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*Research Article*

# Microencapsulation by Spray Coagulation of Diltiazem HCl in Calcium Alginate-Coated Chitosan

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***Abstract.*** The aim of this work was to develop a procedure for encapsulation of diltiazem HCl by spray coagulation. Factors affecting the formulations such as the effect of NaCl on the solubility of diltiazem in alginate solution, surface tension, pH, viscosity of the coagulation medium, and the effect of drug load on drug release were studied. The drug load was increased substantially from 10 up to 320 mg/mL by adding 1.2% *w/v* NaCl in 1% *w/v* alginate solution. More stable microcapsules were obtained at pH 4.6 (acetate buffer) than at a pH 2.8 (lactic acid), and the microencapsulation process was favored by the type of chitosan that produced low turbidity and viscosity in the coagulation medium. A dose of 50 mg/mL of diltiazem HCl, 1.2% *w/v* NaCl, and chitosan CS allowed higher amount of drug to be encapsulated. The high water solubility of diltiazem HCl leads to fast release from the microcapsules.

**KEY WORDS:** calcium alginate; chitosan; diltiazem HCl; microencapsulation; spray-coagulation.

## INTRODUCTION

Numerous factors affecting the manufacturing of calcium alginate-coated chitosan microcapsules (CACC), as drug delivery formulations, have been extensively studied. The effect of the composition of the alginates and the cross-linking ions (1–3) as well as the effect of molecular weight and degree of acetylation of chitosan on the formation of the polyelectrolyte complex (4–7), coagulation time and the pH of the coagulation medium (5,8,9) has been reported. Most of the work has been oriented to the encapsulation of proteins (6–12), but not much work has been devoted to the study of the encapsulation of conventional low-molecular-weight drugs (2,13–15). Although diltiazem HCl microcapsules based on chitosan-alginate has not been described previously, our work on matrix tablets based on chitosan-alginate has yielded good properties for prolonged release systems of diltiazem HCl. Drug release is controlled at low polymer proportions in the formulation, mean dissolving time is high, and different dissolution profiles can be obtained by changing the mode of inclusion of the polymers (16). The release mechanism of diltiazem HCl from the chitosan-alginate matrix tablet is a combined process of diffusion and release. The tablet is able to uptake solvent without disrupting the microstructure due to its high elastic modulus (17).

One of the simplest manufacturing procedures of CACC involves dropping alginate solution into a $CaCl_2$/chitosan solution. In this procedure, alginate diffuses from the droplet core to the interface between droplet and gelling solution to form a membrane with calcium ions and chitosan, which results in a heterogeneous structure (18). There are other procedures such as alginate–chitosan multilayer beads (13,19), emulsification/internal gelation techniques (12), and alginate/poly-L-arginine/chitosan ternary complex (20), but the scaling up is more complicated.

The aim of this work was to develop a procedure for encapsulation of diltiazem HCl by spray coagulation and to study the factors affecting the formulation such as the effect of NaCl on the solubility of diltiazem in alginate solution, surface tension, pH, viscosity of the coagulation medium, and the effect of drug load on drug release.

## MATERIALS AND METHODS

The properties of the three types of chitosan used in this study are shown in Table I. Intrinsic viscosity was determined in 0.1 M acetic acid (HOAc) + 0.2 M NaCl at 25°C. Viscosity average molecular weight was determined by using the Mark–Howink constants in this solvent, $K=1.81$ E−03 mL/g, $a=0.93$ (21). The degree of acetylation (DA) was determined by $^1$H-NMR (22). The results are shown in Table I.

Alginic acid sodium salt of medium viscosity from Macrocystis pyrifera (AS) was from Sigma, USA. Viscosity of 2% solution at 25°C=3,500 mPas.

Diltiazem hydrochloride was from Dr. Reddy's Laboratory, India.

All other chemicals used were analytical grade.

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**ABBREVIATIONS:** A, Amplitude; CACC, Calcium alginate-coated chitosan; CLMW, Chitosan Aldrich, low molecular weight; CMMW, Chitosan Aldrich, medium molecular weight; CS, Chitosan Sigma, practical grade; DA, Degree of acetylation; HOAc, Acetic acid; Mv, Viscosity average molecular weight.

**Table I.** Viscosity Molecular Weight Average (Mv) and Degree of Acetylation of the Chitosan Used

| Type of chitosan | ηsp/c (mL/g) | Mv (kDa) | DA (%) |
|---|---|---|---|
| CS | 453 | 637 | 22.8 |
| CLMW | 203 | 269 | 21.7 |
| CMMW | 594 | 854 | 36.0 |

### Preparation of Alginate Solutions with NaCl and Dispersion of Diltiazem HCl

Two hundred fifty milliliters of alginate solution (Sigma, 3,500 mPas) at 1% *w/v* + 1.5% or 2.5% NaCl (*w/v*) was prepared. The solution was pressure filtered through a 0.65 μm membrane (30 psi, Sartorius, model SM 16249, Gottingen, Germany). The true concentration of alginate and NaCl in the solution was estimated as follows: 5 mL of the solution were dried at 70°C to constant weight (total solids weight), and then 10 mL of water was added to the residue. The water was evaporated, and the solid residue was calcined at 500°C. Finally, the residue was weighed (NaCl weight). The difference between the total solids weight and the NaCl weight gave the alginate weight. Each experiment was repeated 12 times.

Twenty milliliters of alginate solution was added by dropping from a 50-mL burette to different amounts of solid diltiazem HCl contained in a 250-mL Erlenmeyer flask. Diltiazem HCl dispersions were prepared in the range of 10 to 400 mg/mL, and the degree of dispersion of the drug in the solution was measured by turbidimetry (turbidimeter, Hanna HI 93703, Portugal).

