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Elaboration and "In Vitro" Characterization of 5-ASA Beads

A. Iruín, M. Fernández-Arévalo and J. Álvarez-Fuéntes
Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain

A. Fini
Instituto di Scienze Chimiche, Universitá di Bologna, Bologna, Italy

M. A. Holgado
Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain

ABSTRACT The purpose of this research was to perform the design and in vitro evaluation of alginate beads containing 5-ASA in order to achieve an oral system that protects the drug until it reaches the colon. Alginate beads were prepared by the well-known ionic gelation reaction (Ca^{2+}). The influence of the incorporation of several polymers (Eudragit FS 30D, Eudragit S100, and chitosan) in the initial formulation was studied. In all formulations, entrapment efficiencies of the drug higher than 70% were obtained. The scanning electron microscopy (SEM) study of beads showed homogeneous sizes and shapes in all cases. Finally, the release behavior of these polymeric beads were also studied and compared. The results indicated that Eudragit FS 30D (26%) showed the most favorable dissolution behavior in terms of achieving a controlled release of 5-ASA. To determine the mechanism of drug release from these beads, the Korsmeyer equation was applied. $Q_t/Q_\infty < 0.9$ can be described using a Higuchi model and $Q_t/Q_\infty = 0.7$ showed a zero-order release period. This formulation was assayed at other different pH values (pH=6; 6.8; 7.2) to assure that there is no release of 5-ASA until the system reaches the colon. No release was observed at pH 6.0. Release was very slow at pH 6.8; averages about 20% an hour at pH 7.2 and was complete within 4 hour at pH 7.4. So, these Eudragit FS beads exhibited interesting dissolution profiles for the therapy of colon pathologies.

KEYWORDS 5-ASA, Ulcerative colitis, Alginate beads, Eudragit® FS 30D, Eudragit® S100, Chitosan

INTRODUCTION

Although some years ago inflammatory bowel disease was more frequent in northern countries, recent studies prove that the number of patients with this disease is increasing all over the world due to contamination, industrialization, and changes in lifestyle.

Presently, there are many researchers trying to find a good system for directing a specific drug to the colon. Some studies in the U.S. have shown that 5-aminosalicylic acid (5-ASA), an anti-inflammatory agent, is the preferred treatment for Crohn's disease and ulcerative colitis because of its

Address correspondence to A. Iruín, Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, C/Profesor Garcia González no-2, Sevilla 41012, Spain; Fax: 0034-95-55-6726; E-mail: holgado@us.es

effectiveness and safety http://www.el_mundo.es/anuncios/GrupoSB/noticias/1999/octubre/intestinal.html, (1999).

In recent years, multiparticulate systems have received considerable interest. The production of microparticle systems seems to be equally promising in the development of dosage forms in order to reduce the dosage frequency (Lamprecht et al., 2000). The advantage of using microparticles is their small size. Furthermore the influence of gastric emptying time and intestinal motility on intra- and inter-subject variation in the rate and the extent of availability can be largely avoided by the use of multiple-unit dosage forms (Clarke et al., 1993; Coupe et al., 1991; Folkier & Doelker, 1980; Hogan & Auton, 1995).

On the other hand, it is important to devise a strategy to ensure that the unalterable drug arrives at the colon after crossing the gastrointestinal tract. A well-known commercial prodrug, sulfasalazine, is a 5-ASA prodrug that is reduced by colonic bacteria into 5-ASA and sulfapyridine. 5-ASA is largely unabsorbed from the colon and acts as a topical anti-inflammatory. In contrast, sulfapyridine is well absorbed, giving to side effects and toxicity, and as many as 30% of patients are unable to tolerate the use of this prodrug. It would be interesting to develop a system containing a suitable carrier for 5-ASA with minimum adverse effects (Wiwattanapatapee et al., 2003). The efficacy of this drug in the treatment of inflammatory bowel disease may be optimized with a controlled-release drug delivery system that maximizes topical exposure of the drug to the diseased tissue and minimizes systemic absorption of the drug (French & Mauger, 1993).

