Carbohydrate Polymers 74 (2008) 283–289

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Influence of the composition and preparation method on the morphology and swelling behavior of alginate–chitosan hydrogels

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ARTICLE INFO

Article history:
Received 13 December 2007
Received in revised form 27 February 2008
Accepted 28 February 2008
Available online 6 March 2008

Keywords:
Alginate–chitosan hydrogels
Statistical design
Microparticles
Drug delivery

ABSTRACT

In this study, a 2^4 factorial experimental design was employed in order to evaluate the influence of the reaction conditions and preparation method on alginate–chitosan hydrogel properties. Alginate content, pH, chitosan molecular weight and the hydrogel preparation method were the independent variables and the reaction yield, particle size, swelling degree and point of zero surface charge were the dependent variables. The results showed that hydrogels were spherical with an average diameter of 5.0 ± 2.0 μm. Reaction yield varied according to the parameters, and chitosan molecular weight showed the greatest influence. Furthermore, the swelling degree and point of zero surface charge showed a linear dependence on the alginate content. In this regard, the study showed that hydrogels with a specific charge and swelling degree can be obtained by controlling the alginate content using the equation here provided to give an enhanced and site-specific controlled drug release.

1. Introduction

Hydrogels made of polysaccharides, such as chitosan and alginate, have been proposed for many biomedical and pharmaceutical purposes in recent years (Drury & Mooney, 2003; Lin, Liang, Chung, Chen, & Sung, 2005; Lin-Gibson, Walls, Kennedy, & Welsh, 2003; Zhou, Deng, & Li, 2001). Chitosan (CHI) is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells, and alginate (ALG) is a polyanionic copolymer of mannuronic and guluronic sugar residues extracted from brown algae. Both polymers are biodegradable and non toxic, and are used to produce hydrogels with well known properties which are employed for peptide and protein drug delivery (Dai, Wang, & Zhao, 2005). These polymers are polyelectrolytes and form ionic complexes through hydrogen bonding or electrostatic interactions (Berger, Reist, Mayer, Felt, & Gurny, 2004). In the last decade, different alginate–chitosan hydrogels have been produced for drug delivery applications in micro or nano scale using the polyelectrolyte complexation (PEC) technique (Anal & Stevens, 2005; De & Robinson, 2003; Hari, Chandy, & Sharma, 1996; Mi, Sung, & Shyu, 2002; Zheng, Gao, Zhang, & Liang 2004). There are numerous factors affecting the relevant properties of the alginate–chitosan capsules (George & Abraham, 2006). Among these factors are the composition, molecular weight and deacetylation degree of chitosan (Alsarra, Betigeri, Zhang, Evans, & Neau, 2002; Becherán-Marón, Peniche, & Argüelles-Monal, 2004; Boontheekul, Kong, & Mooney, 2005; Liu et al., 2004). Several methods have been developed in which the hydrogel particle size and its related properties are quite distinct, according to the preparation method and reaction conditions employed. De and Robinson (2003) prepared chitosan–alginate nanospheres using different reaction conditions and the optimal mass ratio of sodium alginate:$CaCl_2$:chitosan was 100:17:10. Liu et al. (2004) evaluated the influence of chitosan molecular weight and concentration, along with pH, on the swelling behavior of alginate–chitosan microcapsules, and they postulated that all the factors have an affect on the hydrogel swelling behavior. Wang and He (2002) tested the introduction of konjac glucomannan and $CaCl_2$ into alginate–chitosan hydrogels for improved protein release. Sarmento, Ferreira, Veiga, and Ribeiro (2006) conducted a series of experiments with varying pH and investigated the stoichiometric relationship between polyelectrolytes of opposite charge to monitor their complexation. Although some of these parameters have been studied to varying extents, the hydrogel preparation method is a factor that can influence the final properties but has seldom been tested as a parameter. Also, few attempts have been made to correlate statistically the reaction conditions with the final hydrogel properties. An experimental design can be used to reduce the number of variables to a manageable size so that further experiments can be performed using these key variables for a better understanding of a process (González-Rodríguez, Barros, Palma, González-Rodríguez,

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doi:10.1016/j.carbpol.2008.02.017

& Tabasco, 2007). This strategy of reducing the number of variables allows experiments to focus process improvement efforts on the key factors and permits the union of various factors of interest in a single formulation (Cunha, Melo, Veronese, & Forte, 2004; González-Rodríguez et al., 2007). Statistical analysis using linear regression not only highlights the significant reaction parameters, but can also provide an equation for each targeted property. The equations allow an optimization of the hydrogel properties taking into account only the significant parameters. In this study, ALG–CHI hydrogels were prepared under different reaction conditions, and the effects were statistically investigated aiming to predict and modulate the hydrogel properties for controlled drug delivery systems.

