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Study of the prolonged release of theopylline from polymeric matrices based on grafted chitosan with acrylamide

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The aim of this work was to study the performance of chitosan (CB) grafted with acrylamide (CB-g-A) as prolonged drug release matrix as compared with unmodified chitosan. A non-pH dependent swelling behaviour for the matrix tablets based on grafted chitosan was observed. The overlaping between degree of swelling measured by weighing (DS_w) and measured by increase of diameter (DS_d) up to 240 minutes showed that the swelling process could be isotropic. The non-pH dependent swelling behaviour of these matrices could be explained by the partial substitution of amine groups of the chitosan chain by acrylamide. The grafting reaction provides an ionizable amine group by a neutral amide group which make the matrix non pH-dependent. On the contrary, the matrix tablet based on chitosan showed a pH dependent swelling behaviour where the swelling process could be anisotropic. The higher degree of erosion and swelling of the formulation based on CB-g-A600 (%G = 600) compared with the formulation based on chitosan and CB-g-A418 (%G=418) could explain the higher fraction of theopylline released. For all formulations studied in this work, the amount of theopylline released from the matrix tablets was found to be controlled by a combination of the diffusion process and relaxation of the polymeric structure. These results match with the controlled swelling behaviour and low degree of erosion observed for these systems.

1. Introduction

Chitosan, and other natural polymers, such as cellulose and pectin can be modified by graft copolymerisation with vinyl monomers [1]. The grafting reaction is performed in aqueous solution using a redox type initiator such as potassium persulfate. Free radicals generated by decomposition of the initiator are capable of abstracting hydrogen atoms from chitosan and therefore producing radical sites for grafting on chitosan macromolecular backbone. The grafting process consists of three different steps, initiation, propagation and termination. Hydrogels from chitosan have been prepared in our laboratories through grafting with acrylic acid [2].

Acrylamide is a vinyl monomer, which copolymerizes with other vinyl monomers and produces hydrogels with anionic or cationic characteristics. The ionic nature of the hydrogels produced depends on the comonomer used. Some specific characteristics of a hydrogel can be designed by controlling parameters such as degree of cross-linking and molecular weight.

Chitosan, and chitosan in mixtures with anionic polymers such as alginate and pectin have shown good properties to be used as matrix system for prolonged drug release [3–7]. The matrix systems as oral dosage forms for prolonged drug release are widely studied because they offer good flexibility in the drug dissolution profiles. However, the design of the matrix is complicated because its behaviour depends on the properties of the materials that compose the matrix and the properties of the drug used. In general, when hydrogels are exposed to the dissolution media, the drug liberation is controlled by two main processes: a) by swelling of the matrix and b) by the dissolution/erosion in the periphery of the matrix [8].

The properties and the use of chitosan grafted with acrylamide as prolonged drug release matrix system have not been described in the literature. The mechanical and viscoelastic properties of a polymeric matrix and its microstructure are important properties that affect swelling behaviour, erosion and drug diffusion through the matrix. All these factors define the drug dissolution profile from the matrix. Also, as the polymer used as matrix has ionizable groups its behaviour is affected by the pH and ionic strength of the dissolution media.

The aim of this work was to study the perfomance of chitosan grafted with acrylamide as prolonged drug release matrix as compared with unmodified chitosan.

2. Investigations and results

Chitosan is a material with good compression properties [9]. Chitosan tablets obtained by direct compression have been used as polymeric matrix for prolonged drug release. It has been observed that when the percentage of chitosan in the formulation is increased, the drug release from the matrix is more prolonged but the percentage of drug retained inside the matrix is increased [10].

