Drug Development and Industrial Pharmacy, 28(2), 217–224 (2002)

RESEARCH PAPER

Study of the Influence of the pH Media Dissolution, Degree of Polymerization, and Degree of Swelling of the Polymers on the Mechanism of Release of Diltiazem from Matrices Based on Mixtures of Chitosan/Alginate

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ABSTRACT

The dissolution profiles of formulations based on mixtures of chitosan/alginate depend on the pH. It is possible to distinguish two processes: (a) a fast kinetic drug release up to 180 min, where the pH value changes from 1.17 to 2.21 and the drug released is controlled by the degree of polymerization and the quantity of chitosan in the formulation; (b) a low kinetic drug release between 210 and 480 min, where the pH value changes from 5.52 to 8.72 and the drug release from the matrix is controlled by the interpolymeric complex. In all formulations the order of release, according to Peppas's model in the range of fast kinetic drug release, was between 0.5 and 1.0. The mechanism of release was non-fickian diffusion, which corresponds to a coupling mechanism of diffusion and relaxation of the polymer.

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INTRODUCTION

Biopolymers are widely used in pharmaceutical systems because they give important functional properties to formulations. Between them polysaccharides have many characteristics, such as a wide availability from a variety of sources, inexpensive, safe, nontoxic, and biodegradable, that make them attractive for controlled release (1). In particular chitosan and alginate have been proposed as hydrophilic matrices for prolonged drug release (2–4). Chitosan is a partially *N*-deacetylated product from the natural polymer Chitin, which is found widely in nature. Alginates are obtained from brown algae. Chitosan is produced in Chile as a byproduct of the sea-spider industry (Punta Arenas, Chile). We have described in a previous paper dissolution studies developed at pH 7.4 with previous activation in acidic media, showing that matrices based on mixtures of chitosan/alginate (C/A) have more prolonged release than matrices based only on chitosan with the same percentage in the formulation (30%). Also, matrices based on C/A mixtures showed less erosion than matrices based on chitosan (5). The aim of this work was to evaluate the effect of the pH dissolution media, degree of polymerization of both polymers, and degree of swelling of the C/A mixtures on the mechanism of releasing Diltiazem from matrices based on C/A mixtures.

MATERIALS AND METHODS

Materials

Chitosan (Bioquímica Austral, Chile) (CB), desacetylation = 81%, viscosity of 1% solution in 1% acetic acid solution at 25°C = 400 mPa.

Chitosan (Sea Cure 242, Protan, Norway) (CP), desacetylation = 80%, viscosity of 1% solution in 1% acetic acid solution at 25°C = 200 mPa.

Alginic acid sodium salt high viscosity from *Macrocystis pyrifera* (AHV) (Sigma, USA), viscosity of 2% solution at 25°C = 1400 mPa.

Alginic acid sodium salt low viscosity from *Macrocystis pyrifera* (ALV) (Sigma, USA), viscosity of 2% solution at 25°C = 250 mPa.

Diltiazem hydrochloride.

Magnesium estearate.

Lactose monohydrate.

All other chemicals were of analytical grade.

Methods

Determination of the Optimal Ratio Between Chitosan and Alginate

Solutions of chitosan and alginate at 0.2% in acetic acid/sodium acetate buffer at pH 4 and 5 were prepared. Both solutions were mixed in different proportions to make 20 mL. The mixtures were incubated at 37°C for 48 hr, then centrifuged at 15,000 rpm for 20 min. Finally, the supernatant viscosity of solution at 25°C was measured using a Cannon-Fenske viscometer (6). The optimal ratio between chitosan and alginate was obtained when the supernatant viscosity was close to the solvent viscosity.

Determination of the Degree of Swelling at Equilibrium of Polymer Mixtures

Mixtures of CB/ALV and CP/AHV were prepared by mixing manually. Then, the mixture of powders was pressed using an infra-red (IR) manual press to obtain tablets of 7.1 ± 0.1 mm diameter and 0.6 ± 0.1 mm thickness ($n = 10$). The tablets were immersed in a dissolution bath which contained 1.0 L of 0.1 N HCl solution at 37°C and 100 rpm. The equilibrium of swelling of the mixtures was evaluated when the weight of swollen tablet reached its maximum value. This equilibrium was expressed as the degree of swelling at equilibrium (DS_e): DS_e = maximum weight of the swollen tablet/weight of the dried tablet.

