Objective: The objective of the present work was to prepare and evaluate a novel oral formulation for systemic delivery of low molecular weight heparin (LMWH). The formulation consisted of Eudragit S 100-coated positively charged liposomes encapsulating LMWH and a penetration enhancer. Materials and Methods: Positively charged liposomes were first prepared by the thin film hydration method using lipid (soy phosphotidylcholine and cholesterol) and stearyl amine (SA) in the optimum ratio of 16:1, along with cetylpyridinium chloride (CPC) as a penetration enhancer. Prepared liposomes were coated with negatively charged Eudragit S 100 (0.3% w/v). The formulations were studied for various in vitro and in vivo properties. Differential scanning calorimetry (DSC), x-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) studies, and in vitro drug release were used for in vitro characterization of the formulations. Ex vivo permeation studies were performed by using distal small intestine of rat. Oral absorption studies were conducted with the rat model. Results: Coating of the liposomes was confirmed by SEM and particle size determination studies. In vitro release studies of coated liposomes have demonstrated that the release of LMWH was in the following order: Stomach < small intestine < distal small intestine < colon. Ex vivo permeation studies have shown a fivefold increase in permeation of LMWH with Eudragit S 100-coated liposomes compared to uncoated, uncharged liposomes. Oral absorption studies have showed that with Eudragit-coated liposomes, the oral bioavailability of LMWH was improved, compared to plain LMWH solution. This is revealed by a threefold increase in the area under the curve (AUC) of the plasma concentration time curve. Conclusion: A novel formulation for oral delivery of LMWH was thus successfully prepared and evaluated.

Key words: Colon targeting, Eudragit S 100, liposomes, low molecular weight heparin (LMWH), oral absorption, permeation

INTRODUCTION

Globally, anticoagulants are widely used in a variety of clinical situations. These drugs are used in a clinical setting for the treatment of two common postsurgical complications, deep vein thrombosis and pulmonary embolism, as well as in the outpatient setting. Oral anticoagulants received particular attention from scientists all over the globe. Of the anticoagulants that have been developed and used, heparins received particular attention. These molecules were discovered in 1916 and were later brought into clinical use. The only problem with heparins is that they need to be administered invasively. They are administered in clinics through the subcutaneous, intravenous, or intramuscular route. The oral bioavailability of these molecules is very low. Clinical levels of the drugs cannot be achieved with such dosing. Yet heparins are used in a variety of disorders. Oral delivery of these molecules has obvious advantages and is clinician-friendly. However, owing to a variety of problems associated with the

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oral route, the administration of these molecules is complicated. Structurally, unbranched acidic glycosaminoglycan (GAG) mixture forms heparin. GAG consists of alternative glucosamine and hexuronic acids modified by salivation and acetylation. Its administration requires hospitalization, which is invariably invasive with the current technologies. Unfractionated heparin (UFH) is not absorbed across the gastrointestinal tract (GIT) and thus has very low oral bioavailability. This has been attributed to its high molecular weight as well as its high negative charge. A high negative charge repels the molecule from negatively charged mucosal and epithelial layers in the GIT. Further, heparin demonstrated significant disadvantages. These include variable anticoagulant and pharmacological properties, among them a highly variable anticoagulant response. Additionally, heparin use is linked to bleeding problems. Low molecular weight heparin (LMWH), which was later developed from heparin, has proved to have less disadvantages. LMWHs are associated with a predictable dose/response and have fewer nonhemorrhagic side effects. Because of these clinical advantages, LMWHs have gradually replaced UFH for most indications. However, LMWH also needs to be administered via the parenteral route. Thus, all the varieties of heparins available are invariably administered using the invasive route. An oral heparin formulation could consequently be a tremendous clinical success.

