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**ORIGINAL ARTICLE**

# Pellets for oral administration of low-molecular-weight heparin

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**Abstract**

*Background*: Oral absorption of low-molecular-weight heparin (LMWH) is limited by its molecular size and negative charge. It has been shown previously that orally administered polymeric nano- or microparticles containing encapsulated LMWH have led to gastrointestinal absorption of heparin in rabbits. *Method*: Based on these investigations, pellets containing two LMWHs, enoxaparin (MW 4500 Da) or bemiparin (MW 3600 Da), and Eudragit®RS30D (ERS), were prepared using extrusion/ spheronization technique. Uncoated or coated (ERS) pellets were evaluated in vitro and in vivo on rabbits. *Results*: Enoxaparin pellets showed fast in vitro release in phosphate buffer (pH 7.4) and prolonged in vivo drug absorption after a single oral dose of 600 anti-Xa IU/ kg of body weight, leading to relative bioavailabilities ranging from 9.7 ± 1.9% to 12.8 ± 2.7% and anti-Xa activity over the curative dose. Bemiparin included in matrix pellets of ERS and coated with ERS exhibited in vitro prolonged release up to 4 hours and in vivo anti-Xa activity below the therapeutic minimum value of 0.1 IU/mL. *Conclusion*: This study presents LMWH in a pellet dosage form, which compared to nano- or microparticles, may offer a more convenient and industrializable way of manufacture leading to an easier scale-up process.

**Key words:** *Anti-Xa activity in vivo; drug release in vitro; Eudragit®RS30D; low-molecular-weight heparin; oral heparin delivery; pellets*

## Introduction

Heparin is a well-known, highly prescribed, anticoagulant antithrombotic agent that was discovered almost 100 years ago and used in clinical practice for more than 50 years. Unfractionated heparin (UFH) is a complex polysaccharide that reduces the incidence of both deep vein thrombosis and pulmonary embolism dramatically in surgical patients, but its use is associated with an increase in hemorrhagic complications. As a result of these complications and the need to administer low doses two or three times daily to achieve therapeutic efficacy, studies in the late 1970s were undertaken to develop safer and more convenient heparin preparations[1]. These studies resulted in the development of low-molecular-weight heparins (LMWHs) that have a molecular weight of 5000 Da or less and still contain the specific pentasaccharide sequence necessary for the inhibition of activated factor X. Their length is, however, insufficient to inhibit thrombin. Thus the rationale behind their development was that with this greater and more specific ability to inhibit activated factor X rather than thrombin, the LMWHs would exhibit greater antithrombotic properties, leading to increased clinical efficacy, but with fewer of the anticoagulant properties and bleeding complications associated with thrombin inhibition[2]. In addition, LMWHs can be administered once daily and clinical studies showed its improved safety profile in the prevention and treatment of venous thromboembolism when compared to UFH[3].

Unfortunately both UFH and LMWH can only be administered parenterally, presumably because of large

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molecule and negative ionic charge[4]. Thus many studies have been done to find a delivery system for the oral heparin administration that could decrease health-care costs and improve patient compliance. They include several strategies such as cell membrane permeabilization, tight junction modifications, increase of drug lipophilicity, or protection against acidic pH of the stomach[5]. Absorption enhancers were one of them. Thanou et al.[6] used the $Ca^{2+}$-chelating ability of a poly(acrylate) derivative Carbopol 934P to interfere with the intercellular junctions and increase the paracellular permeability for heparin. Another strategy to circumvent the barrier function of the gastrointestinal tract is offered by thiol groups of polymeric adjuvants inhibiting protein tyrosine phosphatase involved in the closing process of tight junctions through a GSH-mediated mechanism (GSH = thiolated polymers in combination with reduced glutathione)[7,8]. Absorption-enhancing effects of glycyrrhetinic acid[9], L-arginine[10], or sodium caprate[11] on the intestinal permeability and bioavailability of ardeparin, another LMWH, using rat model and Caco-2 cell culture model were discovered. Some other investigations include chitosan derivatives[12], labrasol[13], or dimethyl sulfoxide and deoxycholic acid conjugates[14]. Currently, the most effective formulation is the coadministration of heparin with *N*-[8-(2-hydroxybenzoyl)amino]caprylate, a carrier molecule that improves the oral absorption of heparin in humans[15].