Evaluation of the Interpolymeric Complex

The precipitate obtained by centrifugation from the mixture at the optimal ratio between chitosan and alginate was washed with distilled water, dried at 105°C for 2 hr, and milled in the mortar. Then, the product was analyzed by differential scanning calorimetry (DSC) at 10°C/min scanning rate (DSC 7, Perkin-Elmer).

Formulation and Preparation of the Tablets

The formulations studied are shown in Table 1. For each formulation the polymers were dry mixed with Diltiazem, lactose, and magnesium stearate to make 300 mg tablets. The tablets were obtained by direct compression (excentrical tabletting machine Wilhelm Fette type EIIN.270). The pressure of compaction was regulated to obtain a hardness of

Table 1

Formulations of Tablets Using as Matrices Mixtures of Chitosan/Alginate

Components	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
Diltiazem hydrochloride	30	30	30	30	30
Lactose	49	49	49	39	9
Chitosan (Sea Cure 242, Protan) (CP)	—	—	—	18	36
Chitosan (Bioquímica Austral) (CB)	6	9	12	—	—
Alginic acid sodium salt high viscosity (AHV)	—	—	—	12	24
Alginic acid sodium salt low viscosity (ALV)	14	11	8	—	—
Magnesium stearate	1	1	1	1	1

tablets over 7 kPa. The dimensions of the tablets were approximately 12 mm diameter and 2 mm thickness.

Dissolution Test

Three tablets of each formulation were assayed using the dissolution test proposed by Das and Gupta (7). This method tried to simulate the physiological pH through the gastrointestinal tract. The dissolution test was developed using the USP basket method at 100 rpm and 37°C (Pharmatest, type PTW Slll). Tablets containing 90 mg Diltiazem were added to 900 mL of solution A (4.2 mL HCl in 1 L, pH 1.4 ± 0.1). Every 30 min samples were taken of 5-mL aliquots which were replaced with solution B (40 g $Na_2CO_3 + 50$ g $NaHCO_3$ in 1 L, pH 9.3 ± 0.1) repeated to complete 10 sample tests. Then, the 5-mL aliquots were replaced by solution C (10 g $Na_2CO_3 + 20$ g $NaHCO_3$ in 1 L, pH 9.3 ± 0.1) up until the experiment completed at 8 hr. The samples were assayed spectrophotometrically at 236 nm (UV/Visible (VIS) spectrometer UV3, UNICAM) after suitable dilution.

RESULTS AND DISCUSSION

Figure 1 shows how the supernatant viscosity changed when the chitosan/alginate weight ratio changed. The optimal ratio between polymers is when the supernatant viscosity is close to 1, which means that both polymers have reacted completely to form an insoluble complex. This value was obtained when the percentage of chitosan in the mixture was between 40 and 50%. This optimal ratio was independent of the degree of polymerization of the polymers used in the mixture (CB/ALV or CP/AHV) and the pH of the dissolution media (pH 4 or 5). The complex formed between both polymers is produced by electrostatic attraction between the amine group of chitosan and the carboxylic group of alginate. Since there is one group for each monosaccharide unit, the molecular weight of each monosaccharide unit is almost the same, and both types of chitosan have a similar degree of polymerization. The optimal ratio between them would then be 1 : 1. In the region of the curve where there is a high excess of chitosan in the mixture, the supernatant viscosity is determined mainly by the ionization degree of chitosan which is not reacted with alginate. Thus, the supernatant viscosity at pH 4 is higher compared with pH 5 for both mixtures,

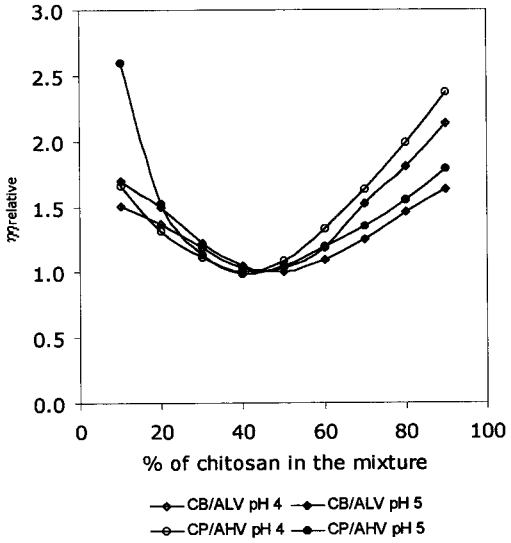

Figure 1. Relative supernatant viscosity of chitosan/alginate mixtures.