Several attempts to develop effective oral heparin formulations have been made. Research on noninvasive modes of delivery of these molecules received considerable attention from scientists. Several other routes and delivery approaches have been investigated to enhance the bioavailability of heparins. In this regard, the enhancement of oral bioavailability is a promising option. Attempts to enhance the oral bioavailability of heparins have included the use of pH-sensitive chitosan nanoparticles, N-trimethyl-O-carboxymethyl nanoparticles, conjugation with deoxycholic acid, bile acid–mediated delivery, alginate-coated nanoparticles, and tricaprilin microemulsion. These techniques led to enhanced oral bioavailability. Although there was an increase in the oral bioavailability, clinical levels could not be achieved. In this regard, we investigated acid-stable oral liposomes containing permeation enhancers to achieve clinical levels of heparins. One area of research involves using liposomes as carriers for LMWH. Liposomes have been widely reviewed in the literature. They have also been widely used as drug delivery systems for many years, offering temporal control of drug release and site-specific drug delivery for an extensive range of drugs. The advantages associated with liposomes for oral delivery are their ability to interact with cells, ease of preparation; good permeation properties through the GIT; and the capacity to incorporate a wide variety of drugs, both hydrophilic and lipophilic. Further, after oral administration it has been demonstrated that heparins permeate into the systemic circulation from the end portion of the small intestine as well as the colon, despite being degraded in the stomach and the earlier portions of the small intestines. Based on this data, we hypothesized that an oral system such as a liposomal formulation that can release the drug at the distal small intestine through the colon can result in successful clinical levels of heparins when administered along with permeation enhancers. Liposomes bind specifically to the membrane, lead to enhancement in residence time at the membrane, and also increase the permeability of drugs across the membrane. Thus, a formulation utilizing permeability enhancers and liposomes have been investigated in this study to achieve clinical levels of heparin after oral administration. LMWH was used as a model heparin in this study. For this molecule, like other heparins, degradation in the stomach as well as permeability is very poor after oral administration. Some absorption has been shown at the distal intestine and significant absorption has been demonstrated at the colon. This permeability can be enhanced with permeation enhancers so as to achieve therapeutic levels of heparins after oral administration. In the present work, attempts were made to coat the liposome with Eudragit S 100, which prevents the release of the drug in the stomach and enhances the release in the distal small intestine and colon, from where the drug is absorbed. The novelty of the work is that the application of liposomes in the oral delivery of LMWH was not previously published. Liposomes for oral delivery were previously reported for UFH and not for LMWH. Further, coating with Eudragit S 100 intended for targeting the lower small intestine and colon and the addition of the penetration enhancer cetylpyridinium chloride (CPC) potentiates the uniqueness of the work. The purpose of this study was to prepare LMWH liposomes coated by Eudragit S 100 and containing permeation enhancers, to evaluate the in vitro performance and in vivo efficacy of the same after oral administration.

**MATERIALS AND METHODS**

**Materials**

Enoxaparin sodium, a LMWH, was purchased from Bharath Biotech, Hyderabad, India. Soybean phosphatidylcholine was purchased from Himedia laboratories Private Limited, Mumbai, India. Cholesterol was obtained from Qualikems Fine Chemicals Private Limited, New Delhi, India. Stearyl amine (SA), CPC, Triton X-100, and Dialysis Membrane (MW 12000 Dalton) were purchased from Sigma Aldrich Private Limited, Mumbai. Eudragit S 100 was supplied by Zebra Technologies and Data Services Private Limited, Bangalore, India. Stachrom heparin was supplied by Diagnostica Stago, Asnieres-sur-Seine, France. All the other chemicals and reagents were of analytical grade and used as supplied.

**Methods**

**Reacting procedure for analysis**

To 1 mL of LMWH standard solution, 1 mL of 1M acetate buffer of pH 5 and 4 mL of CPC solution (0.1%) in sodium chloride (0.94%) were added and reacted for 1 h. Samples were then analyzed at 500 nm using ultraviolet (UV)–visible spectrophotometer.