2. Materials and methods

2.1. Materials

The low viscosity alginic acid sodium salt (Sigma, USA), low and medium molecular weight (MW) chitosan (Aldrich, USA), calcium and sodium chloride (Synth, Brazil) were of analytical grade and were used without further purification. The main characteristics of the materials employed, designated as ALG, LCHI, MCHI, are shown in Table 1. The chitosan deacetylation degree (DA) was determined by FTIR following the procedure described by Brugnerotto et al. (2001), given by Eq. (1).

$$DA = 31.92\left(\frac{A_{1320}}{A_{1420}}\right) - 12.2 \tag{1}$$

where A_{1320} and A_{1420} = chitosan absorption area bands at 130 and 1420 cm^{-1}, respectively.

The viscosimetric average molecular weights (M_v) of the polymers were estimated at 25 °C in 0.1 M NaCl for ALG and at 25 °C in 0.1 M CH_3COOH for chitosan, using known α and κ values from the literature for chitosan (Kumar, 2000) and alginate (Mancini, Moresi, & Sappino, 1996) by the Eq. (2).

$$V = k(M_v)^{\alpha} \tag{2}$$

where V is the intrinsic viscosity of the polymer, α and κ are constants which are dependent on the interactions between the polymer and a specific solvent for a given solution.

2.2. Preparation of alginate–chitosan hydrogels

The ALG–CHI hydrogels were prepared by ionic complexation using alginate as a gel core. The alginic acid solution was dissolved in distilled water to give a final concentration of 0.2% (w/v). Solutions of low and medium M_v chitosan were prepared by dissolving the chitosan in acetic buffer (pH = 4) to give a final concentration of 0.2% (w/v). The hydrogels were prepared through two different procedures, the direct and indirect addition methods. In the direct addition method the CHI solution (0.2% w/v) was added to a beaker containing the $CaCl_2$ solution (0.02% w/v), and the ALG solution (0.2% w/v) was extruded through a 0.45 mm syringe needle at a dropping rate of 1.0 ml/min to give a final molar ratio of 10:1 ALG–Ca. Two different relative composition were used, 35/65 and 75/25 w/v ALG–CHI. In the indirect addition method the alginate solution (0.2% w/v) was added dropwise using a 0.45 mm syringe needle at a dropping rate of 1.0 ml/min into a beaker containing the $CaCl_2$ solution (0.02% w/v) to give a 10:1 alginate:Ca molar ratio. The mixture was left to harden for 30 min, producing ALG–Ca particles. The chitosan solution was then added dropwise into the ALG–Ca solution, and homogenized for 30 min in order to form ALG–CHI hydrogels. The microcapsules were rinsed three times with distilled water by centrifugation at 3500 rpm for 20 min and stored in distilled water.

Table 1
Hydrogel polymer characteristics

	Intrinsic viscosity	Viscometric average M_W	Deacetylation degree (%)
Alginic acid (ALG)	5.25	54,600	–
Low M_W chitosan (LCHI)	6.53	6683	90
Medium M_W chitosan (MCHI)	12.84	13,827	92

2.3. Hydrogel characterization

The ALG–CHI hydrogel formation was characterized by the vibration modes of their main groups using FTIR. The hydrogel yields were evaluated through the weight difference, placing the washed hydrogels into pre-weighed flasks and then into a stove at 50 °C until dryness. The hydrogel particles were visualized and characterized by particle size and size distribution using an inverted optical microscope (Carl Zeiss, Axiovert 200). Axiovision AC Release 4.5 software was used to perform image capture and the measurement of the average diameter of a minimum of 50 particles. Particle surface charge at different pH values and the point of zero charge were determined through zeta potential measurements (ZetaMeter, USA). The swelling degree (% SW) for different time periods and pH values were determined using the Eq. (3), adapted from Liu et al., 2004.

$$\%SW = 100\left[\left(\frac{\overline{d_{hyd}}}{\overline{d_{dry}}}\right)^3 - 1\right] \tag{3}$$

where $\overline{d_{hyd}}$ = hydrated particle size diameter, $\overline{d_{dry}}$ = dried particle size diameter.

