

Preparation, Characterization and in vitro Release Studies of Enoxaparin in Nanoparticle form and Enterically Coated Tablets Containing Different Enhancers

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Abstract

In the past decade, many strategies have been developed to enhance oral drug delivery. Different techniques were investigated, amongst those the use of permeation enhancers such surfactants and biodegradable polymers were studied more extensively. Chitosan derivatives have been studied as permeation enhancer in free soluble form and as nanoparticulate systems. The aim of the current work was to develop a nanoparticulate system based on ionic interaction between Trimethyl Chitosan (TMC) and Enoxaparin as well as enterically coated Enoxaparin tablets containing surfactants such as Tween 80 and Sodium Lauryl Sulfate (SLS) as permeation enhancer. The prepared nanoparticles were characterized for size, zeta potential, loading capacity and in vitro release. The tablets were enterically coated using Acryl-Eze[®], characterized physically and were assayed for their content. The release of Enoxaparin was studied in PBS pH 6.8 corresponding to the pH of small intestine. The result suggested that the nanoparticles were positively charged with a diameter of about 120 nm and loading capacity of around 95%. The tablets contained 60 mg of Enoxaparin, 10 mg of surfactants as enhancer. The in vitro release from tablets showed almost 100% Enoxaparin release in 2 hours; whereas in nanoparticles there was a 67.5% release in 24 hours. The Result suggests that the enhancing effect of the systems may be useful for oral Enoxaparin tablets. In order to better evaluate the enhancing effect of the polymer with the surfactants as well as the oral tablets with nanoparticulate systems further ex vivo and in vivo studies are required.

Keywords: Enoxaparin, Nanoparticles, Enhancer agents, Trimethy Chitosan, Release studies.

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Introduction

Oral drug delivery is the most desirable and preferred method of administering therapeutic agents. It is also the first choice of investigation in the discovery and development of new pharmaceutical formulations due to convenience in administration, patient compliance and cost effective manufacturing process. However, not all new drugs can be administered orally due to their large size and their low bioavailability and poor permeation. Oral drug delivery is frequently impaired by several physiological and pharmaceutical challenges associated with the inherent physicochemical nature of the drugs and/ or the variability in Gastro- Intestinal (GI) condition such as pH, presence of food, transit times and enzymatic activity in the GI tract. In order to overcome the above obstacles, the ideal oral delivery system must release its contents in a pH-dependent fashion only at the optimal target region, remain in the optimal site long enough for the complete drug release to be absorbed across the intestinal epithelium, and have a reproducible therapeutic effect.

Enoxaparin, an acidic mucopolysaccharide, is a medicine known as Low Molecular Weight Heparin (LMWH). Enoxaparin is used as anticoagulant with anti thrombotic activity and is administered subcutaneously. Although this administration is painful and has less patient acceptability, the large molecular size and strong negative charge

resulted in a low bioavailability if used orally. Polymeric compounds are found to be useful carriers for high molecular weight drugs [1]. Chitosan (poly [β -(1-4)-2-amino-2-deoxy-D-glucopyranose]) with excellent biocompatible and biodegradable properties (Figure 1) is a natural cationic polysaccharide derived by partial deacetylation of chitin from crustacean shells [2] and has been used extensively in drug delivery systems [3].

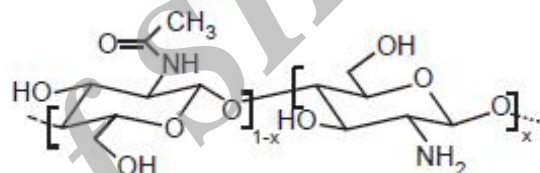


Figure 1. Chemical structure of Chitosan.

Moreover, chitosan has been extensively investigated for its potential as a permeation enhancer across intestinal epithelium. The application of chitosan in biomedical field is limited due to its low solubility at pH above 6.5. Hence, numerous chitosan derivatives were synthesized; among them trimethyl chitosan (TMC) was studied in more detail for its higher solubility in broader pH values and for its permeation enhancing effect [4]. Figure 2 shows the polymeric structure of TMC.

Many strategies have been developed to enhance drug delivery. Among these approaches, nanoparticulate systems have attracted special interests as the nanoparticles are able to protect active agents from degradation, they can improve the drug transport and provide

controlled release properties for encapsulated drugs [5]. In recent years, ion gelation or polyelectrolyte complex formation (PEC) has drawn increasing attention for producing nanoparticles containing peptides [6]. The nanoparticles prepared by this method have

several characteristics favorable for cellular uptake and colloidal stability, including suitable diameter and surface charge, spherical morphology, and a low polydispersity index indicative of a relatively homogeneous size distribution [7].