because chitosan, $pK_a = 6.3$ (8), is ionized more at pH 4 than at pH 5. On the other hand, in the region of the curve where there is a high excess of alginate in the mixture, the supernatant viscosity is determined mainly by the ionization degree of alginate which did not react with chitosan. Thus, the supernatant viscosity at pH 5 is higher compared with pH 4 for both mixtures, because alginate, $pK_a = 3.3$ (9), is ionized more at pH 5 than at pH 4. The greatest difference was for that mixture which contained the high viscosity alginate (CP/AHV).

The interpolymeric complex, formed at optimal weight ratio between CB/ALV (45/55), was separated and purified as described above. The results of DSC analysis are shown in Fig. 2. The DSC of ALV showed one endothermic peak at 109.1°C and one exothermic peak at 244.2°C. The first peak was related to the loss of water absorbed by the polymer, and the second one was related to the decomposition temperature of alginate. The DSC of CB showed one endothermic peak at 83.9°C since, related to the loss of water absorbed by the polymer, chitosan did not decompose in the range of temperature studied (40–250°C). The DSC of the complex showed two endothermic peaks, one at 95.3°C related to the loss of water absorbed by the complex and the other at 213.5°C which could be assigned to the formation of an ionic pair between the carboxylate group ($-COO^-$) of alginate and the ammonium group ($-NH_3^+$) of chitosan.

One of the aspects that is very relevant in the mechanism of drug release from matrix tablets, based on hydrogels, is the behavior of the swelling of the matrix (10). Figure 3 shows the degree of swelling at equilibrium for the mixtures CB/ALV and CP/AHV in three different proportions. It is evident from the figure that the degree of swelling is dependent on the chitosan proportion in the

