

DEVELOPMENT OF ENOXAPARIN SODIUM POLYMERIC MICROPARTICLES FOR COLON-SPECIFIC DELIVERY

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Abstract

Background and aims. Recent studies have shown that low molecular weight heparins are effective in the treatment of inflammatory bowel disease. Therefore, there is considerable interest in the development of an oral colonic delivery pharmaceutical system allowing targeted release of heparin in the inflamed tissue. The objective of this study was to prepare microparticles for the oral administration and colonic release of enoxaparin and to evaluate the influence of certain formulation factors on their characteristics.

Methods. Microparticles were prepared by water/oil/water double emulsion technique followed by solvent evaporation. The influence of several formulation factors on the characteristics of microparticles were evaluated. The formulation factors were alginate concentration in the inner aqueous phase, polymer (Eudragit[®] FS 30D and Eudragit[®] RS PO) concentration in the organic phase and ratios between the two polymers. The microparticles were characterized in terms of morphology, size, entrapment efficiency and enoxaparin release.

Results. The results showed that increasing sodium alginate percentage reduced the encapsulation efficiency of enoxaparin and accelerated enoxaparin release. Regarding the influence of the two polymers, reducing polymer concentration in the organic phase led to a smaller size of microparticles, a lower entrapment efficiency and an important retardation of enoxaparin release. The formulation prepared with Eudragit[®] FS 30D limited the release to a maximum of 3% in gastric simulated environment, a specific characteristic of oral systems for colonic delivery, and fulfilled our objective to delay the release.

Conclusions. Microparticles prepared with Eudragit[®] FS 30D represent a suitable and potential oral system for the colonic delivery of enoxaparin.

Keywords: microparticles, colonic delivery, enoxaparin, Eudragit[®] FS 30D, Eudragit[®] RS PO

Background and aims

Heparin has been used for more than 80 years as an anticoagulant and administered by parenteral route. In the last decades, many groups of researchers attempted to

improve heparin non-invasive drug delivery systems, in order to enhance patient compliance and minimize side effects [1]. Two main strategies have been used to allow oral administration of heparin: increasing the intestinal absorption with permeation enhancers [2,3,4,5] and increasing the stability by the encapsulation of heparin into various pharmaceutical systems – microparticles (MP)

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[6,7], nanoparticles [8,9] or pellets [10].

A few research groups have studied the development of polymeric nano- or micro-systems for the oral delivery of low molecular weight heparins (LMWH). Water/oil/water double emulsion technique followed by solvent evaporation was used for the encapsulation of heparin. This method, developed by Alex and Bodmeier in 1990 [11], allows the encapsulation of hydrophilic active principles in a hydrophobic matrix.

A polymer that gave good results in terms of LMWH's encapsulation efficiency and drug release was Eudragit® RS PO (ERS), a methacrylic cationic copolymer, which allows time controlled release by the presence of ammonium salts that make the polymer permeable [12]. Tinzaparin-loaded nanoparticles which were prepared with ERS had an anticoagulant effect prolonged up to 8 h [9]. Also, ERS microparticles loaded with tinzaparin and nadroparin, had a higher and stable anti-Xa/anti-IIa ratio compared to the commercial ratio [13].

Recently, these studies have become even more interesting since there is proof of other potential applications of heparins, including cancer and various inflammatory diseases, such as inflammatory bowel disease [14]. The treatment of these diseases is based on the immunomodulatory and anti-inflammatory properties, such as modulation of cytokine production, cytotoxic activity of T lymphocytes and inhibition of adhesion, leukocyte activation and transportation [15]. Therefore, there is major interest in the development of an oral colonic delivery pharmaceutical system allowing targeted release of heparin in the inflamed tissue.

One of the most common colonic dosage forms are pH-controlled release systems [16,17,18,19], which use polymers or polymer blends in order to protect the active drug from the gastric and intestinal fluid and at the same time enable its release in specific regions of the gastrointestinal tract [20]. The types of pH controlled release systems range from single-unit tablets or capsules [16] to multiparticulate formulations, such as pellets, granules, microparticles and nanoparticles [21]. Microparticles are one of the pH-dependent systems that showed potential as colonic delivery dosage forms and the methods used for their preparation were commonly double emulsion technique followed either by solvent evaporation or by solvent extraction [13,22], but also spray-drying [23] or emulsion dehydration technique followed by oil-in-oil solvent evaporation method [24].