2.4. Experimental design

In this study, a two-level 2^4 factorial experiment design was employed to evaluate the morphology and related properties of the hydrogel microcapsules. The independent variables evaluated were ALG content, pH, CHI M_W and preparation method. The dependent variables were hydrogel yield, morphology, swelling degree and point of zero surface charge. The experimental matrix containing the independent and dependent variable levels are shown in Table 2 and the factors were defined as follows:

- Factor *A*: relative ALG content in the hydrogel; high level (+) = 75% w/v (*A*75) and low level (−) = 35% w/v (*A*35).
- Factor *B*: solution pH; high level (+) = 6 (*P*6) and low level (−) = 4 (*P*4).
- Factor *C*: CHI molecular weight; high level (+) = Medium M_W chitosan (MCHI) and low level (−) = low M_W chitosan (LCHI).
- Factor *D*: Chitosan coating method; high level (+) = Direct Addition Method (DM) and low level (−) = Indirect Addition Method (IM).

A total of 16 runs in duplicate were performed for each dependent variable tested, designed to be carried out in blocks of 4 experiments in random order to nullify the effect of nuisance variables.

Linear regression attempts to model the relationship between the variables by fitting a linear equation to observed data and was used to point out the significant reaction parameters. The statistical data treatment was carried out using the Excel Program (Ribeiro, Fogliatto, & Caten, 2000) and all the studied independent variables and their possible combinations were normalized for the values −1 and 1, for low and high level, respectively, and then ar-

Table 2
Experimental planning of ALG–CHI hydrogels reaction conditions. Independent variables and levels and dependent variables (yield, particle size, zeta potential and swelling degree)

Run N^o	Hydrogel code	Independent variables[a]				Dependent variables				
		A	*B*	*C*	*D*	Yield (%)	Particle size (μm)	Zeta potential (mV)	Swelling degree (%)	
								pH = 6.8	pH = 1.2[b]	pH = 6.8[c]
1	A35,P4,LCHI,IM	−1	−1	−1	−1	59	7.0 ± 1.7	+17 ± 2	717	146
2	A75,P4,LCHI,IM	1	−1	−1	−1	55	6.8 ± 2.6			
3	A35,P6,LCHI,IM	−1	1	−1	−1	99	3.2 ± 2.6			
4	A75,P6,LCHI,IM	1	1	−1	−1	95	4.8 ± 2.1	−36 ± 3	2386	273
5	A35,P4,MCHI,IM	−1	−1	1	−1	61	4.8 ± 2.8			
6	A75,P4,MCHI,IM	1	−1	1	−1	27	4.6 ± 1.9	−23 ± 2	958	335
7	A35,P6,MCHI,IM	−1	1	1	−1	17	5.5 ± 2.4	+23 ± 2	296	1771
8	A75,P6,MCHI,IM	1	1	1	−1	36	4.6 ± 2.6			
9	A35,P4,LCHI,DM	−1	−1	−1	1	52	4.5 ± 2.5	−31 ± 2	1015	422
10	A75,P4,LCHI,DM	1	−1	−1	1	36	5.3 ± 1.5			
11	A35,P6,LCHI,DM	−1	1	−1	1	92	5.3 ± 1.5	+17 ± 1	700	151
12	A75,P6,LCHI,DM	1	1	−1	1	79	3.3 ± 1.6			
13	A35,P4,MCHI,DM	−1	−1	1	1	47	5.7 ± 2.7	+30 ± 1	657	965
14	A75,P4,MCHI,DM	1	−1	1	1	77	4.0 ± 2.7			
15	A35,P6,MCHI,DM	−1	1	1	1	51	3.8 ± 1.9			
16	A75,P6,MCHI,DM	1	1	1	1	62	7.4 ± 2.1	−24 ± 3	1339	521

[a] A = ALG content; B = pH; C = CHI M_w; D = preparation method; lower (−1) and higher (+1) level of variable.

[b] After 6 h.

[c] After 48 h.

rayed in a matrix. Their coefficient and the significance factor (p) were determined by linear regression. The significant independent variables were selected, considering the significance factor (p) lower than 0.05 for a reliability of 95%. The coefficient is a valuable numerical measure which indicates the strength of the association of the observed data for the variables. Subsequently, a response function's equation for each of dependent variables was produced considering only the significant independent variables, represented by the general equation Eq. (4).

$$Y = k + \alpha A + \beta B + \gamma C + \cdots + \varepsilon AB + \cdots + \phi ABC + \cdots \tag{4}$$

where Y is dependent variable; A, B, C are independent variables and AB, ABC their combined effects; k is the intercept; α, β, γ, ε, φ are the variable coefficients.