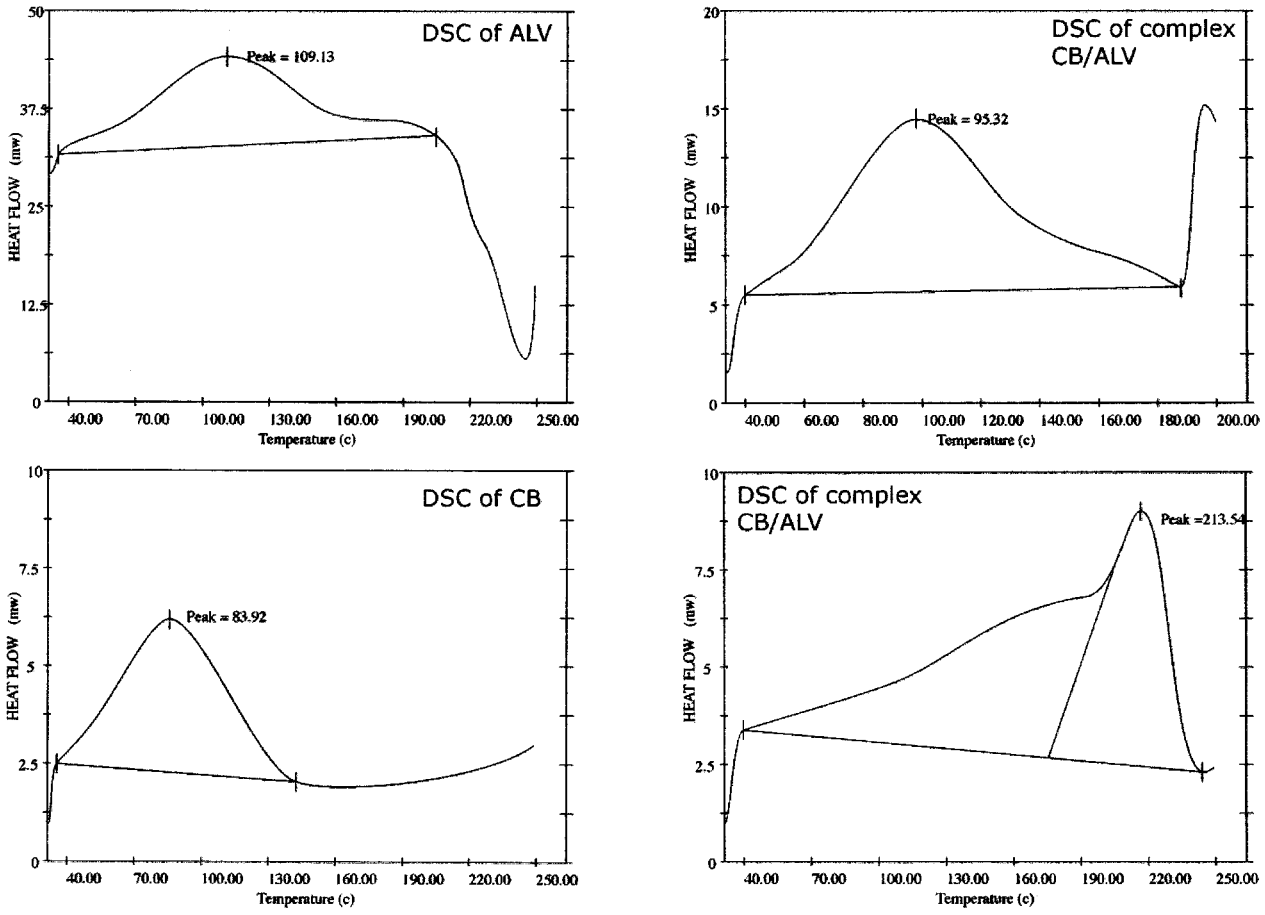

Figure 2. DSC of ALV, CB, and the complex CB/ALV.

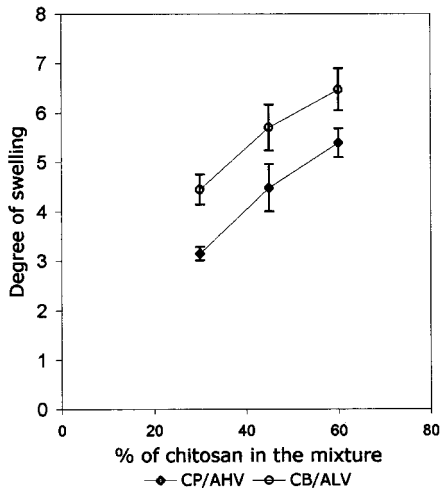

Figure 3. Degree of swelling of the mixtures CP/AHV and CB/ALV.

mixture. For both mixtures, when the proportion of chitosan is increased the degree of swelling is increased, and the mixtures which contain CB show a higher degree of swelling than the mixtures which contain CP. Thus, the degree of swelling obtained is also dependent on the degree of polymerization of chitosan used. It is known that the swelling of polyelectrolytes is controlled by the gel properties (charge of ionizable monomer, pK_a of ionic monomer, degree of ionization, concentration of ionizable monomer) and the swelling solution (pH, ionic strength, counterion). One of the theories which has been developed to explain the swelling equilibrium is based on the Donnan equilibrium (11). The Donnan equilibrium for the swelling experiments developed can be calculated as follows (12):

$$[H^+]_o/[H^+]_i = \left\{1 + ([Q\text{-}NH_3^+]_i/[H^+]_i)\right\}^{1/2}$$

where $[H^+]_o$ = proton concentration out of the gel, $[H^+]_i$ = proton concentration into the gel, $[Q\text{-}NH_3^+]_i$ = protonated chitosan concentration. The ratio $[H^+]_o/[H^+]_i$ estimated for both mixtures is shown in Table 2. As the ratio $[H^+]_o/[H^+]_i$ is greater than 1, the swelling equilibrium for both systems should be due to the Donnan potential.

The percentage of Diltiazem dissolved in all formulations studied showed a dependence on the pH, see Fig. 4. It is possible to distinguish two processes: (a) a fast kinetic drug release up to

Table 2

The Ratio $[H^+]_o/[H^+]_i$ Estimated for the Mixtures CB/ALV and CP/AHV

Mixture	Ratio (w/w)	Swelling Volume (mm^3)	$[H^+]_o/[H^+]_i$
CB/ALV	60/40	185.7	3.3
CP/AHV	60/40	150.8	4.6