There is a large variety of pH-dependent polymers commercially available, among them polyvinyl acetate phthalate and cellulose acetate phthalate or copolymers of acrylic and methacrylic acid, known as Eudragit. Eudragit P-4125F, a pH-dependent polymer used to prepare microparticles for the colonic delivery of enoxaparin, prevented LMWH release at pH<6 and allowed a fast release at pH 7.4 [22]. Eudragit® FS 30D (EFS) is an anionic methacrylic copolymer which allows targeted colon

delivery by dissolving above pH 7.0 by salt formation [25]. This polymer was previously used for colonic drug delivery by other researchers, both in conventional pharmaceutical dosage forms as tablets and pellets [26,27,28], or novel pharmaceutical dosage forms as microparticles [29], but not for the encapsulation of LMWH.

The main purpose of this study was to develop and evaluate the influence of certain formulation variables – type and ratios of polymers (Eudragit® RS PO, Eudragit® FS 30D) used as controlled release polymers, concentration of sodium alginate (NaAlg) introduced in the aqueous phase – on the characteristics of enoxaparin-loaded microparticles: morphology, size, entrapment efficiency and *in vitro* release.

Materials and methods

Materials

Marketed sodium enoxaparin (Clexane® 10000 UI anti-Xa/1 mL) was purchased from Sanofi-Aventis (France). Alginic acid sodium salt from brown algae (medium viscosity, ≥ 2000 cP, 2% (25°C)) and poly(vinyl alcohol) (87-90% hydrolyzed, average mol wt 30000-70000) were purchased from Sigma-Aldrich Chemie GmbH (Germany). Eudragit® FS 30D and Eudragit® RS PO were received by courtesy of Evonik (Germany). All other chemicals were of analytical grade.

Preparation of microparticles

The microparticles were prepared by water/oil/water double emulsion technique and solvent evaporation, as previously described [11].

Table I shows the composition of enoxaparin microparticles. The formulations F1-0 to F8-5000 were intended to study the influence of the concentration of sodium alginate and the type of polymer on the characteristics of microparticles and the formulations F9-0 to F12-5000 studied the influence of EFS/ERS ratio. Drug-free microparticles and enoxaparin-loaded microparticles were prepared by the same method in order to study if drug loading had influence on the characteristics of microparticles.

Briefly, Eudragit® FS 30D, Eudragit® RS PO or their mixture were dissolved in dichloromethane (DCM). Then, water or an aqueous solution of sodium alginate (0.5%, 1% or 1.5%) with or without enoxaparin, was emulsified into the organic polymer solution using an ultrasound probe (Vibracell®, France) for 30 seconds. The resulting w/o-emulsion was then poured into an aqueous solution of poly(vinyl alcohol) (1%) and stirred with a magnetic stirrer for 1.5 minutes at 510 rpm to obtain the w/o/w-emulsion. This emulsion was added to 400 mL water and stirred with a three-blades propeller for 1.5 h at 500 rpm at room temperature in order to allow the evaporation of the organic solvent from the internal phase. During solvent evaporation, the polymers precipitated and the microparticle

Table I. Composition of enoxaparin microparticles.

