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Synthesis of Eudragit® L100-coated chitosan-based nanoparticles for oral enoxaparin delivery

Yuri Basilio Gomes Patriota [a], Igor Eduardo Silva Arruda [a], Antônia Carla de Jesus Oliveira [a], Thaisa Cardoso de Oliveira [a], Eliadna de Lemos Vasconcelos Silva [a], Luíse Lopes Chaves [a,c], Fábio de Oliveira Silva Ribeiro [b], Durcilene Alves da Silva [b], Mônica Felts de La Roca Soares [a], José Lamartine Soares-Sobrinho [a,*]

[a] Quality Control Core of Medicines and Correlates, Federal University of Pernambuco, Recife, PE, Brazil
[b] Research Center on Biodiversity and Biotechnology – BIOTEC, Federal University of Delta of Parnaiba, Parnaiba, PI, Brazil
[c] Department of Immunology, Aggeu Magalhães Institute (IAM), Oswaldo Cruz Foundation (FIOCRUZ), Brazil

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ABSTRACT

Enoxaparin is an effective biological molecule for prevention and treatment of coagulation disorders. However, it is poorly absorbed in the gastrointestinal tract. In this study, we developed an Eudragit® L100 coated chitosan core shell nanoparticles for enoxaparin oral delivery (Eud/CS/Enox NPs) through a completely eco-friendly method without employing any high-energy homogenizer technique and any organic solvents. Spherical nanocarriers were successfully prepared with particle size lower than 300 nm, polydispersity index about 0.12 and zeta potential higher than +25 mV, entrapment efficiency greater than 95% and the *in vitro* release behavior confirms the good colloidal stability and the successful Eudragit® L100 coating process demonstrated by negligible cumulative enoxaparin release (<10%) when the particles are submitted to simulated gastric fluid conditions. Finally, we demonstrated that the core-shell structure of the particle influenced the drug release mechanism of the formulations, indicating the presence of the Eudragit® L100 on the surface of the particles. These results suggested that enteric-coating approach and drug delivery nanotechnology can be successfully explored as potential tools for oral delivery of enoxaparin.

1. Introduction

Enoxaparin (Enox) is the low molecular weight heparin (LMWH) of choice for the prevention and treatment of deep vein thrombosis and coronary syndromes [1]. It exerts your anticoagulant activity by binding to antithrombin (AT), thereby catalyzing the indirect inhibition of factor Xa (FXa) and in lesser extent, factor IIa (FIIa) (Fig. 1) [2].

Enox is only available as parenteral aqueous solution (intravenously or subcutaneously) which limit its clinical applicability. The Enox half-life (4.5 h) requires daily administration, impairing the patient compliance [3]. Moreover, Enox is inactivated in acidic medium and shows poor permeation through the intestinal wall due to high molecular weight, hydrophilicity and high negative charge and consequently, low oral bioavailability [4].

Several micro/nanoformulations for improve oral bioavailability of Enox including liposomes [5], microparticles [6], self-emulsifying drug delivery systems [7] and polymeric nanoparticles (PNPs) [8] have been explored. The development of an oral formulation of Enox would be essential for long anticoagulant therapy in chronic conditions since it would overcome the inconveniences of the daily injections (*e.g.* needle-associated pain, infections, hospitalization, hematomas, *etc.*), reducing side effects and, thus, improving the patient adhesion to treatment [9].

Polymeric nanoparticles (PNPs) have been drawn attention as nanocarriers for oral delivery of hydrophilic macromolecules since they have some remarkable advantages: (1) protection against acidic denaturation and enzymatic degradation; (2) increase the contact and absorption area; (3) increase the intestinal membrane permeability and (4) might show controlled release properties [10].

Chitosan (CS), a natural cationic heteropolysaccharide consisting of *N*-acetyl-D-glucosamine and D-glucosamine linked through (1–4)-

* Corresponding author at: Department of Pharmaceutical Sciences, Federal University of Pernambuco, Avenue Professor Arthur de Sá, s/n, ZIP CODE: 50740-521, Cidade Universitária, Recife, Pernambuco, Brazil.
E-mail address: jose.ssobrinho@ufpe.br (J.L. Soares-Sobrinho).