An oral heparin formulation would alleviate many of the disadvantages of present pharmacotherapy and would offer several other important indications for heparin, representing large market opportunities, for example, additional cardiovascular indications, inflammatory diseases, and cancer[3,16].

Previous results obtained with orally administered polymeric nano- or microparticles containing encapsulated UFH or LMWH[17–20] have led to the gastrointestinal absorption of heparin in rabbits with doses that were similar to those administered by intravenous infusion or subcutaneous injection in humans. Such results were attributed to the potential mucoadhesive properties of the polycationic Eudragit® RS polymer. As relatively similar results were observed for nano- and microparticles (size ratio 1–100), it was thought that particle size was not directly affecting heparin absorption. Therefore, a more industrial manufacturing method was selected to potentially further extend the concept to pellets (size ratio 1–20; microparticles to pellets).

In this experimental work, pellets containing two LMWHs differing in the average molecular weight, that is, enoxaparin or bemiparin, and Eudragit RS30D (ERS), were prepared using extrusion/spheronization technique and evaluated in vitro and in vivo on rabbits. Enoxaparin, with a mean molecular weight of 4500 Da, is derived from UFH by benzylation followed by alkaline depolymerization. This LMWH presents anti-Xa and anti-IIa activities of 100–110 and 25–30 IU/mL, respectively, conferring to an anti-Xa/anti-IIa ratio between 3 and 4. Bemiparin is a new second-generation LMWH with a mean molecular weight of 3600 Da obtained from medical grade porcine UFH by depolymerization in nonaqueous medium. Its anti-Xa and anti-IIa activities are 80–100 and 5–10 IU/mL, respectively, leading to an anti-Xa/anti-IIa ratio of 8[21].

Heparin in a pellet dosage form, compared to nano- or microparticles, may offer a more convenient and industrializable way of manufacture leading to an easier scale-up process. Furthermore, the utilization of polymeric aqueous dispersions allows avoiding the establishment of toxicological data usually related with the use of organic solvents and obtaining a more safe product for the working staff as well as for patients.

## Materials and methods

### *Materials*

Sodium enoxaparin solution ($M_W$ 3500–5500 Da) (Lovenox® 10.000 IU anti-Xa/1 mL) marketed by Sanofi-Aventis (Paris, France) with an anti-Xa/anti-IIa ratio between 3 and 4 was obtained from commercial sources. Bemiparin powder ($M_W$ 3000–4200 Da) with anti-Xa/anti-IIa ratio of 8 was kindly offered by Rovi Pharmaceutical Laboratories (Madrid, Spain). Avicel®PH 101 and Avicel®RC 581 microcrystalline celluloses (MCCs) and α-lactose monohydrate were used as fillers and were, respectively, purchased from Asahi Kasei Chemicals Corporation (Tokyo, Japan) and Cerapharm (Vienna, Austria). An aqueous dispersion of ammonio methacrylate copolymer ($M_W$ 150,000 Da), ERS, from Evonik Industries (Essen, Germany) was used as matrix forming and/or coating polymer together with acetyltriethylcitrate (TEC) delivered from Vertellus Performance Materials Inc. (Greensboro, NC, USA) as a plasticizer, silica oxide Syloid 244 FP (Grace GmbH, Worms, Germany) and talc (Jan Kulich, Hradec Králové, Czech Republic) as coating additives. Hydroxypropylmethylcellulose (Methocel E5) purchased from Dow Chemicals Company (Plaquemine, LA, USA) was used as an additional layer on pellet cores prior to the coating. The reagents used for the measurement of the anti-Xa activity were supplied by Diagnostica Stago (Asnières-sur-Seine, France). All other reagents were of analytical grade and used as supplied.