### Evaluation of the Physicochemical Properties of the Coagulation Media

Different mixtures of ethanol/buffer sodium acetate–acetic acid 0.1 M solution (0:100 to 80:20 *v/v* ethanol/buffer) were prepared and the pH, turbidity, and surface tension of these mixtures were measured. The viscosity, pH, and turbidity of the coagulation media containing a mixture of ethanol/ buffer sodium acetate–acetic acid 0.1 M solution (20:80 *v/v*) with 1% *w/v* of three different chitosans and 1% *w/v* of $CaCl_2$ were measured.

### Preparation of Microcapsules by Spray-Coagulation Method

Aqueous dispersions (20 mL) of alginate (1% *w/v* + NaCl 1.5% *w/v*) and diltiazem HCl (100 mg/mL) were pumped using a peristaltic pump (Masterflex 7523-35, L/S tubing 14, Barrington, USA) at the rate of 1 mL/min into an automatic spray gun (Walther Pilot mod WA-XV, Wupertal, Germany) provided with a 1.5 mm diameter nozzle. The solution was sprayed at a pressure of 0.1 MPa and a variable volumetric air flow into 50 mL of $CaCl_2$ 1% *w/v* in a mixture of water/ethanol 80:20 *v/v* where the water phase was (1) buffer sodium acetate–acetic acid 0.1 M solution (pH 4.6), (2) the same buffer described in (1) + chitosan (Sigma, technical grade; 1% *w/v*), (3) lactic acid 1% *w/v* (pH 2.8) + chitosan (Sigma, technical grade; 1% *w/v*). The microcapsules were separated by centrifugation at 3,000 rpm for 15 min, washed with ethanol, and dried in an oven (Labtech mod LDO-080F, Namyangu, Korea) at 50°C to constant weight. Blank alginate microcapsules were prepared by the same procedure.

### Determination of the Size of the Microcapsules

Forty milligrams of dried microcapsules was dispersed in 25 mL of solvent (pH 5.5 water, pH 1.2 HCl 0.1 M, and pH 8.0 borate buffer), stirring for 1 h. Then, the microcapsules were observed under a microscope (ZEISS model AXIOSTAR PLUS, Oberkochen, Germany, magnification ×10) equipped with a digital camera (Nikon mod E450, Tokyo, Japan). The diameter of the microcapsules was measured using Image J software v. 1.36b. The particle size distribution and amplitude ($A$) were characterized by the ratio of $(D_{90}-D_{10})/D_{50}$, in which $D_{90}$, $D_{10}$, and $D_{50}$ represent the diameter below which 90%, 10%, and 50% by diameter of the particles are found, respectively.

### Drug Release Studies

Drug release studies were carried out in dissolution equipment (Pharmatest, type PTW SIII, Hainburg, Germany) at 37°C and 50 rpm. The paddle method (USP type 2) was used (23). A 200-mg portion of microcapsules was placed in 900 mL of acid medium (0.1 M HCl + 0.2 M KCl, pH 1.2) or basic medium (0.2 M $H_3BO_3$ + 0.2 M KCl) adjusted with 0.1 M NaOH to pH 8.0). Ten-milliliter aliquots were taken at different times. This was replaced with an equal volume of the medium. The diltiazem HCl content of the aliquots was determined by UV spectroscopy (UV/Visible UNICAM UV3 spectrometer, Cambridge, UK) at a wavelength of 236 nm. Each assay was done in triplicate. The dissolution data were analyzed according to Dobashi's model (24). According to the following equation:

$Cs(t) = Cs^{eq}\left(1 - e^{-t/\tau}\right)$ , where Cs is drug concentration in the dissolution medium; $Cs^{eq}$ is drug concentration in global equilibrium; $t$ is elution time; and $\tau$ is a time constant. $Cs^{eq}$ and $\tau$ are related to the chemical potentials and the diffusion coefficient of the drug.

### Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) measurements were made in a Bruker model IFS 32 spectrometer (Ettlingen, Germany). About 2 mg of the samples were ground thoroughly with KBr, and pellets were formed under a hydraulic pressure of $10^3$ kg/cm$^2$. The characteristic absorption bands for the polymers and the drug, respectively, were determined in blank Ca-alginate, chitosan–Ca-alginate microcapsules, and Ca-alginate and chitosan–Ca-alginate microcapsules loaded with diltiazem HCl. The spectra were obtained by averaging 20 scans in the spectral range of 4,000–700 cm$^{-1}$.

### Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was carried out under a nitrogen flow in a Mettler Toledo TC15 TA (Greinfensee, Switzerland) over the 30–250°C temperature range at a heating rate of 10 K/min. The sample weights examined were between 5 and 10 mg. The weight loss during the heating cycle was estimated using the associated software.

### Statistical Analysis

Data were analyzed by analysis of variance and significance of differences between means by the Tukey's multiple-range tests (Statgraphic version 4.0). A $p$ level of 0.05 was used to determine significance.

## RESULTS AND DISCUSSION

### Dissolution Studies of Diltiazem HCl in Alginate Solution

There are three main parameters (25) that control alginate solubility in water: pH, ionic force, and the salting-out effect of gellifying and nongellifying cations. In this work, sodium alginate was dissolved in distilled water (pH 5.5). At this pH, the uronic acid residues of the molecule are fully ionized because the alginate pKa is close to 3.5. Thus, there is a strong repulsion between polymer chains, which contributes to the hydration and solubilization of the polymer. It is also known that polyelectrolyte behavior, solvent, conformation, and viscosity properties of alginate are dependent on the ionic force of the solution. At high ionic force, the molecule adopts a less extended conformation. The solubility of the alginate decreases, and therefore, the viscosity of the solution also decreases, causing the salting-out effect.