**Preparation of Eudragit S 100-coated LMWH liposome**

Liposomes were prepared by the dry film rehydration method by using rotary vacuum evaporator. Briefly, soybean
phosphatidylcholine, cholesterol, SA, and CPC were dissolved in chloroform in a round-bottom flask. The proportion of cholesterol in each of the formulations was changed from 10 mg to 90 mg. Subsequently, vacuum was applied and the organic solvent was evaporated to obtain a thin lipid film. Then LMWH (20 mg) and phosphate-buffered saline solution was added to rehydrate the dried lipid film and rotated at 60°C. Rotation was continued until all the film was rehydrated. The multilamellar liposomes so formed were reduced in size by means of both ultrasonic homogenizer and probe sonicator. The unentrapped drug was separated by using cooling centrifuge, which was operated at 15000 rpm for 20 min at -2°C. The formed pellet was suspended in deionized distilled water. To prepare Eudragit S 100-coated liposomes, the following protocol was used. Briefly, Eudragit S 100 solution was prepared by dissolving different percentages of Eudragit S 100 (0.0125%, 0.025%, 0.05%, and 0.1% w/v) in 3% acetone solution. The required amount of Eudragit S 100 solution was added to the liposomal suspension of equal amount and kept under vortex mixer for 15 min. The solution was then placed in the refrigerator overnight for stabilization of the coating.\textsuperscript{[24,25]} 

**Optimization of liposomes**

Liposomes were optimized for process parameters such as hydration time, sonication time, and rotation speed of the flask. The formulation variables, such as SA concentration and cholesterol concentration, were also considered.

**In vitro characterization of liposomes**

**Encapsulation efficiency**

The amount of LMWH entrapped in liposomes after centrifugation was determined by taking the supernatant (1 mL) or liposomal suspension. To 0.2 mL of liposomal suspension, 1% of Triton X-100 solution was added to disrupt the lipid layer of the liposomes.\textsuperscript{[26]} Then aliquots of 1 mL of each sample were taken and mixed with 1 mL of 1M acetate buffer of pH 5.0. To this solution, 4 mL of 0.1% CPC in 0.94% sodium chloride solution was added and reacted for 1 h. The absorbance of these samples was taken at 500 nm by using UV–visible spectrophotometer. The previous protocol to assay LMWH was used in in vitro samples. The assay was validated in our laboratory. The study was performed in triplicate, and the percentage entrapment efficiency was determined by the direct method:

\[ \text{EE}\% = \frac{\text{Amount of LMWH detected}}{\text{Total amount of LMWH}} \times 100 \]

**Drug–polymer incompatibility studies by Fourier transform infrared spectroscopy (FTIR) analysis**

The FTIR analyses of drug, lipid, Eudragit S 100, Eudragit S 100-coated and uncoated liposomes, and other excipients were performed using Bruker Alpha-E FTIR spectrophotometer (Bruker, Ettlingen, Germany).

**Scanning electron microscope (SEM)**

Shape and surface morphology of liposomes before and after coating with Eudragit S 100 were determined by SEM. The samples were examined under a LEO 435 VP (Eindhoven, Netherlands) at an acceleration voltage of 30 KV, and photomicrographs were taken at 2890×.

**Determination of particle size, zeta potential, and polydispersity index**

The average particle size and the change in zeta potential value in different Eudragit S 100 concentrations (i.e., 0.05-0.1%) of the liposomes were evaluated by a particle size analyzer (Zetasizer, model 3000 HSA, Malvern, WR14, UK). The average particle size was calculated from about 10 determinations and polydispersity index values were displayed. The 20-μL sample was diluted with 8 mL of filtered deionized water prior to the determination of surface properties. All the measurements were carried out at room temperature.

**In vitro release studies**

A comparative LMWH in vitro release study was done at three different pH values of 1.2, 6.4, and 7.2, respectively. A known amount of liposomal solution was transferred into a boiling tube to which a dialysis membrane was attached at one end.\textsuperscript{[27,28]} The tube was then dipped in a beaker containing 50 mL of buffer solution and placed under magnetic stirring at 37°C and 100 rpm. The release studies were performed by using 0.1N HCl (pH 1.2) for 2 h, phosphate buffer of pH 6.4 and pH 7.2 for 3 h and 5 h, respectively. Then 0.5 mL of sample was withdrawn at 5 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, and 240 min, respectively for 10 h, and the withdrawn volume was replaced with fresh prewarmed buffer. It was then diluted with CPC and acetate buffer (as described in the entrapment efficiency study) and allowed to react for 1 h.\textsuperscript{[9]} Then the absorbance was taken in UV–visible spectrophotometer at 500 nm. The study was performed in triplicate.

