Formulation and evaluation of novel chitosan-alginate nanoparticles for oral delivery of low molecular weight heparin

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ABSTRACT:

Background: The aim of this study was to create a new oral delivery system for low molecular weight heparin (LMWH) by employing chitosan-alginate based nanoparticles. Although LMWH is typically given by injection, oral administration may increase patient convenience and compliance. The preparation and assessment of nanoparticles encapsulating LMWH utilizing chitosan-alginate in the polyelectrolyte complexation method were the main objectives of the study.

Materials and methods: Nanoparticles encapsulating LMWH were prepared using the polyelectrolyte complexation method with chitosan-alginate. The size, shape, and zeta potential of the nanoparticles were characterized. Scanning electron microscopy (SEM) was employed to confirm spherical particle formation with a smooth surface. The size of the nanoparticles ranged between 415-485 nm. The % entrapment efficiency of LMWH within the nanoparticles was determined to be between 46.5% to 80.5%. In vitro drug release studies were conducted to assess the release profile.

Results: SEM confirmed spherical nanoparticles (415-485 nm) with high LMWH entrapment efficiency (45.3% to 83.2 %). Optimized formulations achieved 85% drug release within 24 hours. Compared to enoxaparin sodium, these nanoparticles showed improved pharmacokinetics post oral administration.

Conclusion: In efficiently encasing LMWH, chitosan-alginate nanoparticles provide improved oral administration through colon targeting. They have the potential to improve patient convenience without affecting therapeutic efficacy as a substitute to injectable LMWH. It is recommended to carry out considerable optimization and clinical validation.

Key Words: Nanoparticles, LMWH, Scanning electron microscopy, pharmacokinetics

Novel Drug Delivery Systems (NDDS)

Refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body to safely achieve its desired therapeutic effects. NDDS is a system used for delivery of a drug other than conventional drug delivery systems. It is a combination of advance techniques and new dosage forms which are far better than conventional dosage forms. Some advantages of novel drug delivery systems are: optimum dosing at the right time and right location, efficient use of expensive drugs, reduction in production cost, beneficial to patients, and improved comfort and standard of living.

STABILITY AND STORAGE CONSIDERATIONS OF NANOPARTICLES

- The stability of chitosan-alginate nanoparticles is a key concern in their application fordrug delivery systems. Chitosan, a natural biopolymer derived from chitin, and alginate,a polysaccharide extracted from brown seaweed, are often used together to create nanoparticles with desirable properties for drug delivery. These nanoparticles benefit from the synergistic effects of both polymers, which contribute to their stability and efficacy.
- The stability of chitosan-alginate nanoparticles can be influenced by several factors, including storage conditions, the presence of stabilizers, and the intrinsic properties ofthe nanoparticles themselves. Over time, nanoparticles can face issues such as aggregation, which can lead to changes in size, distribution, and overall performance. Aggregation can occur due to various reasons, including interactions between nanoparticles or with external factors such as temperature, pH, and ionic strength.

ANTICOAGULANTS

The clinically useful anticoagulants produce their pharmacological response by interfering with plasma clotting factors, inhibiting platelet aggregation, or dissolving clot. The mechanism of anticoagulation determines the onset and duration of drug action. Heparin has a quick onset and duration of action because the anticoagulant effect occurs as soon as the thromboplastin-drug complex is formed. Warfarin has a long onset and duration of action because it takes days to clear the normal clotting factors before an effect can be observed.

AIM

- The primary aim is to formulate and evaluate an effective oral delivery system for low molecular weight heparin (LMWH) using chitosan-alginate nanoparticles to overcome the limitations of its parenteral administration and improve its bio availability

OBJECTIVE

- To formulate nanoparticle using polyelectrolyte complexation method
- To characterize nanoparticle with respect to particle size, surface morphology, entrapment efficiency, zeta potential.
- To characterize chitosan nanoparticle using polyelectrolyte complexation method with respect to in- vitro drug release.
- To optimize the formulation parameters, including to achieve desired particle size, encapsulation efficiency and drug release profile
- To select the best formulation based on in-vitro studies

To perform stability studies on the most satisfactory formulation

DRUG PROFILE

HEPARIN

- Heparin is a heterogeneous group of straight-chain anionic mucopolysaccharides, called glycosaminoglycans, possessing anticoagulant properties.