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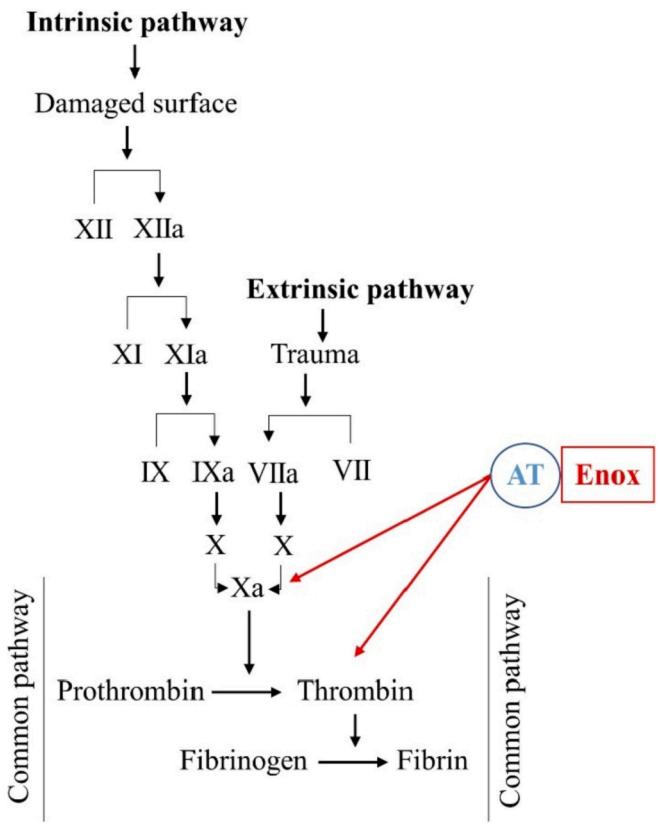

Fig. 1. Coagulation cascade and Enox therapeutic targets.

glycosidic bonds [11], has been extensively explored as a promising biomaterial for delivery of large variety of macromecules [12–14]. CS has excellent biocompatibility, biodegradability, and non-immunogenicity properties [15]. Moreover, CS-based biomaterials exhibit mucoadhesive and absorption-enhancing properties [16]. In fact, CS is capable to interact with the mucus and epithelial cells to prolong the residence time in the small intestine [17] and promote the opening of tight junctions (TJs) reversibly, facilitating the paracellular transport [18,19], respectively. However, oral delivery of CS-based materials is limited due to CS is easily solubilized at gastric pH medium, leading to loss of its mucoadhesive and absorption-enhancing properties [20] and in addition, allowing the leakage the drug to the harsh gastric environment [21].

Eudragit® L100 (Eud) is a synthetic anionic copolymer based on methacrylic acid and methyl methacrylic acid (ratio 1:1) [22]. Eud is an enteric pH-dependent product, soluble above pH 6, that has been widely used as controlled drug release excipient [23]. In fact, the use of Eud in various formulations such as liposomes [24], nanoparticles [21,25] for oral delivery of hydrophilic macromolecules has been reported.

The aim of this study was to develop Enox-loaded Eud-coated CS core/shell nanoparticles through an eco-friendly method for oral delivery.

2. Materials and methods

2.1. Materials

Enoxaparin sodium (Endocrisis® 40 mg/0.4 mL, 4000 IU) was a kind gift from Cristalia (Brazil). Eudragit® L100 (M_w of 135 kDa, apparent viscosity of 50–200 mPa s) was purchased from Evonik (Essen, Germany). Chitosan (M_w of 105 g/mol, degree of deacetylation ~ 81%) was purchased from Polymar (Fortaleza, Brazil). All other chemicals and reagents were of analytical grade and used without any further purification.

2.2. Preparation of CS/Enox nanocomplexes

CS/Enox nanocomplexes (F1, CS/Enox NCs) were prepared through polyelectrolyte complexation (PEC) technique. Briefly, Enox aqueous solution (0.55 mg/mL, pH 6.5) was dripped into 2 mL of CS aqueous acetic acid solution (0.5 mg/mL, pH 4.5) under magnetic stirring for 30 min at room temperature [10]. The system pH was adjusted with 1 N HCl or 1 N NaOH as required. Formulation variables such as initial CS pH and Enox to CS weight ratio (w/w) were studied on the basis of their effect on particle size and drug entrapment.

2.3. Preparation of Eud coated core shell CS nanoparticles

Eud coating on CS/Enox NCs was carried out in accordance with the method described by Xu and coworkers [21]. To prepare Eud-coated CS/Enox NPs, Eud phosphate buffer solution (PBS) (0.05 mg/mL) were added dropwise to CS/Enox NCs under mild agitation at room temperature and left under agitation for 20 min (F2, NC-loading) or Eud PBS solution were premixed with Enox aqueous solution before complexation with CS solution (F3, anion-loading) [3].

2.4. Colloidal dispersions characterization

2.4.1. Particle size (PS), polydispersity index (PDI) and zeta potential (ZP)

The average particle size and zeta potential of undiluted colloidal dispersions were determining by dynamic light scattering (DLS) using ZetaSizer Nano-ZS90 (Malvern Instruments Ltd., UK). Measurements were carried out after an equilibration time of 120 s at a cell temperature of 25 °C with a detection angle of 90°. Particle size distribution was reported as a polidispersity index (PDI) [8].

