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Research Article

NOVEL NANOPARTICLES FOR THE ORAL DELIVERY OF LOW MOLECULAR WEIGHT HEPARIN: *IN VITRO* AND *IN VIVO* ASSESSMENT

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ABSTRACT

Objective: The objective of the present study was to prepare and evaluate a novel oral formulation of nanoparticles for the systemic delivery of low-molecular-weight heparin (LMWH).

Methods: Nanoparticles were prepared by polyelectrolyte complexation method using polymers, i.e., sodium alginate and chitosan (CH). Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), entrapment efficiency, *In vitro* release and scanning electron microscopic studies were carried out for nanoparticles. *Ex vivo* permeation studies were performed with optimized formulation using small intestine of rat and *in vivo* studies were conducted on rat model.

Results: Entrapment efficiency of LMWH in nanoparticles was found to be 88%. *In vitro* release studies demonstrated that the release of LMWH was negligible in the stomach and high in the small intestine. FTIR has indicated that there is no interaction between the ingredients in nanoparticle. DSC and XRD studies confirmed that the amino groups of CH interacted with the carboxylic groups of alginate. *In vitro* % drug release of 95% was shown by formulation AC5. *Ex vivo* permeation studies have elucidated that ~73% of LMWH was transported across the epithelium. Nanoparticles have shown enhanced oral bioavailability of LMWH as revealed by 4.5-fold increase in area under the curve of plasma drug concentration-time curve.

Conclusion: The results suggest that the nanoparticles prepared can result in targeted delivery of LMWH into systemic circulation through intestinal and colon routes. Novel nanoparticles thus prepared in this study can be considered as a promising delivery system.

Keywords: Antifactor Xa activity, Chitosan, Differential scanning calorimetry, Sodium alginate, Low-molecular-weight heparin, Oral bioavailability.

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INTRODUCTION

Low-molecular-weight heparin (LMWH) is used as an anticoagulant in mostly two common post-surgical complications such as deep vein thrombosis and pulmonary embolism. It is used for long-term dosing through parenteral route and hence has reduced patient compliance. This can be improved by administering LMWH through the oral route. However, its clinical application through oral route is limited because of its large molecular size, high negative charge, instability in gastrointestinal region, and high water solubility. However, several studies addressed oral delivery of LMWH into systemic circulation. Various approaches have been investigated such as microemulsions, nanoparticles, microparticles, pellets, and chemical conjugates [1-5]. Yet, no oral formulation is able to produce good oral bioavailability and is not available in the market yet now. Since LMWH is permeable in the lower small intestine and colon, its penetration enhancement can be conveniently used to produce a market viable oral formulation for LMWH. Subsequently, in the present study, attempts were made to prepare and evaluate LMWH nanoparticles.

Nanoparticles were selected as they have the following advantages (a) they offer protection against acidic environment and enzymatic degradation, (b) its small size and the bioadhesive polymers used help in prolonging the gastric transit and permeating the mucous membrane. Particle size (1000 nm) is considered as a crucial parameter for bioadhesion and adsorption through the mucosal membrane. Paracellular and endocytic pathway are the major transport mechanisms for the transport of nanoparticles which majorly depended on particle size [6]. Various types of nanoparticles have been studied earlier [7,8]. Polyelectrolyte complexation (PEC) method is selected for preparation of nanoparticles as these are considered as the emerging delivery systems for the oral delivery of proteins, nucleic acids [9,10], etc. A combination of chitosan (CH) and alginate was used as the polyelectrolyte combination. Previously studies indicated that such nanoparticles are gastric resistant. Further, the natural bioadhesive property of the combination can lead to more residence on the mucosal membrane which in turn can result in significant systemic LMWH.

PEC is formed by spontaneous interaction of oppositely charged polysaccharides in aqueous solution. Polysaccharides are of particular importance due to their desirable biocompatible, biodegradable, hydrophilic, and protective properties. PEC method has the advantage of not using sonication and organic solvents which are harmful for proteins and polysaccharides. Previous investigations confirmed that CH and alginate were widely used for PEC formation and they have profound applications in drug or gene delivery systems in biomedicine [11,12].

CH is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) [13]. It is a biocompatible, biodegradable polymer, having inherent mucoadhesive properties with the capacity to open tight junctions in the mucosal membrane. The positive charge of

CH forms a strong bond with the negatively charged epithelial lining of gastrointestinal tract. This high interaction helps in opening the tight junction and makes the drug to reach the systemic circulation. However, it is mostly soluble in acidic pH conditions of the stomach, which makes it to lose its mucoadhesive and permeation enhancing properties. To overcome this, in the present study, a complex was formed between CH and a natural polyanion to improve the physicochemical property of CH. One of the polyanionic polymers that are widely used is sodium alginate (SA).

