td>
<td>1 152 293</td>
<td>4.1</td>
</tr>
<tr>
<td>AC</td>
<td>56 864</td>
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<td>0.2</td>
</tr>
<tr>
<td>BC</td>
<td>888 612</td>
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<td>888 612</td>
<td>3.2</td>
</tr>
<tr>
<td>ABC</td>
<td>611 444</td>
<td></td>
<td>611 444</td>
<td>2.2</td>
</tr>
<tr>
<td>Error</td>
<td>3 100 916</td>
<td>11.0</td>
<td>281 901</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7 157 516</td>
<td>15.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. Degree of Swelling at (a) pH = 1.2 and (b) pH = 7.4 for ALG-based hydrogels with CHI or CMCHI as a function of composition and pH of preparation.

Figure 6. Optical micrographs of hydrogels hydrated in SIF (pH = 7.4) for 24 h with 40 wt% ALG under different reaction conditions: (a) CMCHI at pH = 4; (b) CMCHI at pH = 6; (c) CHI at pH = 4; (d) CHI at pH = 6.

Pasparakis and Bouropoulos. In summary, the optimized reaction conditions found in this work for the production of ALG-based hydrogels with low SW are those with the lowest ALG content (40 wt%), pH = 6 and CMCHI. The ALG–CMCHI core–shell hydrogels present significantly lower SW than the ALG–CHI ones and the CHI nature modification with carboxymethyl groups ensures a lower SW in SGF, a characteristic advisable for sustained release, which could prevent gastric burst release before reaching the intestine.

SW of the hydrogels in SIF was also evaluated. Figure 6 shows optical micrographs of hydrogels produced with 40 wt% ALG after 24 h in SIF (pH = 7.4). The dehydrated hydrogels show a different size, depending on the reactions conditions, when produced with CHI at pH = 4 or 6 (Figs 6(c) and (d), respectively); they present a larger average size ($8.5 \pm 2.9 \mu m$) than those produced with CMCHI ($4.2 \pm 1.0 \mu m$) (Figs 6(a) and (b), respectively).

The ALG–CHI and ALG–CMCHI hydrogel degree of swelling in SIF is shown in Fig. 5(b), where hydrogels produced with 80 wt% ALG at pH = 4 present the highest swelling, due to an insufficient quantity of amino groups needed to form a protective coating, for both CMCHI and CHI. Hydrogels with 40 wt% ALG when in SIF possess a greater quantity of amino groups and, even with a low degree of protonation, the amino groups still form bonds with ALG. This inhibits the swelling of the calcium alginate core$^9$ and CMCHI hydrogels exhibit similar degree of swelling to CHI ones. ANOVA data analysis confirms statistically the non-influence of the chemical nature of CHI on the swelling ability in neutral SIF, as shown in Table 5 by the non-significance of the $F$ value for all variables and their interactions. Therefore, CMCHI reduces significantly the hydrogel SW in SGF and does not alter notably the SW in SIF. This effect is a reliable indication for the use of CMCHI instead of CHI in ALG-based hydrogels, which could prevent burst release through gastric media, retaining the swelling ability required in the intestinal media for promising drug release.

inhibit the swelling of the Ca–ALG core and both CHI and CMCHI hydrogels have greater SW. The effect of the ALG content on SW of hydrogels in SGF was observed in a previous study$^{27}$ where a linear equation correlated the ALG content increase with the increase of SW. Lin et al.$^{21}$ in a study of the swelling behaviour of ALG–CMCHI hydrogels having different composition highly crosslinked with Ca$^{2+}$ verified that the hydrogel SW is limited by the tightly bound ionic Ca$^{2+}$ crosslinking and the increase in the hydrogel swelling depends on the CMCHI concentration. The synergic effect (AB) in SW in SGF is relevant for ALG–CHI hydrogels when the particle size is more influenced by pH. Since CHI has higher amounts of protonated amino groups entangled in the ALG polymer chains, a repulsion force causes the swelling of the hydrogels in acid media, which is in agreement with the study reported by...
Hydrogel thermal stability