Table 1: Formulations of theopylline using chitosan and grafted chitosan with acrylamide as polimeric matrix

Components	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
Theophylline	20	20	20	20	20
Avicel® PH 101	28	28	28	28	28
Chitosan (CB)	50	–	–	–	–
Bioquímica Austral					
*CB-g-A	–	50	–	–	–
%G = 53; %E = 22.3					
*CB-g-A	–	–	50	–	–
%G = 213; %E = 72					
*CB-g-A	–	–	–	50	–
%G = 418; %E = 96					
*CB-g-A	–	–	–	–	50
%G = 600; %E = 75%					
Magnesium stearate	2	2	2	2	2
Diameter (mm)	12	12	12	12	12
Hardness (Kp)	8.0	4.0	5.7	2.5	3.5
Gelification behaviour	++	–	+	++	++

++ = The tablet gelified in HCl 0.1 N, pH 1.2.
+ = The tablet desintegrated in HCl 0.1 N, pH 1.2. before one hour
– = The tablet desintegrated in HCl 0.1 N, pH 1.2. before 30 minutes
* = CB-g-A= chitosan grafted with acrylamide
$\%G = 100(W_2\text{-}W_1)/W_1$
$\%E = 100(W_2\text{-}W_1)/W_3$

Matrix based on chitosan grafted with acrylamide showed that when the percentage of grafting (%G) increased from 53% to 600%, the perfomance of matrix gelation increases in acid media. Thus, at low percentages of grafting (53% and 213%) the tablets could not form a stable gel layer and therefore these were desintegrated at less than 1 h. At higher percentages of grafting (418% and 600%) the tablets can form a stable gel layer in acid media. At the same time it was observed that the grafted chitosan had less compactibility as compared to that of unmodified chitosan, which is reflected by the lower hardness of formulations F2 to F5 compared to the formulation F1 (Table 1). It has been reported that graft copolymerization of D,L-lactic acid (LA) and/or glycolic acid (GA) onto chitosan by condensation reactions leads to grafted chitosan which is amorphous in nature [11]. Thus, the grafting process affects the compressibility of chitosan which is reflected in the tablet hardness for formulations F2 to F5 compared witth formulation F1 made with unmodified chitosan.
Two formulations, namely F4 and F5, based on grafted chitosan were chosen for the following studies by considering their gelling ability in acid media and the difference in the % G between them. Formulation F1 was also studied as reference.

2.1. Chemical reactions during the grafting process

The possible mechanism for the formation of active sites on chitosan could hence be postulated, by analogy with the cellulose, and by considering that chitosan can act as a weak reducing agent. Therefore, the reactions leading to grafting of a vinyl monomer onto chitosan and its subsequent propagation and termination are based on the primary building of radicals [12] (Scheme 1):

Scheme 1

$$SO_4^{\bullet -} + H_2O \rightarrow HSO_4^- + {}^{\bullet}OH$$
$${}^{\bullet}OH + C{-}H \rightarrow H_2O + {}^{\bullet}C$$

The formation of the chitosan macroradical (formation of active sites for grafting) can occur by hydrogen abstraction possibly from C6 as well as from $-NH_2$ groups. However, to our knowledge no mechanism for grafting of vinyl monomers onto this polyssacharide has been described.
The following reaction show the grafting and propagation of grafted chains (Scheme 2):

Scheme 2

$${}^{\bullet}C + A \rightarrow C\text{-}g\text{-}A^{\bullet} \xrightarrow{nA} C\text{-}g\text{-}(A)_{n+1}$$
(Grafted Chitosan)

Regarding the crosslinking reaction, *N,N*-methylene-bisacrylamide was used for this purpose. Please note that this monomer is of double functionality capable of interconnecting the growing polyacrylamide grafted chains.

2.2. Swelling studies

As can be seen from Fig. 1 the matrix based on chitosan show a pH dependent swelling behaviour. Chitosan has a high ionization degree in acid media (pH = 1.2) since its pKa is 6.3 [13], thus almost all $-NH_2$ groups are in its protonated ($-NH_3^+$) form. Therefore, as the hydrogel is mainly in its ionised form, an electroosmotic effect is produced [14] which permits the entry of water into the tablet producing swelling of the tablet. When the pH changes from 1.2 to 8.0, Chitosan suffers from a great decrease in its ionisation degree accompanied with disappearance of the electrosmotic effect. This stops the water flow to the tablet and the swelling process. At the same time it was observed that the degree of swelling determined by weighing (DS_w) and increase of diameter (DS_d) are not overlaped. This results pointed out that the swelling process could be anisotropic as has been observed in others hydrophilic matrices [15].
A non-pH dependent swelling behaviour for the matrix tablets based on grafted chitosan was observed. The overlaping between the degree of swelling measured by weigh-