SA is an anionic polysaccharide distributed widely in the cell walls of brown algae. It is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D- mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, covalently linked together in different sequences or blocks. The negatively charged carboxylic acid groups of mannuronic and guluronic acid units of alginate interact electrostatically with positively charged amino groups of CH to form polyelectrolyte. At a pH of 2.5, SA is negatively charged. As the pH increases from 2.5, the number of anionic charges on the SA was increased. While at pH <6.5, CH is positively charged. The pH values, i.e. 4.0 for CH and 6.5 for SA ensures an increased charge density on each polymer and leads to intense cross-linking on mixing [14]. SA is mostly used as polyelectrolyte that forms complex with CH because the complex formed between the polymers is still biodegradable, biocompatible, and mechanically stronger in acidic pH conditions where CH dissolves. A research work has demonstrated that CH alone was effectively degraded by lysozymes [15,16]. However, this enzyme effect on PEC is negligible because of the strong interaction between the two polymeric chains.

In this study, PEC is formed by one-stage process. In this method, one polymer solution of a particular pH is added dropwise to the other polymer solution of a certain pH under high shearing conditions. In the present work, LMWH loaded PEC nanoparticles were formulated and it's *in vitro* release and *in vivo* absorption are compared with LMWH CH nanoparticles. Such nanoparticles for oral delivery of LMWH were not reported previously.

MATERIALS AND METHODS

Materials

Enoxaparin sodium (LMWH) was purchased from Bharath Biotech, Hyderabad, India. CH, sodium tripolyphosphate (STPP), dialysis membrane (MW 12,000 Daltons), and cetyl pyridinium chloride were purchased from Sigma-Aldrich Private Limited, Mumbai, India. SA was purchased from SD Fine Chemicals Limited, Gujarat, India. Stachrom Heparin supplied by Diagnostica Stago, Asnieres-sur-Seine, France. All the other chemicals and reagents were of analytical grade and used as supplied. Simulated gastric fluid pH 1.2, simulated intestinal fluid of pH 6.8 and 7.4 were prepared by referring to official methods as specified in USP (XXV).

Methods

Reagents used for analysis

To 1 ml of standard solution, 1 ml of 1 M acetate buffer of pH 5 and 4 ml of cetyl pyridinium chloride solution (0.1%) in sodium chloride (0.94%) were added and reacted for 1 hr. Samples were then analyzed at 500 nm in ultraviolet (UV)-visible spectrophotometer [17].

Preparation of PEC nanoparticles of alginate and CH

SA solution was prepared by dissolving SA in reverse osmosis (RO) water. CH solution was prepared by dissolving CH in 1% acetic acid solution. Both the solutions were placed separately on magnetic stirrer at 100 rpm for 1 hr. LMWH (20 mg) was then added to SA solution and dissolved. SA solution was adjusted to pH 6.5 and CH solution to pH 4.0. SA solution is then slowly added to CH solution at a flow rate of 1 ml/s with the help of high-speed homogenizer (Unidrive X1000D Homogenizer drive, CAT scientific laboratory, California) at 10,000 rpm. Alginate in CH PECs was thus formed [10].

Preparation of CH nanoparticles

Nanoparticles were prepared by ion gelation of CH with STPP aqueous solution. First, CH was dissolved in 1% solution of acetic acid. LMWH was then added to CH solution and mixed. Aqueous solution of STPP was added dropwise to CH solution by magnetic stirring at room temperature using high-speed homogenizer [18].

In vitro characterization of PEC nanoparticles

PEC nanoparticles and its formulation components were subjected to Fourier transform infrared (FTIR) (Bruker Alpha-E spectrophotometer, Ettlingen, Germany), differential scanning calorimetry (DSC) (automatic thermal analyzer, DSC 910S, TA Instruments, America), X-ray diffraction (XRD) (X'Pert-PRO multipurpose X-ray diffractometer, PANalytical, Tokyo, Japan), scanning electron microscopy (LEO 435 VP, Eindhoven Netherlands), and particle size and zeta potential studies (Zetasizer, Model 3000 HSA, Malvern Instrument, WR14 1XZ, UK).

Entrapment efficiency (EE)

The EE of LMWH in PEC nanoparticles was performed by an indirect method. The amount of unentrapped LMWH in the nanoparticles suspension was analyzed by taking the supernatant after centrifugation at 15000 rpm for 20 minutes. Then, aliquots of 1 ml of the supernatant was taken and mixed with reagents as discussed previously in the methods and reacted for 1 hr. Later, the absorbance of these samples was taken at 500 nm using UV-visible spectrophotometer. The study was performed in triplicate, and the percentage EE was determined [19].

$$EE\% = \frac{\text{Total amount of LMWH taken} - \text{Free LMWH in the supernatant}}{\text{Total amount of LMWH taken}}$$

Percentage transmittance

The study was performed 1-3 hrs after the preparation of PECs. RO water was used as the blank and adjusted to 100% transmittance at 800 nm using UV-visible spectrophotometer [10]. The % transmittance of PEC nanoparticles was then measured.

