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Research Article

The Effect of Chitosan as Internal or External Coating on the 5-ASA Release from Calcium Alginate Microparticles

Cristián Tapia,[1,5] Sergio Molina,[1] Alvaro Diaz,[1] Lilian Abugoch,[2] Mario Diaz-Dosque,[3] Fernando Valenzuela,[1] and Mehrdad Yazdani-Pedram[4]

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Abstract. The effect of chitosan as internal or external coating on the mesalamine (5-ASA) release from calcium alginate microparticles (CaAl) was studied, and a delayed release of 5-ASA system intended for colonic drug delivery was developed. The external chitosan coating was developed by immersion of wetted CaAl in chitosan solution and the internal coating by mixing 5-ASA with chitosan solution and drying before the preparation of CaAl. Both systems were coated with Acryl-EZE® using combined fluid bed coating and immersion procedure. The results showed that in phosphate medium (pH 7.5), chitosan as 5-ASA coating promotes a quick erosion process accelerating drug release, but chitosan as external coating (CaAlCS) does not increase the T_{50} value compared with the microparticles without chitosan (CaAl). Chitosan as internal or external coating was not effective to avoid the quick 5-ASA release in acidic medium (pH 1.2). The presence of β-glucosidase enzymes increases significantly the 5-ASA release for CaAl, while no effect was observed with chitosan as internal or external coating. Fourier transform infrared spectroscopy, thermogravimetric analysis, and X-ray data revealed that 5-ASA did not form a solid solution but was dispersed in the microparticles. The Acryl-EZE® coating of microparticles was effective because all the formulations showed a low release, less than 15%, of 5-ASA in acid medium at pH 1.2. Significant differences in the percentage of 5-ASA released between formulations were observed in phosphate buffer at pH 6.0. In phosphate buffer at pH 7.2, all the formulations released 100% of 5-ASA.

KEY WORDS: 5-ASA; chitosan–alginate; colonic drug delivery; delayed drug release; microparticles.

[1] Laboratorio de Operaciones Unitarias, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago 1, Chile.
[2] Laboratorio de Tecnología de Alimentos, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
[3] Laboratorio de Biofísica, Facultad de Odontología, Universidad de Chile, Santiago, Chile.
[4] Laboratorio de Química de Polímeros, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
[5] To whom correspondence should be addressed. (e-mail: ctapia@uchile.cl)

ABBREVIATIONS: 5-ASA, Mesalamine; 5ASAcoCS1, 5-ASA coated with chitosan (1% *w/v* solution); 5ASAcoCS3, 5-ASA coated with chitosan (3% *w/v* solution); AS, Alginate medium viscosity (SIGMA); C1CaAl, 5-ASA coated with chitosan (1% *w/v* solution) loaded Ca-alginate microparticles; C1CaAlAE, 5-ASA coated with chitosan (1% *w/v* solution) loaded Ca-alginate microparticles coated with Acryl-EZE®; C3CaAl, 5-ASA coated with chitosan (3% *w/v* solution) loaded Ca-alginate microparticles; C3CaAlAE, 5-ASA coated with chitosan (3% *w/v* solution) loaded Ca-alginate microparticles coated with Acryl-EZE®; CaAl, 5-ASA loaded Ca-alginate microparticles; CaAlAE, 5-ASA loaded Ca-alginate microparticles coated with Acryl-EZE®; CaAlCS, 5-ASA loaded Ca-alginate microparticles coated with chitosan; CaAlCSAE, 5-ASA loaded Ca-alginate microparticles coated with chitosan and Acryl-EZE®; CaAlCSwD, Ca-alginate microparticles coated with chitosan; CaAlwD, Ca-alginate microparticles; CS, Chitosan (Sigma, USA); PMC1CaAl, Physical mixture of Ca-alginate microparticles and 5-ASA coated with chitosan (1% *w/v* solution); PMC3CaAl, Physical mixture of Ca-alginate microparticles and 5-ASA coated with chitosan (3% *w/v* solution); PMCaAl, Physical mixture of Ca-alginate microparticles and 5-ASA; PMCaAlCS, Physical mixture of Ca-alginate microparticles coated with chitosan and 5-ASA.

INTRODUCTION

Chitosan–alginate drug delivery systems have been described for site-specific drug delivery in the colon (1–7) due to the fact that polysaccharides, calcium alginate, and chitosan are degraded by bacteria in the colon (1,5,8–11). It has been shown that the use of bacteria as a trigger mechanism for colonic drug release shows improved specificity over a pH-responsive approach. These bacteria produce enzymes, which are capable of breaking down undigested polysaccharides in the colonic contents (9). One of the procedures for obtaining chitosan–alginate microparticles is the two-step method, where Ca-alginate microparticles are recovered and subsequently coated with chitosan (12). In this procedure, the reaction occurs mainly on the surface of Ca-alginate beads to form a membrane. The thickness of the membrane depends on the molecular weight (Mw) of chitosan. Low molecular weight chitosan forms a thick membrane with better anti-swelling ability compared with high molecular weight chitosan (13). It has been reported that the mean size of the rehydrated calcium alginate microcapsules at pH 8.0 is significantly much larger than in acid medium (pH 1.2). However, when the microcapsules were coated with chitosan, no significant difference in the mean sizes was observed between both media (14). The aim of this work was to evaluate the effect of chitosan as coating agent for mesalamine (5-ASA) prior to encapsulation and as external coating in calcium alginate microparticles on drug

release and to develop a delayed release of 5-ASA system intended for colonic drug delivery.

MATERIALS AND METHODS

Chitosan (CS) was from Sigma–Aldrich Inc. (St. Louis, MO, USA; $\eta_{sp/c}$=453 mL/g; M_v=637 kDa; DA(%)=22.8). These properties were determined under conditions described elsewhere (14).

Alginic acid sodium salt of medium viscosity from *Macrocystis pyrifera* (AS) was from Sigma–Aldrich Inc. (St. Louis, MO, USA). Viscosity of 2% solution at 25°C equals 3,500 mPa.

5-ASA was from Ferring Pharmaceuticals, Denmark.

β-Glucosidase from almonds were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA).

Calcium chloride was supplied from Scharlau Chemie, Spain.

Acryl-EZE® (AE) was supplied from Colorcon, USA.

All other chemicals used were analytical grade.

Preparation of Microparticles

5-ASA Loaded Ca-Alginate Microparticles (CaAl)

Five grams of 5-ASA were dispersed in 1 L of 1% *w/v* sodium alginate solution with continuous mechanical stirring at 800 rpm (IKA, RW 20 DZM, Germany). The suspension was pumped using a peristaltic pump (Masterflex 7523-35, L/S tubing 14, Barrington, USA) at the rate of 10 mL/min into an automatic spray gun (Walther Pilot mod WA-XV, Wupertal, Germany) provided with a 3.0-mm diameter nozzle. The suspension was dropped at a pressure of 0.1 MPa and minimum volumetric air flow into 1 L of $CaCl_2$ 1% *w/v* in water. The mixture was stirred with a magnetic stirrer until all the suspension was added, and then the mixture was maintained with agitation for 15 min. The microparticles obtained were separated from the solvent using a strainer, washed three times with water, and then left to drain on perforated tray for 12 h. Finally, the microparticles were dried in an oven (Labtech mod LDO-080F, Namyangu, Korea) at 30°C for 12 h and sieved through 20 mesh. Microparticles with moisture content of 10% were obtained.

5-ASA Loaded Ca-Alginate Microparticles Coated with Chitosan (CaAlCS)

The microparticles obtained from the procedure described above were immersed in 1 L of 1% *w/v* chitosan solution in 1% *w/v* acetic acid for 1 h. The separation procedure of the microcapsules coated with chitosan was the same as described above.