Code	Eudragit® FS (%)	Eudragit® RS (%)	Sodium alginate (%)	Clexane® (sodium enoxaparin) 10000 UI/mL (mL)	DCM (mL)
F1-0	100	-	-	-	2
F2-0	100	-	0.5	-	2
F3-0	100	-	1	-	2
F4-0	100	-	1.5	-	2
F1-5000	100	-	-	0.5 mL (5000 UI)	2
F2-5000	100	-	0.5	0.5 mL (5000 UI)	2
F3-5000	100	-	1	0.5 mL (5000 UI)	2
F4-5000	100	-	1.5	0.5 mL (5000 UI)	2
F5-0	-	100	-	-	2
F6-0	-	100	0.5	-	2
F7-0	-	100	1	-	2
F8-0	-	100	1.5	-	2
F5-5000	-	100	-	0.5 mL (5000 UI)	2
F6-5000	-	100	0.5	0.5 mL (5000 UI)	2
F7-5000	-	100	1	0.5 mL (5000 UI)	2
F8-5000	-	100	1.5	0.5 mL (5000 UI)	2
F9-0	100	-	-	-	5
F10-0	75	25	-	-	5
F11-0	50	50	-	-	5
F12-0	-	100	-	-	5
F9-5000	100	-	-	0.5 mL (5000 UI)	5
F10-5000	75	25	-	0.5 mL (5000 UI)	5
F11-5000	50	50	-	0.5 mL (5000 UI)	5
F12-5000	-	100	-	0.5 mL (5000 UI)	5

cores solidified. Microparticles were collected by filtration (Porafil®, CA, 0.2 µm) and frozen at -30°C [11].

Morphology of microparticles

The morphology of microparticles was analyzed by an optical microscope equipped with a camera (Nikon Eclipse Ti-S, France).

Size of microparticles

The mean diameter of microparticles was evaluated by laser light diffraction, using a Mastersizer® 2000 device (Malvern Instruments, UK). In this respect, 20 mg of microparticles were re-suspended in 2 mL aqueous solution of Tween 80 (0.1%) and dispersed in an ultrasonic bath for 5 minutes. Each sample was measured in triplicate.

Encapsulation efficiency

The encapsulation efficiency (%) of enoxaparin was determined from the external aqueous phase by an indirect turbidimetric method based on the quantitative precipitation reaction occurring between sulfate and carboxyl groups of heparin and the amine groups of cetylpyridinium chloride at pH 6.8 [30]. All experiments were performed in triplicate.

Aliquots (500 µL) of each sample were reacted for 1 hour at 37°C with sodium acetate buffer (500 µL) and an aqueous solution of cetylpyridinium chloride 0.1% in NaCl solution (2 mL). The precipitates were assayed by spectrophotometry (Shimadzu, Japan) at 500 nm. The

encapsulation efficiency was expressed as the percentage of enoxaparin entrapped in relation to the theoretical value.

In vitro drug release study

The enoxaparin release from the microparticles was assessed by dissolution testing using a water bath maintained at a temperature of 37°C and placed on a magnetic stirrer adjusted at a rotation speed of 200 rpm. Microparticles were suspended in 20 mL HCl 0.1M or simulated gastric fluid (pH 1.2) for 2 h, then the medium was replaced by 20 mL phosphate buffered saline (pH 6.8) for another 3 h, and finally, by 20 mL phosphate buffered saline (pH 7.4) until the end of the 24 h in order to simulate the pH values in the stomach, the proximal and middle small intestine (duodenum and jejunum), and the distal small intestine (ileum) respectively. Samples of 1.5 mL were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 8 and 24 h time intervals and replaced with an equal volume of fresh medium. The content of enoxaparin sodium in the withdrawn samples was analyzed by the spectrophotometric method described above, at 500 nm.

Statistical evaluation

The results were expressed as mean values ± S.D. Statistical analysis was carried out with the Analysis Toolpak from Excel 2010. Analysis of variance was used to analyze the differences between groups and regression analysis to assess whether there was a linear connection

between independent and dependent variables. In all cases, a probability value of less than 0.05 was considered to be significant.

Results

Influence of the formulation factors on the microparticles morphology and size

Microscope images of enoxaparin-loaded microparticles are presented in Figure 1 and the influence of the concentration of sodium alginate and the type of polymer on the size of microparticles is displayed in Table II. Table III presents the size of microparticles according to the EFS/ERS ratio.

Influence of the formulation factors on the encapsulation efficiency

Table IV shows the influence of the concentration of sodium alginate and type of polymers (Eudragit® FS or Eudragit® RS) on the encapsulation efficiency, whereas Table V presents the encapsulation efficiency values obtained with different mixtures of the two polymers.

Influence of the formulation factors on *in vitro* drug release

The release of enoxaparin from polymeric microparticles, depending on the concentration of sodium alginate and type of polymers, is shown in Figure 2.