Therefore, the objective of the present paper is to design an oral system that protects 5-ASA until it reaches the colon without causing toxicity. In this article the elaboration and release characteristics of beads containing mesalamine with a new polymeric resin composed of mathacrylic acid, methylacrylate, and methylmethacrylate (Eudragit® FS 30D) is described. This polymer dissolves quickly at pH 7.5 (data on file, Degusa Iberia S.A.), which should lead to site-specific release in the ileocecal region in patients with ulcerative colitis (UC). The release behavior of these new polymeric beads as well as those elaborated with the polymer Eudragit S and with chitosan was compared. Chitosan [poly(1,4-β-d-glucopyranosamine)] is yielding increased importance in the pharmaceutical field because of its good biocompatibility, nontoxicity, and biodegradability. It is a cationic polyelectrolyte having the ability to form gels in acidic medium, but at neutral and high pH values this polymer presents poor gel formation ability. Furthermore, chitosan has been presented as useful polymer for colon-specific drug delivery systems because of its specific biodegradation by colonic bacteria (Turkoglu & Ugurlu, 2002). Therefore, the aim of the present study was to develop an oral, multiunit dosage form containing 5-ASA for the treatment of UC. The proposed system consists of beads of several compositions (polymer with different nature and characteristics) introduced in an enteric hard gelatine capsule to obviate the transition across the stomach. Since the gastric residence time of monolithic dosage forms is strongly influenced by the presence and caloric content of food in the stomach and the motility pattern of the different digestive and interdigestive phases, we have decided to elaborate a multiparticulate dosage form design.

MATERIALS AND METHODS

Materials

5-Aminosalicylic acid (5-ASA) was a gift from Schering Plough (Madrid, Spain). Eudragit S 100 and Eudragit FS 30D were received from Degussa Iberia S.A. (Barcelona, Spain) The following materials were obtained from the indicated suppliers and used as received: alginic acid sodium salt (low viscosity) and calcium chloride anhydrous from Sigma (Barcelona, Spain); chitosan, low molecular weight, from Aldrich Chemical Company (Barcelona, Spain); disodium hydrogen phosphate anhydrous, potassium dihydrogen phosphate, sodium chloride, and hydrochloric acid from Panreac Química S.A. (Barcelona, Spain).

Preparation of Beads

Alginate beads were prepared as follows: an alginate solution of 2% w/v was prepared dissolving the sodium alginate in distilled water under stirring. The drug suspension (0.5% w/v) was added to the alginate solution. This mixture was added dropwise, from a

TABLE 1 Composition of the Beads

LOT	Alginate %	5-ASA %	Eudragit® FS 30D %	Eudragit® S100 %	Chitosan %
Lot 1	2	0.5	–	–	–
Lot 2	2	0.5	26	–	–
Lot 3	2	0.5	13	–	–
Lot 4	2	0.5	–	26	–
Lot 5	2	0.5	–	–	0.1
Lot 6	2	0.5	–	–	0.2
Lot 7	2	0.5	–	–	0.5

hypodermic syringe, into 1.4% w/v $CaCl_2$ solution. The gel beads, which formed immediately in the $CaCl_2$ solution, were incubated at room temperature (22°C) in darkness in this solution for 24 h to ensure complete reaction. After this time, the beads were filtered and dried using microwave (3200 W) during 110 minutes with 10% of power.

In order to prepare Eudragit S 100 or FS 30D/alginate beads, the drug suspension (0.5% w/v) was added to the previously lescribed alginate 2% w/v solution. After that, a suspension of Eudragit S 100 (26% w/v) or FS 30D (26% or 13% w/v) was added in the mix under agitation and the reluctant suspension was added dropwise from a hypodermic syringe over a 1.4% w/v $CaCl_2$ solution. The resultant beads were treated as previously described.

Finally, the chitosan/alginate beads were elaborated as follows. Chitosan solutions of 0.1%, 0.2%, and 0.5% (w/v) were prepared by adding chitosan to distilled water containing 1% (v/v) acetic acid. The solution was stirred for 1 h. The 1.4% w/v $CaCl_2$ solution was added to the chitosan solution. An alginate/drug suspension was added to this solution to form the beads. The beads were incubated and dried under the same conditions as described above.