3. Results and discussion

The production of ALG–CHI hydrogels with controlled morphology consisted of addition of the CHI and ALG solution in a chemically controlled environment, which enabled the formation of a complex. The hydrogel properties were determined by the extent of the interaction between the polymers in the solution and the chemical environment. Thus, the hydrogel properties are controlled by the polyelectrolyte complexation. In order to modulate these properties according to the chemical environment, selected parameters were altered and the effects on the microcapsule morphology, swelling degree and point of zero surface charge were observed. The chitosan samples used in this study had a similar deacetylation degree, being 90% for LCHI and 92% MCHI. In this context, it was assumed that the two chitosan types differed only in terms of their molecular weight. CHI dissolved in aqueous solution at pH <6.5, and the amino groups became easily positively charged. ALG, on the other hand, dissolved in a neutral pH solution and the carboxyl groups became negatively charged. In aqueous solutions with pH between 3.5 and 6.5, CHI amino groups interacted with the ALG carboxyl groups to form the ionic polymer complex. The hydrogel formation reaction can be observed through changes in the vibration modes of the main groups observed by infrared spectroscopy. Fig. 1 shows the infrared spectra of chitosan, alginate and some ALG–CHI hydrogels.

For the ALG, characteristic functional groups (COO^- stretching) were present, with a broad asymmetrical band at 1610 cm^{-1} and a narrower symmetrical band at 1418 cm^{-1}. An even broader absorption was observed near 1030 cm^{-1}, which can be attributed to COH stretching. CHI possesses amine charged groups (NH_3^+) in acid solution, which show a broad absorption near 3300 cm^{-1}, due to their stretching vibrations. Their principal vibration modes are asymmetrical and symmetrical bending NH_3^+ vibrations at 1620, 1596 and 1421 cm^{-1}. The ALG–CHI complex formation was evidenced by the sharpening of the bands at 1610 cm^{-1} due to the COO^- groups in the ALG and the disappearance of the CHI amino band at 1596 cm^{-1}. Also, the appearance of a small peak was observed at around 1080 cm^{-1}, possibly as a result of ionic interactions between the COH and NH_3^+ groups at the hydrogel surface.

The significance of the independent variables was evaluated through statistical treatment. The dependent variable equation represents the surface response as a function of only the factors that had a significant influence on each variable, with 95% reliability.

3.1. Hydrogel yield

The hydrogels showed a wide variation in yield (17–99%) as can be seen in Table 2. The best results were obtained for reactions 3, 4, 11 and 12, with LCHI and at pH 6, where the yield was over 80%. Linear regression allowed the identification of the relevant variables and the determination of the yield variation as a function of the independent variables along with the effects of their interactions (Eq. (5)).

$$Y_{\mathrm{ield}}(\%) = 55.4 - 11.7C - 10BC + 9.6CD \quad R^2 = 0.70 \tag{5}$$

where Y_{ield} = yield (% w/w), B = solution pH, C = chitosan M_W, D = addition method.

This equation provides the average yield, which is the intersection value (55.4%), and the factors which contribute to it, explaining 70% of the yield variability related to CHI M_W (C) as being due to the interaction of the solution pH with CHI M_W (BC) and of the CHI M_W with the addition method (CD). Substituting the variables B, C and D in Eq. (5) with normalized values for the independent variables (−1 and +1 for low and high levels), an optimization is achieved. The equation provided yield maximization by replacing

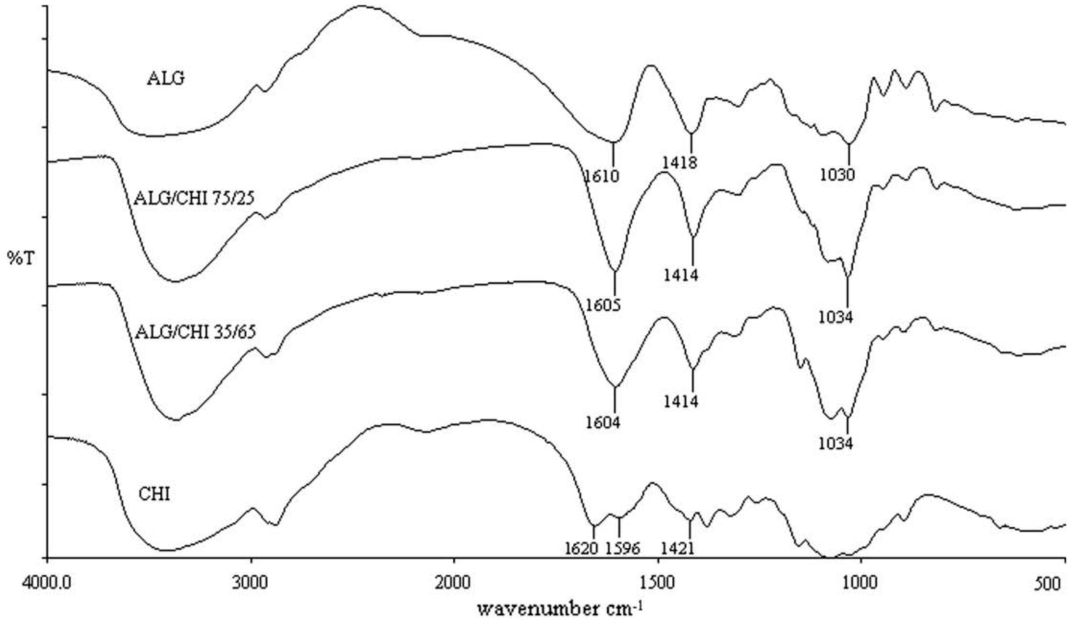

Fig. 1. FTIR spectra of alginate (ALG), chitosan (CHI), and hydrogels ALG–CHI 75/25 and ALG–CHI 35/65.