**Ex vivo permeation studies**

In this study, the amount of LMWH transported across selected gastrointestinal barriers was measured. Distal small intestine of rat was taken and rinsed with normal saline solution. A piece of flattened intestinal membrane slightly larger than the opening of diffusion chamber was cut and mounted between the half cells with the mucosal surface facing the donor compartment and the serosal side facing the receiver compartment. Liposomes that contain positive charge on their surface and CPC (penetration enhancer) are considered as modified liposomes. Liposomes that do not contain positive charge and penetration enhancer are considered as unmodified liposomes. Ex vivo permeation study was performed for both types of liposomes. Briefly, a liposomal sample was placed on the mucosal surface and the receptor compartment was filled with pH 7.2 phosphate buffer solution. At fixed time intervals, 1 mL of the sample from the receptor chamber was withdrawn and replaced with the same volume of fresh buffer solution. The samples were collected at 5 min, 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, and 180 min, and the collected samples were treated with Triton X-100 to lyse the liposomes and extract LMWH.\textsuperscript{[29]} They were further analyzed by UV–visible spectrophotometer at 500 nm, as discussed in entrapment efficiency studies. To determine permeability in the absence of a liposomal
formulation, LMWH solution of a concentration similar to that of \( C_0 \) was used as a control. The apparent permeability coefficient (Papp) was calculated by using the following equation:

\[
Papp = \frac{Q}{A \times C_0 \times t}
\]

where Q is the amount of drug permeated within 3 h (\( \mu \)g), \( C_0 \) is the concentration of LMWH-loaded liposomes taken on the donor compartment (\( \mu \)g/cm\(^2\)), A is the area of the mucous membrane placed between the donor and receptor compartment (cm\(^2\)), and T is the time of sampling (s).

Transport enhancement ratios (ER) were calculated by:

\[
ER = \frac{Papp_{\text{sample}}}{Papp_{\text{control}}}
\]

**Oral absorption studies in rats**

All animal studies were approved by the Institutional Animal Ethics Committee. Male Wistar rats (weighing about 250 ± 20 g) were taken for the study and administered with a single oral dose (50 mg/kg) of LMWH in liposomes. Three groups of animals were taken, each group containing 6 animals. They were fastened overnight with free access to water before the administration of formulations. Group 1 (Control animals) was administered with an oral plain LMWH solution (50 mg/kg), Group 2 received LMWH uncoated liposomal suspension, and Group 3 were administered with LMWH-loaded Eudragit S 100-coated liposomes. LMWH at a dose of 50 mg/kg was administered to all the three groups. Blood samples (200 \( \mu \)L) were collected from the tail veins of Wistar rats at 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, and 8 h intervals, and LMWH was quantified by using an anti-factor Xa chromogenic assay. The anti-factor Xa activity versus time profile of LMWH in the plasma was then plotted and compared with that of the oral LMWH solution. Plasma concentrations of the drug were estimated and different pharmacokinetic parameters were evaluated. Clotting time with a plain LMWH solution, uncoated LMWH liposome, and Eudragit S 100-coated liposomes were determined in male Wistar rats (250 ± 20 g) using a laboratory method as specified in the reference. Typically, the three formulations mentioned above were tested in three animal groups by the capillary method. Clotting time was evaluated at 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, and 8 h time intervals.

**Stability studies**

The LMWH-loaded Eudragit S 100 liposomes were studied for stability studies at various temperatures such as 4 ± 1°C and 28 ± 4°C. Samples withdrawn at specific time intervals such as 1 month, 3 months, and 6 months were visually examined for appearance, ease of reconstitution, sedimentation, particle size, and entrapment efficiency.

**RESULTS AND DISCUSSION**

Liposomes were successfully prepared by using the method discussed in this study [Table 1]. Liposomes were optimized for achieving maximum entrapment efficiency and the desired particle size. Process parameters that were optimized are shown in Table 2. Formulation variables are discussed in the section below.