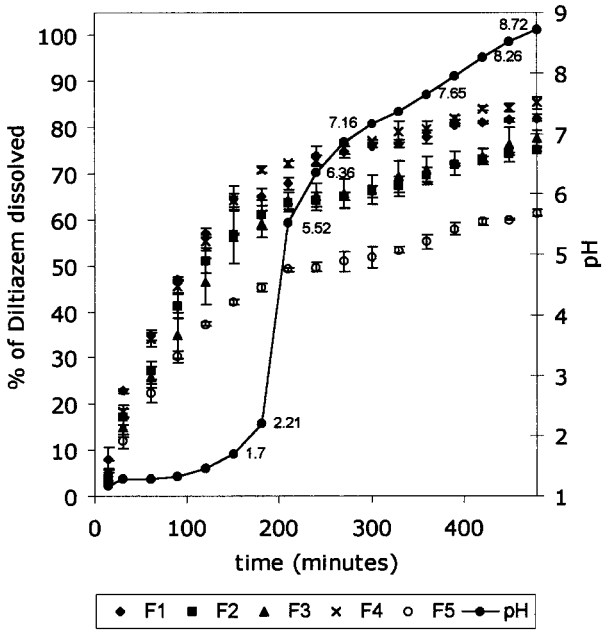

Figure 4. Dissolution profiles of formulations F1 to F5.

180 min, where the pH value changed from 1.17 to 2.21; (b) a low kinetic drug release between 210 and 480 min, where the pH value changed from 5.52 to 8.72. Assume that the complex between chitosan and alginate is in the ratio 1 : 1, due to the electrostatic interaction between one carboxylate group of the anhydromannuronic or anhydroguluronic unit of alginate and one ammonium group of chitosan. It is then possible to estimate the quantity (in millimoles) of chitosan–alginate complex at different pH values. This estimation can be done as shown in Table 3. As is shown in Fig. 5, there would be a very low quantity of complex in the range pH 1.17–2.21 and the polymers would be mainly chitosan in its protonated form and alginate in its non-ionized form, see Fig. 6. Thus, in this range of pH the drug release would be controlled by the

Table 3

Calculations for the Quantity of Ionized Forms of Chitosan and Alginate and Quantity of Interpolymeric Complex at Different pH

Data	Values	Parameters Estimated	Formula	Values
Example formulation F1				
pH	1.17	mmoles C_t	C_t/MwC	0.11
Tablet weight (mg)	300	mmoles A_t	A_t/MwA	0.24
Chitosan (mg) (C_t)	18	C_t	C+CH[a]	1.0
Alginate (mg) (A_t)	42	A_t	A+AH[b]	1.0
Molecular weight of monosaccharide unit of chitosan (MwC)	161.17	CH	$\frac{10^{(6.3-1.17)}}{1+10^{(6.3-1.17)}}$	0.999993
Molecular weight of monosaccharide unit of alginate (MwA)	176.14	A	$\frac{10^{(1.17-3.3)}}{1+10^{(1.17-3.3)}}$	0.007359
pK_a of chitosan	6.3	mmoles CH	CH × mmoles C_t	1.12×10^{-1}
pK_a of alginate	3.3	mmoles A	A × mmoles A_t	1.75×10^{-3}
		mmoles CH–A complex		1.75×10^{-3}

[a]CH = fraction of chitosan ionized; C = fraction of chitosan unionized.
[b]A = fraction of alginate ionized; AH = fraction of alginate unionized.

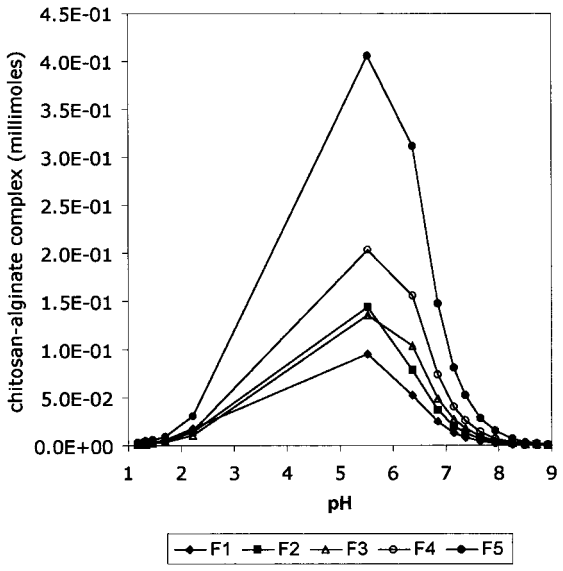

Figure 5. Quantity of interpolymeric complex estimated for formulations at different pH.