In vitro release study

The *in vitro* release study of LMWH from drug loaded PEC nanoparticles was performed using the dialysis membrane method. LMWH PEC nanoparticulate suspension was filled in a dialysis bag (MW-12,000 Da) which was attached to a two-end opened boiling tube [20]. The boiling tube was then dipped in a beaker containing 50 ml of pH 1.2 buffer and placed on a magnetic stirrer for 2 hrs at 37±0.5°C and 50 rpm. The medium was then replaced with pH 6.8 buffer and after 3 hrs with pH 7.4 buffer. The release study was performed for 24 hrs. Aliquots of 0.5 ml were taken at regular intervals, i.e. 1, 2, 3, 4, 5, 6, 8, 10 up to 24 hrs and analyzed by using UV-visible spectrophotometer at 500 nm. The same experiment was performed for plain CH nanoparticles and analyzed.

The release data were then fitted to different kinetic models, and the best fit model was determined based on R^2 values.

Ex vivo drug release study

The amount of LMWH transported across the intestinal barrier was measured. Small intestine of male Wistar rat was removed and rinsed with normal saline solution. LMWH loaded PEC nanoparticulate suspension was filled in the membrane, and both ends were tied with a thread separately and fixed to a stand. The membrane was then introduced into 50 ml of 6.8 pH buffer in a beaker. The beaker was then placed on a magnetic stirrer at 37°C and 100 rpm. Aliquots of 1 ml were taken at intervals of 5, 15, 30, 45, 60, 90, 120 minutes up to 8 hrs. The samples were then analyzed by UV-visible spectrophotometer at 500 nm by the addition of reagents and reacted for 1 hr. Permeation studies were performed in triplicate [21].

Stability studies

The LMWH loaded PEC nanoparticles were studied for stability studies at various temperatures such as 4±1°C and 25±1°C and examined at regular time intervals for any change in particle size and drug content [19].

Oral absorption studies in rats

All animal studies were approved by the Institutional Ethical Committee, Warangal, registered under CPCSEA, India (IAEC no. 1047/ac/07/CPCSEA). Male Wistar rats (weighting 130±20 g) were taken and fastened overnight with free access to water before the administration of formulations. Three groups of animals were taken, each group containing six animals. Group 1 is considered as control which was a plain LMWH solution (50 mg/kg) and given orally to rats. Formulations such as LMWH loaded plain CH nanoparticulate suspension and PEC nanoparticles were administered orally to Group 2 and Group 3 rats, respectively, in the same dose. To Group 3, LMWH loaded PEC nanoparticles were administered orally in the same dose. Blood samples were withdrawn from the retro-orbital plexus at 0, 1, 2, 3, 4, and 5 up to 8 hrs. LMWH was quantified using an anti-factor Xa chromogenic assay. 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 studies were performed with a plain LMWH solution, LMWH CH nanoparticles, and LMWH PEC nanoparticles on Wistar rats using a laboratory method as specified in reference [22,23]. Briefly, formulations were administered to three groups of animals as mentioned above. At regular time intervals, i.e. 0, 1, 2, 3, 4 up to 8 hrs blood samples were collected and their clotting time was determined by capillary method.

RESULTS

PECs were frequently prepared by mixing two electrolyte solutions of opposite charges. Various quantities of alginate and CH were taken at desirable pH conditions and complexes were prepared (Table 1). The formation of PECs was confirmed by FTIR and DSC studies. From the FTIR spectrum of LMWH loaded PEC nanoparticles; it was observed that the asymmetric and symmetric carboxylate anion (COO^-) stretching was shifted from 1661 to 1638/cm and from 1450 to 1439/cm, respectively. Furthermore, the absorption band of CH was shifted from 1582 to 1546/cm and the broadening of band from 1510 to 1400/cm was attributed to the overlapping of amide of CH with carboxyl anion of alginate (Table 2). No characteristic bands of LMWH were observed in the spectrum of nanoparticulate formulation (Fig. 1). DSC confirms the presence of interaction between CH and alginate. As observed from the thermograms shown in Fig. 2, the endotherms of CH and SA were exhibited at 111.63°C and 130°C which indicates the evaporation of absorbed water. An endotherm of PEC nanoparticles was observed at 99.6°C which is lower than the endothermic peaks of CH and SA individually. This indicates that the hydrophilic groups in the PECs were more exposed possibly due to the formation of gaps after complexation. Exothermic peaks registered in 320.54°C, 253.58°C, and 281.4°C for CH, SA, and PEC nanoparticles, respectively, indicate that PEC nanoparticles have a peak value intermediate between the peaks of SA and CH.

This peak value was interpreted as an interaction between the two polymers [24]. XRD spectra of PEG nanoparticles have shown the presence of 2 peaks of SA and a large bump. This indicates that SA was exposed on the outer surface and concealed the CH groups. The large bump indicates the entrapment of drug and formation of PEC (Fig. 3).

Two types of nanoparticles were prepared, i.e., PEC nanoparticles and CH nanoparticles. CH nanoparticles were only used for comparison, in *in vitro* and *in vivo* studies. Morphology of the PEC nanoparticles was determined by scanning electron microscopy (SEM) study. The shape of the PEC nanoparticles was found to be almost spherical (Fig. 4).