Table 1 shows the composition of the different bead formulations.

Thermal Analysis

Differential scanning calorimetry (DSC) analysis was used to characterize the thermal behavior of the different bead components. This analytical method was carried out on isolated substances, 1:1 physical drug-polymer mixtures, as well as on loaded beads. The DSC thermograms were obtained using an automatic thermal analyzer system (*Mettler FP80HT Central Processor and Mettler FP85TA Cell*). A data processing system (*Mettler FP89HT*) was connected to the thermal analyzer. Sealed and perforated aluminium pans were used in the experiments for all the samples. Temperature calibrations were performed using indium as a standard. All samples were run at a scanning rate of 10°C/min, from 30–350°C.

Scanning Electron Microscopy

Scanning electron microscopy (*Philips, XL30*) was used to examine the morphology and surface structure of the beads. A thin coat of gold was applied, under vacuum to each sample prior to examination.

Size and shape parameters for beads were determined using an image analysis system based on Fourier description and connected to the microscope mentioned above. They were obtained using a special computer program based on obtaining the coordinates (x, y) of the particle boundary through the digitization of the particle image. The coordinates were then used to calculate a set of invariant descriptors.

The following parameters were selected to characterize the 5-ASA beads:

- Mean diameter (D).
- Shape factor (S). This parameter provides information about the elongation of the particle: for a circular particle the shape factor is 1, for all other particles the shape factor is smaller than 1:

$$S = 4\pi\lfloor \text{area}/(\text{perimeter})^2 \rfloor$$

- Aspect ratio (a). This is the ratio of the horizontal maximum and the vertical maximum distance of the particle: for a round or a square particle, the aspect ratio is unity, for those elongated in the x-direction the ratio is larger than 1 and particles

TABLE 2 Release Mechanism According to "n" Exponent

Exponent n	Release mechanism
~0.5	Case I or Fickian diffusion
0.5<n<1.0	Anomalous diffusion or no Fickian
~1.0	Case II (zero order)

elongated in the y-direction have an aspect ratio smaller than unity.

Twenty bead particles were employed to accomplish all the measurements.

Calibration Curve of 5-ASA

Before undertaking release assays of 5-ASA beads, a calibration curve of the drug was plotted under the same conditions of the dissolution tests. It has been proved that there is a point at 315 nm (*Hitachi, mod. U-2000*) where the absorbance is not affected by changes in the pH of the medium where 5-ASA is dissolved. Concurrently, with this determination we also established that there was no absorbance at 315 nm by the other components of the formulation.

Entrapment Efficiency

To know the entrapment efficiency of beads, the systems were placed into a 7.4 Sorensen's phosphate buffer during 12 hours by shaking. Aliquots from the filtered solutions remaining after removal of the beads were spectrophotometrically assayed at 315 nm (*Hitachi, mod. U-2000*).

Dissolution Studies

Drug release was determined using 100 mg of beads (introduced in hard gelatin capsules) in 500 mL of different dissolution media, set at 37±0.5°C and a stirring rate of 50 r.p.m. The different dissolution media used were Sorensen's phosphate buffers at different pH values: 6.0, 6.8, 7.2, and 7.4.

In all the studies, the XXVI USP (2003) basket apparatus (Turu Grau, model D-6) was used. Samples (3 mL) were withdrawn at specific time intervals and assayed spectrophotometrically at the wavelength of maximum absorbance (315 nm). From the absorbance values, the cumulative percent released was determined. All the studies were carried out in triplicate.