### *Pellet preparation*

Enoxaparin extrudate mass was prepared from homogenized powders, that is, lactose and MCC (mixer Tefal Kaleo, France) wetted by a mixture of ERS dispersion, TEC, enoxaparin solution, and water. For bemiparin extrudates, as bemiparin was obtained as a powder, it

was added to the fillers before homogenization, and the resulting mixture was subsequently wetted by diluted ERS dispersion with TEC to produce approximately 120 g of mass appropriate for extrusion. The amount of added water was carefully controlled as the wetting step is critical for successful pelletization by extrusion/spheronization technique[22]. Composition of extrudates is summarized in Table 1. One screw extruder (Pharmex 35T; Wyss & Probst, Extertal, Germany) fitted with one axial located die (extrusion perforations 0.6 mm in diameter; thickness of extrusion die 1.0 mm) was used to form extrudates. Wetted mass was fed through the hopper on a rotating screw at standard extruder speed of 110 rpm. Prepared extrudate was subsequently placed into the spheronizer (Pharmex 35T; Wyss & Probst) with a 23-cm diameter serrated plate. The spheronization lasted 15 minutes at rotating speed 640 rpm. Formed pellets were dried in a ventilated oven at 40°C for 48 hours.

### *Coating of pellets*

Pellets of 0.5–0.8 mm size were charged into the process chamber of a bottom-spray fluid bed unit of Multiprocessor (MP 1; Aeromatic Fielder, Bubendorf, Switzerland) and heated up. When the product temperature reached 45°C, the layering solution was sprayed onto the pellets with the aid of the 0.8-mm diameter spray nozzle and peristaltic pump using 170 k*P*a of atomization pressure. The inlet air temperature was 65°C, product temperature was 45°C, and spray rate was kept at 20 g/min. Pellets were dried at a temperature of 45°C. First, methocel layer (6% solution of Methocel E5 in purified water) was applied up to 5% of the total pellet weight, and then 10% ERS layer was sprayed on isolated pellets. ERS layer was formed from ERS aqueous dispersion containing 2.7% of TEC and 4.0% of Syloid and 0.7% of talc. Coated pellets were thermally treated in ventilated oven at 40°C for 24 hours to complete the coating step (as recommended by the producer).

### *Pellet evaluation*

Pellet fractions of 0.5–0.8 and 0.8–1.2 mm were selected using sieves of corresponding apertures (AS 200; Retsch, Haan, Germany), and their characteristics, that is, pellet sphericity, hardness, friability, and drug content, were evaluated.

Pellet shape was observed by an optical microscope (DN 45; Lambda, Prague, Czech Republic) and CCD camera (Alphaphot; Nikon, Tokyo, Japan) and confirmed by the pellet sphericity *S*, calculated from the area and the perimeter determined by image analysis (Leco IA; Leco Instruments, St. Joseph, MO, USA) of 500 pellets according to the following formula[23]:

$$S = \frac{4\pi \times \text{area}}{\text{perimeter}^2}.$$

Mechanical properties were characterized as pellet friability and hardness. Ten grams of pellets were placed into a stainless steel drum of the abrasion tester adapted for pellet testing (Erweka TAR 10; Erweka, Ensenstam, Germany), together with 200 pieces of 4-mm glass beads, and rotated for 10 minutes at 20 rpm. The dust was thereafter removed and pellets were reweighed. The friability, that is, the weight loss after agitation, was expressed as a percentage. Tablet hardness and compression tester fitted with 5-kg load cell (Hardness C5 tester; Engineering System, Nottingham, UK) was used for the determination of pellet hardness. Ten randomly selected pellets of each formulation were tested; the hardness mean value and the standard deviation were calculated.

LMWH content was performed by adding 30 mg of pellets to 10 mL of distilled water. The amount of LMWH included in pellets was determined by turbidimetric method[24] by measuring the amount of free LMWH in the aqueous solution recovered after destruction of the pellets in ultrasonic bath (15 minutes) and centrifugation of the obtained suspension.

Drug content, pellet hardness, and friability measurements were carried out in triplicate and the results were expressed as an arithmetic mean ± SD.