The solubility of sodium alginate in water decreases when NaCl concentration is increased. This effect is accounted for by considering that the process occurs in two steps: (1) swelling and partial hydration controlled mainly by water diffusion and (2) dissolution and disentanglement of polymer chains. The disentanglement of molecules depends on the diffusion of water, ions, and alginate chains. The interaction between low-molecular-weight counter ions ($Na^+$) and large polyions ($Alg–COO^-$) decreases the fast movement of small ions and speeds up the movement of large polyions. Therefore, the second step of the dissolving process will be slower with increasing NaCl concentration because when the ionic force is increased, the electrostatic interaction between the alginate polyion and its counterion ($Na^+$) decreases, reducing the diffusion of alginate chains (26).

Table II shows that, when NaCl concentration was increased from 1.2% to 1.9% *w/v*, the alginate concentration dropped significantly from 0.94% to 0.75% *w/v*, showing a salting-out effect on the alginate molecule.

It is possible to disperse up to 10 mg/mL of diltiazem HCl in 1% *w/v* alginate solution, but at higher concentrations, the drug precipitates. The effect of NaCl on drug dispersion was studied at the two concentration levels, 1.2% and 1.9% *w/v* in 0.94% and 0.75% *w/v* alginate solutions, respectively, using diltiazem HCl concentrations of 10 to 400 mg/mL. Figure 1a shows that up to 320 mg/mL of diltiazem HCl can be dispersed in 0.94% *w/v* alginate + 1.2% NaCl *w/v* solutions, but at higher concentrations, the drug precipitates. When NaCl concentration was increased to 1.9% *w/v*, a maximum of 200 mg/mL of the drug could be dispersed (Fig. 1b).

**Table II.** Effect of NaCl Concentration on Alginate Solubility

| Solutions | Alginate % *w/v* | NaCl % *w/v* |
|---|---|---|
| 1% *w/v* alginate + 1.5% *w/v* NaCl | 0.94±0.04[a] | 1.20±0.03[a] |
| 1% *w/v* alginate + 2.5% *w/v* NaCl | 0.75±0.04[b] | 1.91±0.03[b] |

Different letters means significant differences between column ($p<0.05$)

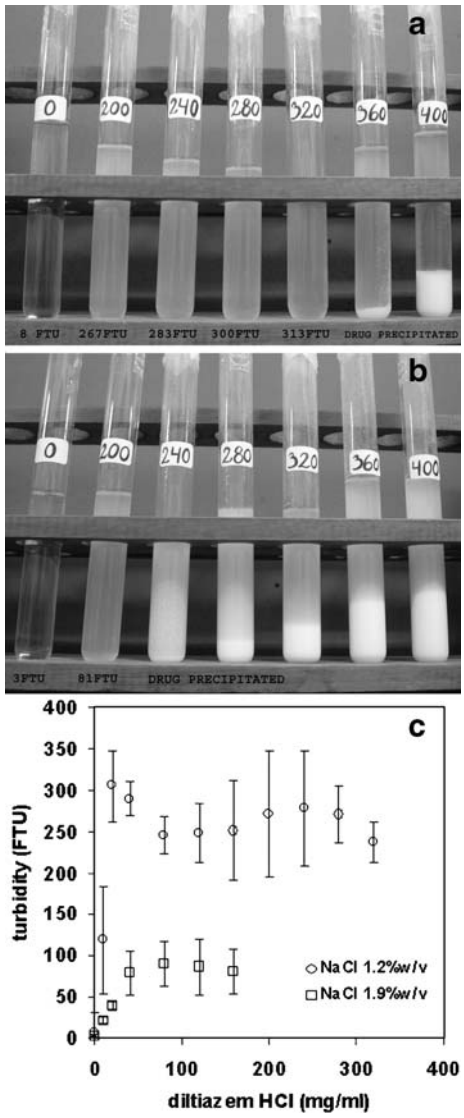


**Fig. 1.** Dispersion of diltiazem HCl in 1% *w/v* alginate solution with **a** 1.2% *w/v* NaCl and **b** 1.9% *w/v* NaCl. **c** Turbidity of 1% *w/v* alginate solution with 1.2% *w/v* NaCl or 1.9% *w/v* NaCl at different diltiazem HCl concentrations

When NaCl concentration was increased from 1.2% to 1.9% *w/v*, the turbidity of the solution decreased significantly ($p=0.05$) at the same concentration of diltiazem HCl, indicating a higher dispersion of the drug in alginate solution (Fig. 1c).

This work showed that low diltiazem HCl concentrations, up to 10 mg/mL, can be dissolved in 1% *w/v* alginate solution in water at pH 5.5, in spite of the high water solubility of diltiazem HCl, 520 mg/mL at 25°C (27), and of its pKa=7.7 (28).

The results showed that the addition of NaCl to the alginate solution increases drug dispersion dramatically, from 10 to 320 mg/mL. As mentioned earlier, 1.2% *w/v* NaCl does not affect the solubility of alginate, but 1.9% *w/v* NaCl causes salting-out. It has been reported that diltiazem HCl reacts with lambda-carrageenan in distilled water forming a complex

**Table III.** Effect of Sodium Acetate–Acetic Acid 0.1 M/Ethanol Mixtures on pH, Miscibility, Turbidity, and Surface Tension of the Mixture

| Ethanol (% *v/v*) | HOAc–NaAc 0.1 M (% *v/v*) | pH | Miscibility | Turbidity (FTU) | Surface tension (D/cm) |
|---|---|---|---|---|---|
| 0 | 100 | 4.41 | Miscible | 0 | 60.8 |
| 20 | 80 | 4.63 | Miscible | 16.2 | 45.4 |
| 30 | 70 | 4.78 | Miscible | 20.9 | 39.0 |
| 40 | 60 | 5.05 | Miscible | 20.4 | 36.0 |
| 50 | 50 | 5.57 | Partially miscible | – | – |
| 80 | 20 | 6.36 | Immiscible | – | – |

– not applicable

with poor solubility (28). Lambda-carrageenan has three sulfate groups per two galactose residues, and it has the highest charge density of the three types of carrageenan (kappa, iota, and lambda) (29,30). The study of the dialysis equilibrium of this complex in buffered media did not show significant differences in the amount of drug bounded to the polymer when the pH was varied between 1.8 and 6.8. However, the interaction decreased when the ionic force of the buffer was increased, pointing to an ionic interaction between the polymer and the oppositely charged drug (28).