Treatment of Data

Among several methods investigated for dissolution profile comparisons, Food and Drug Administration (FDA) guidelines choose f_2 as the simplest. Moore and Flanner (1996) proposed a model-independent, mathematical approach to compare the dissolution profiles using two factors, f_1 and f_2.

$$f_1 = [\Sigma(|R_t - T_t|)/\Sigma R_t)] \cdot 100$$

$$f_2 = 50 \cdot \log -\{[1/(1 + (\Sigma(R_t - T_t)^2)/n]^{1/2} \cdot 100\}$$

where R_t and T_t represent the average percent dissolved at time t for reference and test, respectively, and t is the number of time point tested. The factor f_1 is proportional to the average difference between the two profiles, and factor f_2 is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time points. The factor f_2 measures the closeness between the two profiles. Because of the nature of measurement, f_1 is described as difference factor, and f_2 as similarity factor (Lin & Liaw, 1997).

The FDA has set a public standard f_1 value of 0–15 and f_2 value of 50–100 to indicate similarity between two different dissolution profiles.

Moreover, in order to determine the release mechanism attached from the beads, the experimental data were fitted to the (Korsmeyer equation Korsmeyer et al., 1993):

$$(Q_t/Q_\infty = K_k \times t^n)$$

where Q/Q_∞ is the drug released ratio at different times, Kk, is the Korsmeyer constant, and n is a parameter that defines the release mechanism.

TABLE 3 Entrapment Efficiency of 5-ASA in the Beads

Beads formulations	Percent entrapment (%)±S.D.
Alginate	75.98±0.15
Eudragit® FS 30D (26%)/alginate	78.37±0.19
Eudragit® FS 30D (13%)/alginate	80.96±0.13
Eudragit® S100 (26%)/alginate	72.85±0.13
0.1% Chitosan/alginate	70.56±0.14
0.2% Chitosan/alginate	71.01±0.15
0.5% Chitosan/alginate	82.86±0.13

Table 2 shows the different release mechanisms as a function of the value of n exponent.

RESULTS AND DISCUSSION

Entrapment Efficiency

Total 5-ASA percent entrapment efficiency of elaborated beads is shown in Table 3. The yield of the formulation without polymer was 75.98%, while the best results were obtained by using chitosan 0.5% (w/v) (82.86%). As can be appreciated, the differences between the entrapments of all the beads elaborated neither changing the polymer nor using the same polymer but with different percentage are not of great magnitude. Nevertheless, it has been found that for each polymer used, the higher the mean diameter of particles, the higher the drug content present in the systems. Furthermore, the analysis of the variance carried out with these data showed statistically significant differences among all the batches ($p<0.05$; $F=227.59$; $n=20$). Post-hoc comparison showed that no statistically significant differences between lots 5 and 6 were found for drug entrapment.

Thermal Analysis

In previous works (Fernández-Hervás et al., 1998; Li, 1996; Lin et al., 1997; Polk et al., 1997), some thermal events and interactions between some of the materials used in this paper were indicated. In Fig. 1 are shown the corresponding thermograms of the new products used in this work. As it can be seen, 5-ASA shows a clear melting peak. In this study, no interactions were detected either between alginate and the different Eudragits or between 5-ASA and the employed polymers. 5-ASA and the used polymers were weighed in a 1:1 ratio and then mixed by light trituration in a mortar. The physical mixture showed endothermic peaks corresponding to the isolated substances, indicating no interactions between the original products.

As an example, the thermograms of some beads are shown in Fig. 2. No important interactions can be observed. The endothermic peak of the drug can be