5-ASA Coated with Chitosan (1% and 3% w/v Solution) Loaded Ca-Alginate Microparticles (C1CaAl, C3CaAl)

Five grams of 5-ASA were manually mixed with 4 mL of 1% *w/v* chitosan solution in 1% *w/v* acetic acid (C1CaAl) or 6 mL of 3% *w/v* chitosan solution in 1% *w/v* acetic acid (C3CaAl) until a paste was obtained. Then, the paste was dried in an oven (Labtech model LDO-080F, Namyangu, Korea) at 50°C for 12 h, and then it was powdered in a mortar. The 5-ASA coated with chitosan at a dose of 5 g/L was loaded in alginate solution following the procedure described for CaAl microparticles.

Drug Release Studies

Drug release studies were carried out in a digital water bath (Labtech, LWD-122D, Namyangu, Korea) provided with three glass dissolution vessel (180-mm tall and 95-mm diameter) equipped with a mechanical stirrer (IKA, RW 20 DZM, Germany) with digital speed control. The stainless steel 316L paddle impeller was built with the same dimensions of the apparatus 1 of the USP. Dissolution test was carried out at 37±0.1°C and 50±5 rpm. The microparticles were placed into a basket made of stainless steel mesh no. 40 with the following dimensions: 40-mm long×30-mm width×10-mm tall and submerged into 900 mL of dissolution medium.

The dissolution media were acid (0.1 M HCl+0.2 M KCl, pH 1.2), water (distilled water, pH 5.5), and phosphate (KH_2PO_4 0.05 M, adjusted with 10 M NaOH solution to pH 7.5). Ten-milliliter aliquots were taken at different times between 5 min and 9 h. This was replaced with an equal volume of the medium. The 5-ASA content of the aliquots was determined by UV spectroscopy (UV/Visible UNICAM UV3 spectrometer, Cambridge, UK) at a wavelength of 302 nm for acid, 298 nm for water, and 330 nm for phosphate (15). Each assay was done in triplicate. The dissolution data were analyzed according to Dobashi's model for fast release in acid and phosphate (14) and Weibull's model for prolonged release in water (16). From these models, the mean dissolution time, T_{50}, was estimated.

Evaluation of Swelling Behavior

The same equipment and conditions used in the drug release studies were employed. At each sampling time, the basket containing the microparticles was removed, the dissolution medium was eliminated, and the basket was weighed.

Each assay was done in triplicate. The swelling degree (SD) was estimated as follows:

$$\mathrm{SD}(\%) = \frac{M_t - M_0}{M_0} \tag{1}$$

where M_t is the mass at time t and M_0 is the mass at time zero.

Microparticles Coating Procedure in Fluid Bed Reactor

The coating procedure was performed in a 1-L capacity glass reactor provided with three-necked flask head with a center neck of 31 mm in diameter in line with two side necks of 18 mm of diameter. A suspension 20% Acryl-EZE® was introduced through the center neck, and the side necks were used for inlet and outlet fluidification air.

The suspension was pumped using a peristaltic pump (Masterflex 7523-35L/S 14 tubing, Barrington, USA) at a rate of 3 mL/min to a continuous spray gun (WALTHER PILOT mod. WA-XV, Wupertal, Germany) equipped with a nozzle

of 3 mm in diameter. The spraying was carried out with air at pressure of 0.5 MPa and a flow rate of 25–30 L/min. Of the suspension, 150 mL was applied.

Filtered air was used for the fluidification of microparticles at a pressure of 0.2 MPa and a flow rate of 35–40 L/min. The air was injected through a second continuous spray gun (WALTHER PILOT mod. WA-XV, Wupertal, Germany) provided with a nozzle with 1.5 mm in diameter. Approximately 36 g of microparticles with 13% of humidity on wet basis was introduced in the reactor. The coated microparticles were dried in an oven at 50°C for 2 h and then were dipped for 5 min in 50 mL of Acryl-EZE® 20% suspension. The coated microparticles were sieved through a sieve mesh 14 and subsequently were dried at 50°C in an oven (Labtech, model-150f, Namyangu, Korea) for 2 h. The percentage of coating of the microparticles was estimated as follows:

$$\% \text{ of coating} = 100 - \left[\frac{a \times b}{c}\right] \quad (2)$$

where

a 100 mg of uncoated microparticles
b Milligrams of 5-ASA released at 360 min from 100 mg of coated microparticles in phosphate buffer at pH 7.5.
c Milligrams of 5-ASA released at 360 min from 100 mg of uncoated microparticles in phosphate buffer at pH 7.5.

Evaluation of Dissolution Behavior of Microparticles Coated with Acryl-EZE®

The microparticles coated with Acryl-EZE® were evaluated using the USP dissolution test conditions used for 5-ASA delayed release tablets (17) using the same equipment described for drug release studies section.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) measurements were carried out in a Bruker model IFS 32 spectrometer (Ettlingen, Germany). About 2 mg of the samples was 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 were determined in unloaded and 5-ASA loaded microparticles, respectively. 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.

X-Ray Diffraction

The X-ray diffraction (XRD) measurement was conducted using a Siemens D-5000 powder X-ray diffractometer with CuKα radiation (λ 1.54 Å), and 0.02° step and 2θ range of 1.7–80° were selected to analyze the crystal structure.

Characterization of the Microstructure

The microstructure characterization of microparticles was carried out in the swollen state by using scanning electron microscopy (SEM). Of the microparticles, 100 mg was suspended in 100 mL of dissolution medium used in the dissolution studies, *i.e.*, acid or water or phosphate. The suspension was shaken at 37°C for 1 h in an orbital shaker (PolyScience Shaking Water Bath, USA). Then, the swollen microparticles were separated from the dissolution media. The SEM of swollen microparticles was obtained by using the critical point drying method. In this method, the microparticle is desiccated with acetone, and then it was saturated with CO_2 (16).

Dissolution Studies with β-Glucosidase Enzymes

One hundred milligrams of enzyme (β-D-glucoside glucohydrolase, Fluka) with an activity of 8.92 U/mg protein in 1,000 μL phosphate (KH_2PO_4) 0.05 M pH 7.5 solution was prepared as stock enzyme solution. One hundred microliters of stock solution was diluted to 1 mL, resulting in a concentration of 89.2 U/mL of β-glucosidase. The dilutions prepared were frozen at −5°C until use.

The assay was developed for the microparticles CaAl, C1CaAl, C3CaAl, and CaAlCS in phosphate solution (KH_2PO_4) 0.05 M at pH 7.5, under the same experimental conditions and equipment as described for the drug dissolution studies. One milliliter of β-glucosidase enzyme with a concentration of 89.2 U/mL was added to this dissolution medium. The final enzyme concentration was 0.09911 U/mL.

Stability Studies

The stability of the microparticles coated with Acryl-EZE® designated as CaAlAE, C1CaAlAE, C3CaAlAE, and CaAlCSAE was evaluated at 40±2°C and 75±5% RH (15). The microparticles were placed in plastic containers and then introduced in a desiccator of 5-L capacity containing a recipient with 150 mL of a saturated sodium chloride solution (6.15 M). The desiccator was sealed with silicone and was introduced into an oven (Labtech, model DVR-150F, Namyangu, Korea). The temperature and humidity were monitored with a digital hygrometer/thermometer (Veto Model A603123K, China). The stability was evaluated at 1 and 3 months of storage by dissolution tests in phosphate solution (KH_2PO_4) 0.05 M at pH 7.5. The similarity factor f_2 (18) and T_{50} values were calculated from the dissolution data.

Statistical Analysis

The experimental 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 the significance.