The ternary system studied in this work (alginate-diltiazem HCl-NaCl) resembles the system described above. Alginate, the same as carrageenan, is an anionic polymer fully ionized at pH 5.5 and with a high charge density. At this pH, diltiazem HCl is in its cationic form, pKa 7.7, so there may be an electrostatic interaction between them. This would explain the low solubility of diltiazem HCl in alginate solution. The incorporation of NaCl into the system increases the ionic force, decreasing the electrostatic interaction between the drug and the polymer, thereby allowing the dispersion of a larger amount of drug in the system, but high levels of NaCl cause salting-out.

## Preparation of Microcapsules by Spray-Coagulation Method

### *Modification of Coagulation Media*

Microencapsulation of Ca-alginate by spray coagulation was carried out by spraying 1% *w/v* alginate solution in 1% *w/v* $CaCl_2$ in distilled water. Microcapsules were formed, but they were agglomerated, forming large aggregates. To avoid this problem, the coagulation medium was modified by testing different ethanol/water mixtures. Ca-alginate microcapsules coated with chitosan were prepared in one step by dissolving 1% *w/v* $CaCl_2$ + 1% *w/v* chitosan in a water/ethanol mixture. Since chitosan is not soluble in water, the water fraction in this mixture was replaced by 0.1 M sodium acetate/acetic acid buffer. The effect of the buffer/ethanol ratio on the turbidity, pH, and surface tension of the mixture was measured (Table III).

Table III shows that the mixture is miscible up to 40% *v/v* of ethanol and that the miscibility of the two phases decreases when the fraction of ethanol is increased because the turbidity increased. Surface tension decreased significantly when the ethanol fraction was increased.

It was observed that the addition of 20% *v/v* of ethanol is an adequate amount to avoid the agglomeration of the microcapsules. In fact, the surface tension diminished by 25% compared with buffer without ethanol, and the miscibility of both phases was maintained (see Table III).

The coagulation media was modified with ethanol because the microcapsules formed were agglomerated, forming large clumps. The effect of ethanol was to decrease the surface tension of the mixture and therefore of the contact angle, which suggests that the surfaces became less solvophobic with increasing ethanol concentration. It has been reported that the contact angles fitted well with the change of the adhesive forces when the molar fraction of ethanol was changed, indicating that for these systems the interaction forces are related to the surface wetting properties (31).

The effect of three types of chitosan, low molecular weight chitosan (CLMW), practical grade chitosan (CS), and medium molecular weight chitosan (CMMW), on the turbidity and viscosity of the coagulation medium ($CaCl_2$ 1% *w/v* in a mixture of water/ethanol 80:20 *v/v*, where the water phase was buffer sodium acetate–acetic acid 0.1 M solution + 1% *w/v* chitosan) was studied (see Fig. 2).

Figure 2 shows the viscosity behavior of the coagulation medium as a function of viscosity average molecular weight (Mv) of chitosan. The value of Mv for each type of chitosan is shown in Table I. It is observed from this table that the viscosity of the coagulation medium increases as the molecular weight of the chitosan increases. On the contrary, the turbidity of the coagulation medium decreases as the molecular weight of the polymer increases.

The microencapsulation method requires the migration of calcium to alginate to produce a Ca-alginate gel, as well as a good miscibility of chitosan to attain an electrostatic interaction with alginate. Therefore, the microencapsulation process will be favored by a type of chitosan that produces a low turbidity and viscosity in the coagulation medium. Considering both aspects, the best type of chitosan would be CS.

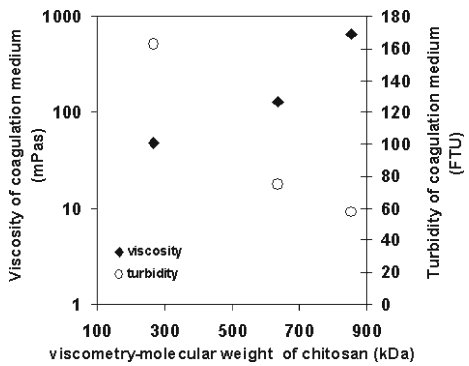


**Fig. 2.** Effect of the viscosity average molecular weight (Mv) of chitosan on the viscosity and turbidity of the coagulation medium

**Table IV.** Effect of the Presence of Chitosan in the Coagulation Medium on the Mean Size and Size Distribution of Microcapsules in Acid and Borate Dissolution Media

| | Acid medium[b] | | Borate medium[c] | |
|---|---|---|---|---|
| Coagulation medium[a] | Mean size (μm) | Amplitude | Mean size (μm) | Amplitude |
| Without chitosan (CS) | 329±17 aA | 1.0 | 402±15 bA | 1.0 |
| With chitosan (CS) | 296±23 aA | 1.4 | 265±24 aB | 0.9 |

Different lowercase letters means significant differences between rows. Different upper letters means significant differences between columns ($p<0.05$)
[a] $CaCl_2$ 1% *w*/*v* in a mixture buffer sodium acetate–acetic acid/ethanol 80:20 *v*/*v* (pH 4.6)
[b] 0.1 M HCl + 0.2 M KCl (pH 1.2), microcapsules loaded with 100 mg/ml diltiazem hydrochloride
[c] 0.2 M $H_3BO_3$ + 0.2 M KCl (pH 8.0), microcapsules loaded with 100 mg/ml diltiazem hydrochloride

### Study of the Effect of Air Flow Rate on Mean Size and Size Distribution of the Microcapsules

Three air flow rates were studied, 16, 20, and 30 mL/min. As air flow increased, the mean particle size of the microcapsules decreased from 1,100 to 200 μm. With respect to the size distribution, an amplitude ($A=D_{90}-D_{10}/D_{50}$) of $A\leq 1.5$ is recommended. At an air flow of 16 or 20 mL/min, acceptable amplitudes of 1.1 and 1.0 were obtained, respectively, but when air flow was increased to 30 mL/min, the amplitude was 1.8. An air flow of 20 mL/min, which gave a mean size of 600 μm and amplitude of 1.0, was chosen.