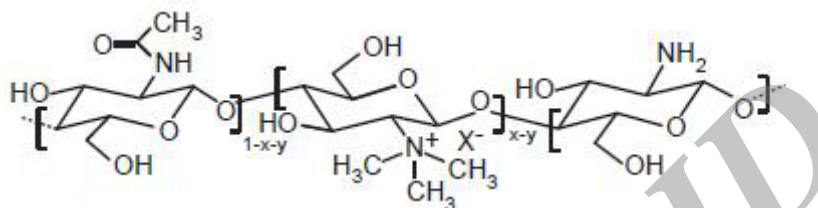


Figure 2. Structural repeating units of TMC.

In addition, this method has the advantage of not necessitating aggressive conditions such as the presence of organic solvents and/or sonication during preparation; therefore, minimizing possible damage to proteins and peptides during ion gelation formation [8]. On the other side, surfactants such as Tween 80 and sodium lauryl sulfate (SLS) were shown to have permeation enhancing effect [9]. Hence, enterically coated tablets containing SLS and Tween 80 were also formulated and were compared with TMC containing tablets to study the permeation enhancing effect of Enoxaparin across the intestinal epithelium.

The aim of the current study was to design and characterize enterically coated tablets containing SLS and Tween 80 as permeation enhancers. Moreover, nanoparticles were prepared and characterized using TMC and Enoxaparin by polyelectrolyte complexation. The release of Enoxaparin from these

nanoparticles was studied at pH 6.8 corresponding to the pH of small intestine.

Experimental

Materials

ChitoClear® chitosan (viscosity 1% (w/v) solution, 22 mPa.s) was purchased from Primex, Iceland. Enoxaparin was purchased from Dongying Tiandong Biochemical, China. Acryl-Eze® was purchased from Colorcon Co. U.K. Methyl iodide (CH₃I), Sodium Iodide (NaI) and N- methyl pyrrolidone (NMP) were purchased from Merck (Darmstadt, Germany), SLS (Sodium Lauryl Sulfate), Tween 80 and the other excipients for tablet preparation were gifts from Hakim Pharmaceutical Co. Iran, Heparin Kit was purchased from HemosIL Co. Spain,

Methods

Preparation and Characterization of TMC

The synthesis of TMC was done according to

method described by Sieval et al [10]. Briefly, Low Molecular Weight Chitosan (1 gram) was mixed with 50 mL N-methyl pyrrolidone (NMP) on a stirrer for 2 hours. Then, NaOH solution (10 ml), CH_3I (14 ml), and NaI (2400 mg) were added to the Chitosan/NMP solution in hot water bath with a temperature of 60° C for 6 hours. Acetone was slowly added to the solution to form a precipitate. In order to exchange I⁻ with Cl⁻, the polymer was dissolved in 5.0% NaCl solution and precipitated with acetone to obtain a water soluble white powder with quantitative yield of 97% and a degree of substitution of 50±5%.

Preparation of TMC/Enoxaparin nanocomplexes

The nanoparticles were prepared by PEC method between positively charged TMC and negatively charged Enoxaparin. Initially, known amount of TMC and Enoxaparin were separately dissolved in water to obtain concentrations of 1mg/mL for each solution. In indirect method, nanoparticles were prepared by adding Enoxaparin solution drop-wise to TMC solution under gentle magnetic stirring at room temperature. Once a stable colloidal suspension was obtained, the samples were centrifuged for 30 min at 30,000 rpm at 25°C, and the supernatant was used for measurement

of free, unloaded Enoxaparin by Heparin HemosIL Kit. The same procedure was used for direct method except TMC solution was added to Enoxaparin solution. Size and zeta potential of the nanoparticles were measured with a Malvern zeta-sizer on the nanoparticle suspensions. The amount of Enoxaparin loaded in the nanoparticles was calculated by measuring the difference between the total amounts of the Enoxaparin added in the nanoparticle solution and the amount of non entrapped Enoxaparin remaining in the clear supernatant after the centrifugation.

Tablets preparation and characterization

The oral tablets contained a known amount of Enoxaparin as active ingredient was prepared by conventional wet granulation method. Two tablet formulations were designed, with 5% SLS and 5% Tween 80 as permeation enhancer (Table 1). The tablets were compacted with single punch press (Erweka, GmbH, Germany). The tablets were sub-coated with 2.0 mg of a 3% polyvinyl pyrrolidone (PVP) for a more modified coating, and then coated with Acryl-Eze® as enteric coating agent. The tablets characterized for physicochemical properties such as weight, hardness, friability as well as assay of Enoxaparin and content uniformity.