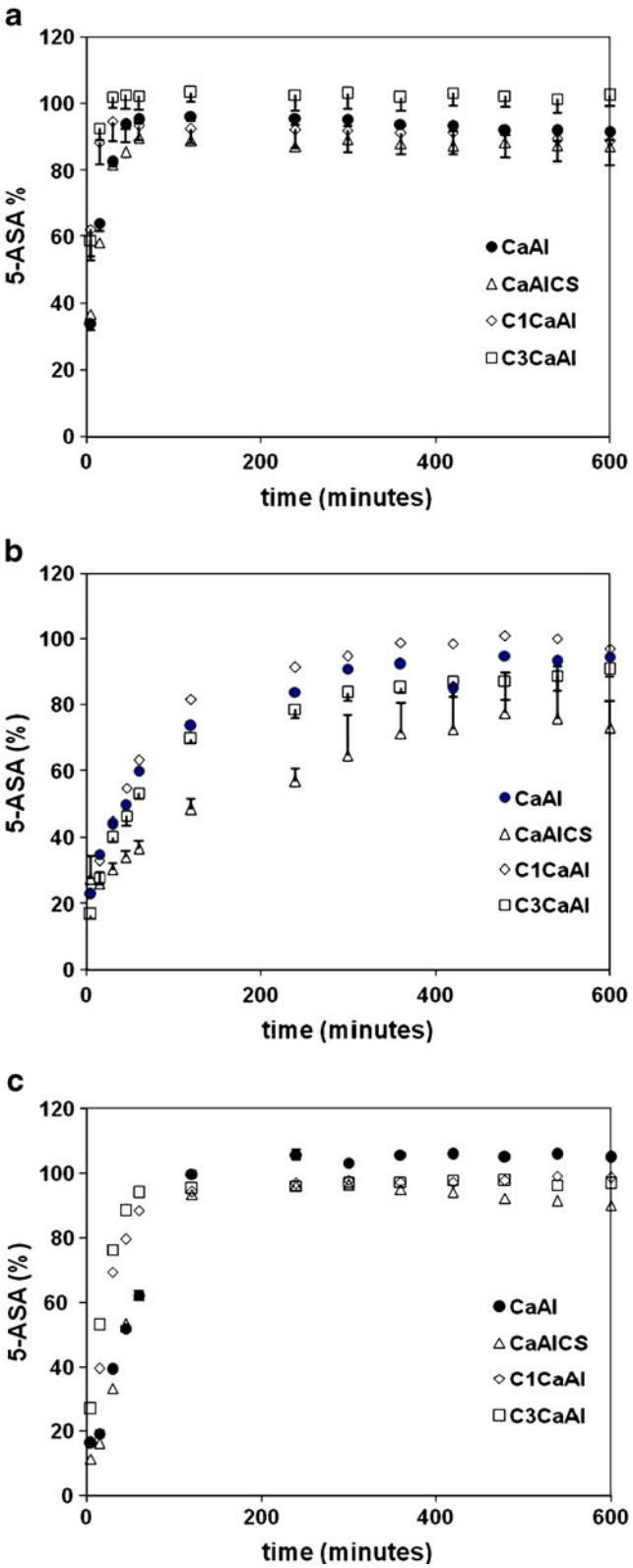

Fig. 1. 5-ASA release behavior in **a** acid pH 1.2, **b** water pH 5.5, and **c** phosphate pH 7.5

RESULTS AND DISCUSSION

Drug Dissolution Behavior of Microparticles

Figure 1a shows the drug dissolution behavior of the four formulations studied in acid at pH 1.2. It is noted that in this medium, 5-ASA is quickly released and that the presence of chitosan both externally (CaAlCS) and internally (C1CaAl and C3CaAl) does not prevent the fast release of the drug in this medium. The mean dissolution time, T_{50}, for all formulations was less than 9 min, see Table I. These results demonstrate the need to protect the microparticles with a coating to prevent loss of drug in acidic media.

Figure 1b clearly shows that the drug release from all formulations is significantly slower in water than in acid. This change in the dissolution behavior is reflected in the dissolution data not fitted to Dobashi's model. In water, the dissolution data were fitted to Weibull's model. The T_{50} values reflect these large differences between both media. T_{50} values were between 62 and 212 min in water medium, whereas T_{50} values were between 3 and 9 min in acid medium, see Table I. In water medium, the coating of Ca-alginate microparticle with chitosan (CaAlCS) allows to retard significantly the release of the drug in water, resulting in a T_{50} of 212 min compared to T_{50} of 62 min for the uncoated Ca-alginate microparticle (CaAl). Only the previous coating of 5-ASA with 3% chitosan solution (C3CaAl) allows slow release of the drug in relation with CaAl, resulting in a T_{50} of 84 min for C3CaAl compared to a T_{50} of 62 min for CaAl. Figure 1c shows the 5-ASA release behavior in phosphate medium that simulates the pH conditions of the colon. It is observed that when 5-ASA was previously coated with chitosan (C1CaAl and C3CaAl), the drug release was faster than the other formulations. Table I shows a T_{50} of 18 min for C1CaAl and a T_{50} of 13 min for C3CaAl. In contrast, T_{50} for CaAl and CaAlCS were significantly higher, 43 and 39 min, respectively.

These results clearly indicate that the rate of drug release depends mainly on the pH of the dissolution medium. Thus, the increasing of mean dissolution time, T_{50}, follows the order: acid medium pH 1.2<phosphate medium pH 7.5< water medium pH 5.5. The incorporation of chitosan to the calcium alginate microparticle, either externally (CaAlCS) or internally (C1CaAl and C3CaAl), does not change this order.

The swelling–erosion behavior of the microparticles, in the same dissolution media used in the dissolution studies, was evaluated by weighing (Fig. 2) and by the observation of the swollen microstructure (Fig. 3). All the formulations in

Table I. T_{50} Values Obtained from Dobashi and Weibull's Model

Formulations	T_{50} acid[a]	T_{50} water[b]	T_{50} phosphate[a]
CaAl	9.1±0.5 aA	62.0±3.8 aB	43.2±2.9 aC
C1CaAl	3.0±0.2 bA	52.9±4.7 aB	18.2±0.6 bC
C3CaAl	4.0±0.1 cA	83.5±4.9 bB	12.7±0.3 cC
CaAlCS	8.2±0.7 dA	211.6±10.0 cB	38.6±3.0 dC

Different lowercase letters mean significant differences between rows. Different upper letters mean significant differences between columns ($p<0.05$)