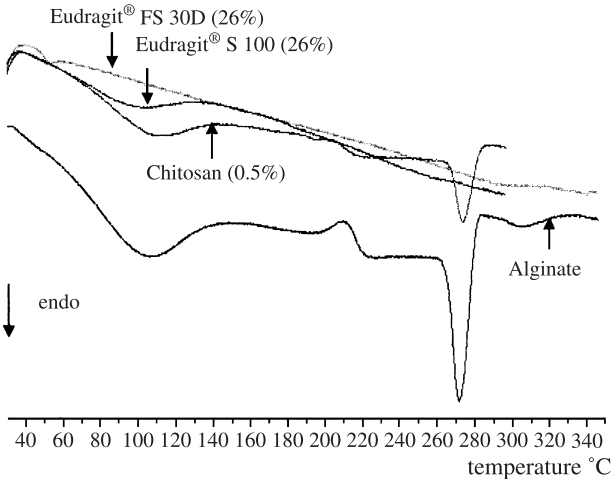

FIGURE 2 **DSC Thermograms of the Indicated Beads.**

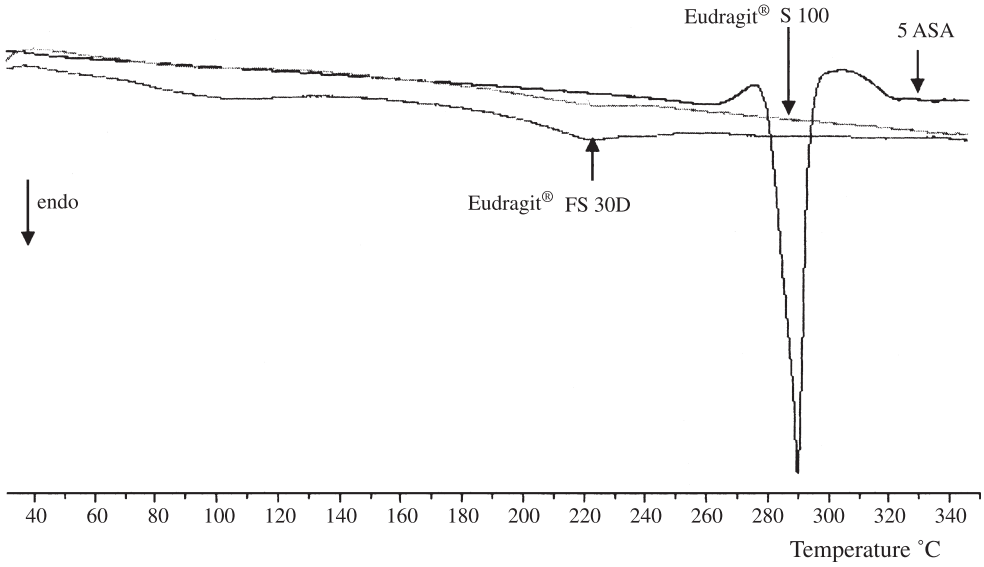

FIGURE 1 **DSC of the Components of the Beads.**

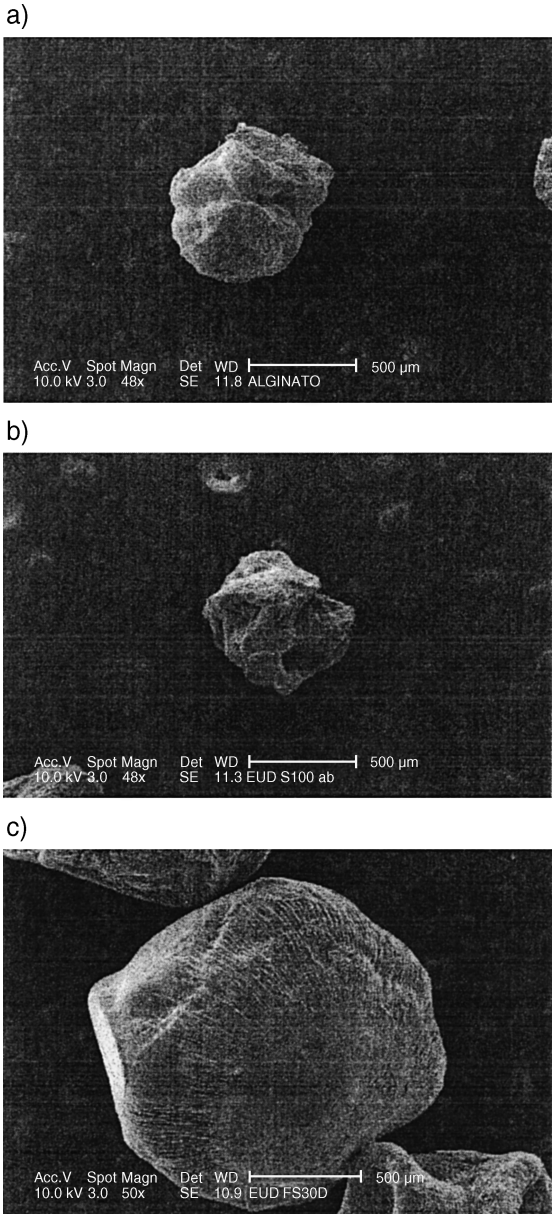

FIGURE 3 **Microphotographs of the Beads Obtained From: (a) Alginate, (b) Eudragit S 100 (26% w/v), and (c) Eudragit FS 30D (26% w/v).**