In earlier studies, it had been reported that the oral stability of liposomes was improved by surface coating of liposomes\(^{[11,12]}\). In the present study, attempts were made to use Eudragit S 100 as the coating polymer. Eudragit S 100-coated liposomes were prepared by forming an ion complex between SA and Eudragit S 100, in a manner similar to the ion complex formed between pectin, SA\(^{[13]}\), and carbopol (CP), SA\(^{[14]}\). Thus, before coating the liposomes, cationic charge was incorporated by using SA in the lipid mixture. Eudragit S 100 is a negatively charged polymer. The Eudragit S 100 coating on the liposomes was considered to be formed via ionic interaction between positively charged SA and negatively charged Eudragit S 100 on the surface of liposomes. In the GIT, Eudragit S 100 coating dissolves at a pH above 7.0 and the remaining positively charged liposomes help in penetrating the mucosal layer, as positively charged liposomes have better penetration power than uncharged liposomes\(^{[15,16]}\).

The ratios of lipid to SA taken were 16:1, 16:2, and 16:3. The zeta potential values were found to increase with increase in concentration of SA, i.e., 22.16 mg, 38.7 mg, and 45.44 mg, respectively. The values were in accordance with the results demonstrated by Takeuchi et al. In the preparation of Eudragit S 100-coated liposomes, core liposomes containing 16:1 ratio was selected, where high amounts of SA resulted in the aggregation of liposomes after coating. In preparation of CP liposomes, Takeuchi et al., used the liposomes in ratio 40:1 (lipid:SA) as core liposomes. Eudragit S 100 solutions of different concentrations, i.e., 0.025%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% w/v were prepared and coated onto the core liposomes (lipid:SA = 16:1), the increase in Eudragit S 100 concentration has led to a shift in zeta potential values toward the negative values [Figure 1].

| Table 1: Composition of liposomes by varying cholesterol concentration |
|---------------------------------------------------------------|
| **Formulation** | **LMWH** | **Soy phosphatidylcholine** | **Cholesterol (%)** | **SA (%)** | **CPC (%)** |
|-----------------|----------|--------------------------|-------------------|-----------|-----------|
| LC1             | 10 mg    | 100 mg                   | 10                | 10        | 10        |
| LC2             | 10 mg    | 100 mg                   | 20                | 10        | 10        |
| LC3             | 10 mg    | 100 mg                   | 30                | 10        | 10        |
| LC4             | 10 mg    | 100 mg                   | 40                | 10        | 10        |
| LC5             | 10 mg    | 100 mg                   | 50                | 10        | 10        |
| LC6             | 10 mg    | 100 mg                   | 60                | 10        | 10        |
| LC7             | 10 mg    | 100 mg                   | 70                | 10        | 10        |
| LC8             | 10 mg    | 100 mg                   | 80                | 10        | 10        |

| Table 2: Values of optimized parameters of LMWH liposomes |
|----------------------------------------------------------|
| **Factors** | **Optimized parameters** |
|------------|--------------------------|
| Hydration time | 80 min                   |
| Sonication time (80% amplitude and 5 cycles) | 2 min                   |
| Rotating speed of flask | 100 rpm                 |
| Data are expressed as mean ± SD (\( n = 3 \)) |
This change in zeta potential values was a result of changes in the charge on the surface of the liposome. The formation of the polymer layer on the surface of liposomes was confirmed by comparing the zeta potential values before and after coating with the polymer. The higher the zeta potential values (negative or positive), the greater was the physical stability of liposomal suspension as an effect of enhanced repulsion between particles, which ultimately reduces particle aggregation in colloidal dispersion. In Figure 1, high negative charge was observed for liposomes coated with Eudragit S 100 at concentrations 0.2% and 0.3% w/v. Further increase in concentration has shown constant values. Therefore, 0.3% w/v Eudragit S 100 concentration was selected for coating the liposomes as it showed negative charge.

In vitro characterization of liposomes

At the end of the fabrication, the chemical integrity of the drug was determined in the formulation. Initially, FTIR studies were performed on all the ingredients and liposomal formulations before and after coating [Figure 2]. The spectra were analyzed for characteristic bands to evaluate the interactions between the ingredients. Table 3 indicates that all the ingredients and formulations have exhibited their characteristic bands and there are no extra peaks observed in the spectra. This confirms that there was no interaction between the ingredients. Eudragit S 100-coated liposomes have shown peaks for C-O at 1166 cm⁻¹, C = O at 1727 cm⁻¹, which are characteristic bands of Eudragit S 100. This indicates that the liposomes were coated with Eudragit S 100. The differential scanning calorimetry (DSC) thermogram of LMWH showed a melting exotherm at 256.77°C. This exotherm was absent in thermogram of Eudragit S 100-coated LMWH liposome [Figure 3]. This indicates that LMWH was dispersed in liposomes in either amorphous or molecularly dispersed form. The endothermic peaks observed in the physical mixture, soybean phosphatidylcholine, and cholesterol were not seen in the formulation, indicating the formation of liposome. The x-ray diffraction (XRD) spectra of LMWH have shown a sharp crystalline peak. Less intense, broader peaks were observed in the spectra of liposomal formulation. This indicates that LMWH is converted to amorphous form in the formulation [Figure 4].