[a] T_{50} values obtained from Dobashi's model

[b] T_{50} values obtained from Weibull's model

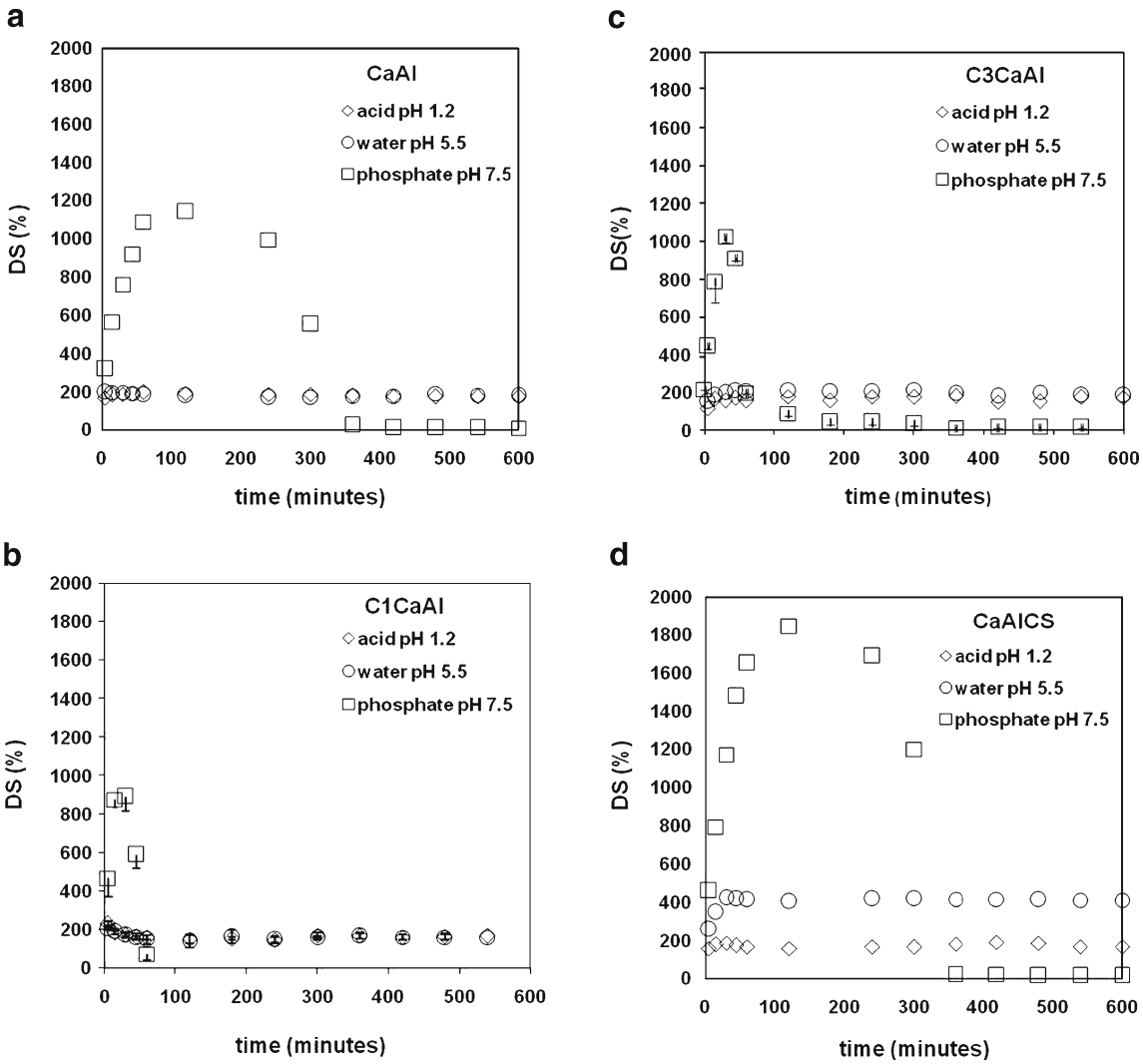

Fig. 2. Swelling–erosion behavior, measured by weighing, in acid, water, and phosphate media for **a** CaAl, **b** C1CaAl, **c** C3CaAl, and **d** CaAlCS

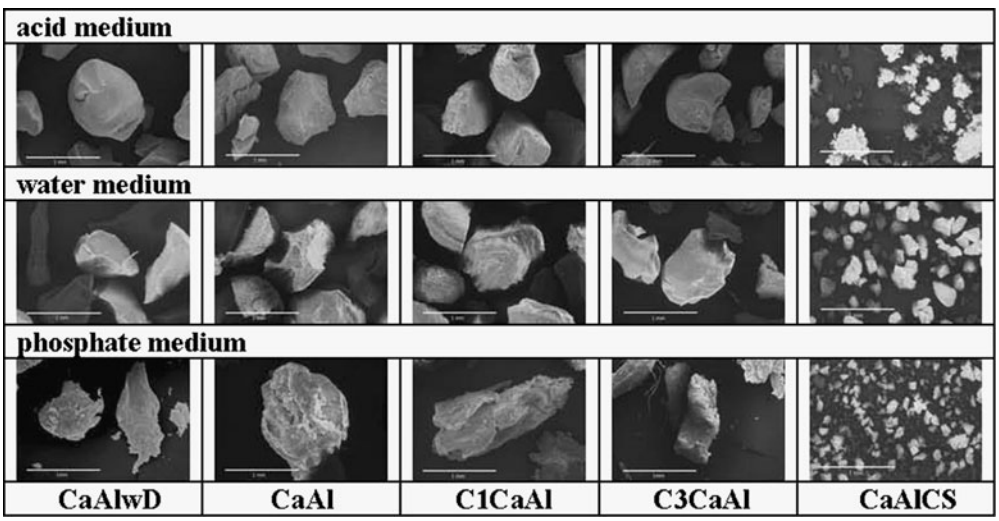

Fig. 3. Microstructure, measured by SEM, for CaAlwD, CaAl, C1CaAl, C3CaAl, and CaAlCS, in acid, water, and phosphate media

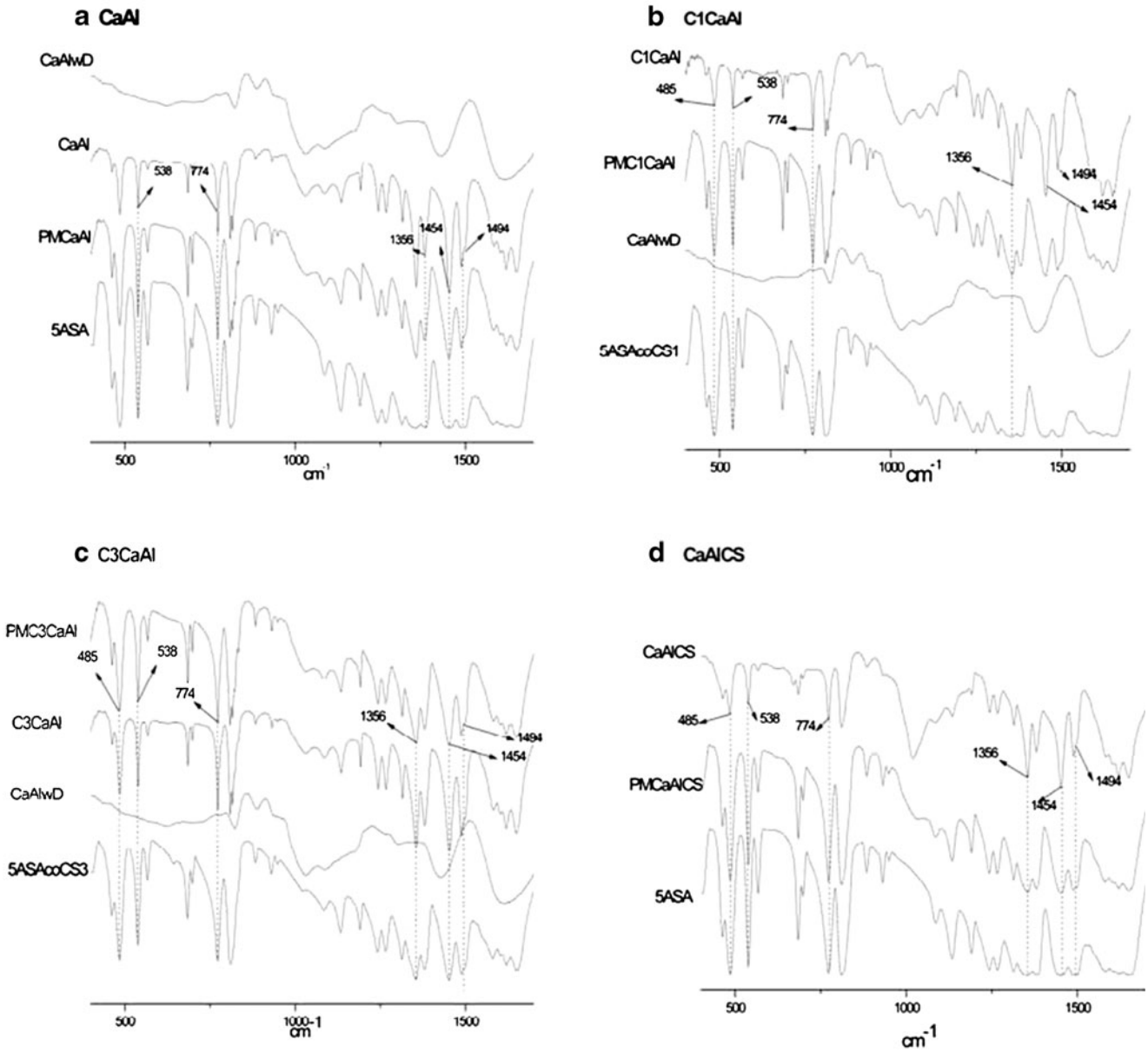

Fig. 4. FTIR spectra of the formulations compared with physical mixture, 5-ASA, and matrices **a** CaAl, **b** C1CaAl, **c** C3CaAl, and **d** CaAlCS