Figure 3 presents the release profile of enoxaparin

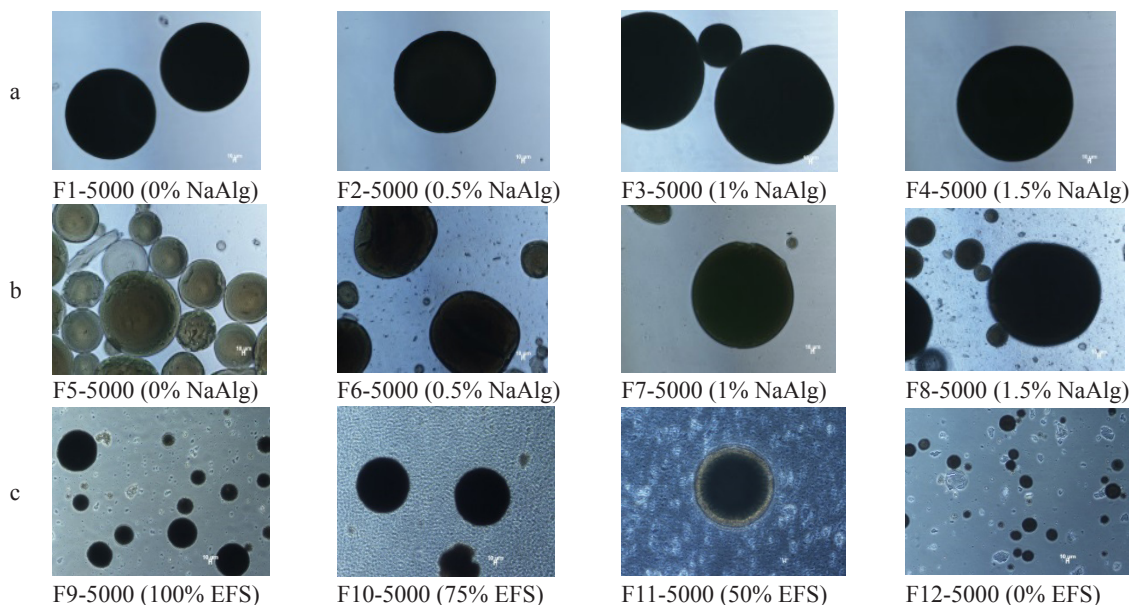


Figure 1. Microparticles analyzed with optical microscope (x10). a – EFS microparticles prepared with different NaAlg concentrations; b – ERS microparticles prepared with different NaAlg concentrations; c – Microparticles prepared with blends of EFS/ERS in different weight ratios.

Table II. Influence of sodium alginate concentration and type of polymer on the size of microparticles (MP).

Conc. of NaAlg (%)	Eudragit® FS ^{a,b}				Eudragit® RS ^{a,b}			
	Code	Drug-free MP Size (µm) ^d	Code	Drug-loaded MP Size (µm)	Code	Drug-free MP Size (µm) ^d	Code	Drug-loaded MP Size (µm) ^c
0	F1-0	221±23	F1-5000	317±17	F5-0	68±11	F5-5000	249±12
0.5	F2-0	221±38	F2-5000	388±119	F6-0	108±1	F6-5000	298±30
1	F3-0	242±64	F3-5000	362±56	F7-0	167±2	F7-5000	344±103
1.5	F4-0	250±29	F4-5000	396±3	F8-0	166±66	F8-5000	359±34

^a Data are shown as mean ± S.D. (n=3);

^b Statistically different for drug-free formulations compared to drug-loaded formulations (p<0.05);

^c Statistically different depending on the concentration of sodium alginate (p<0.05);

^d Statistically different depending on the type of polymer (p<0.05).

from microparticles prepared using different ratios of Eudragit® FS/Eudragit® RS.

Table III. Influence of EFS/ERS ratio on the size of microparticles.