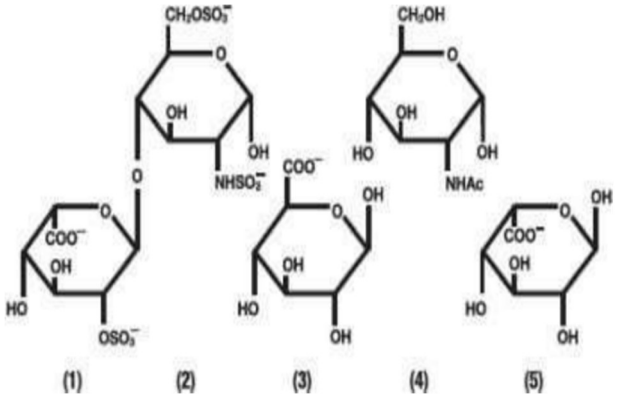
COMPOSITION:

- It is composed of polymers of alternating derivations of α-D-glucosamido (N-sulfatedO-sulfated O-sulfated or N-acetylated) and O-sulfated uronic acid (α-Liduronic acid orβ-D-glucoronic acid).

STRUCTURE

USES:

- To prevent blood clots or keep on exiting clot from getting worse
- Deep vein thrombosis



EXCIPIENTS PROFILE

CHITOSAN

- It is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D- glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).
- Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) andcell walls of fungi.

Non -proprietary names:

BP : chitosan hydrochloride

Synonyms : 2-Amino-2deoxy-(1,4) -d-glucopranan, deacetylated chitin.

Chemical name : Poly-(1-4)-2-Amino-2-deoxy-ß-D-Glucan

Molecular weight : On average, the molecular weight between 3800 and . . . 20,000Daltons.

Molecular Formula : (C6H11O4N) n

SODIUM ALGINATE

SYNONYMS Algin,Alginic acid sodium salt,Kelp gum.

DEFINITION Sodium alginate is the sodium salt of alginic acid.

Structural formula

Structural formula from Phillips, Wedlock and Williams: Gums and Stabilizers for the Food Industry5 (1990) by permission of Oxford University Press.

MATERIALS

Table: 1 list of chemical use

S.NO	INGREDIENTS	VENDOR
1.	Sodium alginate	Sd fine chemicals limited
2.	Chitosan	Sd fine chemicals limited
3.	Acetic acid	Sd fine chemicals limited
4.	Low molecular weight heparin	Gland pharma Pvt Ltd
5.	Sodium tripoly phosphate(STPP)	Sigma-aldrich chemicals limited
6.	Cetyl pyridinium chloride	Sigma-aldrich chemicals limited

Table: 2 list of equipment used

S.NO	EQUIPMENT	MODEL
1.	UV VISIBLE SPECTROPHOTOMETER	THERMO SCIENTIFIC
2.	COOLING CENTRIFUGE	HITTECH
3.	JSM - 5200 SCANNING ELECTRON MICROSCOPE(SEM)	MIKRO
4.	ZETASIZER NANO (ZS)	MALVERN UK
5.	FOURIER TRANSFORMS INFRARED SPECTROPHOTO METER	PERKINEL MERP

EXPERIMENTAL METHODS

PREFORMULATION STUDIES

(a)Fourier transform infrared spectroscopy (FTIR):

- FTIR spectrum of drug, polymers, physical mixture, and formulations were obtained on FTIR instrument. Sample about 2 mg was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 Psi for 3 minutes. The resultant disc was mounted in a suitable holder in Shimadzu IR spectrophotometer and the spectrum was scanned over the wave number range of 4000-400 cm-1. IR helps to confirm the identity of the drug and to detect the interaction of the drug with the carriers.