acid presented a lower degree of swelling. The degree of swelling was reached quickly and did not change during the period studied, also there was no erosion, see Fig. 2. Figure 3 shows the large difference in size and microstructure between the microparticles of calcium alginate without drug (CaAlwD) and (CaAl, C1CaAl, C3CaAl) and those externally coated with chitosan (CaAlCS) in all media tested. The smaller individual size of CaAlCS in acid medium has been described previously. It was found that the presence of chitosan restricts the penetration of the solvent into the core of calcium alginate and thus maintains a smaller size (14). In acid medium, the fast release of 5-ASA from the CaAl would be favored by the high solubility of the drug and because the alginate is in its unionized form so that the matrix would not suffer a swelling–erosion process. Thus, the drug diffuses quickly from the unswelled matrix. The pre-coating of 5-ASA with chitosan (C1CaAl and C3CaAl) increases the charge density inside the microparticle and therefore enhances the penetration of liquid inside the microparticles due to electro-osmotic flow and since chitosan is fully ionized at this pH. This effect increases the drug release from both formulations compared with CaAl, see T_{50} values in Table I. In contrast, microparticles with external coating with chitosan (CaAlCS) show similar drug release behavior as CaAl, because at pH 1.2, the chitosan–alginate polyelectrolyte complex in external layer of microparticle is not soluble at this pH.

In water, CaAlCS showed a higher degree of swelling compared with the other formulations (CaAl, C1CaAl, and C3CaAl). This higher degree of swelling of CaAlCS is correlated with a higher T_{50} value compared with the other formulations. Additionally, the absence of electrolytes in water medium generates a high solvent flux inside the

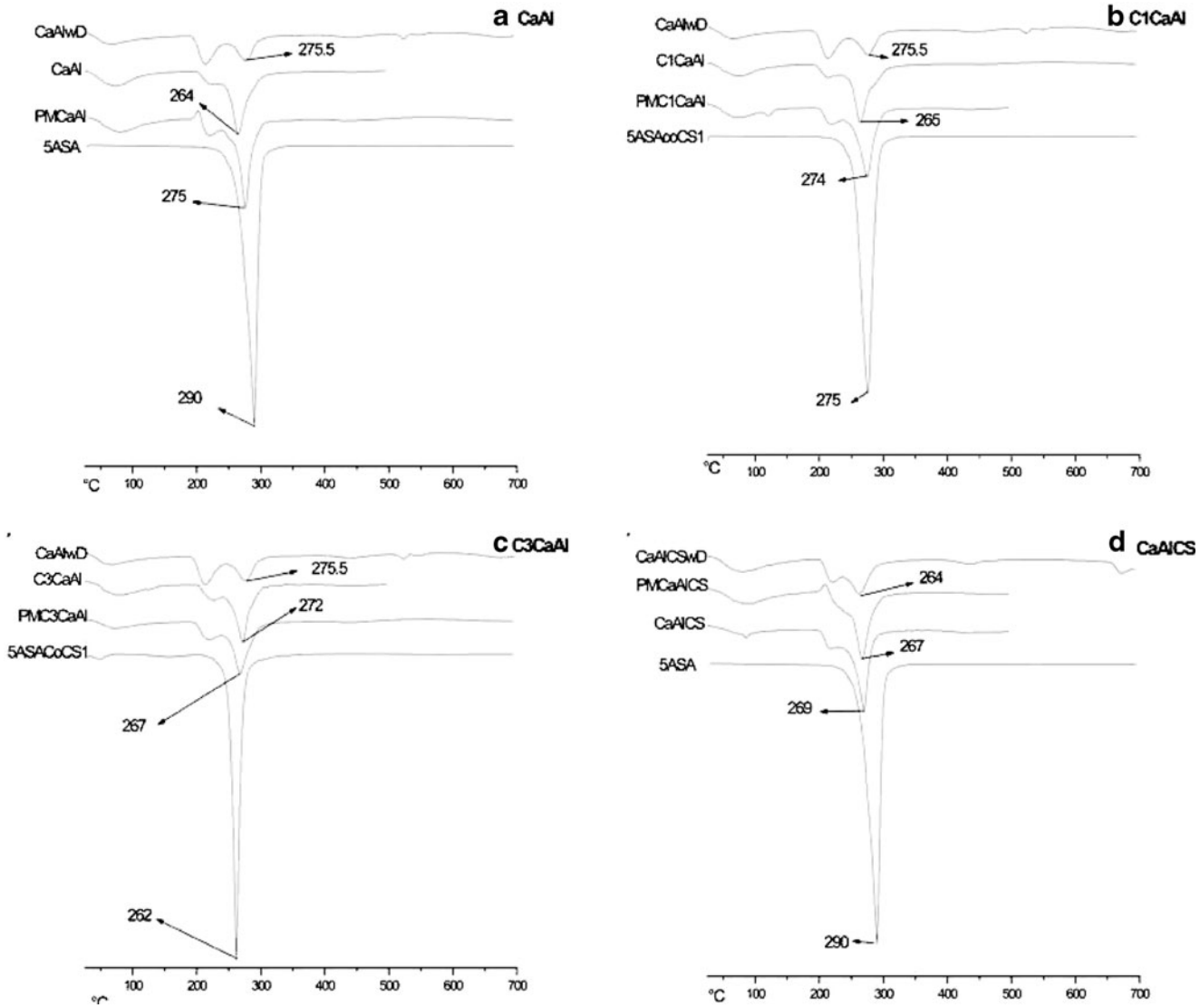

Fig. 5. DTG (%/min) *versus* temperature (°C) of the formulations compared with physical mixture, 5-ASA, and matrices **a** CaAl, **b** C1CaAl, **c** C3CaAl, and **d** CaAlCS

microparticles. In particular, it is observed that the microparticles with chitosan as external coating (CaAlCS) show a highest value of T_{50} compared with the other formulations see Table I. The explanation of this result could be that, at pH 5.5, the highest quantity of chitosan–alginate polyelectrolyte complex is obtained (19), which would contribute to increase the degree of swelling of the matrix. A higher degree of swelling of the matrix will produce slower drug diffusion through the matrix.

In phosphate at pH 7.5, calcium alginate microparticles (CaAl, C1CaAl, and C3CaAl) show a swelling/erosion process completely different from that obtained in acid medium. It is clear from Fig. 2 that the previous coating of 5-ASA with chitosan (C1CaAl, C3CaAl) promotes the erosion process. Large microparticles accompanied by some small pieces produced by the breaking of them were observed in phosphate medium, see Fig. 3. Calcium alginate is completely soluble in phosphate medium which explains the high degree of swelling reached and the subsequent erosion of the microparticles. The quick erosion process for C1CaAl and C3CaAl is probably due to osmotic effect produced by chitosan inside of the microparticle which leads to matrix rupture. T_{50} values are consistent with the behavior of swelling–erosion described in Figs. 2 and 3. The order of values from lowest to highest T_{50} is C3CaAl>C1CaAl>CaAl. CaAlCS system shows a similar behavior of swelling/erosion process as that of CaAl, but it reaches a higher DS.