Ratio of polymers (%)		Drug-free MP		Drug-loaded MP	
Eudragit® FS	Eudragit® RS	Code	Size (μm) ^{a,b,c}	Code	Size (μm) ^{a,b,c}
100	-	F9-0	138 \pm 2	F9-5000	108 \pm 18
75	25	F10-0	156 \pm 12	F10-5000	133 \pm 42
50	50	F11-0	222 \pm 33	F11-5000	244 \pm 26
-	100	F12-0	108 \pm 4	F12-5000	79 \pm 12

^a Data are shown as mean \pm S.D. (n=3).

^b No statistical difference for drug-free formulations compared to drug-loaded formulations ($p>0.05$);

^c No statistical difference depending on the EFS/ERS ratio ($p>0.05$).

Table IV. Influence of the concentration of sodium alginate and type of polymer on encapsulation efficiency (EE).

Concentration of sodium alginate (%)	Eudragit® FS ^{a,b,c}		Eudragit® RS ^{a,b,c}	
	Code	EE (%)	Code	EE (%)
0	F1-5000	47 \pm 7	F5-5000	88 \pm 1
0.5	F2-5000	48 \pm 11	F6-5000	81 \pm 5
1	F3-5000	45 \pm 10	F7-5000	85 \pm 4
1.5	F4-5000	39 \pm 5	F8-5000	74 \pm 10

^a Data are shown as mean \pm S.D. (n=3);

^b No statistical difference related to the concentration of sodium alginate ($p>0.05$);

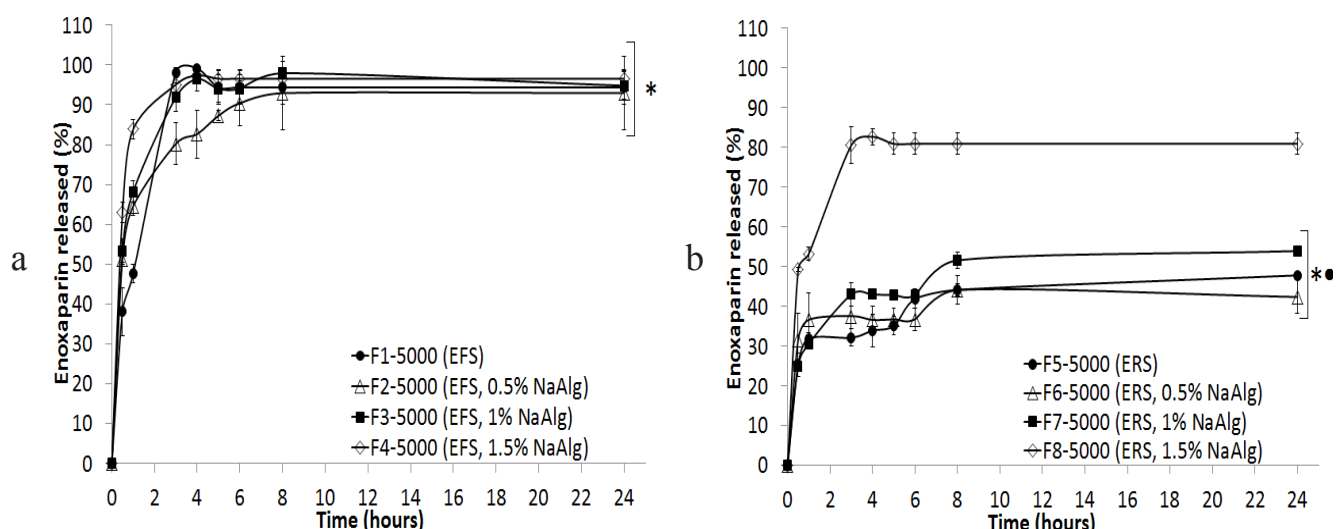
^c Statistically different depending on the type of polymer ($p<0.05$).

Table V. Influence of EFS/ERS ratio on encapsulation efficiency (EE).