recognized in all the thermograms, except those containing Eudragit FS and S 100. This circumstances can be explained considering that the high proportion of polymers in beads containing both types of Eudragits (26%) masks the thermal behavior of the drug.

Morphology of the Beads

All the obtained beads produced were more or less spherical in form. Scanning electron micrographs show that all samples maintain this spherical form after drying, and the size is similar after this process (Fig. 3).

In relation to SEM parameters, Table 4 shows data obtained for each bead formulation. As a whole, and considering the size parameter mean diameter (D) (mm), very few differences were found among the different formulations. The statistical study did not indicate differences between formulations prepared with the same type of polymer ($p<0.05$). The shape factor (S) data obtained for the analyzed beads yielded values less than unity, demonstrating few variations in the particles' silhouette. On the other hand, the aspect ratio (a) data obtained show similar values close to unity, also indicating regular forms for all the formulations. Therefore, the shape of the beads is not affected by the type of polymer or by the polymer concentration used.

In relation to the beads containing chitosan, the shape of the microparticles is less regular, especially when the concentration of chitosan is increased (Fig. 4). Although it has been reported that the impossibility of using this polymer in concentrations over 0.25% is due to the high viscosity of its solution, which shows a tendency to form agglomerates (20), a higher concentration (0.5% w/v) has been employed in the present study. The less regular shape of this type of bead can be explained on the basis of the high viscosity of the chitosan-calcium chloride solution

TABLE 4 **Size and Shape Descriptors of Beads Formulations (Mean Values ± Standard Deviations)**

Bead formulation	D (mm)	S	a
Alginate	0.820±0.114	0.593±0.148	0.972±0.117
Eudragit® FS 30D (26%)/alginate	0.795±0.086	0.741±0.121	1.075±0.174
Eudragit® FS 30D (13%)/alginate	0.902±0.159	0.727±0.098	1.004±0.148
Eudragit® S100 (26%)/alginate	0.761±0.097	0.668±0.196	1.084±0.199
0.1% Chitosan/alginate	0.699±0.120	0.573±0.178	1.132±0.117
0.2% Chitosan/alginate	0.718±0.103	0.496±0.134	0.914±0.142
0.5% Chitosan/alginate	0.895±0.132	0.454±0.153	0.907±0.198