In summary, in relation with the effect of chitosan on the value of T_{50}, it can be stated that, in phosphate medium, the presence of chitosan within the microparticles promotes a quick erosion process accelerating the drug release. However, the presence of chitosan as external coating (CaAlCS) does not increase the T_{50} value compared with the microparticles without chitosan (CaAl). In water, the effect of the external coating with chitosan (CaAlCS) significantly retards drug release compared with CaAl. The effect of chitosan as coating of 5-ASA on T_{50} will depend on the concentration of chitosan solution used for coating. A higher chitosan concentration (C3CaAl) retards drug release compared with CaAl. In acid, there was no effect of chitosan to avoid the 5-ASA release, so

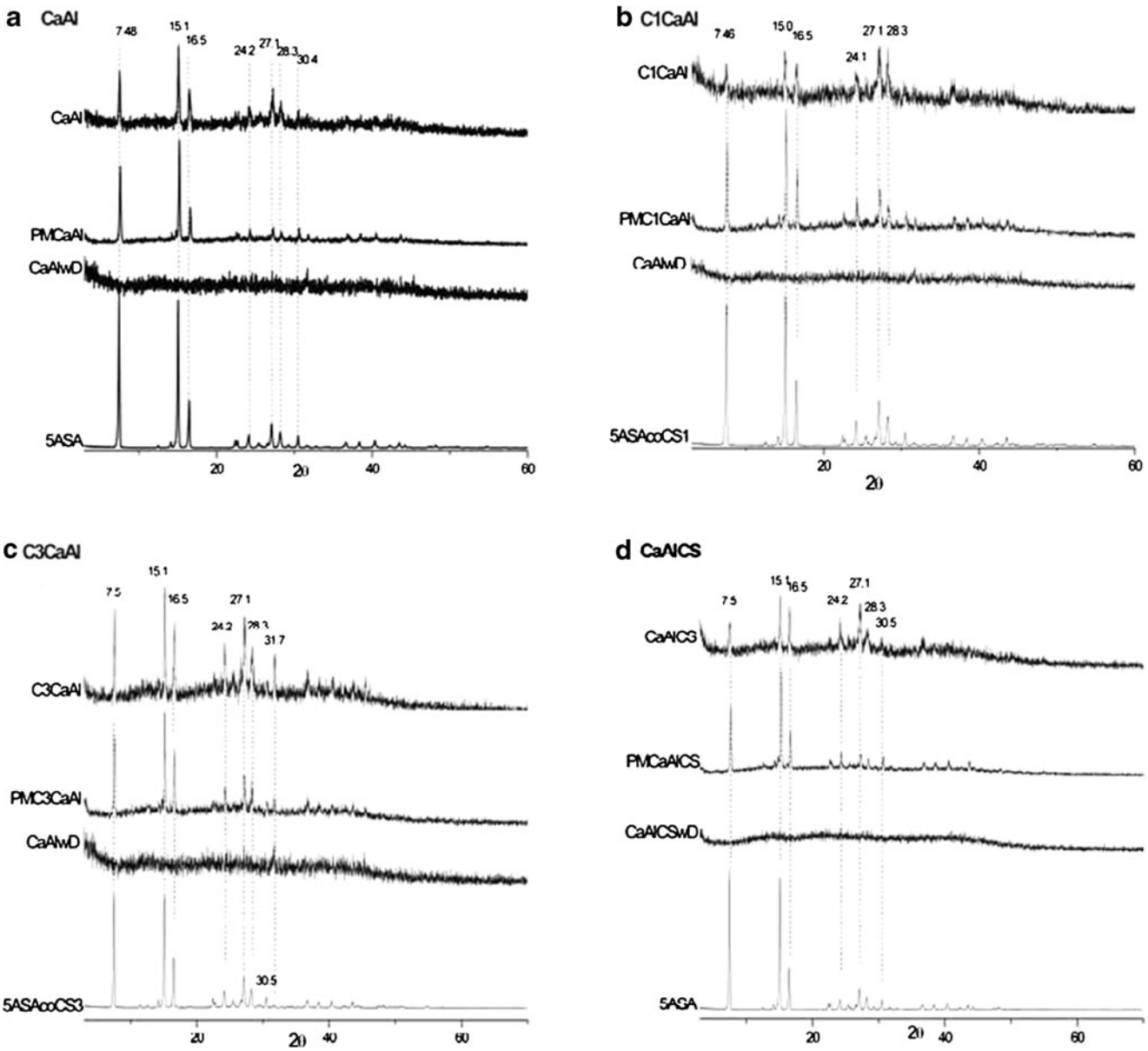

Fig. 6. XRD of the formulations compared with physical mixture, 5-ASA, and matrices **a** CaAl, **b** C1CaAl, **c** C3CaAl, and **d** CaAlCS

additional coating of microparticles is required in order to obtain a 5-ASA delayed release formulation.

Microparticle Characterization by FTIR

According to the literature (4), the characteristic infrared absorption bands of 5-ASA are present at 1,494, 1,454, 1,356, 774, 538, and 485 cm^{-1}. In this region, chitosan only shows absorption bands related with its saccharide structure (1,154 cm^{-1} due to the asymmetric stretching vibration of C–O–C bridge, 1,083 and 1,038 cm^{-1} related with skeletal vibration involving stretching of the C=O bond). It was observed (results are not shown) that the intensity and position of the absorption bands of 5-ASA were not changed when 5-ASA was coated with 1% CS solution (5-ASAcoCS1), while the intensity of these absorption bands decreased when 5-ASA was coated with 3% CS solution (5-ASAcoCS3), which is in agreement with the lowest proportion of 5-ASA in the mixture, 95% to 98%.

For the system CaAl, see Fig. 4, a clear decrease in the intensity of the absorption bands associated with 5-ASA at 538, 774, 1,356, 1,454, and 1,494 cm^{-1} in relation to the physical mixture (PMCaAl) is observed, indicating the interaction of 5-ASA with the calcium alginate matrix. Decrease in the intensity of the absorption bands associated with 5-ASA was also observed in the case of 5-ASA coated with chitosan prior to encapsulation in calcium alginate for the system C1CaAl, while the diminishing of the absorption bands associated with 5-ASA was lesser for the system C3CaAl. This indicates that an increase in the concentration of chitosan in the coating reduces the interaction of 5-ASA with the calcium alginate core. When the coating of chitosan is external to the calcium alginate core (CaAlCS), a significant diminish of the signals associated with 5-ASA