the variables *B*, *C* and *D* with the values 1, −1 and −1, respectively, giving a theoretical yield of 87%. These conditions correspond to the hydrogel produced with LCHI, at pH 6, using the two-step method, as in runs 3 and 4.

The CHI M_W was the main factor which influenced the yield. The ALG–CHI complexes are formed due to co-operative binding between calcium and ALG carboxyl groups, followed by CHI coating. LCHI has a lower M_W, which implies shorter chains and less available amino groups per molecular chain. Consequently, a greater quantity of LCHI chains will be used to bind the amino groups to the acid groups present in the ALG chains, resulting in a higher yield. The interactive effect between pH and CHI M_W (*BC*) is due to the stronger binding of chitosan to alginate at pH 6 that at pH 4, which is in agreement with Gåserød, Smidsrød, and Skjåk-Bræk (1998) Finally, the interaction effect between CHI M_W and preparation method (*CD*) may be attributed to the higher CHI coating efficiency after the ALG–Ca pre-gel formation. In a recent publication Becherán-Marón et al. (2004) have reported that the composition of the chitosan–alginate polyelectrolyte complex is independent of the molecular weight of chitosan. However, the addition method may have influenced on yield. In the indirect addition method, first a pre-gel is formed between ALG and Ca, followed by the chitosan coating which may contribute to yield maximization. In the direct addition method, due to the simultaneous hydrogel formation and chitosan coating, the ALG–Ca pre-gel complex formation step is skipped, lowering the yield.

3.2. Hydrogel morphology

The ALG–QUI hydrogels had spherical or spheroidal morphology, with variations in the particle size distribution. Fig. 2 shows the scanning electron micrographs of ALG–CHI hydrogel particles produced using the indirect addition method with 35% wt ALG, for different pH values and chitosan M_W. Hydrogels produced at pH 6 with MCHI (Fig. 2a) had spherical particles and a narrower size distribution, while those produced at pH 4 with LCHI (Fig. 2b) had spherical particles with a wider variation in their size. The particle size (Ps) data for the dried hydrogels are given in Table 2. The average size of the dried microspheres ranged from 3.1 to 7.4 μm, with an overall average of 5.1 ± 2.1 μm. It is important to note that considering the particle size deviation, the average diameter of all hydrogels was not significantly affected by the reaction conditions. Although the particle sizes were different from those obtained in our study, Wittaya-areekul, Kruenate, and Prahsarn (2006), also tested the effect of several parameters on ALG–CHI hydrogels, and found no influence of the reaction conditions on the average particle size. However, in our study, some conditions provided hydrogels with more homogeneous size, especially those produced by the direct addition method and LCHI, as in runs 10, 11 and 12. Thus, particle morphology was dependent on the reaction conditions.

3.3. Swelling behavior

The swelling degree of the hydrogels was evaluated at pH 1.2 simulating gastric media and at pH 6.8 simulating intestinal media, in order to exclude hydrogels produced with an excessive swelling degree under these pH conditions, which may cause the burst release of drugs. A fractionated factorial 2^{4-1} was conducted, in duplicate, in order to obtain reliable information about the main effects.

The results showed that the hydrogels had different swelling degrees at pH 1.2 according to the exposure time. Fig. 3 shows the average diameter variation of the hydrogels at pH 1.2 and pH 6.8 with exposure time. As observed in Fig. 3a, the average diameter at pH 1.2 after 1 h 30 min did not show a variation in size, with values around 5.0 μm. After 6 h, all hydrogels showed an increase in the average size, with diameter values from 10 to 14 μm. Finally, after 24 h of exposure, the average diameter was 19 μm with a wide standard deviation (±8 μm) and an irregular morphology.