2.4.2. Morphological characterization

The morphology of the colloidal dispersions were observed by atomic force microscopy (AFM) using a TT-AFM instrument (AFM workshop, USA) in intermittent contact mode using TED PELLA tips (TAP300-G10) at an amplitude frequency of approximately 239 kHz. The samples were diluted with ultra-pure water and left in ultrasound bath for 30 min. Further, 10 μL of diluted samples was applied to a freshly cleaved mica surface and oven dried at 36 °C for 15 min. Images were analyzed using Gwyddion software 2.45 [26].

2.4.3. Stability study

To evaluate the stability of CS/Enox NCs and Eud/CS/Enox NPs in gastrointestinal tract (GIT), the colloidal dispersions were submitted to simulate GIT pH conditions (0.1 M HCl, pH 1.2, PBS pH 6.8 and 7.4). Briefly, 0.5 mL of colloidal dispersions was dispersed into 2.5 mL of simulated GIT fluids under continuous stirring at room temperature. The integrity of nanoformulations was monitored at predetermined time intervals through any change of PS, PDI and ZP using ZetaSizer Nano-ZS90 (Malvern Instruments Ltd., UK) [21].

2.4.4. Estimation of Enox entrapment efficiency (EE)

The EE of Enox was calculated based in the difference between the total amount of Enox added to the colloidal dispersions and the untrapped drug amount remaining in the aqueous supernatant after the centrifugation step. The EE was determined by an indirect turbidimetric/nephelometric method based on the quantitative precipitation reaction occurring between Enox's sulfate and carboxyl groups and the amine groups of cetylpyridinium chloride. Briefly, 250 μL of each sample was reacted for 1 h at room temperature with 250 μL of sodium acetate buffer (1 M, pH 4.8) and 1 mL of cetylpyridinium chloride (0.1% w/v) in NaCl aqueous solution (0.94% w/v). The precipitates were assayed spectrophotometrically (Varian, 50 UV-VIS, Australia) at 290 nm [6]. This method was validated according to the ICH guide [27]

through parameters such as linearity, specificity, precision and accuracy, and robustness (data not shown).

2.5. Enox in vitro release study

The Enox *in vitro* release studies were performed through dialysis bag method [28]. The Enox *in vitro* release from selected colloidal dispersions was determining in enzyme-free and gradient pH medium in air shake incubator at 150 rpm. Briefly, Eud/CS/Enox NPs and CS/Enox NCs were placed inside a dialysis membrane (MWCO 12–14 kDa). The dialysis membrane was suspended in 5 mL of simulated gastric fluid (SGF, pH 1.2) at 37 ± 0.5 °C for 2 h. Then, the SGF was replaced by 5 mL of PBS pH 6.8 for 4 h and finally, for PBS pH 7.4 until the end of 12 h both at 37 ± 0.5 °C. Aliquots (500 μL) were collected at predetermined time intervals and replaced with equal volumes of respective medium. The concentration of Enox released was determined by turbidimetric/nephelometric method previously described.

2.6. Evaluation of the mechanism of drug release

To examine the drug release behavior from nanoformulations, it was evaluated changes in the PS upon incubation in simulated GIT conditions. For this study, the nanoformulations were submitted to the same protocol described in Section 2.5. The volume-based mean diameters (Dv_t) of the nanoformulations were measured at predetermined time intervals using ZetaSizer Nano-ZS90 (Malvern Instruments Ltd., UK). The Dv_t was subtracted from that volume-based mean diameters observed at time zero (Dv_0) and the differences between the diameters were then plotted against time [29].

2.7. Statistical analysis

All results were expressed as the mean value ± standard deviation (SD) from at least three measurements. Significance of difference was evaluated using one-way ANOVA at the probability level of 0.05.

3. Results and discussion

3.1. Preparation of CS/Enox NCs

3.1.1. Effect of system pH

Since the NCs formation is mainly driven by electrostatic interactions, both polymers have to be ionized and own opposite charges. The self-assembly reaction should be performed at pH values close to the pKa interval of the two polymers (pK_a of Enox is approximately 3.1 and the pK_a of CS is 6.5) [30]. Thus, this investigation was performed in pH range of 3–7 for CS since the pH of CS solution will influence its charge density and therefore, the properties of resulting NCs.

To study the influence of changing CS solution pH values on PS and ZP of CS/Enox NCs (Fig. 2), both Enox to CS weight ratio and concentration were kept constant.

As depicted in Fig. 2, the PS and ZP of CS/Enox NCs is dependent on the initial CS solution pH. It was observed that rising the CS solution pH values, an increase in the PS of colloidal dispersions accompanied by a corresponding decrease in their positive surface charge was obtained.