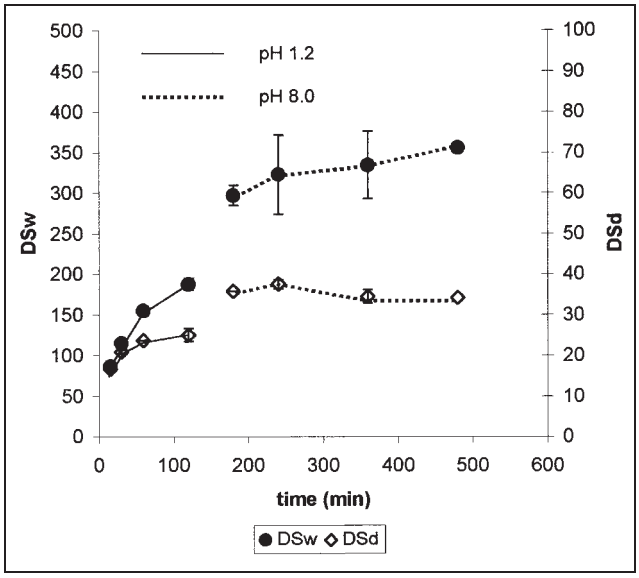

Fig. 1: Degree of swelling measured by weighing (DS_w) and by increase of diameter (DS_d) for matrix tablet based on CB as function of time and pH

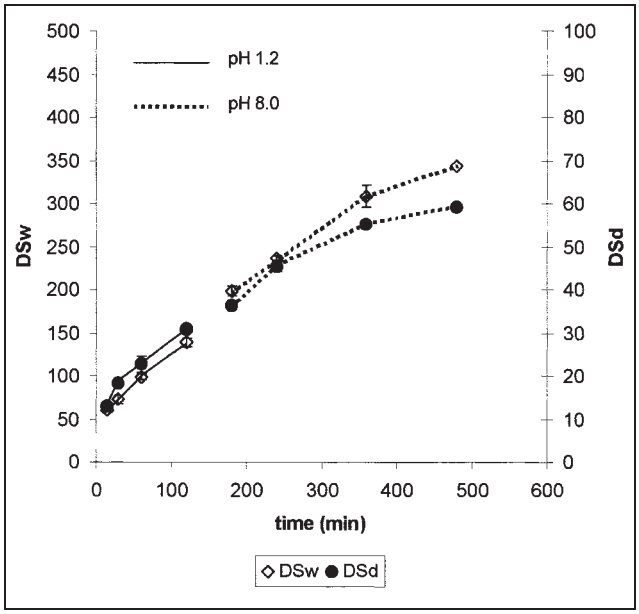

Fig. 2: Degree of swelling measured by weighing (DS_w) and by increase of diameter (DS_d) for matrix tablet based on CB-g-A-418 as function of time and pH

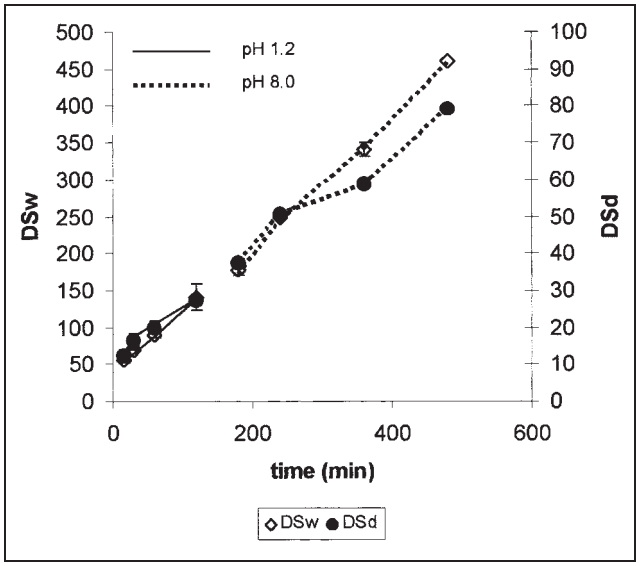

Fig. 3: Degree of swelling measured by weighing (DS_w) and by increase of diameter (DS_d) for matrix tablet based on CB-g-A-600 as function of time and pH