The high size variation of the particles occurred due to the hydrogels rupturing into several pieces, in some cases followed by their agglomeration. This agglomeration may be caused by two effects. Firstly, the chitosan chains at low pH take expanded forms due to the intermolecular repulsions between the positively charged amino groups, leading to the network expansion followed by chitosan solubilization. This process would cause CHI leaching out of the microcapsule walls, and the remaining alginate–Ca particles could reorganize into smaller or bigger domains, causing the

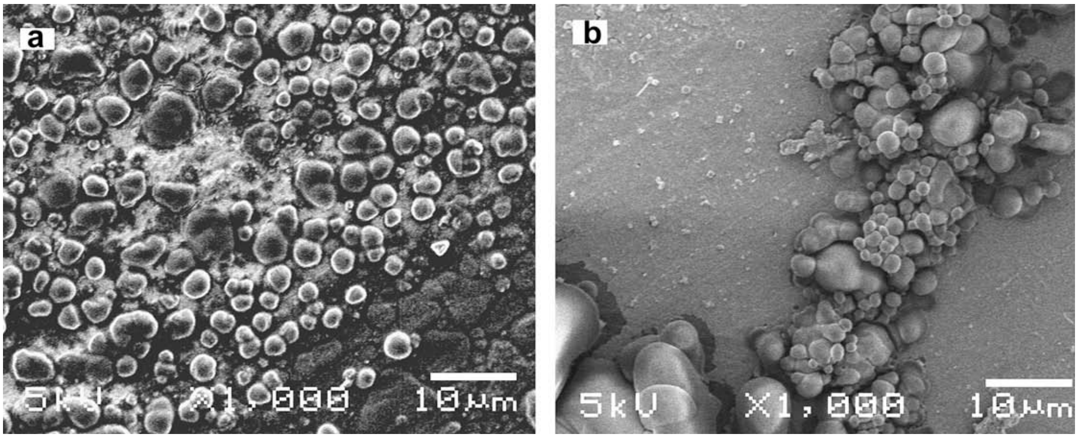

Fig. 2. SEM micrographs of ALG–CHI hydrogels produced by two-step addition under different reaction conditions: at pH 4 with MCHI (a) and at pH 6 with LCHI (b).

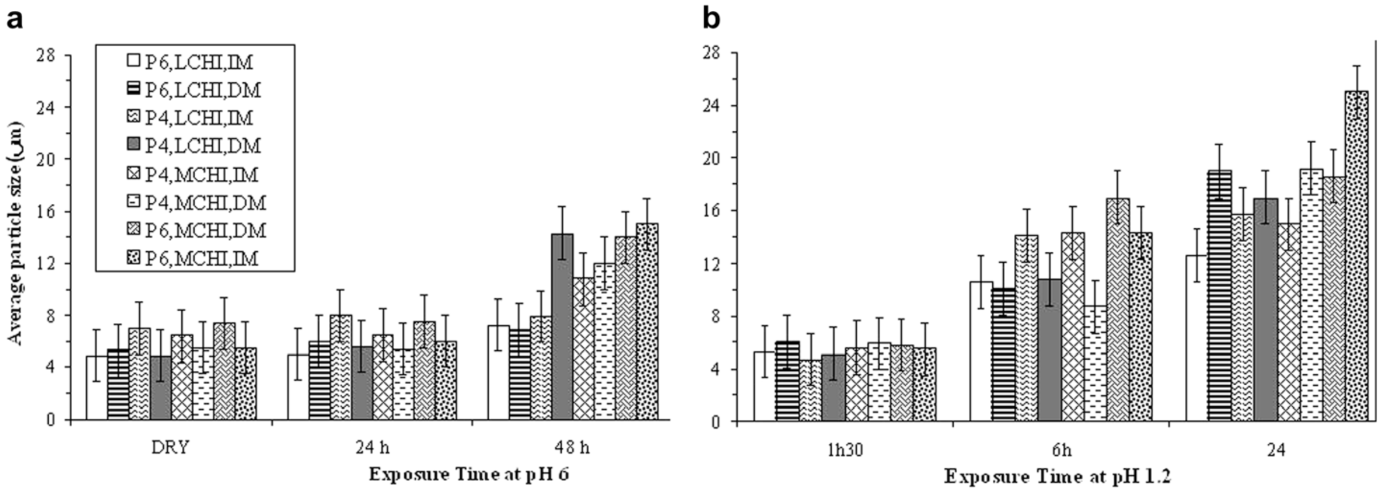

Fig. 3. Hydrogel average diameter in dried state, and submitted to pH 6.8 with time of exposure 24 and 48 h, and at pH 1.2 with time of exposure 1 h and 30 min, 6 and 24 h.

agglomeration between the particles or their rupture. Subsequently, ALG–CHI hydrogels showed a swelling degree dependent on the exposure time, showing good resistance to swelling in short periods of time. After a 6 h period, hydrogel swelling was dependent on the ALG content. The swelling degree of the hydrogels in water at pH 1.2 and pH 6.8, calculated according to Eq. (3), are shown in Table 2. Lower ALG content hydrogels have lower swelling degrees than those with higher ALG content (500% and 1500%, respectively), and it was possible to predict their behavior. Based on the calculated swelling degree values, a correlation was made between ALG content and the swelling degree at pH 1.2 after 6 h using linear regression (Eq. (6)):

$$\mathrm{Sw}_{\mathrm{pH1,2}}(\%) = 1009 + 416A \quad R^2 = 0.59 \tag{6}$$

where % Sw = swelling degree, A = alginate content.