### *Dissolution studies*

LMWH pellets (50 mg) were suspended in 20 mL of phosphate buffer saline ($Na_2HPO_4$ 0.64%, $KH_2PO_4$

**Table 1.** Composition of LMWH extrudates.

| Sample[a] | Drug[b] (%) | MCC (%) | Lactose (%) | ERS[b] (%) | TEC (%) | Water (%) |
|---|---|---|---|---|---|---|
| ME20 | 2.5[c] | 55.2 | 21.1 | 20.8 | 0.4 | 70.0 |
| MBe20 | 3.0 | 55.2 | 20.6 | 20.8 | 0.4 | 74.8 |
| MBe5 | 3.0 | 55.5 | 36.1 | 5.0 | 0.4 | 85.2 |
| MBe15 | 3.0 | 55.5 | 26.1 | 15.0 | 0.4 | 73.0 |
| MBe25 | 3.0 | 55.5 | 16.1 | 25.0 | 0.4 | 76.3 |

[a]M, matrix pellets; E, enoxaparin; Be, bemiparin; 5, 15, 20, or 25: percentage of Eudragit RS included in pellets.
[b]Calculated as dry substance.
[c]Lovenox® inj., enoxaparin sodium 10,000 IU in 1 mL.

0.06%, NaCl 0.59%; pH 7.4). The suspension was incubated in a water bath at 37°C under magnetic stirring at 200 rpm. At different times (5, 10, 15, 30, 45, 60, 120, and 240 minutes) samples of 1.5 mL were withdrawn and replaced by 1.5 mL of fresh phosphate saline buffer. Each sample was filtered through Porafil® cellulose-mixed ester membrane with a pore size of 0.20 µm (Macherey-Nagel, Düren, Germany). The amount of heparin released from pellets was determined by turbidimetric method[24]. Aliquots of 0.5 mL of filtered samples were added to 0.5 mL of sodium acetate buffer (acetic acid 1.22%, sodium acetate 10.75%; pH 4.4) and mixed with 2 mL of 0.1% cetylpyridinium chloride in 0.94% NaCl aqueous solution. Samples were incubated at 37°C for 1 hour and then assayed at 500 nm by UV spectroscopy (Uvikon 922; Kontron, Eching, Germany). All experiments were performed in triplicate.

### *In vivo experiments*

Experiments were carried out according to the French legislation on animal experiments. Pellets were filled into hard gelatine capsules and orally administered to male New Zealand rabbits (3478 ± 395 g) housed in separate cages, fasted overnight with water ad libidum. The administration was performed by oral gavage at a dose of 600 anti-Xa IU/kg of body weight. Blood samples (1.5 mL) were taken from the marginal ear vein at different times (2, 4, 6, 8, 10, and 24 hours) and then added to a constant volume of sodium citrate 0.129 M (0.17 mL) and centrifuged at 3000 × *g* for 10 minutes.

The concentration of LMWH was determined automatically (STA Compact Automate; Diagnostica Stago) with a factor Xa chromogenic assay (Stachrom Heparin, Diagnostica Stago)[25]. Each of the LMWH standards and plasma samples (25 µL) were mixed with 50 µL of antithrombin III solution. This solution was mixed with 100 µL of bovine factor Xa and incubated for 90 seconds at 37°C. Factor Xa chromogen substrate (100 µL) was then added and incubated at 37°C. The absorbance was determined at 405 nm every 2 seconds for 10–30 seconds of incubation. A linear relationship between Δabsorbance/min and the concentration of LMWH in the range of 0.0–0.8 anti-Xa IU/mL was obtained. This assay had a coefficient of variation of <7% at a limit of detection of 0.02 anti-Xa IU/mL.

To calculate the relative bioavailability (*F*) of LMWH pellets, the commercial solution of enoxaparin was administered by subcutaneous injection to overnight fasted rabbits at a dose of 300 anti-Xa IU/kg of body weight. The anti-Xa activity of LMWH was measured as described above. After the determination of the area under the curve (AUC) of the concentration/time profile by the linear trapezoidal method, the relative bioavailabilities were calculated by the ratio of the respective AUC corrected by the administered doses. All experiments were performed in triplicate.

### *Statistical analysis*

The results were expressed as mean values ± SD. For the pairwise comparison, the Mann–Whitney test was used to investigate differences statistically. In all cases, $P < 0.05$ was considered to be significant.