(b) Differential Scanning colorimetry (DSC):

- Differential Scanning colorimetry was used to determine drug excipient compatibility studies and used to observe more phase changes such as glass transition, crystallization, amorphous forms of drugs and polymers. The physical state of drugs and polymer was analyzed by Differential Scanning calorimeter (Schimadzu). Approximately 5 mg of sample was analyzed in an open aluminum pan and heated at scanning rate of 10°C/minbetween 0°C and 400°C. Magnesia was used as the standard reference material.

Solubility studies

- Solubility in different solvents:determine the solubility of LMWH in various solvents to select appropriate solvents for nanoparticle preparation
- Ph-solubility profile : assess the solubility of LMWH at different pH values to understand its behavior in the gastrointestinal tract

Powder blending

- blend physical mixtures of LMWH and excipients changes (color, odor, phase, separation) over time

PREPARATION STUDIES

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%) wereadded and reactedfor 1 hr. Samples were then analyzed at 500 nm in ultraviolet (UV)-visible spectrophotometer.

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. Boththe solutions were placedseparately on magnetic stirrer at 100 rpm for 1 hr.LMWH (20 mg) was then added to SA solution and dissolved. SA solutionwas adjusted to pH 6.5 and CH solution to pH 4.0. SAsolution is then slowlyadded 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

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 solutionand mixed. Aqueous solution of STPP was added dropwise to CH solution by magnetic stirring at room temperature using high-speed homogenizer.

Table 3: Formulation of LMWH Nanoparticles

Formulation	F1	F2	F3	F4	F5	F6
LMWH (mg)	20	20	20	20	20	20
Chitosan (mg)	100	50	100	100	150	150
Sodium Alginate (mg)	0	50	50	125	75	125
Acetic acid (V/V)	1	1	1	1	1	1
Water (ml)	50	50	50	50	50	50

EVALUATION STUDIES

Size and surface analysis:

- The mean diameter of nanoparticles and their surface potential were evaluated with zeta sizer. Surface morphology was evaluated using scanning electron microscopy (SEM).

Scanning Electron Microscopy:

- To study the particle surface morphology and shape of the chitosan-alginate nanoparticles, scanning electron microscopy (SEM) was employed. The process began by preparing a concentrated aqueous suspension of the nanoparticles. This suspension was carefully spread over a flat slab and allowed to dry under a vacuum, ensuring the removal of any excess moisture and reducing the risk of sample contamination or distortion.
- After drying, the sample was subjected to a process called shadowing in a cathodic evaporator. This involved depositing a thin gold layer, approximately 20 nanometers in thickness, onto the sample. The gold layer serves as a conductive coating that enhances the contrast in SEM images, allowing for clearer visualization of the particle surfaces and morphology.
- The SEM analysis was conducted using a JSM-5200 Scanning Electron Microscope, manufactured by JEOL in Tokyo, Japan. The microscope was operated at an accelerating voltage of 20 kV, which provides high-resolution imaging of the nanoparticles. To ensure accurate assessment of the surface morphology, the smallest size nano suspension was utilized, as it offers the most detailed representation of particle shape and structure. The resulting photographs from the SEM provided critical insights into the surface characteristics and uniformity of the nanoparticles, which are essential for evaluating their suitability for drug delivery applications.

Zeta Potential:

- The zeta potential is a crucial parameter used to assess the electric charge on the surface of particles, providing valuable information about the physical stability of colloidal systems. In this study, the zeta potential was measured to evaluate the stability and dispersion characteristics of the chitosan-alginate nanoparticles.
- To determine the zeta potential, the electrophoretic mobilityof the nanoparticleswas firstmeasured. This involved analyzing the movement of the particles in anelectric field, which reflects their surface charge and interaction with thesurrounding medium. The zeta potential gives insight into the electrostatic repulsion or attraction between particles, which is fundamental in predicting thelikelihood of aggregation or dispersionin a colloidal system.
- For the measurement, a Zetasizer Nano ZS instrument from Malvern Instruments (Malvern, UK) was employed. This device is designed to provide precise and reliable zeta potential measurements. The procedure involved preparing a suspension of the nanoparticles and then adding this suspension to a sample cell, specifically a quartz cuvette, which is designed to minimize

interference with the measurement. Once the sample was loaded into the cell, itwas placed in the sample holder unit of the Zetasizer Nano ZS. The instrumentthen applied an electric field to the sample and measured the resulting electrophoretic mobility of the nanoparticles. From this data, the zeta potentialwas calculated, providing insights into the electrostatic stability of the nanoparticles. A higher absolute value of zeta potential generally indicates better stability and reduced risk of particle aggregation, crucial for ensuring the effective performance of the nanoparticles in drug delivery applications.