From the Table 1, it was observed that most of the alginate (AG), CH PEC nanoparticles have good % entrapment efficiencies. With increase in concentration of CH, the EE was increased. This was because of the increased number of complexes that are formed between CH and AG. The formulation AC6 has shown higher % EE. One of the reasons behind this is the interaction of CH with AG, and the other was the interaction of CH with LMWH which was negatively charged leading to higher EE. For obtaining a qualitative measure of the size of PECs, percentage transmittance studies were performed for formulations AC3 to AC9 at 800 nm. At this wavelength, both AG and CH do not absorb any light [10]. Decrease in % transmittance was a measure of increased particle size (Table 3). This may be because of the repulsion between the excess positive charges provided by the CH molecule in nanoparticles.

From the *in vitro* release studies, it was observed that <1% of drug was released within 2 hrs in pH 1.2 buffer (Fig. 5). The complex was tough enough in the stomach because of the insolubility of AG at the particular pH [25]. In pH 6.8 and pH 7.4 buffer, a constant release of the LMWH was observed with a maximum release of 90% of the initial amount. CH nanoparticles have shown 31.45% release of the drug in 1.2 pH buffer, which was not seen in PEC nanoparticles. The results of release kinetics revealed that the mode of drug release from PEC nanoparticles followed the Higuchi model with r^2>0.8654 (Table 4). Data were also fitted into the Korsmeyer–Peppas equation to determine the drug release mechanism further. The n value indicates super case-II transport [26]. Formulation AC3 was considered less stable as it has shown aggregation and increased particle size within 10 days. Taking into consideration EE, *in vitro* release the formulations AC4, AC5, AC7, and AC8 were evaluated for particle size, zeta potential, and polydispersity index (Table 5). Particle size was found to increase with increase in CH concentration. The zeta potential values have shown a shift in charge on the surface of nanoparticles from higher to lower negative values. Polydispersity

Table 1:Composition and % EE of PEC nanoparticles

Formulation	LMWH (mg)	CH (mg)	SA (mg)	Acetic acid (%v/v)	Water (ml)	% EE
AC1	20	100	100	1	100	45.5±2.65%
AC2	20	200	100	1	100	50.5±2.73%
AC3	20	200	250	1	100	77.25±0.8%
AC4	20	300	150	1	100	72.26±1.1%
AC5	20	300	250	1	100	84.88±0.87%
AC6	20	300	400	1	100	88.15±0.9%
AC7	20	350	100	1	100	67.43±0.48%
AC8	20	350	200	1	100	80.86±0.63%
AC9	20	400	300	1	100	87.26±0.7%
AC10	20	500	100	1	100	Viscous fibrous preparation
AC11	20	500	200	1	100	Viscous fibrous preparation
AC12	20	500	300	1	100	Viscous fibrous preparation

Data are expressed as mean±SD (n=3). SD: Standard deviation, LMWH: Low molecular weight heparin, CH: Chitosan, SA: Sodium alginate, EE: Entrapment efficiency, PEC: Polyelectrolyte complexation