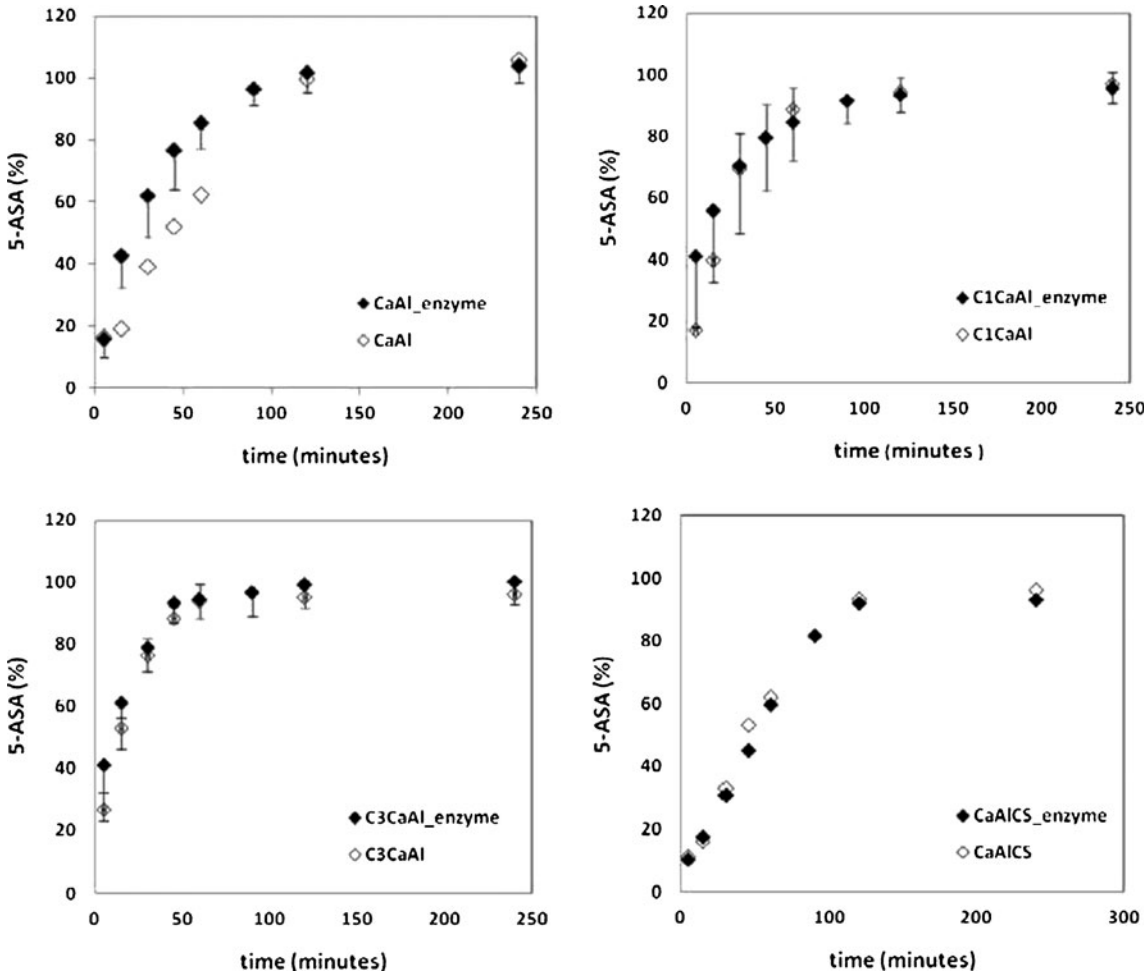

Fig. 7. Effect of β-glucosidase enzymes over 5-ASA released from the microparticles

also occurs, similar to that observed for CaAl. This is probably because 5-ASA is dispersed in the internal calcium alginate nucleus, and therefore, the external chitosan coating does not alter this interaction.

Microparticle Characterization by TGA

Two main weight losses at 213°C and 275.5°C were observed for calcium alginate microparticles without 5-ASA (CaAlwD). These correspond to the decomposition of the polymer (20). 5-ASA shows a thermal transition at 290°C, which corresponds to its melting temperature (21).

Figure 5 shows the results of derivative thermogravimetry (DTG) *versus* temperature for the formulations studied. All formulations showed thermal transition associated with the melting temperature of 5-ASA indicating that 5-ASA or 5-ASA pre-coated with chitosan was not completely soluble in the calcium alginate matrix (CaAl, C1CaAl, C3CaAl) or in the calcium alginate matrix coated with chitosan (CaAlCS), maintaining its crystalline state. A decrease in the decomposition temperature of 5-ASA was observed by comparing the microparticles with the physical mixture for the system CaAl (CaAl, 265°C; PMCaAl, 275°C), C1CaAl (C1CaAl, 265°C; PMC1CaAl, 274°C), and C3CaAl (C3CaAl, 267°C; PMC3CaAl, 272°C). The lower decomposition temperature of the microparticles compared with the physical mixture pointed out that the coacervation procedure allows the incorporation of the matrix in the crystal structure of 5-ASA. In contrast, for CaAlCS, similar decomposition temperature of 5-ASA was observed for the physical mixture (PMCaAlCS, 269°C) and the microparticles (CaAlCS, 267°C). It is also observed that the drug coating with chitosan produced a significant decrease in the decomposition temperature of 5-ASA, and that is dependent on the concentration of chitosan solution used for coating (5-ASA, 290°C; 5-ASAcoCS1 275.3°C; 5-ASAcoCS3, 262.1°C).

Microparticle Characterization by XRD

As seen in Fig. 6, characteristic diffraction peaks of 5-ASA observed at 2θ angles equal to 7.5°, 15°, 16.5°, 24.2°, 27.1°, 28.3°, and 30.5° (4) were observed in both physical mixtures and microparticles, demonstrating that the 5-ASA crystal structure remained unchanged. Drug crystallinity was shown to be less intensive after coacervation procedure for the microparticles CaAl, C1CaAl, and CaAlCS as a result of the partial solubilization of 5-ASA in the amorphous calcium alginate and calcium alginate–chitosan matrix. C3CaAl shows less interaction with the matrix, as it was found from FTIR

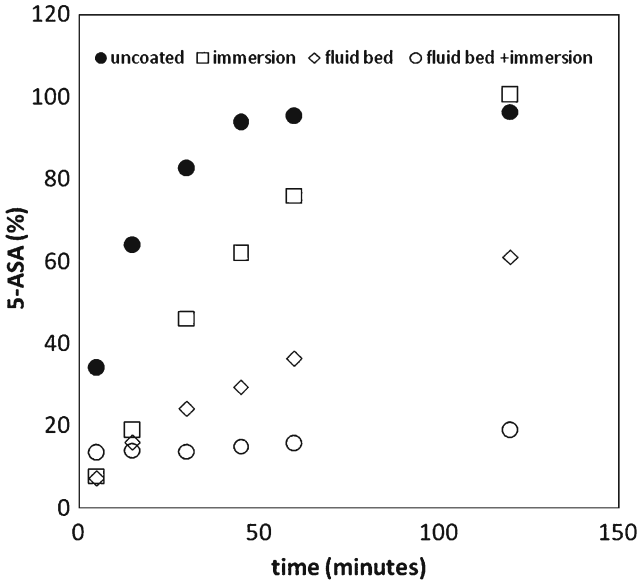

Fig. 8. 5-ASA released in acid medium from microparticles of CaAl coated with Acryl-EZE® using different procedures of coating

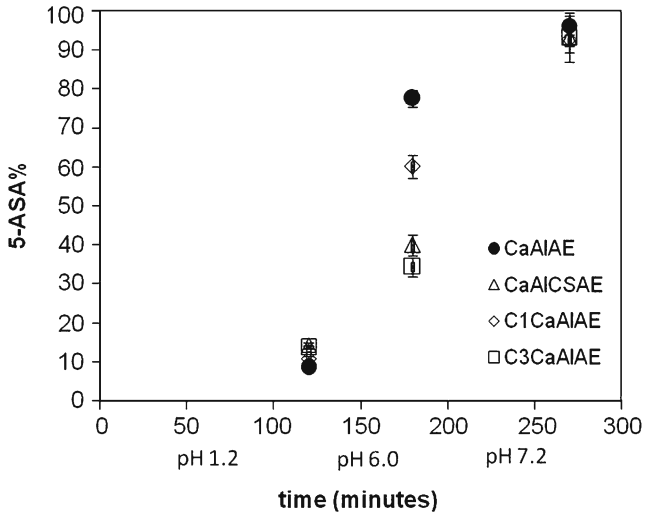

Fig. 9. 5-ASA delayed release test for the microparticles coated with Acryl-EZE®

analysis. The drug crystallinity in the microparticle was similar to that of physical mixtures.