The thermal stability of CHI, CMCHI and their hydrogels produced with 40 wt% ALG at pH = 6, which had the best swelling properties, were evaluated through weight loss curves as function of temperature, as shown in Fig. 7. A first weight loss around 70 °C in the CHI and CMCHI curves can be associated with water evaporation, and the second weight loss at 227 and 290 °C, respectively, corresponds to the degradation process including dehydration of the saccharide rings, depolymerization and decomposition of the acetylated and deacetylated units of the polymer. The presence of the carboxymethyl groups increases the degradation temperature almost 80 °C, improving the polymer stability in accordance with Miranda et al. On the one hand, the ALG–CHI hydrogel shows a weight loss at 70 °C due to water evaporation and two weight losses at 224 and 290 °C due to hydrogel degradation. On the other hand, the ALG–CMCHI hydrogel shows water evaporation at 109 °C and hydrogel degradation shifts towards higher temperatures at 229, 350 and 440 °C. The shift of degradation to higher temperature means a better thermal stability of the ALG–CMCHI hydrogels due to the insertion of carboxymethyl groups and an increase of the intermolecular interactions via hydrogen bonds between the hydroxyl and amino groups in CMCHI and carboxyl groups in ALG.

Zeta potential

The relative charge below the hydrodynamically stagnant layer of the ALG–CHI and ALG–CMCHI hydrogels produced with 40 wt% of ALG at pH = 6 with good balance of properties in SGF was determined from zeta potential measurements. Figure 8 shows the zeta potential as a function of pH showing significant differences in the global charge of these hydrogels. Both hydrogels show a decrease in the potential with the increasing pH, but the ALG–CHI hydrogels present a higher positive zeta potential due to the high charge density of protonated amino groups on the particle surfaces in acid pH. Around neutral pH, the CHI deprotonates and the negative carboxyl groups of the ALG cause a shift in the potential to negative values. In contrast, the ALG–CMCHI hydrogels present a lower positive potential in acid pH due to the smaller amount of amino groups in the polymer, and at pH = 4.5 all the carboxyl groups present in the hydrogel become negatively charged, shifting the zeta potential from positive to negative values.

The isoelectric point shows when the hydrogels have a balance of positive and negative charges, and can be estimated from the slope of the zeta potential curve. The pH of the isoelectric point for ALG–CHI and ALG–CMCHI hydrogels is ca 7 and 4, respectively, showing that the insertion of the carboxyl acid groups causes the equivalency of negative and positive charges at a lower pH, where ALG–CMCHI would have higher binding efficiency with molecules of positive charge and ALG–CHI with molecules of negative charge, in the pH interval from 4 to 6.5, for drug delivery.

CONCLUSIONS

Optimized hydrogels with reduced degree of swelling in gastric media and high thermal stability were obtained. The partially carboxymethylated CMCHI can replace CHI in the production of core–shell hydrogels with improved properties since the negative charge surrounding the particles favours a lower degree of swelling. In the ALG–CMCHI hydrogel particles with core–shell structure, the positive charge of the amino groups of the CMCHI molecules tends to stay in the boundary of the ALG core during the formation of the ionic complex. The ALG–CMCHI core–shell hydrogel or polyelectrolyte complex prepared with 40 wt% of ALG at pH = 6 presented the smallest microparticles and the lowest degree of swelling when in SGF. The improvement in the swelling properties may be an indication that the ionic hydrogels prepared could prevent burst release in gastric media, sustaining the same swelling ability in the intestinal media as ALG–CHI.
hydrogels, being promising for drug release. The independent variables that were investigated were successfully correlated with the hydrogel particle size and degree of swelling. The statistical analysis through ANOVA allowed correlation of the $F$ value and interactions between the variables with the influence on the hydrogel properties. Thus, ALG–CMCHI hydrogels with a good balance of properties, i.e. particles size and distribution, thermal stability and degree of swelling in SGF and SIF, can be obtained by appropriate choice of reaction parameters.

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