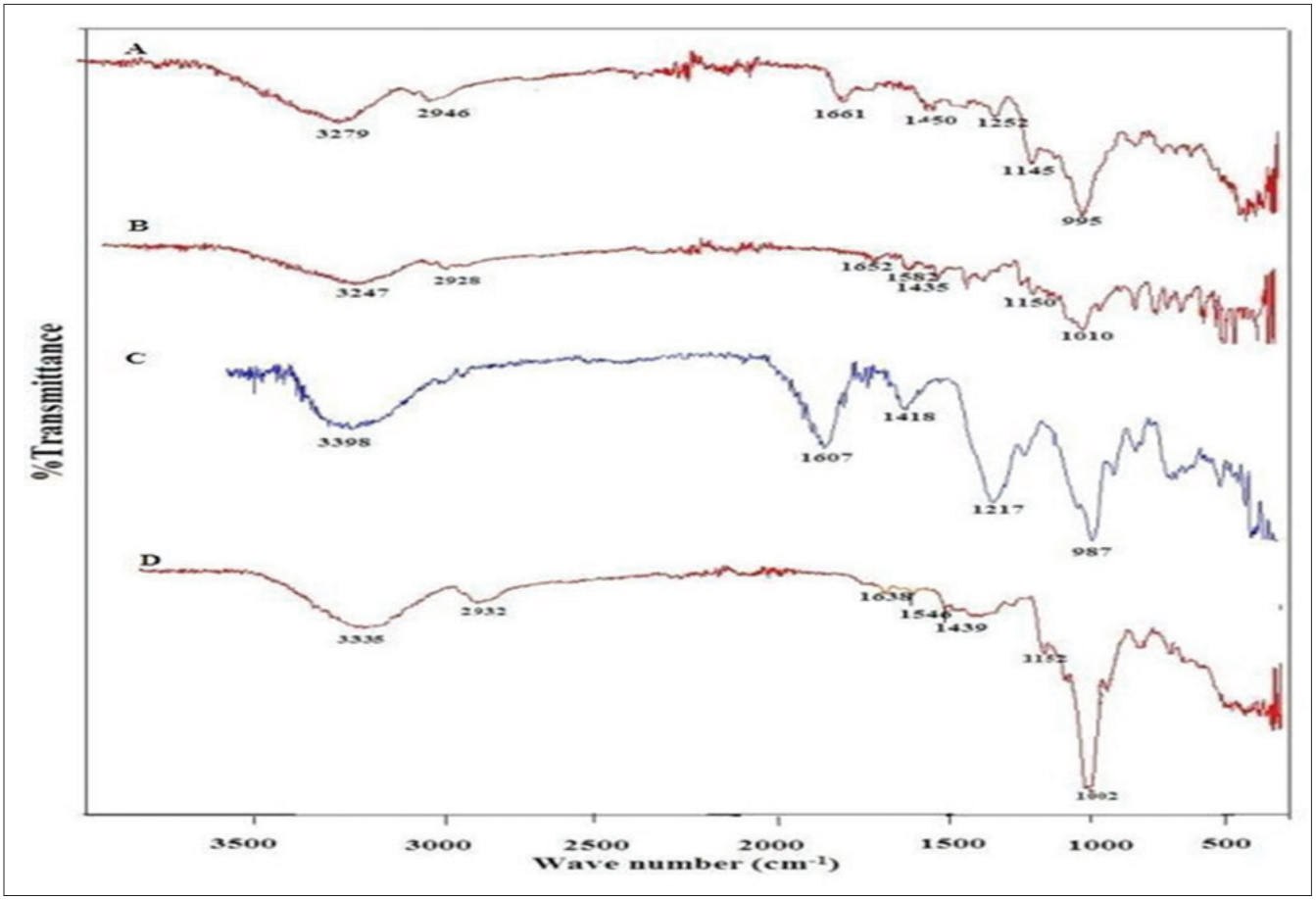

Fig. 1: Schematic diagram showing Fourier transform infrared spectra's of (a) Pure sodium alginate, (b) pure chitosan, (c) low-molecular-weight heparin (LMWH), (d) polyelectrolyte complexed nanoparticles of LMWH

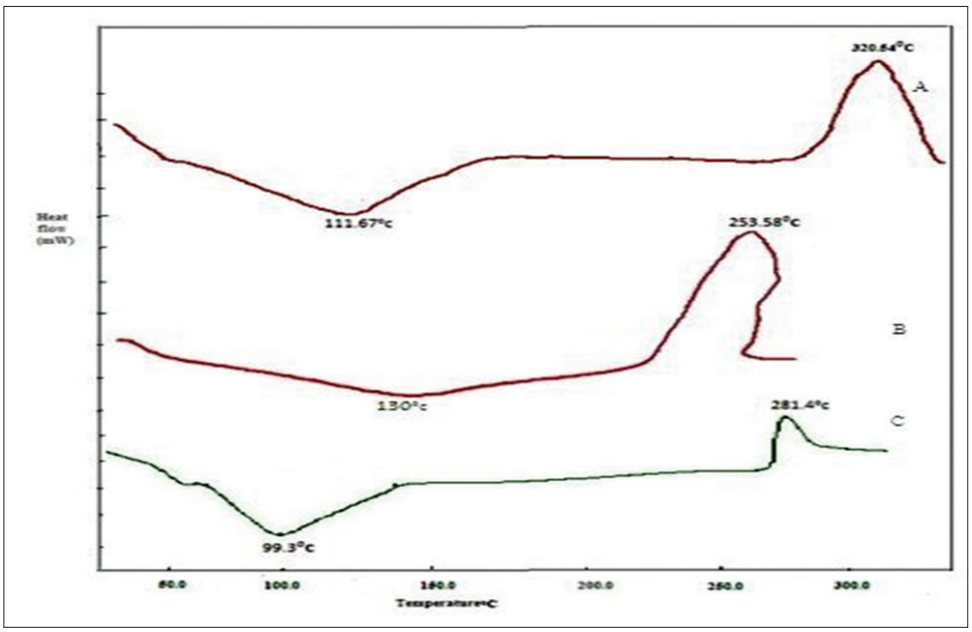

Fig. 2: Differential scanning calorimetry thermograms of (a) pure chitosan (CH), (b) pure sodium alginate, (c) polyelectrolyte complexed alginate/CH nanoparticles

index values <0.5 indicate that the formulation was homogeneous in nature.

In the results of *ex vivo* studies, 73% and 62% of the drug were found to cross the intestinal membrane for AC4 and AC5 formulations, respectively, and reach the buffer in 6 hrs time period (Fig. 6). The optimized nanoparticle formulation was subjected to stability studies at different storage temperatures in terms of particle size and EE. The nanoparticles showed better stability at 4°C than at 25°C in terms of increased particle size and decreased EE (Table 6).