Ratio of polymers (%)	Eudragit® RS	Code	EE (%) ^{a,b}
Eudragit® FS			
100	-	F9-5000	38 \pm 1
75	25	F10-5000	46 \pm 2
50	50	F11-5000	50 \pm 4
-	100	F12-5000	60 \pm 1

^a Data are shown as mean \pm S.D. (n=3);

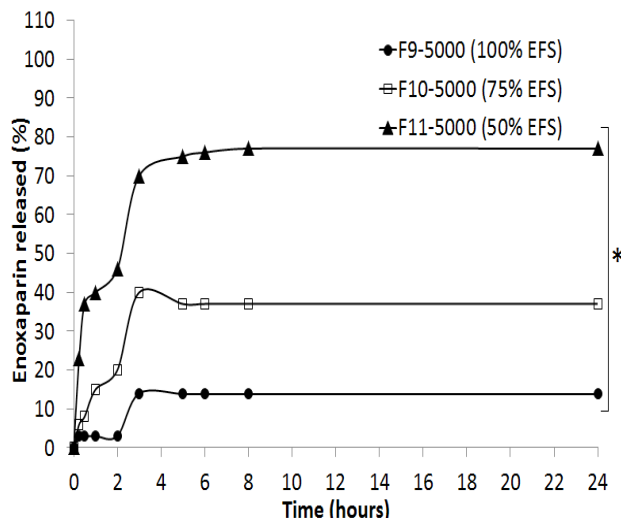
^b Statistically different depending on the ratio of EFS/ERS ($p<0.05$).



* Statistically different at all times depending on the type of polymer ($p<0.05$);

● Statistically different at all times from F8-5000 (ERS, 1.5% NaAlg) ($p<0.05$).

Figure 2. *In vitro* release profile of enoxaparin from microparticles. a – Influence of sodium alginate on enoxaparin release in particles prepared with Eudragit® FS. Data are presented as mean \pm S.D. (n=2); b – Influence of sodium alginate on enoxaparin release in particles prepared with Eudragit® RS. Data are presented as mean \pm S.D. (n=2).



* Statistically different at all times depending on the ratio of EFS/ERS ($p < 0.05$).

Figure 3. *In vitro* release profile of enoxaparin from microparticles – Influence of Eudragit® FS/Eudragit® RS ratio on enoxaparin release (NaAlg=0%)

Discussion

Influence of the formulation factors on the microparticles morphology

The images analyzed under the microscope revealed that the enoxaparin-loaded microparticles had a fairly spherical shape and a dense aspect. The differences in size between different types of microparticles could also be seen, and the visual observations were correlated with the size determination. The increase of the concentration of sodium alginate increased the size of particles, while Eudragit® FS determined the formation of particles greater in size than Eudragit® RS (Figure 1a-b). The microparticles prepared with one type of polymer (F9-5000 and F12-5000) had a smaller size compared to the ones prepared with the mixture of EFS/ERS (F10-5000 and F11-5000) (Figure 1c). Figure 1 also showed that the increase of DCM volume, which consequently decreased the viscosity of the polymeric organic solution, led to a reduction of the size of particles: F1-5000 formulation presented smaller particles compared to F9-5000 formulation and F5-5000 formulation showed smaller particles compared to F12-5000 formulation.

Influence of the formulation factors on the microparticles size

As shown in Table II, the results indicated that drug-free microparticles had a smaller diameter (68-250 μm) compared to enoxaparin-loaded microparticles (249-396 μm). Other researchers obtained similar results for Eudragit® RS microparticles, unloaded (27 μm) and loaded with other low molecular weight heparins, tinzaparin (49 μm) and nadroparin (53 μm) [13]; therefore, it may be concluded that encapsulation of enoxaparin increased the

size of particles. These studies suggested that the surfactant properties of Eudragit® RS determined the small diameter of the unloaded particles [13]. In the case of heparin-loaded particles, the electrostatic interactions between positively charged Eudragit® RS and negatively charged enoxaparin sodium led to a decrease of the surfactant properties of the polymer and the formation of larger particles.

Another factor which influenced the size of the microparticles was sodium alginate: overall, as the concentration of sodium alginate was higher, the microparticles were larger (Table II). However, its influence was statistically significant only for Eudragit® RS enoxaparin-loaded microparticles; the explanation is the existence of electrostatic interactions mentioned before. The combination of positively charged Eudragit® RS and negatively charged sodium alginate leads to a combination similar to the one of Eudragit® RS and enoxaparin, resulting in reduced surfactant properties of the polymer and formation of increasingly larger particles with increasing concentrations of alginate. Although the results were not significant in the case of Eudragit® FS, the size values increased from no alginate to 1.5% alginate; it is hypothesized that negatively charged Eudragit® FS and negatively charged sodium alginate rejected each other and therefore the particles size increased.