The effect of chitosan coating on the mean size of the rehydrated microcapsules loaded with 100 mg/mL of diltiazem HCl, in acid and borate, was studied.

Table IV shows the effect of the addition of chitosan to the coagulation medium on the mean size of the microcapsules rehydrated in acid (0.1 M HCl + 0.2 M KCl pH 1.2) and borate (0.2 M $H_3BO_3$ + 0.2 M KCl pH 8.0) medium. It was observed that the mean size of the rehydrated microcapsules not containing chitosan in borate is significantly much larger than in acid medium. On the contrary, when the microcapsules were coated with chitosan, no significant difference in the mean sizes was observed in either acid or borate medium. On the other hand, when microcapsules were rehydrated in acid medium, no significant difference between coated and uncoated microcapsules was detected. However, in borate medium, uncoated microcapsules have significantly larger size than those coated by chitosan.

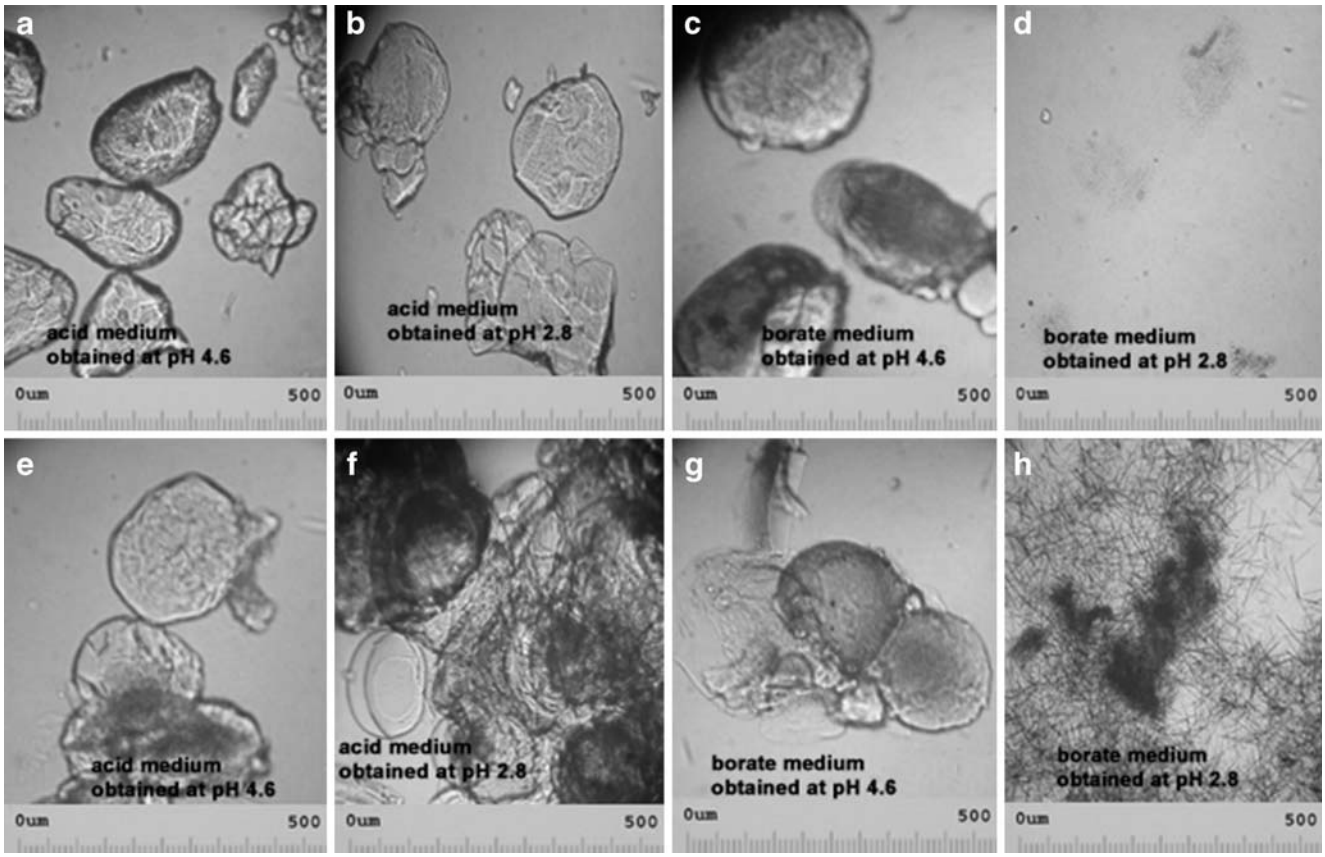


**Fig. 3.** Microcapsules of Ca-alginate loaded with 100 mg/mL of diltiazem (**a**–**d**). Microcapsules of Ca-Alginate coated chitosan loaded with 100 mg/mL of diltiazem (**e**–**h**)

The microcapsules prepared without chitosan corresponds to a Ca-alginate gel which is known to be soluble above pH 7, so in the borate medium, they should undergo greater swelling/dissolving than in the acid medium in which the gel is insoluble.

The preparation of microcapsules in the presence of chitosan at pH 4.6 promotes electrostatic interaction between alginate and chitosan, forming a polyelectrolyte complex. Therefore, under these conditions, a calcium alginate core coated with a layer of chitosan–alginate polyelectrolyte complex would be formed, probably with a layer of unreacted chitosan on top of this layer. This last chitosan layer may restrict the swelling of the microcapsule in borate medium, therefore leading to a smaller mean size compared to the uncoated microcapsules.