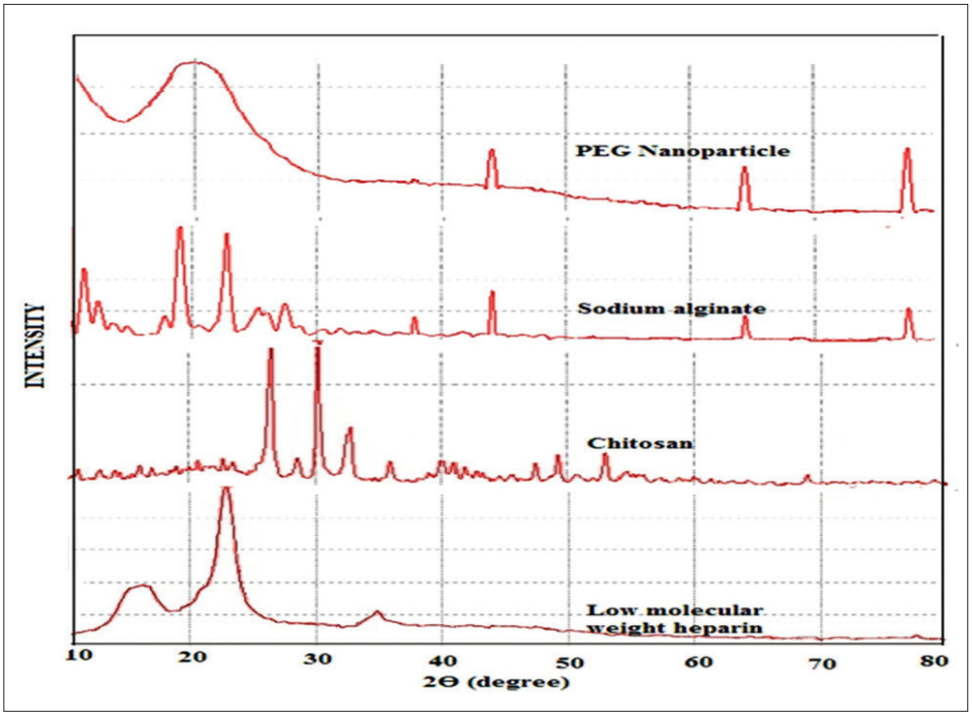

Fig. 3: X-ray diffraction patterns of polyelectrolyte complexation nanoparticles and its formulation ingredients

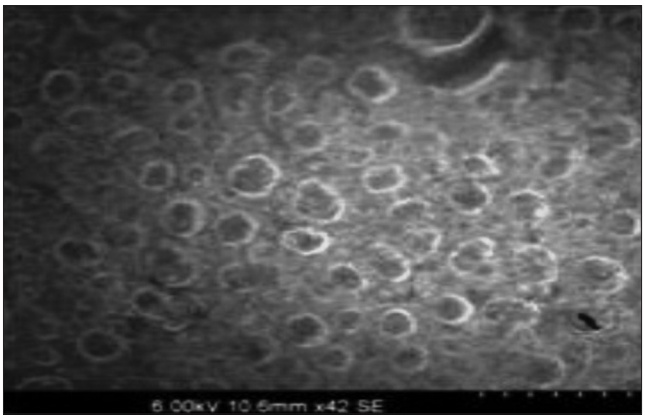

Fig. 4: Scanning electron microscopic micrographs of low-molecular-weight heparin loaded polyelectrolyte complexation nanoparticles

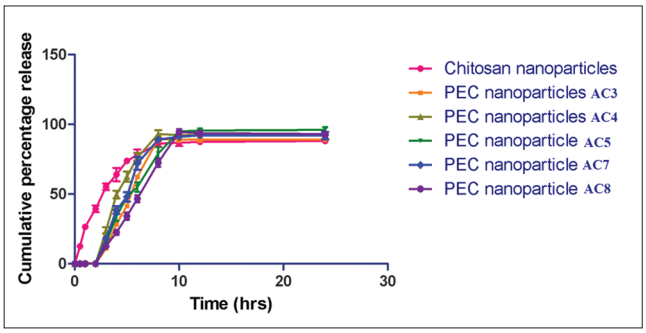

Fig. 5: *In vitro* release profile of low molecular weight heparin loaded polyelectrolyte complexation nanoparticles in different pH environments. Data represent mean±satndard deviation (n=6)

Pharmacokinetic parameters were determined from the plasma concentration-time profile of different formulations (Table 7).

Table 2: Important band frequencies in FTIR spectrum of drug, pure polymer, and nanoparticle

Wave number (cm^{-1})	Interpretation
Pure SA	
3279	O-H stretch
2946	C-H stretch
1661	Asymmetric carboxylate anion (COO^-) stretch
1450	Symmetric carboxylate anion (COO^-) stretch
1252	C-O stretch
1145	Bridge-O-stretch
997	C-O-C stretch due to saccharide structure
Pure CH	
3247	O-H stretch and N-H stretch
2928	C-H stretch
1652	Amide I NH CO stretch
1582	Amide II N-H bend
1435	C-N stretch
1150	Bridge-O-stretch
1010	C-O stretch
LMWH	
3398	O-H stretch
1607	N-H broadening
1418	C-H stretch of methyl group
1217	C-O stretch
987	
PEC nanoparticles of LMWH	
3335	O-H stretch
2932	C-H stretch
1638	Asymmetric carboxylate anion (COO^-) tretch
1546	Amide II N-H bend
1439	Symmetric carboxylate anion (COO^-) stretch
1152	Bridge-O-stretch
1002	C-O-C stretch