Addition of Enox solution led to a turbid aggregating dispersion when the initial CS solution pH range was 6 to 7. Even adjusting the initial CS solution pH to 5.5, the precipitation was observed. This can probably be attributed to the fact that in the formulation prepared at pH values near to CS isoelectric point, the poor ionization of CS amino groups, reduced its charge density, consequently, decreasing the electrostatic interaction with Enox molecules, resulting to unstable dispersions [3].

On the other hand, when the CS solution pH was adjusted to 5 and 4.5, spontaneously formed an opalescent and stable colloidal dispersions. At these pH values, the CS amino groups were more protonated,

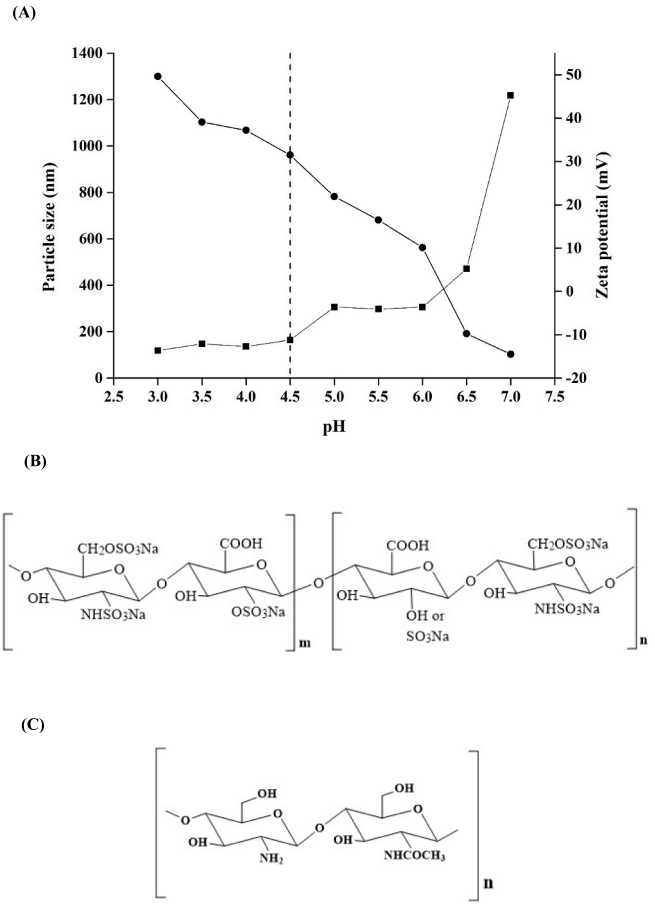

Fig. 2. Influence of CS pH values on PS (■) and zeta potential (●) for CS/Enox NCs (A) and chemical structure of Enox (B) and CS (C) showing their ionization sites.

highly positively charged, favoring the inter-cross-linkages with negatively charged Enox molecules [14].

The PS is a crucial parameter regarding efficient uptake by intestinal cells [31]. In fact, smaller particles can penetrate the gastrointestinal (GI) mucus gel layer and reach the underlying epithelium to a higher extent than larger particles [32]. In addition, surface charge is another parameter that affects the intestinal absorption [31]. Positive charge nanoparticles interact with negatively GI mucus layer, hence, enhancing the intestinal residence time, increasing particle uptake, as well as improving drug absorption [8]. Thus, the formulation prepared with the initial CS solution pH of 4.5 was selected to further studies.

3.1.2. Effect of Enox to CS weight ratio

The drug/polymer ratio is another parameter that has significant influence on the properties of NCs [30]. Therefore, the influence of the stoichiometry on PS, ZP, PDI and EE was investigated at pH 4.5 (Fig. 3).

As shown in Fig. 3, an increase in the PS and EE of CS/Enox NCs were observed with successive increase in the Enox concentration. As the Enox concentration is increased, CS molecules could be linked with more Enox molecules, thereby increasing the PS as well as the EE of the CS/Enox NCs [33], until that a critical point was achieved (Enox to CS mass ratio of 0.6 and 0.5, respectively), from which the PS increased suddenly and the EE reach a plateau phase, in which, it is worth nothing that all CS/Enox NCs showed high EE values (>95%).