ing (DS_w) and measured by increase of diameter (DS_d) up to 240 min showed that the swelling process could be isotropic (Figs. 2, 3). The non-pH dependent swelling behaviour of these matrices could be explained by the partial substitution of amine groups of the chitosan chain by acrylamide. The grafting reaction provides an ionizable amine group by a neutral amide group which make the matrix non pH-dependent.

2.3. Dissolution studies

Fig. 4 shows the fraction of theopylline released as a function of time for the three formulations studied. The release curves of all formulations show a prolonged drug release, where 50–70% of drug were released in a period of 8 h.
In acid media, a higher but not significant drug release from the matrix based on CB-g-A600 as compared with that of CB-g-A418 and CB was observed. When the pH changed from 1.2 to 8.0, all formulations showed a decreased theophylline release rate. In alkaline media, after 240 min, the fraction of drug released from the matrix based on CB-g-A600 was significantly higher than the drug released from the matrices based on CB-g-A418 and CB. No significant difference in the drug release behaviour between formulations F4 and F1 was observed.
Since theophylline is soluble in water its release from the hydrogel matrix is controlled mainly by the swellling of the matrix and the dissolution/erosion in the periphery of the matrix. The swelling and erosion behaviour of the formulations could explain the dissolution profiles observed.

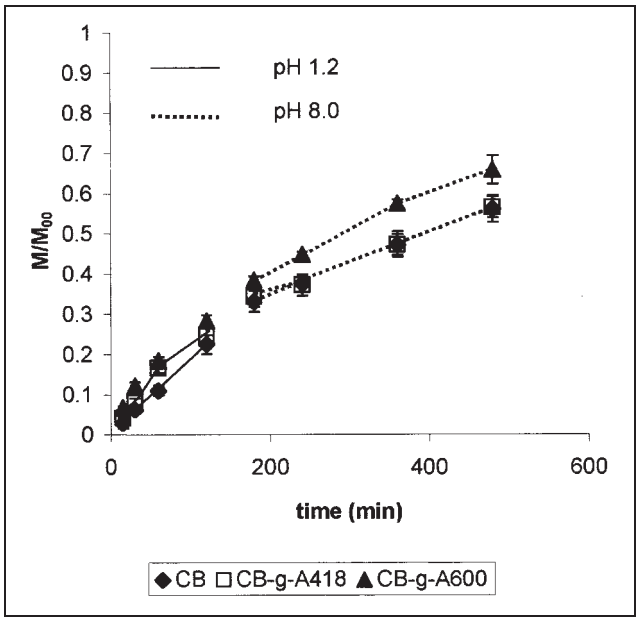

Fig. 4: Dissolution profiles for formulations based on chitosan and grafted chitosan with acrylamide as function of time and pH

2.4. Drug release mechanism

For polymeric hydrogel matrix systems, the most used models for the interpretation of the drug release mechanism are those proposed by both Higuchi and Peppas. These models are both based on the second Fick's law [16]. For the systems based on hydrogels is recommended to interpretate the dissolution data according to Peppa's model because in this systems the relaxation and diffusion mechanisms are important. The dissolution data fitted to Peppa's model are shown in Table 2.