When observed under binocular microscope, the prepared liposomes appeared spherical [Figure 5]. The surface coating and the morphology of liposomes were also confirmed by SEM.

![Figure 1: Change in zeta potential upon coating with Eudragit S 100 solution. Data represent mean ± SD (n = 3)](image)

![Figure 2: FTIR spectra of (a) Soybean phosphatidylcholine (b) Cholesterol (c) SA (d) LMWH (e) Eudragit S 100 and (f) Eudragit S 100-coated liposomes)](image)

| IR spectra | Peak of Functional groups (wavelength cm⁻¹) |
|------------|------------------------------------------|
| LMWH       | C-H (alkane) 1418, C-H (CH₃) 1217, >C = O 3398, 0-H 1600 |
| SPC        | C-H 2915, C-H (CH₃) 2915, 1467, 1230, 1736 |
| SA         | C-H 1471, 1471, 1054, 1315 |
| Cholesterol| C-H 2928, 1434, 1150, 1724 |
| ES 100     | C-H 2950, 1448, 1166, 1727 |
| Uncoated   | C-H 2919, 1467, 1166, 1219 |
| Liposome   | C-H 2950, 1467, 1166, 1727 |

LMWH: Low molecular weight heparin, SPC: Soybean phosphatidylcholine, SA: Stearyl amine, ES 100: Eudragit S 100.
The results confirmed that the shape of the liposomes was spherical before and after coating with Eudragit S 100 [Figure 6]. The surface coating of the polymer was confirmed by the appearance of a white-colored layer on the surface and around the liposome, which was not seen in uncoated liposomes. Particle size determination studies revealed an increase in particle size upon coating with Eudragit S 100 [Table 4], which confirmed the polymer coating on liposomes. Polydispersity index values showed that the sizes of all the liposomal particles were homogeneous in nature.

LMWH standard graph was plotted by using different buffer solutions at pH 1.2, 6.4, and 7.2. The standard graph demonstrated linearity and was conveniently used in the in vitro assays to determine the drug levels. Entrapment efficiency of LMWH was determined by assay, where the amount of drug entrapped in liposomes was compared with that of the initial dose. The entrapment efficiency was found to be increased with the increase in concentration of cholesterol in the lipid mixture.
The bulky steroid rings of cholesterol increase the rigidity and stability of the lipid player and also enhance the permeability barrier function of the player with a role in increasing the entrapment efficiency of drugs.\textsuperscript{[10]} Cholesterol concentration above 60% decreased the entrapment efficiency of the drug; this might be an effect of the formation of a separate layer inside the phospholipid bilayer, which also influences drug release. Entrapment efficiency was also determined by changing the dose of LMWH. Two doses were selected, i.e., 10 mg and 20 mg. A minor change in the entrapment efficiency was observed with increase in the LMWH dose [Table 5]. This may be due to limited space available in the interior of the liposome.

\textit{In vitro} drug release studies were conducted on formulations containing 30%, 40%, 50%, and 60% of cholesterol, with and without Eudragit S 100 coating. The percentage drug release was evaluated in pH 1.2, pH 6.4, and pH 7.2 conditions at predetermined time intervals. The drug release rate was observed to be high for uncoated liposomes at pH 1.2 and 6.4, in contrast to coated liposomes [Figure 7]. Higher percentage drug release was demonstrated by coated liposomes at pH 7.2; this was a result of alkaline pH-led dissolution of Eudragit S 100 coating to expose cationic charged liposomes and to initiate drug release at the distal small intestine [Table 6]. An enhanced drug release was found in the formulations at pH 7.2. However, the percentage drug release was found to decrease with an increase in cholesterol concentration.