The positive ZP values remained unchanged almost all investigation ranging from +21.3 to +16.6 mV (Fig. 3), except in the final mass ratio, where a lower ZP value was observed (+7.4 mV), probably due to the large proportion of free negatively charged Enox groups [34]. On the

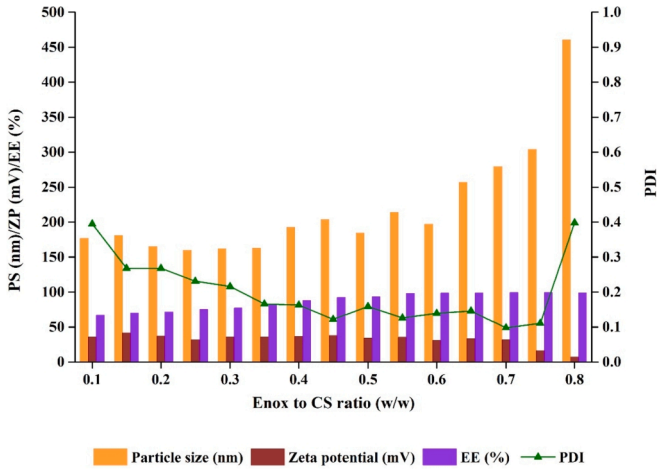

Fig. 3. Influence of Enox to CS mass ratio on the PS, ZP, PDI and EE for CS/Enox NCs.

other hand, the PDI showed a decreasing behavior until an Enox to CS mass ratio of 0.7, from which the PDI increased abruptly, possibly related to a decrease in the ZP values due to excess of Enox molecules, favoring agglomeration of the particles [33].

According to these results, it was defined that Enox to CS mass ratio of 0.55 is the optimal proportion to further studies as it produced colloidal dispersions with appropriate PS (208 ± 21.7 nm), minimum PDI (0.125 ± 0.01) and a maximum EE (98.3 ± 1.1).

3.2. Preparation of Eud/CS/Enox NPs

In order to protect the integrity of Enox in the harsh gastric acid milleu, optimized CS/Enox NCs formulations were coated with Eud layers through electrostatic interaction (Table 1). Several Eud solution concentrations were previously evaluated to potentialize the benefit of Eud in the electrostatic interaction equilibrium (data not shown). It is worth to mentioning that Eud concentrations as high as utilized on this work resulted in unstable aggregated colloidal dispersions leading to precipitation. In addition, it is noteworthy that this method was completely eco-friendly without employing any high-energy homogenizer methods and any organic solvents, contributing to the physical-chemical stability of the drug.

Two Eud loading approaches were applied to investigate the influence of the order of addition of the reactants on nanoformulation characteristics. Eud (0.05 mg/mL) was dissolved in PBS pH 6.8 where the carboxyl groups become negatively charged facilitating the adsorption to the F1 positive amino groups. As the addition of Eud PBS solution, the pH value of the final dispersion raised to 4.7 and as consequence, the solubility of Eud decreased and formed the protective layer, forming the Eud/CS/Enox NPs [21].

NC-loading method showed an increase in particle size which clearly indicates the presence of Eud on the surface of nanoparticle. This effect was mediated through the reduction of F1 positive surface charge density by neutralization of CS amino groups by negatively charged Eud through electrostatic interaction [35].

On the other hand, Eud coating through anion-loading method produced NPs much smaller which could be explained by the competitive ionic interaction between negatively charged carboxyl and carboxyl/sulfate groups of Eud and Enox, respectively on positively charged amino groups of CS. In fact, as Eud was utilized on pH which it would be negatively charged, hence, Eud could compete with Enox to interact with CS electrostatically. This interaction, provided by the multi-ionic sites of the large CS molecules, may have collaborated to the more compact nanoparticles [36].

F2 and F3 showed non-aggregating nanosized colloidal dispersions with positive ZP values (Table 1). The surface charge is an important parameter since it influences not only the stability of the colloidal preparation but also can affects the *in vivo* fate of the nanocarriers, influencing the opsonization process, blood circulation time as well as biodistribution [37]. In addition, the surface charge can be tuned to improve the nanocarries-mucin interaction prolonging the transit and retention times, allowing more time for drug release in the target site [38].

The EE for all colloidal dispersions was comparable and nearly 100% (Table 1), which indicates a strong ionic interaction between CS and Enox molecules. Once the interaction between those entities are driven by electrostatic interaction and an exceeding amount of CS was used in the formulation (weight ratio of CS to Enox was 2:1), therefore, the chance of all Enox molecules being ionically complexed with CS counterparts is too high, leading to a high EE [28]. All formulations showed in Table 1 were chosen for the subsequent studies.

Table 1
Characterization of different formulations.

Code	Formulations	PS (nm)	PDI	ZP (mV)	EE (%)
F1	CS/Enox NCs	208.4 ± 21.7	0.125 ± 0.01	+ 28.0 ± 1.8	98.3 ± 1.1
F2	Eud/CS/Enox NPs[a]	293.6 ± 5.7	0.103 ± 0.03	+ 25.6 ± 0.9	96.2 ± 1.0
F3	Eud/CS/Enox NPs[b]	200.4 ± 4.2	0.124 ± 0.01	+ 31.0 ± 0.4	95.2 ± 1.5

[a] NC-loading: Eud PBS pH 6.8 solution was added to the freshly prepared CS/Enox NCs.

[b] Anion-loading: Eud PBS pH 6.8 solution was premixed with Enox aqueous solution before complexation with CS solution.