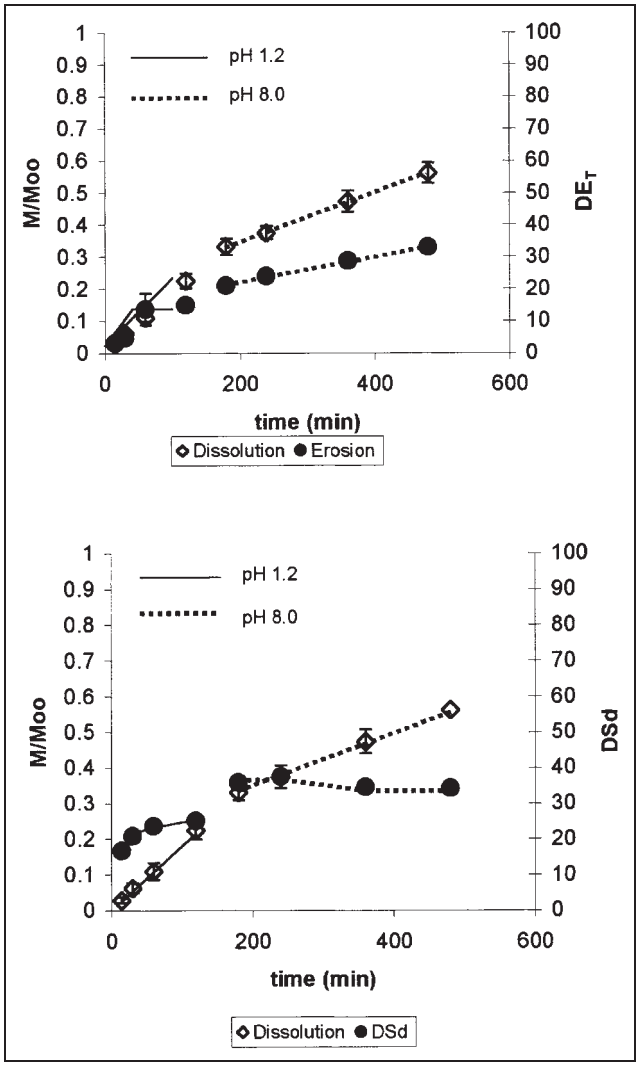

Fig. 5: Correlation between dissolution/erosion and dissolution/swelling behaviour for matrix tablet based on CB as function of time and pH

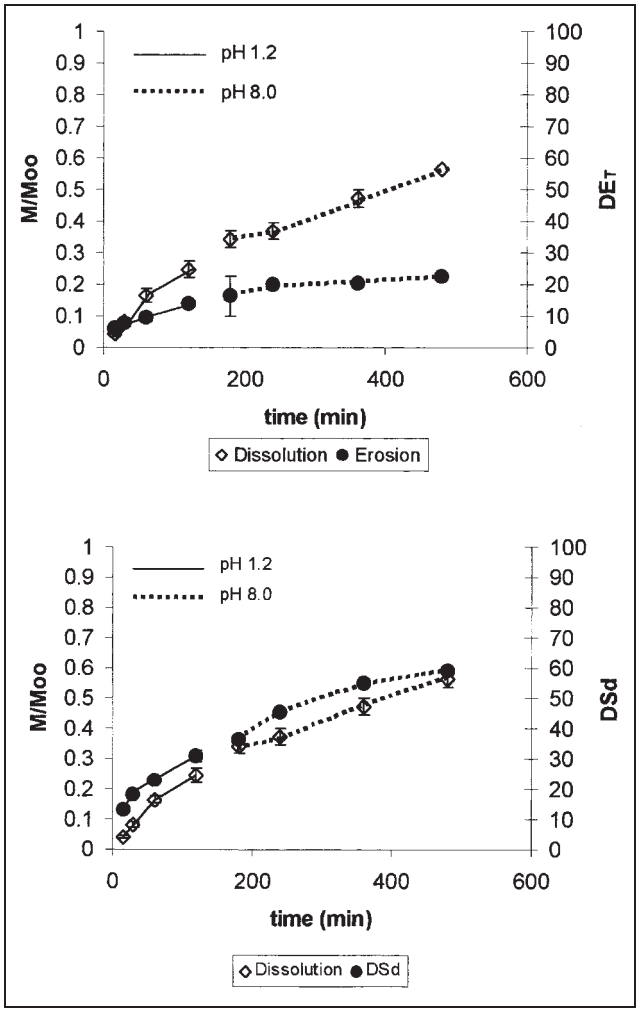

Fig. 6: Correlation between dissolution/erosion and dissolution/swelling behaviour for matrix tablet based on CB-g-A-418 as function of time and pH