Data on release kinetics revealed that the kinetics of LMWH from uncoated liposomes followed zero order with $r^2 = 0.993$. Data were then fitted into the Koersmeyer–Peppas equation to determine the release mechanism [Table 7], found with $r^2 = 0.998$ and $n > 1$. This indicates super case II transport mechanism of LMWH through liposomes.\textsuperscript{[10]}

\textit{Ex vivo} permeation studies

Previous investigations showed that liposomes with a penetration enhancer help in enhancing the permeability of liposomes through the intestinal membrane.\textsuperscript{[11]} In the present study, after the Eudragit S 100 coating was removed, the liposomes charge again changes from negative to positive. The positively charged liposome gets adhered to the negatively charged mucous layer and the penetration enhancer CPC helps in penetration by opening the tight junctions, thus enhancing the permeation of liposomes through the colon region.\textsuperscript{[12]} Hence, in this experiment, permeation study through the intestinal tissue was carried out by using modified liposomes and unmodified liposomes. The cumulative percentage of LMWH transported from the liposomes across rat intestinal tissue was determined [Figure 8]. The apparent permeability coefficient and transport enhancement ratio was calculated after the study [Table 8]. From the table it is evident that the unmodified liposomes also showed a little permeability. This may be because of the interaction of phospholipid head groups with glycoproteins of the mucus, which lead to restructuring of mucous membrane, causing a change in its viscosity and an easier penetration of the drug.\textsuperscript{[13]}

However, the cumulative amount of drug transported from modified liposomes increased by fivefold when compared to the unmodified liposomes. The results obtained were in accordance with the results obtained by Li \textit{et al}., where the PF127-modified liposomes showed five- to sevenfold higher diffusion efficiency than unmodified liposomes.\textsuperscript{[29]} These results strongly supported

| Cholesterol (%) | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 |
|-----------------|----|----|----|----|----|----|----|----|
| EE (%) with 10 mg dose | 11±0.2 | 28±0.45 | 49±0.23 | 58±0.5 | 67±0.62 | 75±0.21 | 65±0.43 | 54±0.22 |
| EE (%) with 20 mg dose | 13±0.11 | 30±0.03 | 52±0.045 | 60±0.34 | 68±0.65 | 76±0.33 | 66±0.07 | 54±0.87 |

Data represent mean ± SD ($n = 3$)
the importance of liposomes to increase the colon penetration of LMWH. In the present study, CPC was applied both as a penetration enhancer and as a reagent. An experiment was conducted to ensure that CPC was only used as a penetration enhancer and that it did not interfere with the absorbance when observed by UV–visible spectrophotometer. Dissolution studies were performed for liposomes with CPC and liposomes without CPC for 3 h in pH 7.2 buffer. It was observed that there was no major change in the absorbance between the two types of formulations, as shown by their percentage release values [Table 9]. The results indicated that CPC did not affect the absorbance of LMWH but was only used as a penetration enhancer.

**Oral absorption studies in rats**

LMWH-loaded Eudragit S 100 liposomal suspension (containing 40% cholesterol), LMWH uncoated liposomes, and plain LMWH solution were administered separately to three groups of Wistar rats, as discussed in Methods. Blood samples were collected at regular intervals and LMWH levels in the plasma were determined by anti-factor Xa assay [Figure 9]. Various pharmacokinetic parameters were evaluated by using Kinetica software version 5.0 (Sherwin K Sy) [Table 10]. The pharmacokinetics parameters such as the area under the curve (AUC), maximum concentration (C_max), time taken to reach maximum concentration (T_max), mean residence time (MRT), and half life (t_half) were analyzed for the plain LMWH oral solution, LMWH uncoated liposomes, and Eudragit S 100-coated LMWH liposomes. Plain oral LMWH solution showed a C_max of 0.10 ± 0.007 IU/mL after 2 h of oral administration. While LMWH-entrapped uncoated liposomes have shown a C_max of 0.115 ± 0.002 IU/mL, LMWH-loading Eudragit S 100 liposomes have shown the highest C_max of 0.345 ± 0.002 IU/mL. Similarly, AUC_0-8h of LMWH was increased by 1.48 times with uncoated liposomes and 3.34 times with Eudragit S 100-coated liposomes. Eudragit S 100-coated liposomes also have shown increased values for MRT and t_half. Clotting time studies indicated that the clotting time of blood was increased by 1.07 times for uncoated liposomal formulation and 3.22 times for Eudragit S 100-coated liposomes when compared with an oral LMWH solution [Figure 10]. Stability studies demonstrated that the liposomes