LMWH: Low molecular weight heparin, CH: Chitosan, SA: Sodium alginate, PEC: Polyelectrolyte complexation, FTIR: Fourier transform infrared

For LMWH plain oral solution, the C_{max} value was observed to be 0.10±0.007 IU/ml after 2 hrs of oral administration, while administration of CH nanoparticles enhanced the C_{max} to 0.16±0.02 IU/ml. However,

Table 3: Percentage transmittance study of alginate/CH formulations

Alginate/CH formulations	AC3	AC4	AC5	AC6	AC7	AC8	AC9
Percentage transmittance	90±1.23%	94±2.6%	92±1.79%	84±3.21%	89±1.4%	90±2.9%	80±1.1%

Data are expressed as mean±SD (n=3). SD: Standard deviation, CH: Chitosan

Table 4: Comparison of estimated parameters deduced from *in vitro* LMWH release curve of PEC nanoparticles in mixed pH media by fitting to various kinetic models

Formulation	Zero order	First order	Higuchi model	Hixon- crowell	Korsmeyer Peppas	
	r^2	r^2	r^2	r^2	r^2	n
AC3	0.4821	0.5692	0.8774	0.1753	0.9917	1.21
AC4	0.3991	0.4054	0.8654	0.1759	0.9802	0.89
AC5	0.5537	0.8772	0.9112	0.2862	0.9935	1.07
AC7	0.6029	0.5524	0.9019	0.2862	0.9848	1.51
AC8	0.5833	0.7472	0.8965	0.1926	0.9903	1.32

Data are expressed as mean±SD (n=3). SD: Standard deviation, LMWH: Low-molecular-weight heparin, PEC: Polyelectrolyte complexation

Table 5: Particle size, zeta potential, and polydispersity index of LMWH loaded PEC nanoparticles

Formulation	Particle size	Zetapotential	Polydispersity index
AC4	119±1.3 nm	−22±0.6	0.25±0.02
AC5	142±0.8 nm	−08±0.4	0.33±0.03
AC7	195±0.3 nm	−24±0.8	0.41±0.01
AC8	253±2.5 nm	−12±0.2	0.11±0.06

Data are expressed as mean±SD (n=3). SD: Standard deviation,
LMWH: Low-molecular-weight heparin, PEC: Polyelectrolyte complexation

the maximum value was observed for LMWH loaded PEC nanoparticles, which was 0.45±0.03 IU/ml. Similarly, area under the curve$_{0\text{-}8\,h}$ of LMWH was increased by 1.8 times for LMWH CH nanoparticles and 4.5 times for LMWH PEC nanoparticles when compared with a plain oral LMWH solution (Fig. 7). The results of clotting time studies have revealed an enhancement in clotting time (Fig. 8).

DISCUSSION

FTIR and DSC studies confirmed that the PEC was formed between AG and CH. DSC and XRD studies indicated that AG and CH were transformed to amorphous forms on complexation and LMWH was entrapped. SEM studies revealed that the formed nanoparticles were of spherical shape and the micrographs did not show the appearance of aggregated particles. Based on % EE and % transmittance studies, the formulations AC4, AC5, AC6, AC7, and AC8 were optimized. *In vitro* release studies indicate that PEC nanoparticles were successful in retarding the release of drug in acidic pH conditions and capable of releasing 90% of the drug in simulated intestinal fluid within 10 hrs time period. This release of LMWH in small intestine could be because of the interaction of AG with alkaline media and an increase in the solubility of AG [27].

The possible mechanism of drug release from LMWH PEC nanoparticles was found to be super case-II transport, which was considered as swelling-controlled release. It was observed that with an increase in concentration of CH the particle size was increased and the zeta potential value has shifted toward lower negative charges. The negative charge demonstrates that AG has formed complex with CH and helped in concealing CH from degradation in the gastric environment. *Ex vivo* permeation studies indicated that the enhanced transport of PEC nanoparticles was ascribed to the mucoadhesive property of CH and the size of nanoparticles [28].

One of the research articles demonstrated that LMWH loaded trimethyl CH nanoparticles have shown 2.4 times bioavailability of LMWH in comparison to plain LMWH solution [19]. Pharmacokinetic studies of the present study have shown an enhancement in oral

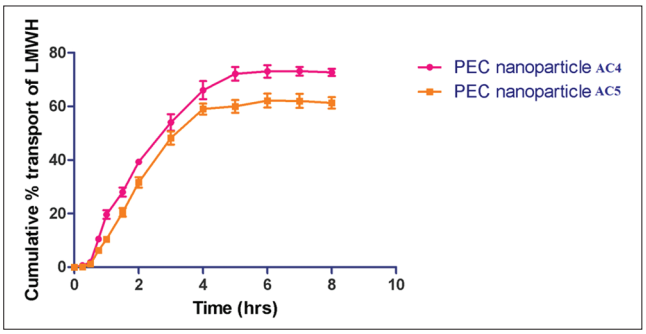

Fig. 6: Cumulative percent transport of low molecular weight heparin from polyelectrolyte complexation nanoparticles AC4 and AC5. Data represent mean±standard deviation (n=6)