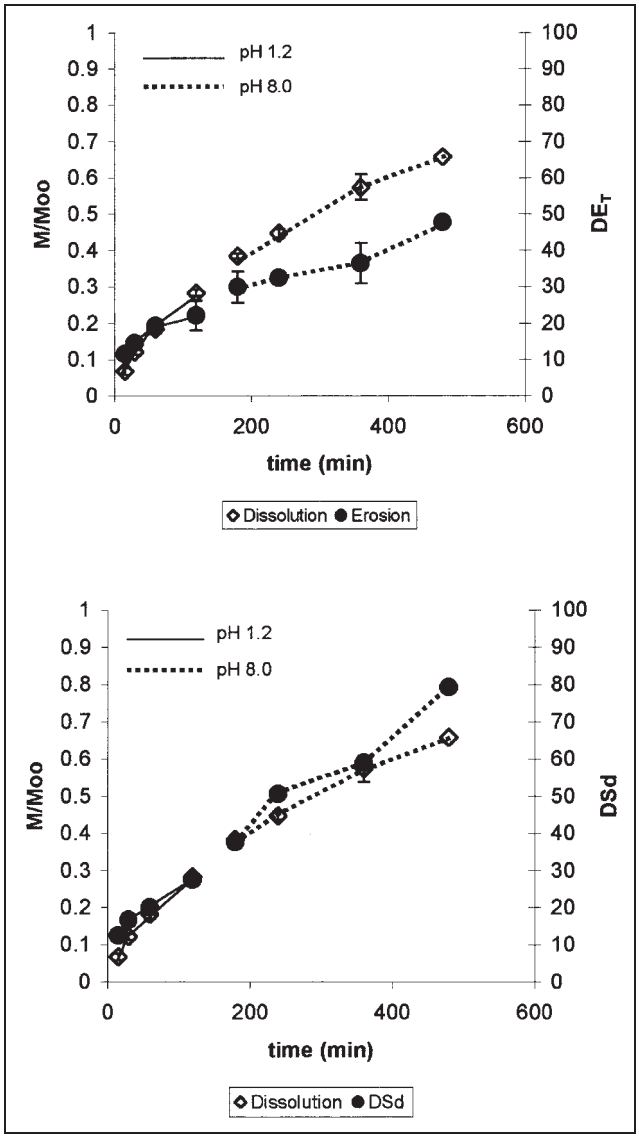

Fig. 7: Correlation between dissolution/erosion and dissolution/swelling behaviour for matrix tablet based on CB-g-A-600 as function of time and pH

The results obtained for the order of release, n, were 0.70, 0.62, and 0.64 for formulations F1, F4, and F5, respectively. The values of n obtained for different formulations studied indicated the existence of deviation from simple Fickian diffusion.

The degree of swelling and erosion of the matrix tablets was correlated with their dissolution profiles. The drug release from the matrix tablet based on CB (F1) was shown to be erosion controlled during the first hour. Thereafter, from 120 to 480 min the drug release was found to be controlled mainly by the swelling behaviour. Between 120 and 240 min, where the pH changes from pH 1.2 to 8.0, the drug release profile overlaped the DSd profiles, then the drug release from the matrix tablet was controlled by swelling. The final drug release period (360–480 min), would be controlled by the combined mechanism of erosion and diffusion through the constant gel layer formed since during this period the matrix tablet suffer erosion and the DSd is constant (Fig. 5). The drug release from the matrix tablet based on CB-g-A-418 (F4) was controlled by erosion for the first 30 min. For the period from 60 to 480 min the drug release is mainly controlled by the swelling behaviour of the tablet (Fig. 6). As can be seen from Fig. 7, the drug release from the matrix tablet based on CB-g-A-600 (F5) seems to be controlled by a combined erosion-swelling mechanism of the matrix tablet during all period studied.