In addition, microparticles prepared with Eudragit® FS were larger than those prepared with Eudragit® RS (Table II). This might be due to the different charge of the two polymers: positively charged Eudragit® RS probably tends to attract negatively charged enoxaparin and alginate, resulting in more compact organization of the particles and thus to a reduced size, while negatively charged Eudragit® FS probably rejects negatively charged enoxaparin and alginate, leading to a looser structure, therefore a larger particle size.

Regarding the influence of the mixture of the two polymers (Eudragit® FS and Eudragit® RS), the results were not statistically significant (Table III), but particles consisting exclusively of one type of polymer were inferior in size (79-108 μm) to those formulated by mixing the two polymers (133-244 μm). In this case, the difference in the level of encapsulation could be at the origin of heterogeneity in size of formulations consisting of the two polymers (Table V). The formulation prepared with 100% Eudragit® FS (F9-5000) presented the lowest encapsulation efficiency (38%) and a diameter of 108 μm , while the formulation prepared with 100% ERS showed the highest encapsulation efficiency (60%) and a diameter of 79 μm . Normally, the expected results would have been a decrease of the size of particles with the addition of ERS and an increase of the encapsulation efficiency, but the formulations prepared with 75%/25% and then 50%/50% EFS/ERS ratios led to an increase of both encapsulation efficiency (46% and 50%, respectively) and size (133 μm and 244 μm , respectively).

Finally, increasing the volume of DCM from 2 mL

to 5 mL (Table I) led to smaller particles due to the decrease of the viscosity of the polymeric organic solution. For example, microparticles prepared with Eudragit® FS 100% and 2 mL DCM (F1-5000) were greater than microparticles prepared with Eudragit® FS 100% and 5 mL DCM (F9-5000). The same can be observed for drug-free Eudragit® FS microparticles (F1-0 and F9-0) and for drug-free (F5-0 and F12-0) and drug-loaded (F5-5000 and F12-5000) Eudragit® RS 100% microparticles. A small volume of organic solvent led to a more viscous solution that determined the formation of larger drops during emulsification, which became large microparticles, probably having a porous structure. Increasing the volume of organic solvent conducted to a low viscosity solution, which formed small drops that became small microparticles, probably with a more compact organization.

Influence of the formulation factors on the encapsulation efficiency

Heparins, being compounds which are soluble in water, have a tendency to pass into the aqueous outer phase prior to the precipitation of the polymer, which lowers entrapment efficiency [13]. For this reason, an excipient, which is capable to retain enoxaparin inside the microparticles, is highly desirable.

An increase of the concentration of sodium alginate decreased the entrapment percentage by approximately 10% in the formulations prepared using no sodium alginate to the ones obtained with 1.5% sodium alginate ($p < 0.05$) (Table IV). The intermediate values of 0.5% and 1% had no significant influence.

The type of polymer had an important effect. Indeed, there was a more efficient encapsulation of the drug for Eudragit® RS (74-88%) than for Eudragit® FS (39-47%) (Table IV). These findings can be explained based on the structure of the two polymers and on the data obtained by other researchers. Hoffart et al stated that in the case of Eudragit® RS, polycationic quaternary ammonium groups of the polymer, mainly oriented towards the continuous aqueous phase, may interact with anionic sulfate and carboxylate chains of enoxaparin, which results in a reduction of the migration of the embedded substance in the external aqueous phase prior to the precipitation of the polymer [9]. The same researchers believe that the encapsulation of enoxaparin can occur by two mechanisms: encapsulation in the internal aqueous phase and adsorption on the surface of particles through the electrostatic interactions mentioned above. Similar percentages were obtained for Eudragit® RS microparticles loaded with other low molecular weight heparins, tinzaparin (72%) and nadroparin (85%) [13]. On the other hand, polyanionic groups of Eudragit® FS rejected probably the negatively charged chains of enoxaparin, which tended to migrate towards the external aqueous phase prior to precipitation of the polymer, resulting in lower entrapment efficiency.