Table 1. Used ingredients in Enoxaparin tablets.

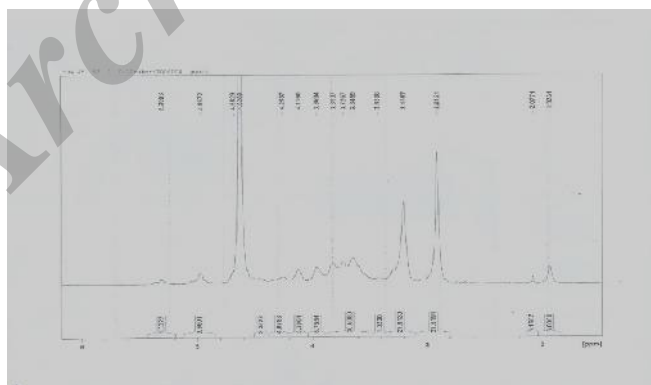
Ingredients	Amount (mg)	Percent in Tablet
Enoxaparin	60	30%
Avicel PH102	116	58%
PVP K30	10	5%
AcDiSol	2	1%
Tween 80 / SLS	10	5%
Magnesium Stearate	2	1%
Total	200mg	100%

In-vitro release studies

The release studies were done on freeze-dried nanoparticles as well as on tablet formulations. The Enoxaparin release of different dosage forms was done in an Erweka dissolution apparatus in phosphate buffer solution (PBS) at pH 6.8. The temperature and rotation were set at 37°C and 75 rpm, respectively. The samples were taken at predetermined time intervals. The amount of Enoxaparin released in the dissolution media was measured by the Heparin HemosIL kit and release profile for every formulation was drawn.

Results and Discussion

The ¹H-NMR spectra of TMC is presented in Figure 3. As shown in this figure the signal at 1.9 ppm is related to the acetyl group of the chitin; the peak at 3.6 represents the N(CH₃)₃ group together with a smaller peak at 3.4 ppm assigned to the N(CH₃)₂ group. According to the depicted peaks and intensity, the degree of quaternization was calculated to be 50±5%. In TMC structure, NH₂-groups were converted to a quaternary ammonium group and that is the why that TMC has higher positivity in comparison to the chitosan itself.

**Figure 3.** The ¹H-NMR spectrum of TMC in D₂O.

Nanoparticle delivery system, which is determined to protect peptide and protein drugs from degradation in the GI tract and increase the contact between drug and mucus membrane layer at the absorption sites, therefore improve the bioavailability of these hydrophilic macromolecule drugs. Although a wide variety of techniques and methods such as

solvent evaporation, interfacial polymerization and emulsion polymerization methods are available for nanoparticles preparation, but in most of these methods we need to use of organic solvents, heat or vigorous agitation which are basically harmful to sensitive biomolecules. In recent years, ion gelation or polyelectrolyte complex formation (PEC) has drawn increasing attention for producing nanoparticles containing peptides [11]. It was shown that chitosan is positively charged at $\text{pH} < 6.5$ due to the protonation of the amino groups and Enoxaparin is negatively charged material that related to carboxylic groups.

Therefore, electrostatic interactions between both entities can be used as a driving force for PEC formation. It seems that nanoparticles prepared by this procedure have several advantages for cellular uptake and colloidal stability, suitable diameter and surface charge, spherical morphology, and a low polydispersity index indicative of a relatively homogeneous size distribution [12].

It was used two procedures, direct and indirect method for nanoparticles preparation. The result for average nanoparticle size and zeta potential is presented in Table 2.

Table 2. Characteristic of the TMC nanoparticles containing Enoxaparin for different formulations (n=3).

Method	Mean Diameter (nm)	PDI	Mean Zeta Potential (mV)	Encapsulation Efficiency
Indirect	120	0.095	+27.0	95%±2
Direct	125	0.084	-13.0	94%±2

The respective average diameters, measured by Zetasizer, and zeta potential for TMC nanoparticles containing Enoxaparin in indirect method were 120 nm and +27 mV respectively. The values for direct method were reported 125 nm and -13 mV. The range for the PDI is from 0 to 0.5 that Values close to zero indicate a homogeneous dispersion and those greater than 0.5 indicate high heterogeneity. Regarding to data in Table 2 the PDI for direct and indirect method is smaller than 0.5 which indicates a relative homogeneous dispersion. As shown in Table 2, two different zeta potential is obtained due to manner of adding solution together. Moreover, in indirect method

the negatively charge of enoxaparin solution is added to TMC solution with positive charge. It seems the whole of positive charge of TMC was not neutralizing with negatively charged of Enoxaparin when the nanoparticles were formed, therefore the resultant is nanoparticles with positive zeta potential. As contrary, when we added the TMC solution to Enoxaparin solution in direct method, the nanoparticles were formed immediately before to completion of interaction between different charges and hence it seems to obtain nanoparticles with negative zeta potential.