![Figure 9](image_url)  
**Figure 9:** Anti-factor Xa activity versus time profile for LMWH oral solution, LMWH-uncoated liposomes, LMWH Eudragit S 100-coated liposomes after oral administration in equivalent dose of 50 mg/kg in Wistar rats. Data represent mean ± SD (n = 6)

![Figure 10](image_url)  
**Figure 10:** Clotting time profile for LMWH oral solution, LMWH-uncoated liposomes, LMWH Eudragit S 100-coated liposomes after oral administration in equivalent dose of 50 mg/kg in Wistar rats. Data represent mean ± SD (n = 6)
Table 9: Effect of cetlypyridinium chloride (CPC) on the release of LMWH

| Time (h) | Percentage release |
|---------|---------------------|
|         | With CPC            | Without CPC          |
| 1       | 9.9±0.01%           | 10.2±0.04%           |
| 2       | 22.7±0.06%          | 22.7±0.03%           |
| 3       | 44.1±0.11%          | 44.1±0.11%           |

Data represent mean ± SD (n = 3)

Table 10: Pharmacokinetic parameters of LMWH-loaded liposomes after oral administration in Wistar rats

| Pharmacokinetic parameters | LMWH oral solution | LMWH-uncoated liposomes | LMWH-Eudragit-coated liposomes |
|----------------------------|---------------------|--------------------------|--------------------------------|
| Dose (mg)                  | 50 mg/kg            | 50 mg/kg                 | 50 mg/kg                       |
| C_{max} (IU/mL)            | 0.10±0.007          | 0.115±0.002              | 0.345±0.006                    |
| T_{max} (h)                | 2                   | 3                        | 4                              |
| AUC_{0-6h} (μg/h/mL)       | 0.25±0.006          | 0.37±0.09                | 0.836±0.014                    |
| MRT (h)                    | 2.11±0.014          | 3.5±0.013                | 4.33±0.5                       |
| t_{1/2} (h)                | 0.36±0.002          | 0.64±0.06                | 0.929±0.07                     |

Data represent mean ± SD (n = 6)

Table 11: Stability studies of Eudragit S 100-coated LMWH liposomes

|                      | 28°C  | 28 ± 4°C |
|----------------------|-------|----------|
| Mean particle size (nm) | 105±0.5 | 112±1.3  |
| Drug leakage (%)      | 11±0.6 | 23±1.5   |
| Ease of dispersibility| Redispersible upon gentle shaking | Redispersible upon gentle shaking |

stored at 2-8°C are stable for the duration of 60 days [Table 11]. These findings suggest that Eudragit S 100 liposomes can be used as a potential delivery system for the oral delivery of LMWH. One of the reasons for the increased bioavailability was ascribed to Eudragit S 100, which prevents degradation of the drug in the stomach and small intestine, and increased the availability of the drug in the distal small intestine and colon. In this region, the Eudragit S 100 coating was dissolved, but the positive charge present on the surface of liposomes and the penetration enhancer CPC helped in infiltration through the mucous layer and penetration through the tight junctions of epithelial cells.

CONCLUSION

Oral delivery of LMWH was challenging owing to its low bioavailability, but oral delivery was required to a great extent to increase patient compliance. Liposomes were chosen in this regard, but it was observed that there was not much increase in bioavailability. As a result, Eudragit S 100-coated LMWH liposomes were formulated, which helped in preventing the effect of acidic pH conditions, in enzymatic activity, and improved drug permeation. They have also proven their utility as oral delivery systems with a significant increase in bioavailability. Liposomes suitably interacted with the mucous layer and further opened the tight junctions with the help of a penetration enhancer to improve the therapeutic effect. The study suggests that Eudragit S 100-coated liposomes could be successfully explored for the oral delivery of LMWH.

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Conflicts of interest

There are no conflicts of interest.

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