3.3. Morphological characterization

The morphology of Enox-loaded nanoformulations was observed with atomic force microscopy (AFM) and is shown in Fig. 4. All the nanoformulations are spherical or sub spherical in shape and well

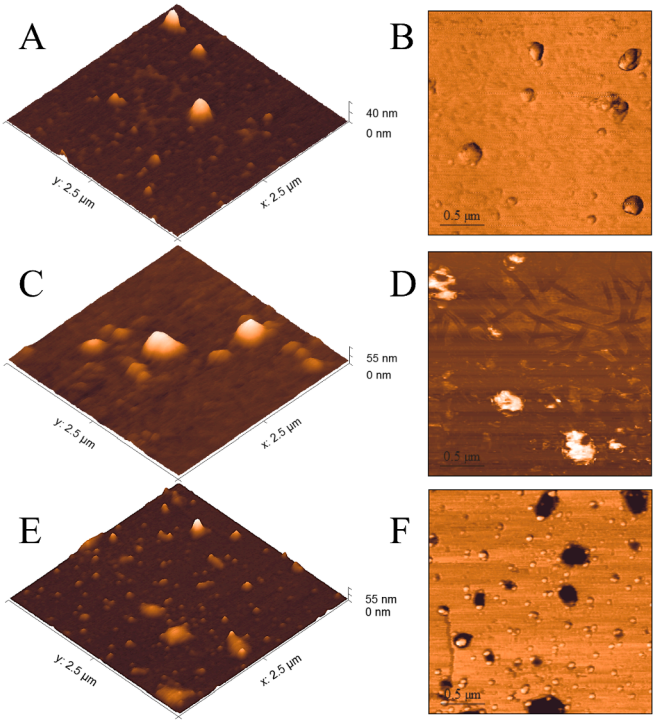

Fig. 4. AFM images of Enox-loaded nanoformulations: F1 (A–B), F2 (C–D) and F3 (E–F).

separated from each other. In addition, the PS obtained from AFM is smaller than that determined by DLS technique (Table 1) which can be attributed to the different measurement mechanism and sample treatment. For DLS, nanoformulations were evaluated in the hydrated environment, so the polymer chains were well swelled. On the other hand, for AFM visualization, nanoformulations were previously dried and possibly the nanocarriers shrunk [39].

3.4. Stability study

The nanoformulations were evaluated for stability at different pH conditions by detecting their particle sizes as depicted in Fig. 5. As showed in Fig. 4A, the PS of F2 and F3 had a negligible increase after 2 h of incubation in SGF indicating that they were able to maintain the integrity, which could be attribute to the Eud layers upon particle surface. On the other hand, when the same formulations were dispersed in pH 6.8 and 7.4 (Fig. 4B and C, respectively), the PS decreased which could be related to the beginning of Eud layers erosion. Once the Enox is inactivated in acidic medium, it is highly desirable that nanocarriers maintain the structure and hence could protect the stability of the drug when passing in the stomach [21,40].

For F1, a more pronounced PS change was observed in all pH conditions comparatively with F2 and F3. However, in pH 7.4 a sudden increase in PS was observed which could be related to the destabilization and consequently, the disintegration of the system. As the CS amino groups become deprotonated, the electrostatic interaction between Enox and CS is weakened, leading the systems to swelling and destabilization process.

3.5. Enox in vitro release study

Fig. 6 shows the Enox *in vitro* release profile from selected colloidal dispersions in simulated GIT fluid at different pH. The Enox release from all nanoformulations was similar ($p < 0.05$), low and incomplete after 12 h.