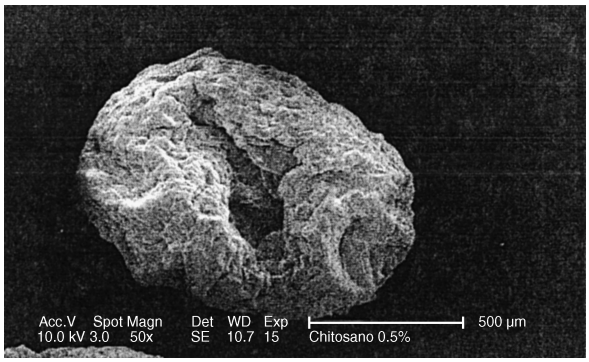

FIGURE 4 **Microphotograph of the Beads Containing Chitosan (0.5% w/v).**

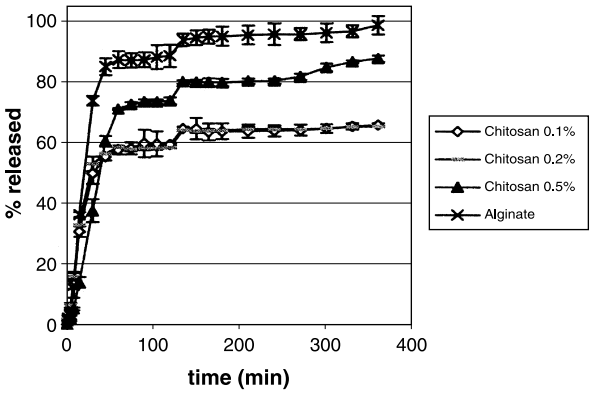

FIGURE 6 **5-ASA Released From Indicated Beads (pH=7.4).**

that prevents the complete interaction between Ca^{+2} and alginate. The nonreacting chitosan is deposited on the bead surface during the incubation period.

Dissolution Studies

Firstly, the release studies were carried out for all the formulations in pH 7.4 buffer dissolution medium as indicated in a previous section. The release profiles appear in Figs. 5 and 6.

As it can be appreciated from these figures, beads containing Eudragit FS 30D (26%) show the most favorable dissolution behavior in terms of achieving a controlled release of 5-ASA. The release rate of drug is slow (averages about 30% an hour) and complete within the assay period, and exhibits a more controlled release profile than Eudragit S 100 at the same concentration. This different behavior is related to the different pH-dependent solubility of both Eudragits: Eudragit S 100 starts to dissolve above pH 6, while Eudragit FS 30D dissolves at pH greater than pH 7.

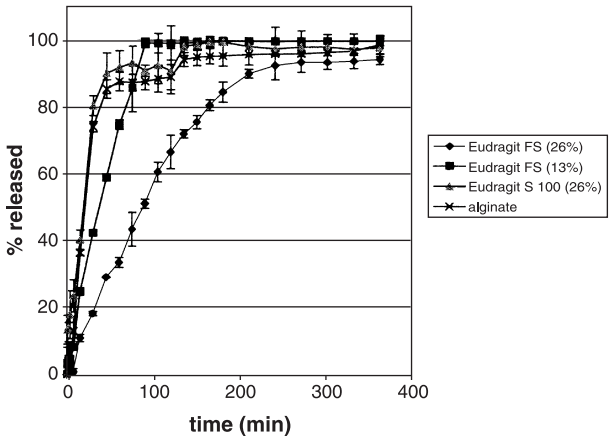

FIGURE 5 **5-ASA Released From Indicated Beads (pH=7.4).**

The dissolution behavior of chitosan formulations differs from the Eudragit beads. Chitosan at 0.1% and 0.2% exhibits a controlled and fast but not complete release process. Perhaps due to an interpolymeric complex formed between alginate and chitosan the drug release rate was reduced (Fernández-Hervás et al., 1998; Tapia et al., 2002). The complex formed between both polymers is produced by electrostatic attraction between the amine group of chitosan and the carboxylic group of alginate. NO excess of chitosan was observed by SEM in the morphology of these formulations (0.1–0.2% chitosan), as it was found that the surfaces of these beads were clearly smooth. Moreover, at the end of the dissolution studies, some beads were found in the basket, indicating that the dissolution process was not finished.

When the proportion of chitosan increases (0.5%), an excess of polymer appears. This excess cannot react with alginate to form a complex. The resultant beads (Fig. 4) appear with a modified structure and a very rough surface. This situation can be responsible for the faster release profile in comparison with the other formulations containing the same polymer. This fact is explained by the poor gel formation ability of chitosan in the pH 7.4 medium (Mi et al., 1997), in comparison

TABLE 5 **f_1 and f_2 Obtained Values for the Formulations Indicated**

	f_1	f_2
Eudragit® FS (26%)	26.754	29.067
Eudragit® FS (13%)	10.105	48.841
Eudragit® (26%)	6.683	60.612
Chitosan 0.1%	32.048	29.385
Chitosan 0.2%	32.025	29.418
Chitosan 0.5%	18.758	40.562

with the chitosan-alginate complex. So, this excess of chitosan in the bead structure cannot form a gel that would act as a barrier to decrease the drug release. In this situation, as there are no swollen particles of chitosan present in the bead network, the dissolved drug diffuses out of the beads at a greater dissolution rate.