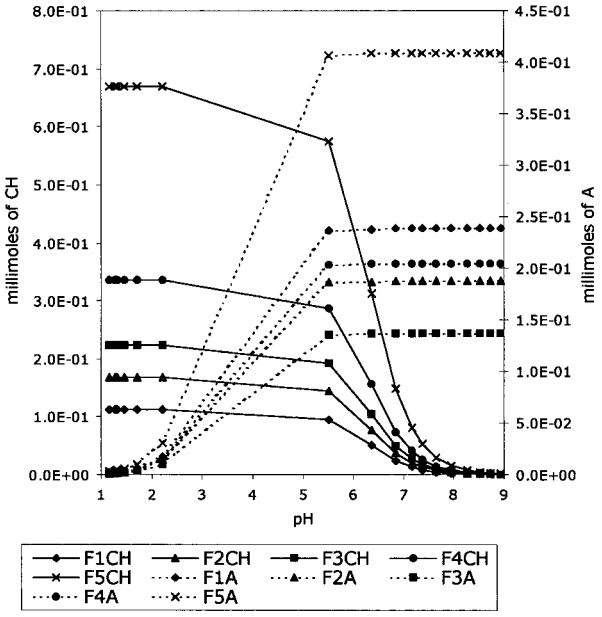

Figure 6. Quantity estimated of ionized forms of chitosan (CH) and alginate (A) at different pH.

degree of polymerization and the quantity of chitosan in the formulation. Thus, F5, which has the highest quantity of chitosan, mainly in its protonated form (see Fig. 6), shows the lowest percentage of drug release, see Fig. 4. Formulations F1 to F3, which have the same type of chitosan but in different proportions in the formulation, show that when the proportions of chitosan are increased the percentage of drug release is slightly decreased, but not significantly, see Fig. 4. This fact should be due to the degree of swelling of the matrix that is increased when the proportion of chitosan is increased, see Fig. 3. F4 showed a similar profile of release to that of F1, in addition to F4 having more chitosan in the mixture than F1. This result could be

explained based on the lower degree of swelling of CP/AHV compared with CB/ALV, see Fig. 3. As observed in Fig. 4, there is a shift from a fast kinetic drug release to a slow kinetic drug release at pH 5.5, which is the pH where the maximum quantity of chitosan–alginate complex is formed, see Fig. 5. At this pH the drug release from the matrix would be controlled by the interpolymeric complex, which would explain the diminished rate of drug release in all formulations. The percentage of drug released by F1 is significantly higher than by F2 and F3. This result correlates with the fact that the quantity of complex for F1 is lower compared with F2 and F3, see Fig. 5. On the other hand, F4, though it has a higher quantity of complex than F2 and F3, shows a similar release profile as F1. This result could be explained based on the lower degree of polymerization (*DP*) of the chitosan used in the formulation F4, CP, compared with formulations F2 and F3, CB. In the F5 formulation, the effect of the lower *DP* of CP would be compensated by the large quantity of matrix used, 60%, in the formulation.

The precise determination of the mechanism of drug release from the swelled matrix is complex, especially when there is more than one polymer as matrix. lt has been described that the performance of the hydrophilic swelled matrices as a prolonged drug release system is dependent on the hydration properties of the polymers, gel-forming properties, and relaxation of polymer chains when the fluid gets into the matrix (13). For these systems, it is recommended to analyze the dissolution data using exponential models. Thus, Peppas's model (14) was applied to the set of dissolution data, using a non-linear regression (Table Curve vers. 1.0, Jandel Scientific), for the formulations F1 to F5. The results are shown in Table 4.

As shown in Table 4, formulations F1 to F4 were fitted to Peppas's model during the fast kinetic drug release period, where chitosan is in its ionized form and alginate is in its non-ionized form. In the case of formulation F5, when all the dissolution data were considered they did not fit into Peppas's model. This may be because, in the fast kinetic drug release period, the release of Diltiazem is controlled by the swelling properties of chitosan and in the slow kinetic drug release period, the release of Diltiazem is controlled by the interpolymeric complex. Thus, only the fast kinetic drug release period was considered to fit Peppas's model.

In all formulations the order of release was between 0.5 and 1.0. The mechanism of release was non-fickian diffusion, which corresponds to a coupling mechanism of diffusion and relaxation of the polymer (15).