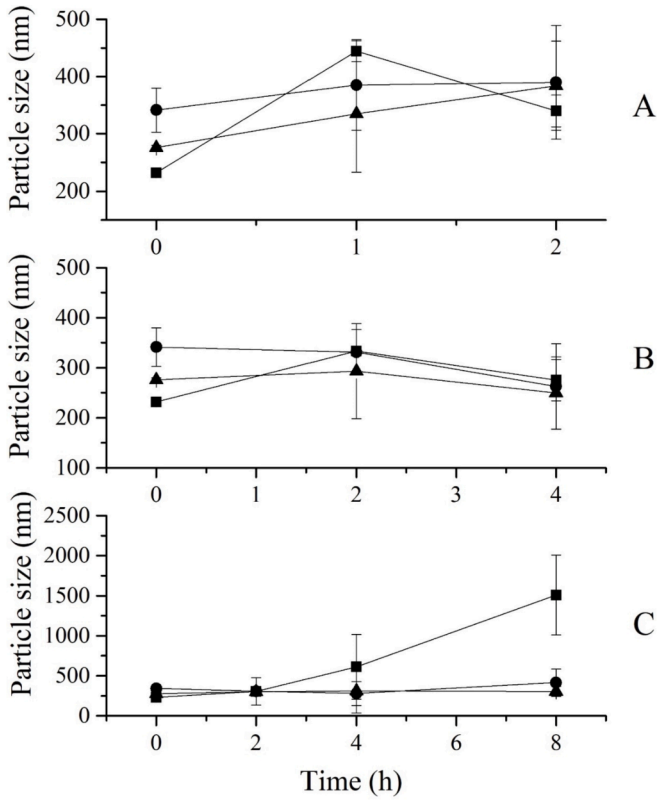

Fig. 5. Particle size monitoring of Enox-loaded nanoformulations in different simulated pH conditions: (A) pH 1.2, (B) pH 6.8, and (C) pH 7.4. (■) F1, (●) F2 and (▲) F3.

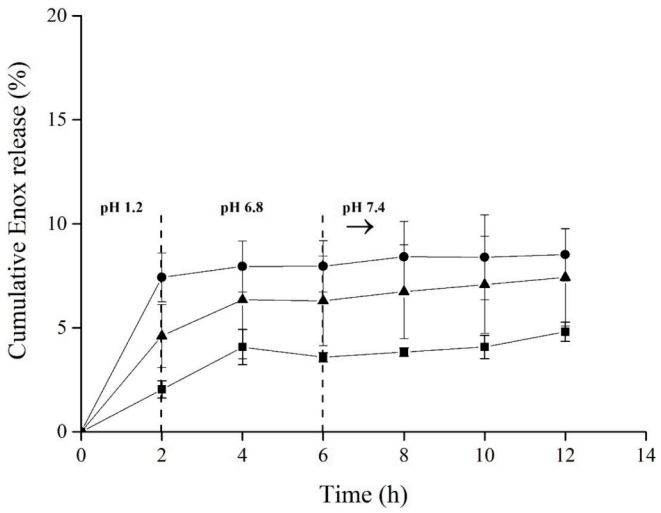

Fig. 6. *In vitro* release profile of Enox-loaded nanoformulations in different simulated pH environments. (■) F1, (●) F2 and (▲) F3.

All nanocarriers exhibited a very poor cumulative Enox release at gastric medium. In addition, at pH 6.8 and 7.4 the cumulative Enox release showed a slight increase, but still it was very slow, achieving a cumulative Enox release less than 10% for all nanoformulations studied *i.e.* in formulations F1, 4.8% (13.2 μg); F2, 8.5% (23.4 μg) and F3, 7.4% (20.4 μg).

In comparison with F2 and F3, F1 showed lower cumulative Enox release. This might be attributed to acid mucopolysaccharide nature of Enox molecules, which are able to maintain the electrostatic attraction with positively charged CS molecules in a wider pH range, and hence maintaining the F1 stability even at pH 1.2 [10]. Furthermore, it is worth to note that the Enox was exposed directly to acidic medium on F1 formulation and it may have suffered inactivation by low pH condition since this formulation is not able to protect the drug from acidic environment [41].

Regard to F2 and F3, slight increase in cumulative Enox release was noticed, which might be related to the Eud-coating procedure. In fact, the addition of Eud PBS pH 6.8 solution possibly weakened the interaction force between positively charged CS molecules and negatively-charged Enox molecules [35]. In addition, the slower cumulative Enox release showed by F3 in comparison with F2, could be attributed to the more compact structure formed by the polymers difficulting the penetration of the incubating medium into nanocarrier matrix [3].

A slow Enox release behavior could be of interest for oral Enox administration. Once Enox must exert your anticoagulant effect in the bloodstream, it is interesting that the release phenomena take place only when the nanocarriers achieve and interact with the proximity of absorbing intestinal epithelium [10].

As a strategy for prolonging the drug residence time of formulations at intestinal mucosal surface, CS, a well-known mucoadhesive biomaterial, was applied to allow more time for drug release at the target site, resulting in improved bioavailability [38,42]. In fact, CS presents strong bioadhesiveness properties which is related to the interaction between sialic acid residue of mucus intestinal barrier and amino groups of CS molecules [42]. It is worth mentioning that a similar Enox release behavior was reported by other authors [3,28,43].

3.6. Mechanism of drug release

The changes in mean volume diameter of nanocarriers suspended in simulated GIT fluids are depicted in Fig. 7.