FTIR, TGA, and X-ray data supported the conclusion that 5-ASA did not form a solid solution but was dispersed in the amorphous calcium alginate (CaAl, C1CaAl, C3CaAl) and calcium alginate–chitosan (CaAlCS) matrices.

Effect of β-Glucosidase Enzymes over 5-ASA Released from the Microparticles

The effect of β-glucosidase enzyme in the hydrolysis of calcium alginate core (CaAl) and the effect of chitosan as 5-ASA coating (C1CaAl, C3CaAl) and as external coating of calcium alginate microparticles (CaAlCS) on the kinetics of 5-ASA release from the microparticles were studied. There is evidence in the literature that both polysaccharides, namely calcium alginate and chitosan, are degraded by bacteria in the colon (1,5,8–11). The test was developed in phosphate buffer at pH 7.5, simulating the colonic pH of the medium, in the absence and presence of 9.9×10^{-2} U/mL β-glucosidase enzymes. As shown in Fig. 7, only the system CaAl shows a significant increase in the release rate of 5-ASA in the presence of β-glucosidase within the first 60 min. In contrast, C1CaAl and C3CaAl did not show significant differences on drug release. This result could be explained by the fast release of 5-ASA due to the fact that the microparticles are quickly eroded in this medium, see Table I and Fig. 2.

Also, significant difference on drug release was not observed for CaAlCS in the presence of β-glucosidase. Although CaAlCS has similar value of T_{50} and swelling–erosion behavior than that of CaAl, CaAlCS microparticles have significantly smaller size due to the external coating with chitosan, which would make the microparticle less sensitive to enzyme action in relation to CaAl.

Microparticles Coated with Acryl-EZE®

Based on the results of 5-ASA release in acid medium, see Fig. 1, it is clear that the addition of chitosan to the core of calcium alginate, internally, as a coating of 5-ASA (C1CaAl, C3CaAl), or externally (CaAlCS), does not prevent the quick release of 5-ASA in acid, T_{50}<10 min for all formulations. In order to reduce the release of drug in acidic medium, the microparticles were coated with Acryl-EZE®, methacrylic polymer type, which has a dissolution pH of 5.5. Procedures by immersion and spray coating were tested for CaAl. The procedure of immersion used 20% Acryl-EZE® suspension, at three different contact times (20, 40, and 60 min). The Acryl-EZE® coating drastically reduced the burst, from 38% to 8% release of 5-ASA, but did not avoid completely the release of 5-ASA within 120 min. An

Table II. Percentage of Acryl-EZE® Coating in the Formulations Using the Combined Spray Coating and Immersion Method

Formulations	% of coating±IC (n=9)
CaAlAE	54.6±0.7 a
C1CaAlAE	47.2±3.8 b
C3CaAlAE	44.1±0.4 b
CaAlCSAE	61.4±0.0 c

Different lowercase letters mean significant differences between rows (p<0.05)

Table III. f_2 Values for Formulations Coated with Acryl-EZE® at 1 and 3 Months of Storage at 40±1°C and 78±1% RH

Formulations	f_2 between 0 and 1 month	f_2 between 0 and 3 month
CaAlAE	52.8	47.6
C1CaAlAE	57.7	49.4
C3CaAlAE	39.1	40.7
CaAlCSAE	75.5	55.0

immersion time of 40 or 60 min was more effective than 20 min (results are not shown). The coating procedure by spraying in a fluid bed system was more effective than immersion procedure. Figure 8 shows that after 120 min, 60% of 5-ASA was released, but this is still insufficient. The combined fluid bed coating and immersion procedure was chosen because less than 20% of 5-ASA was released in acid medium.

Table II shows the percentage of Acryl-EZE® coating, using the combined procedure of coating for the formulations studied. For all formulations, a high percentage of coating is required to prevent the loss of 5-ASA in acid medium. The formulation with external coating of chitosan (CaAlCSAE) required more coating, probably due to the more irregular surface of the microparticles.

The Acryl-EZE® coating diminished significantly the swelling in phosphate buffer for all formulations. The swelling was also diminished with the coating in the case of CaAlCS that showed swelling in water (results are not shown). The effectiveness of the Acryl-EZE® coating was evaluated using the 5-ASA tablets delayed released test described in USP 27 (17), see Fig. 9. All the formulations showed a low release of 5-ASA, less than 15%, in acidic medium at pH 1.2. No significant differences were observed between the formulations, demonstrating the effectiveness of the coating with Acryl-EZE®. Significant differences in the percentage of 5-ASA released from different formulations were observed in phosphate buffer at pH 6.0. At this pH, Acryl-EZE® coating is dissolved and the drug release is restricted by chitosan. The decreasing order of 5-ASA release was as follows: CaAl (78%)> C1CaAl (60%)>CaAlCSAE (39%), C3CaAlAE (34%). In phosphate buffer at pH 7.2, 100% of 5-ASA was released from all formulations satisfying the test requirement.

Stability Studies

The stability of the dissolution characteristics of dosage forms during storage can be affected by formulation components and processing. The stability of the formulations coated with Acryl-EZE® at 40±1°C and 78±1% RH for periods of 1 and 3 months was evaluated using the similarity factor (f_2) to compare 5-ASA released from freshly prepared microparticles and microparticles stored for 1 and 3 months. The results presented in Table III show that the formulations studied show a low stability after the first month of storage, f_2 value near or below 50, except CaAlCSAE (f_2=75.5), and after 3 months of storage, the formulations were not stable. Since the microparticles are made of hydrogels, they are highly sensitive to moisture, and thus, the period of stability is limited. To achieve a more prolonged period of stability, the microparticles should be stored in packages containing desiccant.

CONCLUSIONS

The effect of chitosan on the release of 5-ASA from calcium alginate microparticles was studied. The results showed that the release behavior depended on the dissolution media and the location of chitosan in the microparticle. In phosphate medium, the presence of chitosan as 5-ASA coating inside the microparticle promoted a quick erosion process accelerating drug release; however, the presence of chitosan as external coating (CaAlCS) did not increase the T_{50} value compared with the microparticles without chitosan (CaAl). The external coating of CaAl microparticles with chitosan (CaAlCS) retarded significantly the drug release in water compared with CaAl microparticles. The effect of chitosan as coating of 5-ASA on T_{50}, depended on the concentration of chitosan solution used for coating. A higher chitosan concentration (C3CaAl) retards drug release compared with CaAl. In acid medium, additional coating was required in the microparticle for obtaining a 5-ASA delayed release formulation.

FTIR, TGA, and X-ray data supported the conclusion that 5-ASA did not form a solid solution but was dispersed in the amorphous calcium alginate (CaAl, C1CaAl, C3CaAl) and calcium alginate–chitosan (CaAlCS) matrices.

The effect of β-glucosidase enzymes on 5-ASA release was only observed for CaAl. This formulation showed a significant increase in the release rate of 5-ASA within the first 60 min. In contrast, C1CaAl, C3CaAl, and CaAlCS did not show significant differences on drug release.

The Acryl-EZE® coating of microparticles was effective because all the formulations showed a low release, less than 15%, of 5-ASA in acid medium at pH 1.2. Significant differences in the percentage of 5-ASA released between formulations were observed in phosphate buffer at pH 6.0. At this pH, Acryl-EZE® coating is dissolved, and the drug release is restricted by chitosan. The decreasing order of 5-ASA release was as follows: CaAl (78%)>C1CaAl (60%)> CaAlCSAE (39%)>C3CaAlAE (34%). In phosphate buffer at pH 7.2, all the formulations released 100% of 5-ASA.

The formulations studied showed a low stability after the first month of storage, except CaAlCSAE (f_2=75.5). Since the microparticles are made of hydrogels that are highly sensitive to moisture, the period of stability was limited.

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