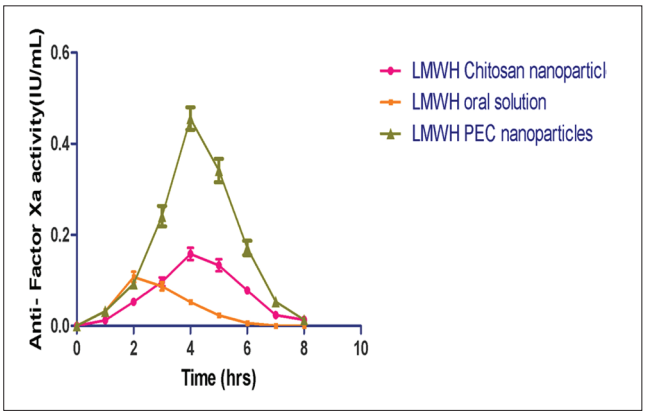

Fig. 7: Antifactor Xa activity profile of low-molecular-weight heparin (LMWH) oral solution, LMWH chitosan nanoparticles, and LMWH polyelectrolyte complexation nanoparticles after oral administration in equivalent dose of 50 mg/kg in Wistar rats. Data represent mean±standard deviation (n=6)

bioavailability of LMWH by 4.5 times when compared with a plain LMWH solution. This enhanced oral bioavailability was attributed to the interaction of AG and CH, which prevented the degradation of drug in gastric fluid. PEC nanoparticles then enter the small intestine where the AG gets slowly dissolved exposing the amino groups of CH. These amino groups get attached to the negative charge of epithelial lining and help with paracellular transport across the intestinal epithelium. This results in delivery of the drug to systemic circulation without damaging the intestinal epithelium. The *in vitro* and *in vivo* evaluation of PEC nanoparticles demonstrates that the

Table 6: Effect of storage temperature on particle size and EE of LMWH loaded PEC nanoparticles

Formulation	Particle size (nm)			EE (%)		
	0 day Room temperature	After 60 days		0 day Room temperature	After 60 days	
		4±1°C	25±1°C		4±1°C	25±1°C
PEC nanoparticle	105±1.3	119±3.9	276±2.6	80±3.4	74±2.1	66±4.2

Data are expressed as mean±SD (n=3). SD: Standard deviation, LMWH: Low-molecular-weight heparin, PEC: Polyelectrolyte complexation, EE: Entrapment efficiency

Table 7: Pharmacokinetic parameters of LMWH nanoparticles after oral administration in Wistar rats

Pharmacokinetic parameters	LMWH oral solution	LMWH CH nanoparticle	LMWH PEC nanoparticle
Dose (mg)	10	10	10
C_{max} (IU/ml)	0.10±0.007	0.16±0.02	0.45±0.03
T_{max} (h)	2	4	4
$AUC_{0-8\,h}$ (µg h/ml)	0.31±0.006	0.56±0.04	1.397±0.24
MRT (hr)	2.81±0.014	4.24±0.012	4.36±0.11
t_{half} (hr)	0.56±0.002	0.72±0.03	0.744±0.06

Data are expressed as mean±SD (n=6). MRT: Mean residence time, AUC: Area under the curve, LMWH: Low-molecular-weight heparin, PEC: Polyelectrolyte complexation, CH: Chitosan

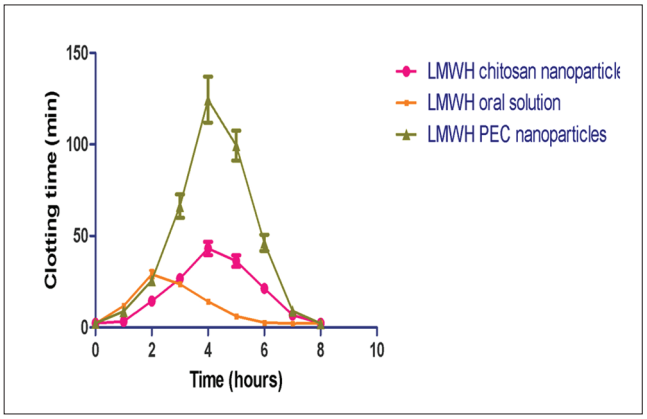

Fig. 8: Clotting time profile of low molecular weight heparin (LMWH) oral solution, LMWH chitosan nanoparticles and LMWH polyelectrolyte complexation nanoparticles after oral administration in equivalent dose of 50 mg/kg in wistar rats. Data represents mean±standard deviation (n=6)

nanoparticulate system can be considered as a useful oral delivery system for enhancing the bioavailability of LMWH.

CONCLUSION

The approach to the oral delivery of LMWH is of utmost importance to prevent the invasive delivery. CH nanoparticles can be considered for oral delivery, but the high solubility of CH in acidic pH conditions makes it less suitable for oral administration. Hence, PEC nanoparticles were developed with AG and CH to overcome the limitations associated with plain CH nanoparticles. The developed PEC nanoparticles have shown an enhanced oral bioavailability in comparison with a plain LMWH solution. The present investigation suggests that the AG complexed CH nanoparticles could be effectively explored for the oral delivery of LMWH.

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