## Results and discussion

### *Pellets evaluation*

From extrudates of different ERS concentrations (Table 1), pellets of good mechanical properties (Table 2), that is, hardness from 6.16 ± 2.99 to 8.66 ± 0.32 N and low friability from 0.04 ± 0.04% to 0.33 ± 0.23%, were prepared (friability value of 1.7% is considered as a limit[26]). It can be observed that pellet hardness increased with increasing concentration of ERS that could be because of its matrix-forming properties. The same tendency is

**Table 2.** Characteristics of pellet samples.

| | | | | Drug content (mg/g) | |
|---|---|---|---|---|---|
| Sample[a] | Sphericity[b] | Hardness[c] (N) | Friability[c] (%) | Theoretical | Practical[d] |
| ME20 | 0.882 ± 0.029 | 8.66 ± 0.32 | 0.07 ± 0.09 | 25.1 | 24.7 ± 1.5 |
| MBe20 | 0.860 ± 0.026 | 7.51 ± 0.62 | 0.08 ± 0.06 | 30.0 | 28.5 ± 0.1 |
| CBe20 | 0.901 ± 0.040 | ND[e] | ND | 26.0 | 25.2 ± 0.1 |
| MBe5 | 0.838 ± 0.012 | 6.16 ± 2.99 | 0.33 ± 0.23 | 30.0 | 29.0 ± 3.0 |
| MBe15 | 0.860 ± 0.028 | 6.49 ± 1.31 | 0.10 ± 0.11 | 30.0 | 25.0 ± 1.0 |
| MBe25 | 0.856 ± 0.004 | 7.63 ± 3.85 | 0.04 ± 0.04 | 30.0 | 26.0 ± 3.0 |

[a]M, matrix pellets; C, coated pellets; E, enoxaparin; Be, bemiparin; 5, 15, 20, or 25: percentage of Eudragit RS included in pellets.
[b]$n = 500$.
[c]$n = 3$.
[d]$n = 6$.
[e]ND = not determined.

apparent within the range of friability values of pellet samples. Higher values were obtained when lower amount of polymer was included in matrix pellet core. Drug content of 83.3–96.6% of the theoretical amount was measured, which is in the range for content uniformity limits. Pellet sphericity within the interval 0.838 ± 0.012–0.901 ± 0.040 indicates regular spherical shape of particles[27].

### *Drug release in vitro*

Drug dissolution from either uncoated or coated pellets was carried out in phosphate buffer of pH 7.4 and the amount of released drug in predetermined time intervals 5, 10, 15, 30, 45, 60, 120, and 240 minutes was measured using turbidimetric method. Figure 1 shows the dissolution profile of enoxaparin pellets of two different sizes 0.5–0.8 and 0.8–1.2 mm, respectively. Both profiles did not differ significantly; however, faster release was observed from smaller pellets. This result could be explained due to larger surface area of the same dose of smaller pellets which ensures faster release of the drug.

Figure 2 compares bemiparin release from matrix pellets without a coating and pellets coated with ERS (10% coating). From this graph it can be seen that bemiparin dissolution from coated pellets was significantly slower with 30 minutes of lag time. Coated pellets thus prolonged the drug release in vitro up to 4 hours. Bemiparin dissolution from uncoated pellets was fast showing burst effect followed by a plateau. The majority of bemiparin was released from pellets without a coating within first 0.5 hour.

The amount of bemiparin released from ERS pellets in vitro is, however, dependent on ERS concentration in pellets: the higher is the concentration, the lower is the drug release (Figure 3). When 5% of ERS is included in pellets, bemiparin dissolution is immediate and complete; the entire drug present in pellets is liberated in first 5 minutes. Pellets containing 15% of ERS released only 66 ± 3.7% of the drug included and those with 25% of ERS only 47.9 ± 3.3% of bemiparin within 2 hours. These differences in bemiparin release from pellets could be explained by possible interaction of negatively charged sulfate and carboxyl groups of heparin and positively charged quarternary ammonium groups of ERS that could lead to an incomplete drug release. However, these differences in the release of the active substance may be also concomitantly related with the amount of lactose incorporated in the matrix of the pellets. When the amount of ERS was lower in the composition of the matrix, the amount of lactose was higher. Indeed, a higher amount of lactose in the matrix of the pellets could lead to a faster hydration of the pellets improving its disintegration and the release of the bemiparin. Finally, it has to be noticed that the release of

