DEVELOPMENT OF COLONIC 5-ASA BEADS

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Introduction

Recent studies prove that the number of patients with inflammatory bowel disease is increasing all over the world due to contamination, industrialization and changes in the style of live. Some studies have shown that 5-aminosalicylic acid (5-ASA), an anti-inflammatory agent, must be the election treatment for these pathologies. In recent years, the production of microparticles systems seems to be equally promising to develop dosage forms in order to reduce the dosage frequency (1). On the other hand, the efficacy of this drug in the treatment of inflammatory bowel diseases may be optimized with a controlled release drug delivery system which maximizes topical exposure of the drug to the diseased tissue and minimizes systemic absorption of the drug (2).

Therefore, the objective of the present paper is to design an oral multi-unit dosage form containing 5-ASA that protects drug up to colon without causing toxicity. The proposed system consists of beads of several compositions (polymers with different nature and characteristics) introduced in an enteric hard gelatin capsule to obviate the transition across the stomach.

Materials and Methods

5 – Aminosalicylic acid (5- ASA) was a gift from Schering Plough (Madrid, España). Eudragit® FS 30D was received from Degussa Iberia S.A. (Barcelona, España). 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); di-sodium hydrogen phosphate anhydrous, potassium di-hydrogen phosphate, sodium chloride and hydrochloric acid from Panreac Química S.A. (Barcelona, España).

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 mix was dropped, from a 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 ºC) and in darkness in this solution for 24 h to ensure complete reaction. After this time, the beads were filtered and dried using microwaves (3200 W) during 110 minutes with 10% of power. In order to prepare Eudragit FS 30D/alginate beads, a suspension of the polymer (26% or 13% w/v) was added in the mix previously described under agitation, before dropping over a 1.4% w/v $CaCl_2$ solution. Finally, the chitosan/alginate beads were elaborated by adding chitosan (0.1, 0.2 and 0.5 % w/v) to distilled water containing 1 % (v/v) acetic acid. 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 beads formulations.

Alginate %	*5-ASA %*	*Eudragit® FS 30D %*	*Chitosan %*
2	*0.5*	---	---
2	*0.5*	*26*	---
2	*0.5*	*13*	---
2	*0.5*	---	*0.1*
2	*0.5*	---	*0.2*
2	*0.5*	---	*0.5*

Table 1. Composition of the beads

Scanning electron microscopy (*Philips, XL30*) was used to examine the morphology and surface structure of the beads. Size and shape parameters for beads were determined using an image analysis system. The following parameters were selected: mean diameter (D), shape factor (S) and aspect ratio (a). 20 beads particles were employed to accomplish all the measurements.

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 assayed spectrophotometrically at 315 nm (*Hitachi, mod. U-2000*).

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 ºC 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 basket apparatus (*Turu Grau, model D-6*) was used. Samples (3 mL) were withdrawn at specific time intervals and spectrophotometrically assayed at the wavelength of maximum absorbance (315 nm). All the studies were carried out in triplicate.

Among several methods investigated for dissolution profiles comparison, FDA guideline chooses f_2 like the simplest. Moore and Flanner (3) proposed a model independent mathematical approach to compare the dissolution profiles using two factors, f_1 and f_2.

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

$$f_2 = 50 \cdot \log \{[1/ (1+ (\Sigma (R_t - T_t)^2) / n)^{½}] \cdot 100\}$$

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* (4).

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

Results and Discussion

Total 5-ASA percent entrapment efficiency of elaborated beads is shown in Table 2.

Beads formulations	*Entrapment (%) ± S.D.*
Alginate	*75.98 ± 0.15*
Eud. FS 30D (26%)	*78.37 ± 0.19*
Eud. FS 30D (13%)	*80.96 ± 0.13*
Chitosan (0.1%)	*70.56 ± 0.14*
Chitosan (0.2%)	*71.01 ± 0.15*
Chitosan (0.5%)	*82.86 ± 0.13*

Table 2.- Entrapment efficiency of 5-ASA

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 it 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 mean diameter of particles is, the higher drug content present 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 containing 0.1% and 0.2% chitosan were found for drug entrapment.

All the obtained beads produced were more or less spherical in form. Scanning electron micrographs show that all the samples maintain this spherical form after drying and the size is similar after this process (Figure 1).

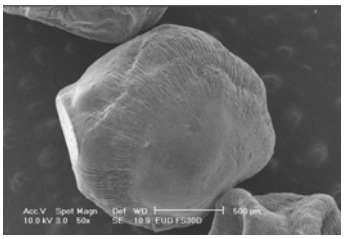

Figure 1.- Microphotograph of the beads containing Eudragit FS 30D (26% w/v)

In relation to SEM parameters, table 3 shows data obtained for each beads formulation. As a whole and considering the size parameter mean diameter (D) (mm), very little 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 little 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.