Depending on the EFS/ERS ratio (Table V), the

lowest entrapment percentage of enoxaparin was observed for microparticles containing 100% Eudragit® FS. An increase of the entrapment percentage of the drug in the presence of Eudragit® RS was systematically observed, as described in the literature [9,13].

Regarding the decrease of organic solutions viscosity, the entrapment efficiency was lowered with the reduction of the viscosity of the solution. The results could be correlated to the size of microparticles, so that a smaller size led to a lower percentage of encapsulated drug.

Influence of the formulation factors on *in vitro* drug release

The release profiles of enoxaparin were influenced by the amount of sodium alginate (0-0.5-1-1.5%) and by the type and ratio of the polymer used (Eudragit® RS or Eudragit® FS).

Regarding the concentration of sodium alginate, the release was different depending on the type of polymer. For Eudragit® FS (Figure 2a), the influence of alginate on release was not significant, while for Eudragit® RS (Figure 2b) it was found that increasing the amount of sodium alginate resulted in increased percentages of enoxaparin released. The formulation prepared with 1.5% alginate (F8-5000) significantly distanced from the other three, showing the highest percentages of enoxaparin released (80% vs. 40-50%). For this last formulation, the interactions between the active ingredient and the polymer were probably disrupted by the presence of large quantities of sodium alginate, leading to increased release rate.

Depending on the influence of polymers, it was found that enoxaparin sodium release after 24 h from the microparticles prepared with Eudragit® RS was incomplete (48-81%), compared with the percentages released from the microparticles prepared with Eudragit® FS (93-97%). Other studies have also reported an incomplete release in the case of Eudragit® RS, making the assumption that certain polysaccharide chains interacted strongly with Eudragit® RS, which resulted in incomplete release [9,13]. As regards Eudragit® FS, the presumed repulsions between the polymer and the drug determined the release of the entire amount after about 4-5 h. However, there was no statistically significant influence of the type of polymer on the release ($p > 0.05$) between formulations prepared with 1.5% sodium alginate, which could mean that a high concentration of sodium alginate accelerated the release of enoxaparin, irrespective of the type of polymer.

Enoxaparin release profiles were also influenced by the EFS/ERS ratios introduced in the formulation (Figure 3). First, there was a rapid release from the formulation prepared with 50%/50% EFS/ERS (F11-5000): 46% of the total amount of enoxaparin was released after 2 h, in HCl pH 1.2. This burst effect diminished with the increase of the amount of Eudragit® FS in the formulation: 20% after 2 h for the formulation with 75%/25% EFS/ERS (F10-5000) and 3% after 2 h for 100% EFS formulation (F9-5000). A

colonic drug delivery system must be capable of allowing maximum 10% drug release in the gastric environment, therefore the formulation that meets this requirements is the F9-5000 formulation.

Comparing F1-5000 and F9-5000 formulations, prepared with 2 mL DCM and 5 mL DCM respectively, the release of enoxaparin decreased considerably for the F9-5000 formulation. The microscope images and the size values also showed an important size decrease for the particles prepared with a higher volume of DCM. The delayed release for smaller particles was probably due to a more compact organization of the polymer that prevented fast drug release, compared to particles greater in size, which probably presented a more porous structure, a closer contact with the medium and facilitated the drug release.

Conclusions

This study presented the development of enoxaparin-loaded polymeric microparticles using a water/oil/water double emulsion technique followed by solvent evaporation, and the evaluation of certain formulation factors – concentration of sodium alginate, type of polymers and ratios of polymers – on enoxaparin particles morphology, size, entrapment and release. Sodium alginate had a negative influence on microparticles characteristics, i.e. increasingly concentrations of alginate decreased drug encapsulation and accelerated the release. The polymer type that ensured higher encapsulation efficiency was Eudragit® RS. The most important achievement was that the colon specific polymer, Eudragit® FS (100%), prevented the release in the gastric simulated fluid. In conclusion, this study proved that Eudragit® FS 30D is a suitable polymer for the preparation of colon-specific oral delivery systems of enoxaparin.

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