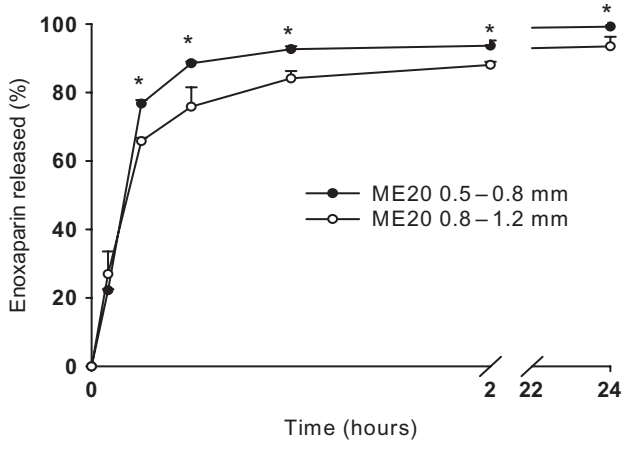


**Figure 1.** Enoxaparin release from matrix pellets of size 0.5–0.8 and 0.8–1.2 mm (phosphate buffer, pH 7.4).

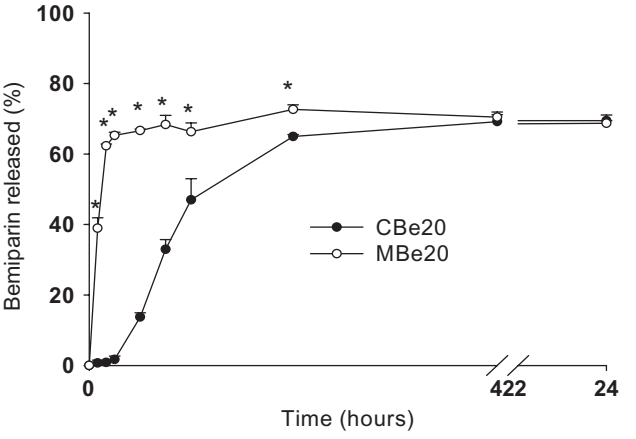


**Figure 2.** Bemiparin release from uncoated MBe20 and coated pellets CBe20 (phosphate buffer, pH 7.4; pellet size 0.5–0.8 mm).

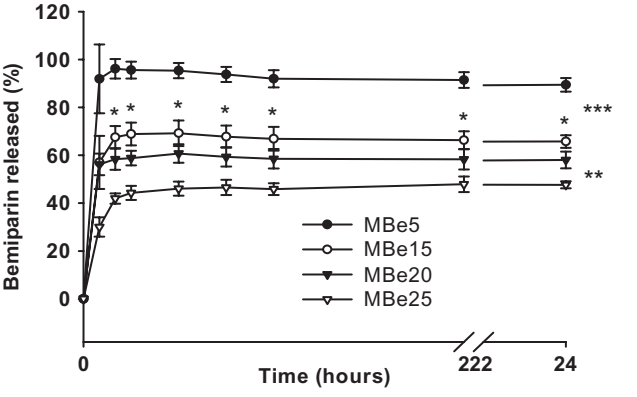


**Figure 3.** Bemiparin release from matrix pellets containing different concentrations of ERS (phosphate buffer, pH 7.4; pellet size 0.5–0.8 mm).

bemiparin from the pellets was lower than the release of enoxaparin. Indeed, for the same percentage of 20% of ERS included in the pellets formulation, bemiparin pellets exhibited only an in vitro release of 70% of LMWH, whereas enoxaparin pellets exhibited a complete release. Lower release of bemiparin could be explained because of the different molecular weight, the charge density, and/or affinity for the Eudragit polymer.

Based on the obtained results, the ratio of both ERS/lactose inside the matrix pellet cores and ERS coating could be used to control heparin release from pellets.