The mucoadhesive properties are based on cationic character of polymers that used in

nanoparticles. The mucus gel layer exhibits anionic substructures in the form of sialic acid and sulfonic acid substructures. Based on ionic interactions between the cationic groups of TMC and anionic substructures of the mucus, mucoadhesion can be achieved. In comparison with various anionic polymeric materials such as carbomer and polycarbophil [13] it seems that mucoadhesive properties of TMC due to high positive charge for more interaction with mucus gel layer can be improved. Regarding to obtained data and above explanation, we

selected indirect method for nanoparticles preparation. The other parameters such as size distribution and encapsulation efficiency in two methods are close together (Table 2).

The release of Enoxaparin from nanoparticles is shown in Figure 4. The release profile for Enoxaparin nanoparticles was obtained in PBS pH value 6.8. Each experiment was done three times. As shown in Figure 4, it was not observed burst effect of Enoxaparin release in the initial steps of the study for two nanoparticles which prepared with direct and indirect method.

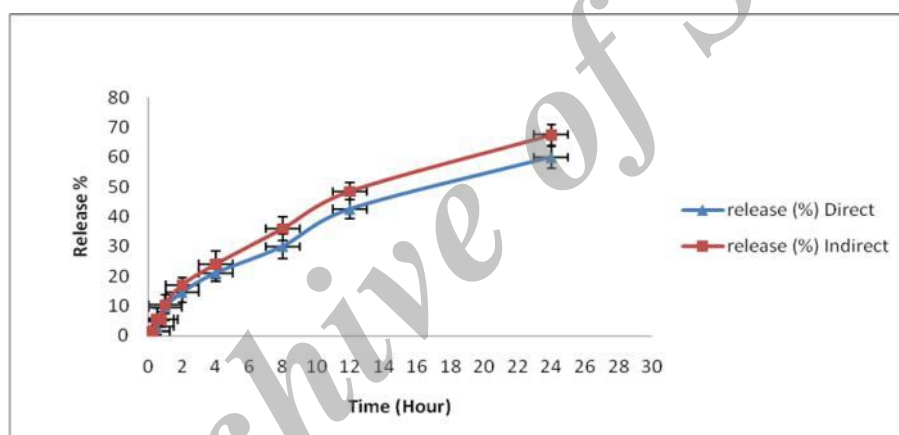


Figure 4. Release profile of Enoxaparin from nanoparticles in direct and indirect method n=6.

According to the figure, the release was sustained over 24 hours; it is indicated that almost 68% of the Enoxaparin in indirect method formulation and almost 60% of Enoxaparin in direct method formulation was released into the medium during 24 hour. This slow release of Enoxaparin may be related to drug entrapment. Furthermore, the interaction between positively charged of TMC and Enoxaparin with negative charge in nanoparticles produce a polymeric

network that resulted in the entrapment of the Enoxaparin in polymeric matrix. It seems the release rate is dependent to complexing forces between two polymers, therefore the solubility and diffusivity of Enoxaparin in polymer membrane becomes the determining factor in drug release. In the first hours, PBS may be diffused in polymeric network and later the polymeric chains go to relaxation and swelling behavior and finally Enoxaparin was dissolved in PBS and diffused into the medium. Fickian

and nonFickian behavior have been used for determining the mechanism of drug release. Equation $Mt/M_{\infty} = kt^n$ was used, where Mt is the amount of released enoxaparin in a given time, M_{∞} is the total amount of Enoxaparin within nanoparticles, k and n are the equation constants, and t is the time. Data has shown for kinetic constant, diffusional constant, and type

of release mechanism in two formulations in Table 3. It seems that value of 0.781 for direct formulation and 0.769 for indirect formulation are related to nonFickian transport [14]. The result suggests that transport is possibly controlled by diffusion and/or swelling of the polymer chains.

Table 3. Kinetic constants (k), diffusional (n) and determination coefficient (r^2) determined by the linear regression of $\ln(Mt/M_{\infty})$ against $\ln t$.