The dissolution data considered up until 8 hr were fitted to Weibull's model (16) using a non-linear regression (Table Curve vers. 1.0, Jandel Scientific) without considering a latency period because the drug is evenly distributed in the matrix. The dissolution times estimated, t_d, with a 95% level of confidence for all formulations are shown in Fig. 7. The formulations F5 and F2 had the highest dissolution times, 5.1 and 4.3 hr, respectively. F2's is comparatively longer than that of F5 because, over a period of 24 hr, it releases over 90% Diltiazem compared with 76% F5, and the quantity of matrix used in F2 is much lower compared with F5, 20% and 60%, respectively.

CONCLUSIONS

The optimal ratio between polymers was obtained when the percentage of chitosan in the

Table 4

Dissolution Data Fitted to Peppas's Model

Formulation	k (min^{-n})	n ($n \pm 5\%$ CI[a])	R^2	M/M_∞ (%)	pH
F1	2.27	0.67 ± 0.07	0.9970 ($n=4$)	57.1	1.28–1.46
F2	1.31	0.76 ± 0.18	0.9786 ($n=5$)	56.9	1.28–1.70
F3	1.11	0.77 ± 0.15	0.9800 ($n=6$)	59.1	1.28–2.21
F4	1.47	0.76 ± 0.17	0.9880 ($n=4$)	55.4	1.28–1.46
F5	1.37	0.68 ± 0.14	0.9779 ($n=6$)	45.4	1.28–2.21

[a]Confidence interval.

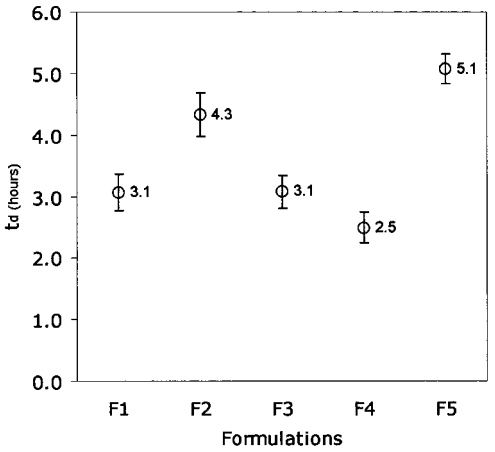

Figure 7. Dissolution time estimated according to Weibull's model.

mixture was between 40 and 50%, which corresponds approximately to a 1 : 1 ratio between chitosan and alginate due to the electrostatic interaction between one carboxylate group of the anhydromannuronic or anhydroguluronic unit of alginate and one ammonium group of chitosan. The optimal ratio was independent of the degree of polymerization of the polymers used in the mixture (CB/ALV or CP/AHV) and the pH of the dissolution media (pH 4 or 5). By DSC the complex showed a second endothermic peak at 213.5°C, which could be assigned to the formation of an ionic pair between a carboxylate group ($-COO^-$) of alginate and an ammonium group ($-NH_3^+$) of chitosan. The degree of swelling, in acidic media, was dependent on the chitosan proportion in the mixture. For both mixtures, when the proportion of chitosan was increased, the degree of swelling increased, and the mixtures which contained CB showed a higher degree of swelling than the mixtures which contained CP. Thus, the degree of swelling obtained is also dependent on the degree of polymerization of chitosan used. The equilibrium of swelling for both mixtures was controlled by the Donnan potential. The dissolution profiles of formulations showed a dependence on the pH values. It is possible to distinguish two processes: (a) a fast kinetic drug release up to 180 min, where the pH value changed from 1.17 to 2.21 and the drug release is controlled by the degree of polymerization and the quantity of chitosan in the formulation; (b) a low kinetic drug release between 210 and 480 min, where the pH value changed from 5.52 to 8.72 and the drug release from the matrix is controlled by the interpolymeric complex. In all formulations the order of release, according to Peppas's model in the range of fast kinetic drug release, was between 0.5 and 1.0. The mechanism of release was non-fickian diffusion, which corresponds to a coupling mechanism of diffusion and relaxation of the polymer. The formulations F5 and F2 had the highest dissolution times, 5.1 and 4.3 hr, respectively. F2 is comparatively better than F5 because in a 24-hr period over 90% Diltiazem is released compared with 76% F5 and the quantity of matrix used in F2 is much lower compared with F5, 20% and 60%, respectively.

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