### *In vivo experiments*

Results obtained in vivo are presented in Figure 4 and Table 3. After subcutaneous injection of a single dose of Lovenox® solution (300 anti-Xa IU/kg of body weight) to overnight fasted rabbits, a peak concentration of $1.59 \pm 0.08$ IU/mL 1 hour after application was reached. Compared to subcutaneous application lower $C_{max}$ values and a shift in $T_{max}$ were observed when pellets (dose of 600 anti-Xa IU/kg of body weight) were administered orally. Anti-Xa activities $0.31 \pm 0.06$ and $0.21 \pm 0.04$ IU/mL, respectively, were determined after 4 hours from pellet application. Compared to the subcutaneous injection which exhibited a duration of biological activity of 6 hours, anti-Xa activity after pellets administration lasted longer (up to 7 and 8 hours); the anti-Xa value was kept above the curative dose of 0.2 anti-Xa IU/mL[28] during 6 and 2 hours for ME20 0.5–0.8 and ME20 0.8–1.2 mm, respectively. Nevertheless, it has to be kept in mind that the oral dose was twofold the subcutaneous injection. This anti-Xa activity may be because of a contact between ERS and mucus resulting from electrostatic interactions between positive charges of ERS and negative charges of mucus especially related to sialic acid residues[29]. This interaction may also lead to a local concentration of heparin helping its diffusion and absorption through the mucocellular wall. Heparin is known to be sensitive to acidic stomach conditions[30] or enzymatic environment[31], so it is possible that protection inside pellets may still increase the amount of heparin available for absorption through a high local concentration gradient. As presented for polycationic chitosan[12,32,33], ERS may also interact with the surface of cells and modify the structural organization of the tight junction-associated proteins, allowing an increased absorption of enoxaparin by paracellular way. Finally, with regard to the disintegration of pellets during the in vitro release study, it might be assumed that such phenomenon could happen during the gastrointestinal transport leading to smaller particles with a size much closer to the size of nano- and microparticles favoring their mucoadhesion to the gut wall. Pellets of smaller size, that is, 0.5–0.8 mm in diameter did not show better pharmacokinetic parameters: $AUC_{0\to8}$ ($0.56 \pm 0.12$ IU h/mL kg) and bioavailability $F = 12.8 \pm 2.7\%$ compared to pellets of 0.8–1.2 mm having $AUC_{0\to8}$ ($0.42 \pm 0.08$ IU h/mL kg) and bioavailability $F = 9.7 \pm 1.9\%$. This is not very surprising as LMWH release profiles were still fairly similar and in vivo, the disintegration of the pellets may lead to similar surface contact area.

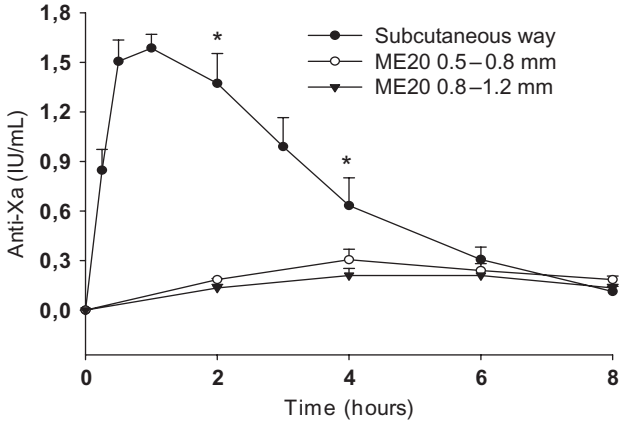


**Figure 4.** Anti-Xa activity of enoxaparin pellets in vivo. Comparison of subcutaneous application at a dose of 300 anti-Xa IU/kg and a single oral administration of pellets to rabbits at a dose of 600 anti-Xa IU/kg. Data are mean ± SD ($n = 3$).

The bioavailability figures obtained with the two types of pellets can be compared with our previous results obtained with nano- and microparticles based on Eudragit RS. The best results were obtained by Hoffart et al. (absolute bioavailability of 59%) with the LMWH tinzaparin using Eudragit RS/poly(ε-caprolactone) 50/50 nanoparticles. By using smaller particles of Eudragit RL/poly(ε-caprolactone) 50/50 but with unfractioned heparin, maximum absolute bioavailability of 22% was reported by Jiao et al. In another study, Jiao et al. prepared microparticles ranging from 71 to 282 μm and observed an absolute bioavailability between 4.7% and 48.3%, whereas gelatin microparticles

**Table 3.** Pharmacokinetic parameters of enoxaparin pellets after a single oral administration to rabbits compared with subcutaneous administration of Lovenox® ($n = 3$).