2.5. Mean dissolution time

The mean dissolution time for all formulations was estimated using the Weibull's model [17]. Table 3 shows the parameters obtained for each formulation when the Weibull model was applied.

Table 2: Dissolution data fitted to Peppa's model

Formulations	k (min^{-n})	n (n ± 5% ci)	R^2DF_{adj}
F1	0.00774	0.70 ± 0.12	0.9801 (n = 8)
F4	0.01248	0.62 ± 0.09	0.9866 (n = 8)
F5	0.01358	0.64 ± 0.04	0.9979 (n = 7)

Table 3: Dissolution data fitted to Weibull's model

Formulations	β	t_d (t_d ± 5% ci) min	R^2DF_{adj}
F1	0.84	611 ± 51	0.9927 (n = 8)
F4	0.77	612 ± 51	0.9931 (n = 8)
F5	0.82	442 ± 12	0.9986 (n = 8)

It can be seen from this table that formulation F1 based on CB and formulation F4 based on CB-g-A418 had a very similar t_d value, 63.2% of the drug was released after 10 h. On the contrary, formulation F5, based on CB-g-A600, had a lower t_d compared with the values obtained for the other two formulations. This indicates that this matrix has lower ability to prolong drug release since 63.2% of the drug was released after 7 h.

3. Discussion

The higher degree of erosion and swelling of the formulation F5 (CB-g-A600) compared with the formulation F1(CB) and F4 (CB-g-A418) could explain the higher fraction of theopylline released. For all formulations studied in this work, the amount of theopylline released from the tablets was found to be controlled by a combination of diffusion process and relaxation of the polymeric structure. These results match with the controlled swelling behaviour and low degree of erosion observed for these systems.

4. Experimental

4.1. Materials

Chitosan was a gift from Bioquímica Austral (Chile). The degree of deacetylation and molecular weight of chitosan were determined according to the procedures described by Rinaudo and Domard [18]. The degree of deacetylation was 81% as determined by ^{1}H NMR spectroscopy. The average molecular weight of chitosan was estimated as 3.5×10^5 by combined viscosity-light scattering measurements. Acrylamide 97% p.a. was purchased from ALDRICH (USA). Potassium persulfate and *N,N′*-methylene-bis-acrylamide were purchased from BDH Chemicals (UK). Theophylline anhydrous, Avicel® type PH101, and Magnesium stearate were a gift from Laboratorio Saval (Chile). All other chemicals were of analytical grade.

4.2. Graft copolymerisation

Graft copolymerisation were carried out in 50 cm^3 stoppered flasks and under atmospheric oxygen by first dissolving an exact amount of chitosan, previously dried at 50 °C under reduced pressure to constant weight, (W_1) in a determined volume of aqueous 2% W/V acetic acid solution, followed by the addition of the monomer, *N,N*-methylene-bis-acrylamide (crosslinking agent) and initiator, all previously dissolved in 2% acetic acid solution. Then the flask was placed in a thermostated bath at 60 °C. Polymerization was started and continued for 60 min. The reaction was stopped by rapidly cooling down the reaction. The product was precipitated, by pouring the polymerization mixture, into a large amount of acetone. The precipitate was filtered, washed thoroughly with acetone and dried under vacuum at 60 °C to constant weight (W_2). The dry sample was extracted with water in a Soxhlet for 24 h in order to remove unreacted monomer, initiator and polyacrylamide homopolymer that eventually could form during the grafting reaction. The dried remaining product was considered to be a graft copolymer. The efficiency of grafting (%E) can be calculated as the weight ratio of the increase in weight of the extracted copolymer sample and that of the initial monomer. This can be calculated by using the relation: $\%E = 100(W_2 - W_1)/W_3$, where, W_1, W_2 and W_3 denote the weight of initial dry chitosan, grafted chitosan after water extraction and drying and the weight of monomer respectively. As is already known the composition of the grafted chitosan can be estimated in terms of the so-called percentage of grafting. This can be expressed on the basis of percent weight increase related to the initial weight of chitosan. Therefore, the extent of grafting can be calculated as percentage of grafting $\%G = 100(W_2\text{-}W_1)/W_1$.