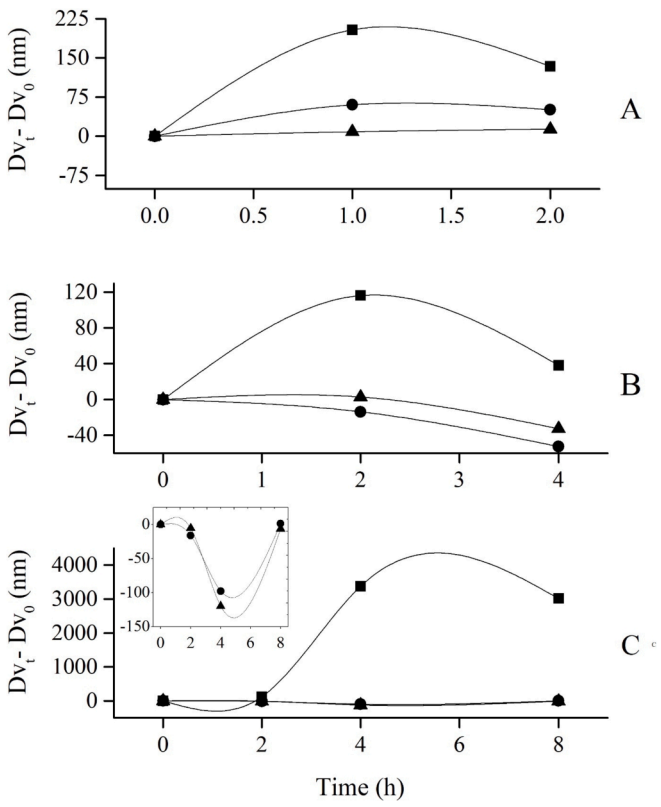

Fig. 7. Mean volume diameter behavior of Enox-loaded nanoformulations in different simulated pH environments: (A) pH 1.2, (B) pH 6.8, and (C) pH 7.4. (■) F1, (●) F2 and (▲) F3. Inset graph emphasizes the F2 and F3 behavior.

Among all conditions, the F1 showed a higher increase in the mean volume diameters compared to F2 and F3. In fact, acrylic polymers such as Eud are pH-dependent polymers extensively used for enteric effect that dissolves at pH 6 above. It is expected that they withstand the lower gastric pH values and the degradation process takes place at neutral or slightly alkaline pH [23].

For F1, at pH 1.2 and 6.8, initially an increase in the mean volume diameters was observed followed by a little reduction in size. However, at pH 7.4, there was a rapid increase in mean volume diameters followed by a significant reduction. On the other hand, for F2 and F3, at pH 1.2, nanocarriers showed a smooth change in mean volume diameters demonstrating the gastric resistance which might be attributed to the Eud coating process. Further, at pH 6.8 and 7.4, the nanocarriers showed a more pronounced reduction in the mean volume diameters followed by a considerable increase in the diameter. In fact, as Eud has a pH-dependent solubility, its erosion and swelling increase as the pH increases [44].

Several studies demonstrated that the mechanism that controls/governs the drug release from CS-containing systems is the diffusion or swelling/erosion or both mechanisms [45]. In the swelling/erosion-controlled release, the system exhibits an increase in particle size followed by a reduction in the diameters [46]. This behavior could be observed in the F1 suggesting that the drug was released by this manner. However, for F2 and F3, a different mechanism could be observed suggesting an initial erosion process and as the medium reaches the more hydrophilic core of the particle begins the swelling process [29].

4. Conclusion

In this study, we developed an spherical Eud-coated nanoformulations using an eco-friendly method which could entrapped high amount of drug and maintain the integrity against gastric acid environment. In addition, the *in vitro* release performance confirms the stability and the ability of Eud-coated nanocarriers to protect the drug from low pH conditions, demonstrated by negligible cumulative Enox release when the particles are submitted to SGF conditions. Finally, we demonstrated that the core-shell structure of the particle influenced the drug release mechanism of the formulations. Based on our study, it suggests that the combination of enteric-coating approach and drug delivery systems could be successfully explored for the oral delivery of Enox.

CRediT authorship contribution statement

Yuri Basilio Gomes Patriota: Formal analysis, Methodology, Investigation, Writing - Review & editing, Visualization, Validation. **Igor Eduardo Silva Arruda:** Methodology, Formal analysis. **Antonia Carla de Jesus Oliveira:** Methodology, Formal analysis. **Thaisa Cardoso de Oliveira:** Methodology, Formal analysis. **Eliadna de Lemos Vasconcelos Silva:** Methodology, Formal analysis. **Luíse Lopes Chaves:** Writing - Review & editing, Visualization, Supervision. **Fábio de Oliveira Silva Ribeiro:** Methodology, Formal analysis. **Durcilene Alves da Silva:** Methodology, Formal analysis, Writing - Review & editing. **Monica Felts de La Roca Soares:** Conceptualization, Validation, Formal analysis, Investigation, Writing - Review & editing, Visualization; Supervision, Resources, Project administration. **José Lamartine Soares-Sobrinho:** Conceptualization, Validation, Formal analysis, Investigation, Writing - Review & editing, Visualization; Supervision, Resources, Project administration.

Declaration of competing interest

The authors have no conflict of interest.

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