Formulation	n ($X \pm SD$, $n = 3$)	k ($X \pm SD$, $n = 3$)	r^2
Direct method	0.781 \pm 0.0452	0.0399 \pm 0.0015	0.9595
Indirect method	0.769 \pm 0.0413	0.0529 \pm 0.0026	0.9485

Two tablet formulation using SLS and tween80 were prepared. The ingredients for two formulations were presented in Table 1. The physical characteristics such as hardness,

friability and disintegration time of two groups of tablets prepared by wet granulation method were done (Table 4).

Table 4. Physical tests for two formulations containing 5% SLS and 5% Tween80.

Formulation	Hardness (Kp)	Friability	Disintegration Time in PBS pH=6.8
Containing 5% SLS	6.2-8.3	0.2%	1:11 min
Containing 5% Tween80	5.5-8.1	0.3%	1:45 min

As indicated, we use two different enhancers in formulation. Polyoxyethylene sorbitan fatty acid esters (polysorbates or tweens) are a series of partial fatty acid esters of sorbitol that copolymerized with ethylene oxide. This agent may be used as solubilizing agent for a variety of substances and as wetting agent in the formulation oral drug delivery system. They have been found to be useful in improving and increasing oral bioavailability when they use in optimum percent in formulation. Moreover, this agent is a nonionic surfactant that is

widely used in oral dosage form formulation that generally regarded as nontoxic substance. The other enhancer that used in this project is sodium lauryl sulfate (SLS) that regarded as anionic surfactant. This agent is employed in a wide range of nonparenteral formulations. It is determined that SLS as wetting agent could be effective in both alkaline and acidic condition. The initial studies showed the optimum concentration (5%) of these two enhancing agent (data has not shown). The other ingredients in formulation are usual substances

that can be used in oral dosage form formulation as filler, binder, disintegrating agent, lubricant or enteric coating agent. Two optimized formulations were chosen for dissolution studies. The parameters for selection of the formulations were good compressibility and

hardness at the range of 4-10 Kp, Friability < 0.5%, suitable disintegration time for tablets and assay of Enoxaparin according to standard guidelines. The release profile of Enoxaparin from tablet formulations is presented in Figure 5.

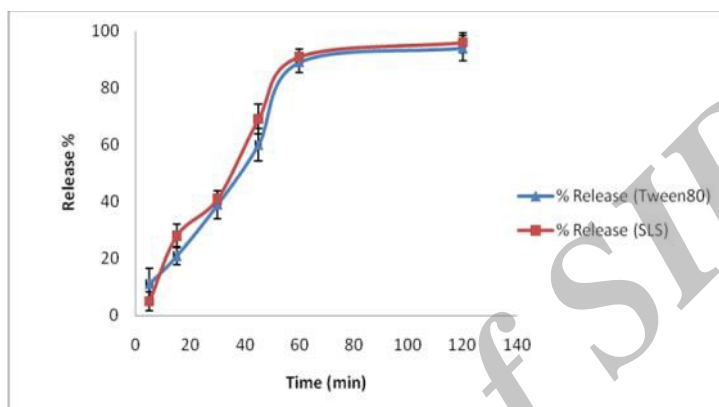


Figure 5. Comparative release profile of Enoxaparin from tablet formulations n=6.

Accordingly, almost all of the Enoxaparin was released within 2 hours in pH 6.8. This is due to the immediate release of the Enoxaparin from enteric coated tablets that dissolve at pH 6.8 corresponding to the pH of the small intestine. Indeed, regarding to analogous formulations (only different in type of surfactant agent) it is predicted to observe the same Enoxaparin release profile. According to data has obtained with the release profile tests, it seems that the release of Enoxaparin in stimulated intestine medium for tablets is fast whereas has retarded for TMC nanoparticles containing Enoxaparin. Furthermore, formulations which used nanoparticles for oral delivery system are able to release the Enoxaparin in slow model and increase the time for remaining in absorption site. Moreover, regarding to transit time of

GI tract, nanoparticles with 24 hour release pattern may be good choice for oral drug delivery systems for peptides, proteins and macromolecular drugs such as Enoxaparin.

Conclusion

In conclusion it was shown that Enoxaparin nanoparticles were prepared using TMC by Polyelectrolyte complexation method. The nanoparticles were characterized and were shown to have a sustained release profile in pH 6.8. Enterically coated Enoxaparin tablets were also prepared by wet granulation containing permeation enhancers. The Enoxaparin release from these tablets was shown to be immediate and almost the entire drug was released within 2 hours. In order to better investigate the activity of the two systems, one has to compare

their enhancing activity in vitro by cell culture in Caco-2 cells or by ex vivo studies where the absorption of Enoxaparin across the intestinal membrane will be monitored.

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