4.3. Formulation and preparation of the tablets

The formulations studied are listed in Table 1. The chitosan grafted with acrylamide was milled at −5 °C in a knife mill (Janke & Kunkel.IKA.Labortechnik.Model A-10). The material used was classified by sieving through mesh 120 sieves. For each formulation the polymers were dry mixed with anhydrous theophylline, Avicel® PH 101 and magnesium stearate to make 400 mg tablets. The tablets were obtained by direct compression with a Wilhelm Fette type EIIN.270 excentrical tabletting machine. The compression pressure was adjusted depending on the compactibility of the formulation studied.

4.4. Estimation of the degree of erosion of the tablet

The erosion study was carried out in a dissolution equipment at 50 rpm and 37 °C using the paddle method (USP Type 2) [19]. The tablets were placed over a container made of stainless steel N° 18 mesh size and then submerged into 900 ml of 0.2N HCl and 0.2N KCl solution (pH 1.2) for 2 h. The tablets were then transferred to an alkaline solution (0.2 N boric acid + 0.2 N KCl solution, pH 8.0) and were left in this media for a period of 6 h until completion of a total of 8 h. At different times the tablets were removed and then dried in a vacuum oven at 60 °C to constant weight. Each assay was done in triplicate 20. The degree of total erosion of the tablet (DE_T) was estimated as follows:

$$(DE_T) = [(W_0 - W_t)/W_0] \cdot 100(\%) \quad (1)$$

Where: W_0 = initial weight of the tablet; W_t = weight of the tablet at time t

4.5. Determination of swelling degree of the tablet

4.5.1. By gravimetry (weighing)

The same equipment and procedure as that for the estimation of the degree of erosion was used except that the stainless steel container was made with 14 mesh size. The tablets were removed at different times and then the degree of swelling was determined by weighing. Each assay was done as triplicate. The degree of swelling by weighing was defined as follows:

$$(DS_w) = [(W_t - W_0)/W_0] \cdot 100(\%) \quad (2)$$

Where: W_0 = initial weight of the tablet; W_t = weight of the tablet at time t

4.5.2 By increase in diameter

The same equipment and procedure as that for the estimation of the swelling degree by weighing was used here, except that in this case the container had in its bottom a plastic coated sheet with a millimetre scale. The tablets were removed at different times and then the swelling degree was determined by observation of the change of the diameter of the tablet by using a magnification lens (Wild M3, Heerbrugg, Germany, magnification 6.4). Each assay was done as triplicate. The swelling degree was measured as increase in diameter of the tablet.The following relation was used to calculate the swelling degree:

$$(DS_d) = (D_t - D_0)/D_0^{\bullet}100(\%) \quad (3)$$

Where: D_0 = initial diameter of the tablet; D_t = diameter of the tablet at time t

4.6. Dissolution test

This was performed at 50 rpm and 37 °C in a dissolution equipment. The paddle method (USP type 2) [19] was used. The tablets were submerged into 900 ml of 0.2 N HCl and 0.2 N KCl solution (pH 1.2) for 2 h. These were then transferred to an alkaline solution (0.2 N boric acid + 0.2 N KCl solution, pH 8.0) and left in this media for another 6 h until completion of a total of 8 h. Aliquots of 10 ml were taken at different times and the content of theopylline was determined by UV spectroscopy at 272 nm. Since the matrix based on grafted chitosan interfered with the absorption at 272 nm, tablets without theopylline were prepared as blank samples. In these cases, the quantity of drug released at each interval of time was estimated as the difference between the absorbance obtained at each time for the tablet with theopylline minus the absorbance of the blank tablet without theopylline. Each assay was done as triplicate.

Acknowledgements: The authors wish to thank FONDECYT for financial support through research grant project N° 1981405 and DID University of Chile.

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Received November 5, 2001
Accepted June 7, 2002

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