The equation allows some important considerations, indicating that almost 60% of the variability in the swelling degree is attributed exclusively to the ALG content. Hydrogels with a lower swelling degree, which is desirable, can be theoretical obtained by replacing the factor A with -1, corresponding to hydrogels produced with a lower content of ALG. This means that after 6 h at pH 1.2 the hydrogels produced with a lower ALG content had a lower swelling degree than those with a higher ALG content.

The hydrogels were also submitted to pH 6.8 and their average diameter was measured after different time periods, as shown in Fig. 3b. It can be seen that all hydrogels had a similar and non significant swelling degree after 24 h, due to the proximity of the hydrated average diameter with those obtained under dry conditions. However, after 48 h at pH 6.8, some hydrogels showed a significant variation in their size, it being possible to estimate reliably the swelling degree. Hydrogels produced with CHI of medium molecular weight had a larger particle size, with a swelling degree higher than 500%. MCHI hydrogels may undergo more swelling due to the larger and more entangled chains, which may lead to a greater expansion of the network.

3.4. Hydrogel zeta potential

The relative charge beyond the hydrodynamically stagnant layer of the hydrogels was determined by zeta potential measurements. Results showed that there were significant differences in the resulting zeta potentials on varying the ALG content. The zeta potential values obtained for ALG–CHI hydrogels at pH 6.8 are shown in Table 2. The zeta potential at pH 6.8 was negative (-23 to -36 mV) for hydrogels with high alginate content and positive for those with low-alginate content (+17 to +30 mV). The hydrogels with higher potential values have a higher charge density of the amino groups on the surface, as in the case of hydrogels produced with higher chitosan content, in particular with MCHI (runs 13 and 7). Fig. 4 shows the pH dependent zeta potentials of some ALG–CHI microcapsules. All hydrogels showed a decrease in the potential with increasing pH. The point of zero charge indicates the pH that provides the balance between positive and negative charges. For all formulations, two distinct behaviors were observed according to

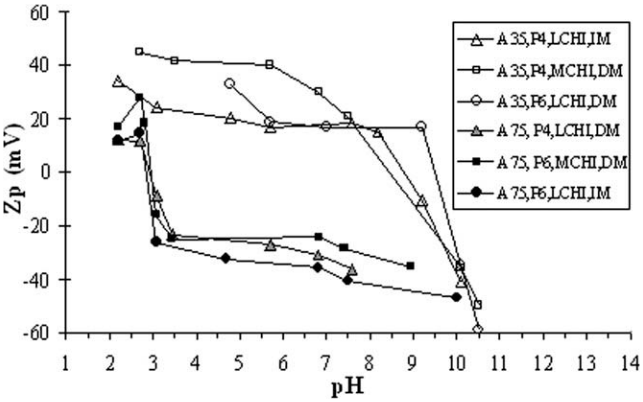

Fig. 4. Zeta potential variation with pH for ALG–CHI hydrogels produced under different reaction conditions.

the relative ALG content. For high alginate content hydrogels, the point of zero charge was situated at around pH 3.0, and for low-alginate content hydrogels it was at around pH 9.0. This observation is in agreement with another study, in which the hydrogel charge showed a variation with composition, showing a neutral charge at the pH values around 2 or 8 (Alsarra et al., 2002).

The statistical study using linear regression provided an equation to show the point of zero charge (Zp_{charge}) dependence on the ALG content (Eq. (7)).

$$Zp_{charge} = 5.43 - 2.85A \quad R^2 = 0.96 \tag{7}$$

This equation shows that the hydrogel point of zero charge is related to the alginate content of the hydrogels. Increasing the quantity of alginate, lower isoelectric points are achieved. Low ALG content hydrogels have a greater content of CHI (35/65 ALG–CHI), subsequently the negative-charged surface of the ALG–Ca internal core was coated by CHI protonated amino groups. This caused the neutralization of the ALG negative charges and shifted the hydrogel zeta potential to positive values until the pH reached around 8, which is the point of zero charge. In the acidic region, the chitosan amino groups are protonated, resulting in relatively high values of potentials. As the pH increases, the protonated amino groups are deprotonated and the alginate carboxylic groups are ionized, leading to a decrease in the potentials. Therefore, low ALG content hydrogels will have adhesive properties, since their positively charged surface favors the adhesion to the cell membranes, which are normally negatively charged (Alsarra et al., 2002; Liu et al., 2004).