### Study of the Effect of the pH of the Coagulation Medium on Mean Size and Size Distribution of the Microcapsules

The microcapsules prepared in the acetate medium rehydrate and swell, while those obtained in the lactic acid medium dissolve completely in borate medium, as shown in Fig. 3c, d. Figure 3e, f shows that, when the microcapsules coated with chitosan obtained in lactic acid are rehydrated in acid, there is more aggregation of the microcapsules and more unreacted chitosan compared to those obtained in acetic medium, see Fig. 3a, b. In borate medium, Fig. 3g, h, the microcapsules obtained in lactic coagulation medium dissolve completely, leaving insoluble diltiazem crystals in an undissolved chitosan matrix.

The effect of pH of the coagulation media was evaluated. The pH of the alginate solution is 5.5, and therefore, the carboxyl groups of alginate are fully ionized. When the acetic acid–sodium acetate buffer in the coagulation medium was replaced by lactic acid, the pH dropped from 4.6 to 2.8. When the alginate spray solution came in contact with the coagulation medium, the degree of ionization of the alginate decreased substantially, probably decreasing the electrostatic interaction between the guluronic sites of the alginate and $Ca^{2+}$ and forming a mechanically weaker gel. This difference between the beads consisting of Ca-alginate obtained in acetic and lactic media becomes evident when the microcapsules are rehydrated in borate medium, where the gel is soluble and undergoes greater swelling. By reducing the pH of the coagulation medium, only the degree of ionization of alginate is affected but not that of chitosan. This lower ionization in alginate would have the effect of reducing the previously described interaction between guluronic sites and $Ca^{2+}$, and the interaction of the carboxyl groups of alginate with the protonated amino groups of chitosan would be disfavored. This is likely to produce a mechanically weak Ca-alginate core and lowers the formation of chitosan–alginate polyelectrolyte complex on the core.

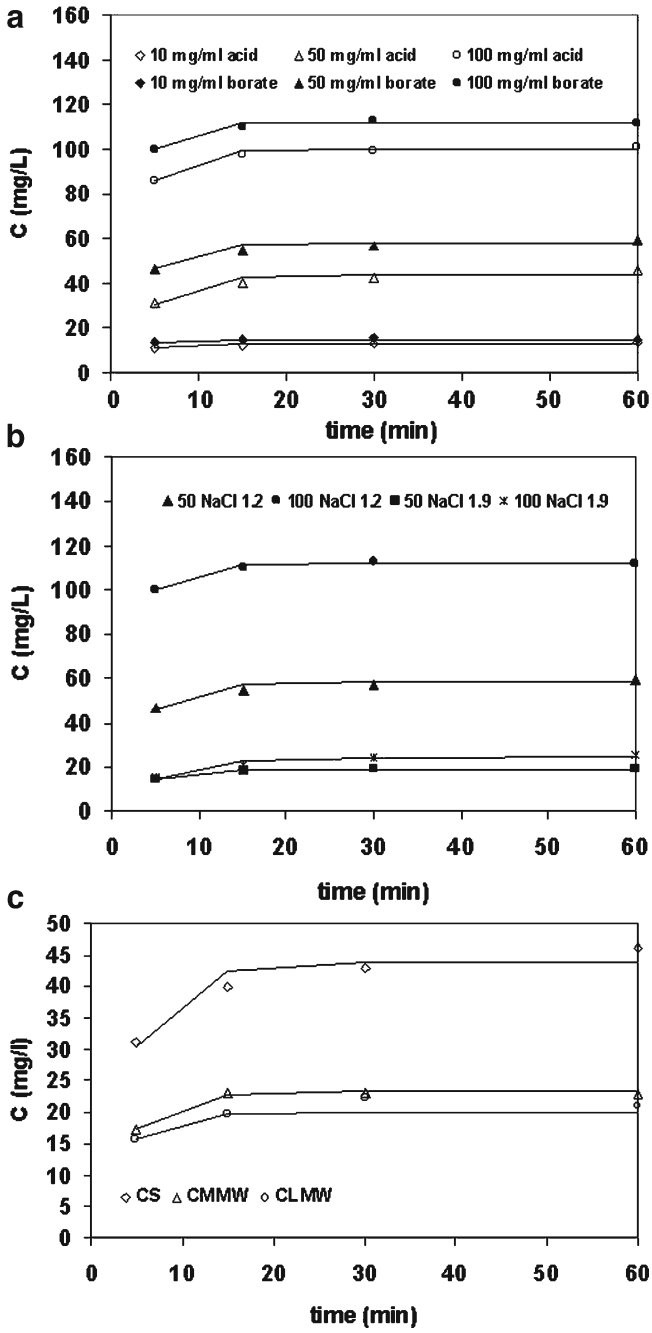


**Fig. 4.** Dissolution profiles of microcapsules coated with chitosan **a** at three different doses, **b** at two NaCl concentrations, and **c** with three types of chitosan. Each curve represents the data that are fitted to Dobashi's model by nonlinear regression analysis

### Drug Release Studies

The dissolution studies were carried out in two different media: acid (pH 1.2) and borate (pH 8.0). The effect of dose, type of chitosan, and NaCl concentration on drug release was evaluated. Microcapsules loaded with three different doses of diltiazem HCl, namely 10, 50, and 100 mg/mL, were prepared by the spray-coagulation method. The drug release data in both dissolving media are shown in Fig. 4a. This figure shows that the equilibrium concentration of different formulations increases when the dose is increased, and also that in the borate medium, the equilibrium concentration is higher compared to the acid medium for any dose. At low dose, 10 mg/mL, the drug was released immediately in both media, indicating that there was no drug encapsulation. For higher doses, 50 and 100 mg/mL, the drug is released quickly, reaching the equilibrium concentration after 30 min.