Beads	*D ± S.D.*	*S ± S.D.*	*a ± S.D.*
Alginate	*0.820 ± 0.114*	*0.593 ± 0.148*	*0.972 ± 0.117*
Eud. FS 30D (26%)	*0.795 ± 0.086*	*0.741 ± 0.121*	*1.075 ± 0.174*
Eud. FS 30D (13%)	*0.902 ± 0.159*	*0.727 ± 0.098*	*1.004 ± 0.148*
Chitosan (0.1 %)	*0.699 ± 0.120*	*0.573 ± 0.178*	*1.132 ± 0.117*
Chitosan (0.2 %)	*0.718 ± 0.103*	*0.496 ± 0.134*	*0.914 ± 0.142*
Chitosan (0.5 %)	*0.895 ± 0.132*	*0.454 ± 0.153*	*0.907 ± 0.198*

Table 3.- Size and shape descriptors of beads

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 figures 2 and 3.

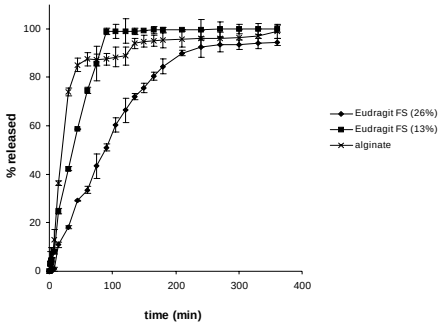

Figure 2. 5-ASA released from indicated beads

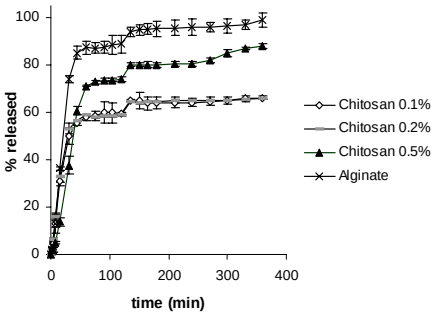

Figure 3. 5-ASA released from indicated beads

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. The dissolution behaviour 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 (5, 6). The complex formed between both polymers is produced by electrostatic attraction between the amine group of chitosan and the carboxylic group of alginate. Moreover, at the end of the dissolution studies, it has been found some beads in the basket, indicating that the dissolution process has not finished.

When the proportion of chitosan increases (0.5%), an excess of polymer appears. This excess can not react with alginate to form the complex. This situation can be the responsible of 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 (7), in comparison with the chitosan-alginate complex. So, this excess of chitosan in the bead structure can not 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 beads network, the dissolved drug diffuses out of the beads at a greater dissolution rate.

For comparison purpose, the difference factor f_1 and the similarity factor f_2 (table 4), have been calculated. Alginate beads without another polymer have been employed as reference.
Table 4 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).

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

Table 4. f_1 and f_2 obtained

This indicated that formulation with the lower content of Eudragit® FS (13 %) shows any advantage with respect 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 that indicate that these dissolution profiles are different with respect the alginate one but allowing an incomplete release process.
So, the formulation elaborated with Eudragit® FS (26%) was then assayed at others different pH values (pH= 6; 6.8; 7.2) to assure that there is not release of 5-ASA until the system reaches the colon. pH=6 corresponding approximately to duodenum, pH=6.8 reflects the jejunal region of the small intestine and pH=7.2 corresponding to the transition from the jejunal to the ileal segment (8). Figure 4 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 is very slow at pH 6.8; averages about 20 % an hour at pH 7.2 and is complete within 4 hour at pH 7.4. This release behaviour 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 (8). So, these Eudragit® FS beads containing 5-ASA exhibit interesting dissolution profiles for the therapy of ulcerative colitis.

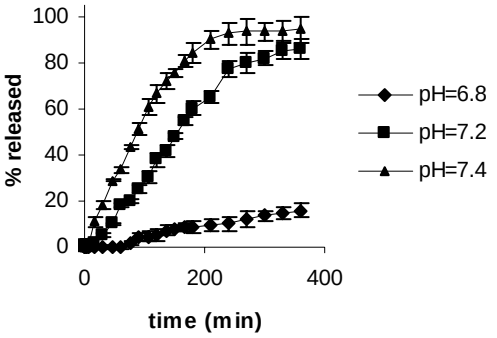

Figure 4. 5-ASA released from the formulation elaborated with Eudragit® FS (26%)

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