For comparison purposes, the difference factor f_1 and the similarity factor f_2 (Table 5), recommended by FDA to compare dissolution profiles, have been calculated. Alginate beads without another polymer have been employed as reference.

Table 5 shows that the dissolution profiles of the lots elaborated with Eudragit FS 30D (13%) and S 100 (26%) are similar to the one of reference (alginate). This indicates that neither formulation with the lower content of Eudragit FS (13%) nor with Eudragit S 100 (26%) shows any advantage with respect to the alginate beads (reference formulation). On the other hand, the three lots elaborated with different percentages of chitosan have values of f_1 and f_2, indicating these dissolution profiles are different with respect to that of alginate, but allowing an incomplete release process.

Finally, on the basis of the obtained results, the formulation elaborated with Eudragit FS (26%) was selected due to its more adequate release behavior. So, a more detailed study of its dissolution profile applying the Korsmeyer equation has been made to determine the mechanism of drug release from these beads. Table 6 shows the results of the fitting of the experimental release data to the Korsmeyer equation.

The results (n=0.575, r=0.9480) indicate that the whole release mechanism ($Q_t/Q_\infty < 0.9$) can be described using a Higuchi model ($n \approx 0.5$). On the other hand, the 5-ASA beads showed a zero-order release period up to $Q_t/Q_\infty = 0.7$ (r=0.9962, n=0.9116) corresponding to the 2 first hours of the release study. It can be concluded that during this period (≈2 h in intestinal medium), most of the dose has been released fitting to a zero-order model.

TABLE 6 Statistical Parameters of Eudragit® FS 30D (26%) Bead Release

Release drug	
10–90%	K=0.038
	n=0.5750
	r=0.9480
10–70%	K=0.009
	n=0.9116
	r=0.9962

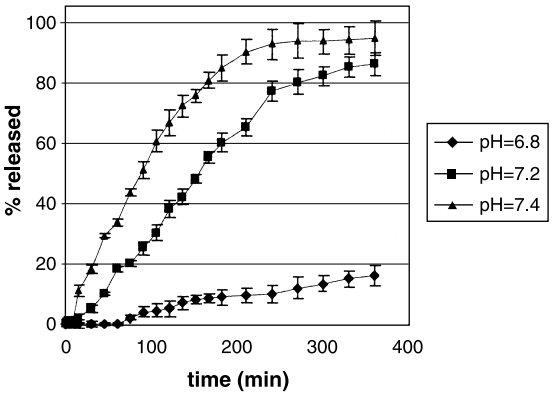

FIGURE 7 5-ASA Released From the Formulation Elaborated with Eudragit® FS (26%) at Different pH Values.

So, the formulation elaborated with Eudragit FS (26%) was then assayed at other different pH values (ph=6; 6.8; 7.2) to assure that there was release of 5-ASA until the system reached the colon. pH=6 corresponds approximately to duodenum, pH=6.8 reflects the jejunal region of the small intestine, and pH=7.2 corresponds to the transition from the jejunal to the ileal segment (Rudolph et al., 2001). The acidic medium is not assayed because the multiparticulate system is introduced in an enteric hard gelatin capsule to obviate the acidic conditions of the stomach as was indicated previously. Figure 7 shows the release profiles at different pH values from 5-ASA beads elaborated with Eudragit FS (26%). No release was observed at pH 6.0. Release was very slow at pH 6.8; averaging about 20% an hour at pH 7.2 and was complete within 4 hour at pH 7.4. This release behavior should lead to a minimal absorption of 5-ASA from the small intestine. Moreover, in contrast to earlier findings, it now appears that the pH in the ileum and proximal colon of ulcerative colitis patients tends to be higher than in healthy individuals (Rudolph et al., 2001). So, these Eudragit FS beads containing 5-ASA exhibit interesting dissolution profiles for the therapy of ulcerative colitis. Further studies will be developed in order to realize a comparative study between this proposed system and the different marketed products based on polymers of a different nature.

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