The effect of NaCl concentration on drug release was evaluated in borate (pH 8.0) for two different doses. From Fig. 4b, it is clear that the microencapsulation with 1.2% NaCl was better than with 1.9% NaCl. When the drug dose was increased from 50 to 100 mg/mL at 1.9% *w/v* NaCl, the equilibrium concentration only increased from 18.7 to 24.3 mg/L.

Based on previous results, a drug dose of 50 mg/mL and a concentration of 1.2% *w/v* NaCl were set. The effect of chitosan on drug release in acid medium was evaluated. It is clear from Fig. 4c that coating with CS chitosan was more efficient compared to that with other chitosans. This is in agreement with the results described above on the effect of the chitosans on the turbidity and viscosity of the coagulation media.

The dissolution data of microcapsules coated with chitosan in acid medium (pH 1.2) and borate medium (pH 8.0), were fitted to Dobashi's model (24) by nonlinear regression analysis. This model assumes that the drug distributes itself uniformly in the inner medium of the microcapsule and in the dispersing medium, but in the membrane, un-uniformness of the drug concentration appears; in the inside and the outside of the microcapsule, a "transient" equilibrium state appears. The boundary regions between the inside and the membrane wall and between the outside and the membrane wall are also in equilibrium. The gradient of the concentration in the membrane induces the drug flow from the inside of the microcapsule to the outside. The drug flow gradually changes the transient equilibrium state and finally makes the system to be in the "global" equilibrium state. Based on this model, the concentration in the global equilibrium, $Cs^{eq}$, was estimated. The $Cs^{eq}$ increased when the drug dose was increased; see Fig. 5a. This means that the diffusion velocity of the drug in the membrane is controlled by the concentration gradient. The increase of NaCl concentration from 1.2% to 1.9% *w/v* allows the dispersion of a larger amount of drug in the system due to the decrease of electrostatic interaction between the drug and the polymer, but high levels of NaCl cause salting-out. Thus, the equilibrium concentration diminished significantly when the NaCl concentration was increased; see Fig. 5b.

Figure 5c shows that $Cs^{eq}$ is dependent on the type of chitosan used in the coagulation media. The microencapsulation method requires the migration of calcium and chitosan to alginate to produce a chitosan-coated Ca-alginate gel. Thus, the combination of low viscosity of the coagulation media as well as good miscibility of chitosan from Sigma would explain the higher $Cs^{eq}$ value compared with the other types of chitosan.

## Ca-Alginate and Ca-Alginate–Chitosan Drug Interaction Analysis Using FTIR Spectroscopy and TGA

To evaluate the interaction between the drug and both matrices, the FTIR spectral region between 1,700 and 900 $cm^{-1}$ was analyzed, since the main absorption bands of diltiazem (1,682, 1,512, 1,252, and 1,033 $cm^{-1}$) are in that range (23). The presence of the characteristic IR bands of

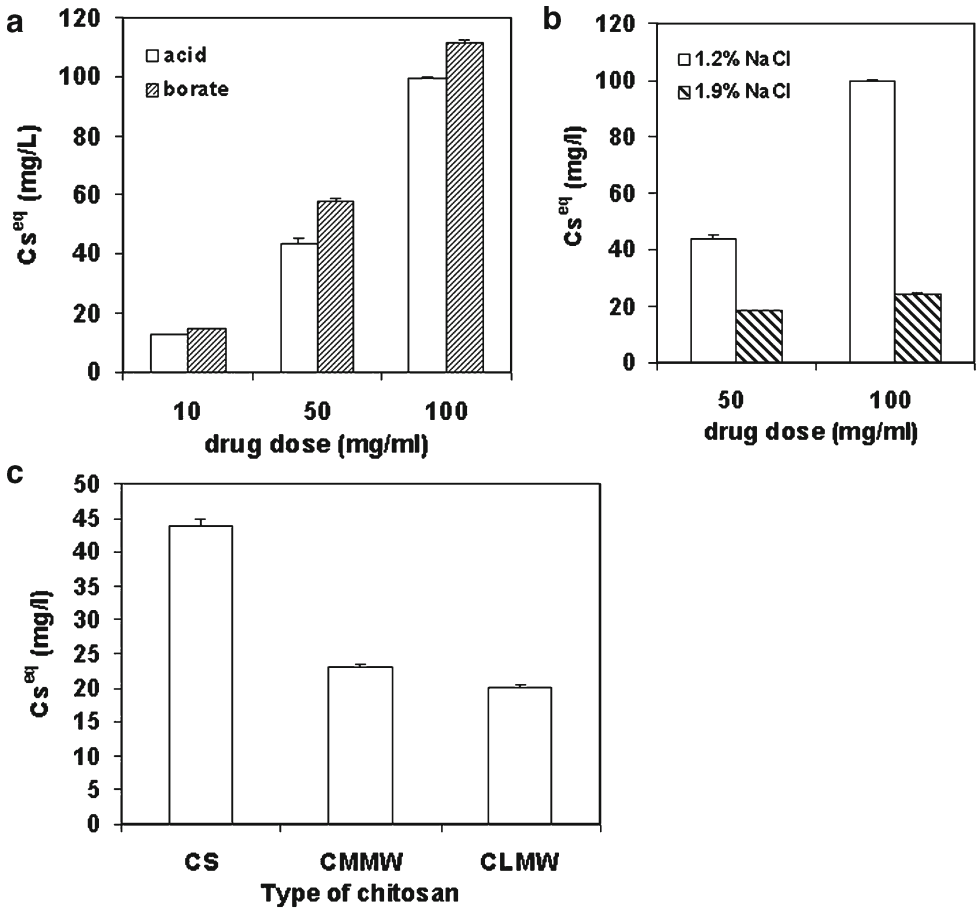


**Fig. 5.** Estimation of $Cs^{eq}$ from Dobashi's model of microcapsules coated with chitosan **a** at three different doses, **b** at two NaCl concentrations, and **c** with three types of chitosan. Each *bar* represents the standard deviation