3.5. Global balance of variables

The experimental design and the statistical analysis provided three linear equations, in which yield, swelling degree and surface-charge properties were described as a function of the significant parameters used. It was revealed through applying Eq. (5) that there is a direct dependence of chitosan M_W on the yield. Considering a lowest acceptable yield limit of 80%, reactions 3, 4 and 11 and 12 fit the general requirements. The equation fitted the experimental data and was able to predict that reactions performed at pH 6 with LCHI by the indirect addition method maximized the hydrogel yield. On the other hand, our results showed that swelling degree and zeta potential are both directly dependent on the ALG content. Eqs. (6) and (7) were successfully applied to predict the swelling degree behavior at pH 1.2 and the point of zero charge pH. The experimental data and the predicted equation for both properties are given in Fig. 5. Considering that the normalized values −1 and 1 correspond to the ALG content of 35% and 75%, respectively, it is possible to predict the hydrogel swelling behavior and surface charge using the proposed equations. Following this procedure, it is possible to produce hydrogel with positive charge on the surface and with a suitable swelling degree at a certain pH, by modulating the ALG content, i.e. by designing the reaction conditions, suitable hydrogels for site-specific drug delivery can be produced.

4. Conclusions

This study demonstrated that the independent variables tested (ALG content, solution pH, CHI M_W and preparation method) have an influence on the dependent variables (yield, particle size, swelling degree and zeta potential). Although the average particle size of the hydrogels remained unchanged on altering the reaction condi-

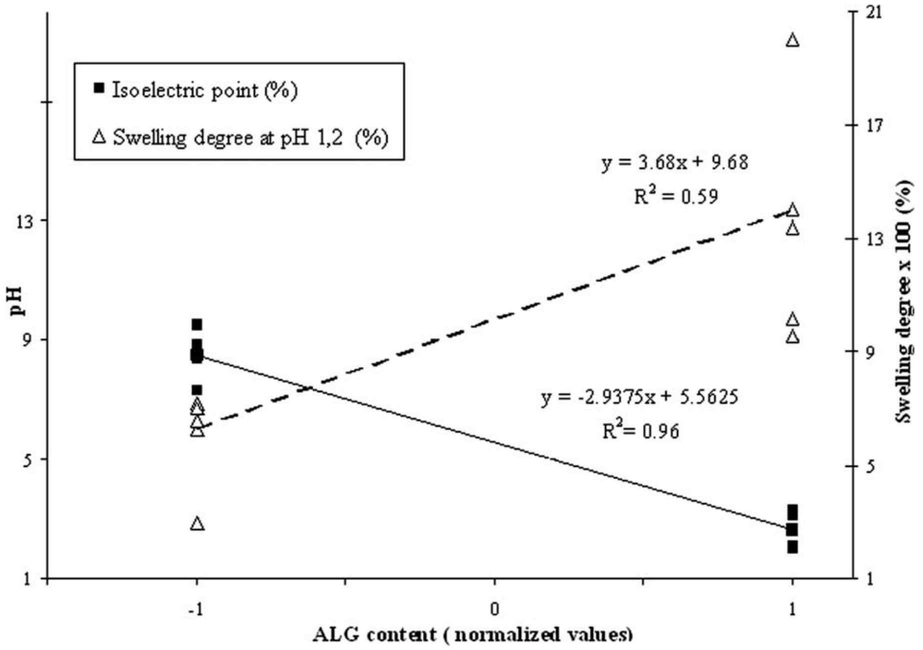

Fig. 5. Experimental data (points) and the equation with the predicted results (lines) for swelling degree and point of zero charge.

tions, hydrogels produced with LCHI using the direct addition method had a more homogeneous particle size. The statistical analysis was a useful tool for the interpretation of the results. The equations provided an estimation of the hydrogel yield, surface-charge properties and swelling behavior according to the variables studied. The variables must be carefully selected in order to produce hydrogels with an acceptable balance of properties. Hydrogels produced with LCHI at pH 6 by indirect addition provided yield maximization. The hydrogel swelling behavior and surface-charge properties were shown to be dependent on the ALG content which can be altered to produce hydrogels suitable for the site-specific drug delivery. In particular, hydrogels produced with LCHI, at pH 6, and with low ALG content are expected to present a higher yield and lower swelling degree when exposed to pH 1.2, and thus can have adhesive properties in intestinal media.

Acknowledgements

The authors thank the Governmental Agencies CNPq and CAPES for financial support.

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