| Formulations | $T_{max}$ (hours) | $C_{max}$ (anti-Xa IU/mL) | $AUC_{0\geq8\ hours}$ (IU h/mL kg) | $F_{8\ hours}$ (%) |
|---|---|---|---|---|
| Enoxaparin solution | 1 | $1.59 \pm 0.08$ | $2.19 \pm 0.14$ | 100 |
| ME20 0.5–0.8 mm | 4 | $0.31 \pm 0.06$ | $0.56 \pm 0.12$ | $12.8 \pm 2.7$ |
| ME20 0.8–1.2 mm | 4 | $0.21 \pm 0.04$ | $0.42 \pm 0.08$ | $9.7 \pm 1.9$ |

of tinzaparin displayed a relatively low bioavailability (<5%). It appears that the increase in bioavailability is more related to the type of heparin than the particles' size. It is difficult to compare our previous results with Eudragit RS pellets as the size reduction during the gastrointestinal tract transport is not easily measurable. However, it can be assumed that the pellets size reduction, as it is observed in the dissolution tests, leads to much smaller particles that can potentially interact in a closer way with the gastrointestinal membrane, the same way that previous nano- and microparticles did.

Compared to previous studies made with microparticles or nanoparticles[17,18], pellets of enoxaparin present a less intense but more sustained anti-Xa activity released profile. This feature can offer together with well-established pelletization technique more convenient, industrializable, and robust way of production leading to an easier scale-up process.

According to the results obtained for enoxaparin, pellets of smaller size, that is, 0.5–0.8 mm, were used for bemiparin administration. During preparation, all parameters were kept constant for better comparison of both pellet samples containing different heparins. When compared to enoxaparin pellets, bemiparin particles showed lower activity on the one hand and, on the other, a larger anti-Xa activity, that is, 24 hours (Figure 5). Maximum concentration $C_{max}$ was reached faster already 2 hours after administration and $T_{max}$ was not dependent on the fact whether pellets were coated with ERS or not. Anti-Xa activity of both, coated or uncoated pellets was below 0.1 IU/mL, that is, below the therapeutic minimum value. Because of its lower molecular weight, bemiparin was expected to exhibit better anti-Xa activity but it was not the case. This lower anti-Xa activity of bemiparin in comparison with enoxaparin could be explained, as shown in the dissolution studies, by an incomplete release from the pellets which may be related to a difference in charge density and/or in affinity with ERS. Thus, further studies on bemiparin pellets should take into account this phenomenon and the appropriate dose of bemiparin should also be found for optimal biological activity.

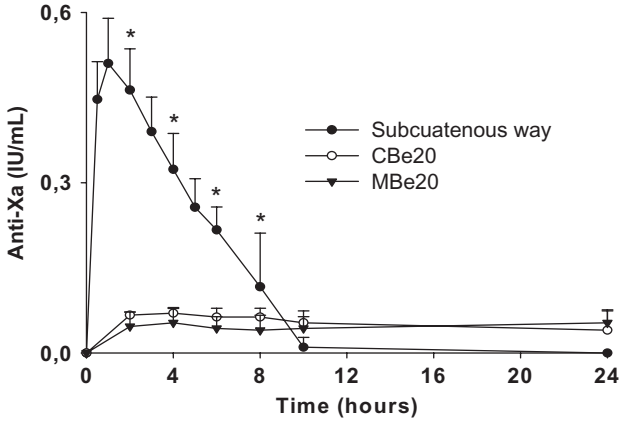


**Figure 5.** Anti-Xa activity of coated and uncoated bemiparin pellets in vivo. Comparison of subcutaneous application at a dose of 150 anti-Xa IU/kg and a single oral administration of pellets to rabbits at a dose of 600 anti-Xa IU/kg. Data are mean ± SD ($n = 3$).

## Conclusion

An oral LMWH formulation would be very useful in the improvement of present heparin therapy: it could reduce inconvenient injections, decrease the health-care costs, improve patient compliance, and offer several other important indications. Furthermore, as pellets as a dosage form are well established in pharmacotherapy as well as in pharmaceutical industry, they can be easily produced, especially when their manufacture abandons organic solvents. Contrary to most of the studies made on the oral absorption of heparin, our study allowed showing an anti-Xa activity level over the curative dose after oral administration to rabbits probably related with the mucoadhesive properties of Eudragit RS. If the pellets size did not seem to be a critical parameter for the absorption of LMWH through the gut wall, various factors have to be considered as the LMWH used and polycationic nature of the polymer.

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