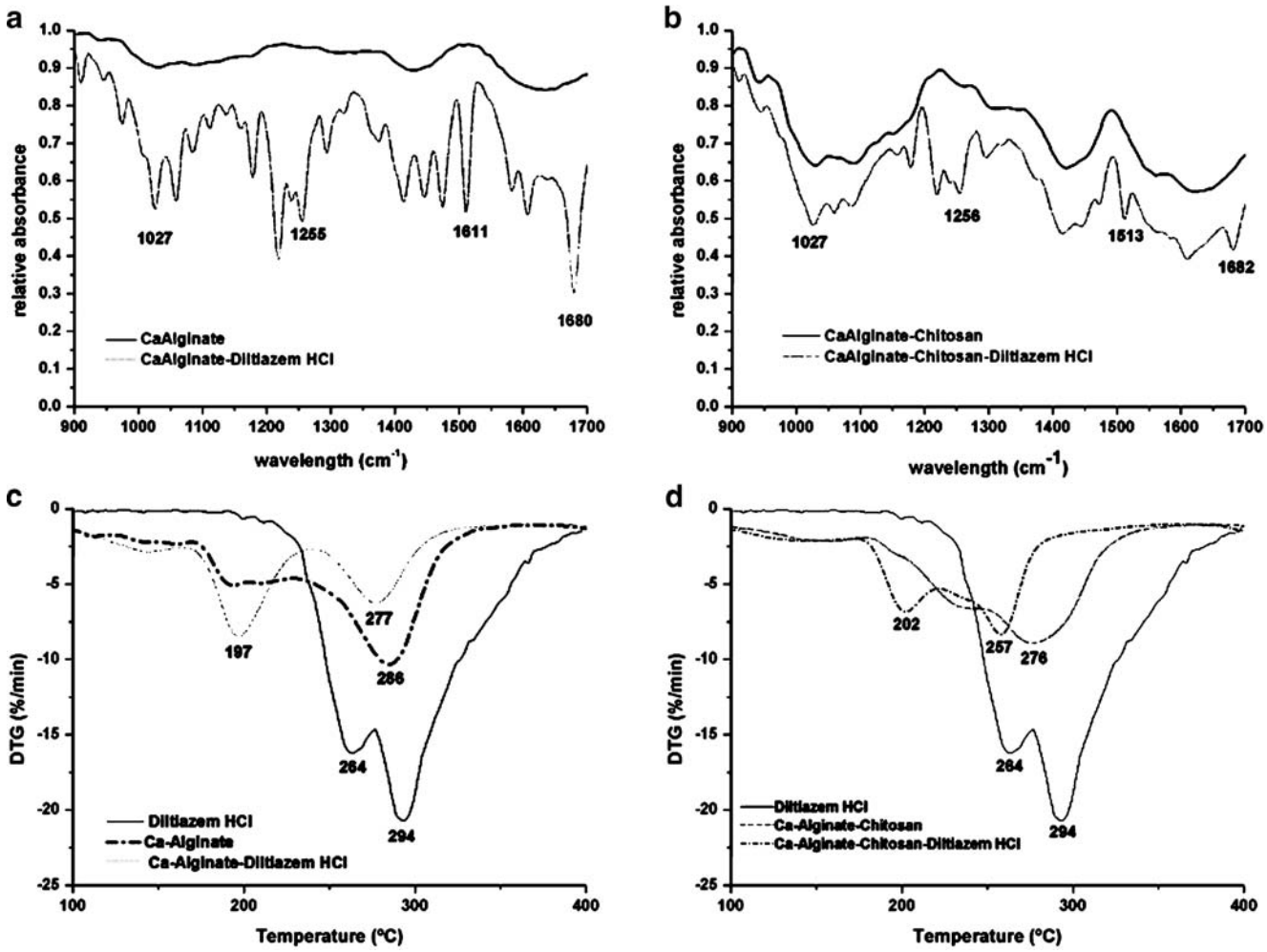


**Fig. 6.** FTIR spectra of **a** Ca-alginate and **b** Ca-alginate–chitosan microcapsules. Thermograms of **c** Ca-alginate and **d** Ca-alginate–chitosan microcapsules

diltiazem at 1,680, 1,511, 1,255, and 1,027 $cm^{-1}$ in the spectra of Ca-alginate microcapsules loaded with diltiazem suggests the absence of a high affinity and interaction between alginate and diltiazem in the microcapsule. The same situation was observed in the spectra of Ca-alginate–chitosan microcapsules loaded with diltiazem, as shown in Fig. 6a, b.

Figure 6c, d shows the differential thermogram of diltiazem HCl and the microcapsules described above in the temperature range at which diltiazem HCl shows two broad peaks (264°C and 294°C). In this region, Ca-alginate microcapsules showed one peak at 286°C, while Ca-alginate microcapsules loaded with diltiazem HCl showed two peaks at 197°C and 277°C. Ca-alginate–chitosan microcapsules showed one peak at 276°C, while Ca-alginate–chitosan loaded with diltiazem HCl showed two peaks at 202°C and 257°C. The difference in the thermal degradation behavior of unloaded microcapsules and drug-loaded microcapsules indicated that diltiazem HCl recrystallizes in the microcapsules.

The FTIR and TGA analysis of Ca-alginate–chitosan microcapsules loaded with diltiazem indicated that diltiazem HCl recrystallizes in the microcapsules.

## CONCLUSION

The surface tension, viscosity, and pH of the coagulation medium are very important factors for controlling agglomeration of the microcapsules, drug encapsulation, and stability of the microcapsules. The high water solubility of diltiazem HCl leads to fast release from the microcapsules.

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