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 takingthe supernatant after centrifugation at 15000 rpm for 20 minutes. Then, aliquots of 1 mlof the supernatant was taken and mixed with reagents as discussed previously in the methods and reacted for 1 hr. Later, theabsorbance of these samples was taken at 500 nm using UV-visible spectrophotometer. The study was performed in triplicate, and thepercentage EE was determined.

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

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. The boiling tube wasthen dipped in a beaker containing 50 mlof pH 1.2 buffer and placed on a magnetic stirrer for 2 hrs at 37±0.5°C and 50 rpm. Themedium was then replaced with pH6.8 buffer and after 3 hrs with pH7.4 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 todifferent kinetic models,and the best fit model was determined based on R2values

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 anychange in particle size and drug content.

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 1661to 1638/cm and from 1450 to 1439/cm, respectively. Furthermore, the absorption bandof 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 PECnanoparticles, 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 XRD spectra of PEG nanoparticles have shown the presence of 2 peaks of SA anda 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 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 thatare formed between CH and AG. The formulation AC6 has shown higher % EE. One ofthe 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 AGand CH do not absorb any light. 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 within2 hrs in pH 1.2 buffer (Fig. 5). The complex was tough enough in the stomach becauseof the insolubility of AG at the particular pH . 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 ofdrug 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. Formulation AC3 was considered less stable as it has shown aggregation and increasedparticle 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 (Tab.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 index values <0.5 indicate that the formulationwas homogeneous in nature.

Table 4 :Composition and % EE of PEC nanoparticles

Formulation	LMWH (mg)	CH (mg)	SA (mg)	ceticacid Wa (%v/v)	Ter (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			250	1		
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

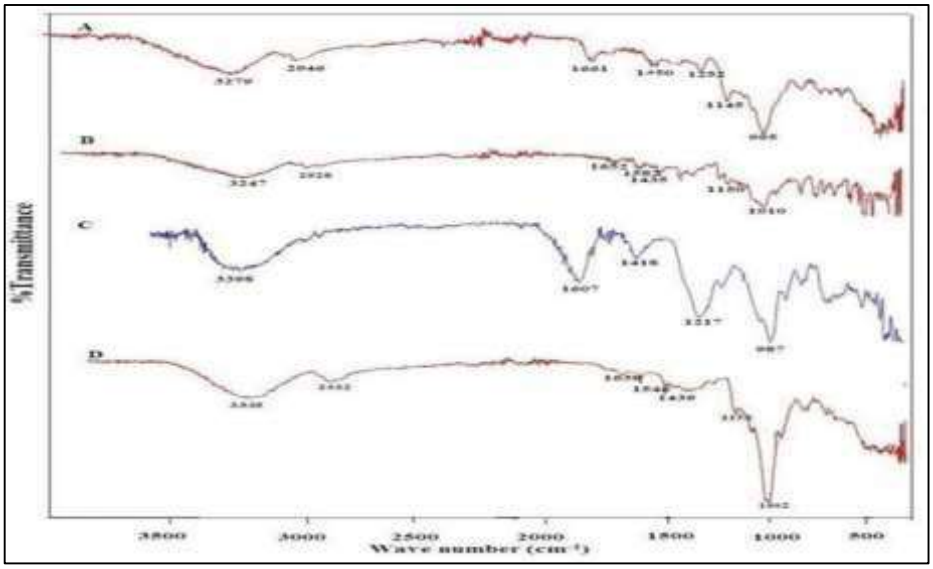


Fig. 1: Schematic diagram showing Fourier transform infrared spectra's of (a) Pure sodiumalginate, (b) pure chitosan, (c) low-molecular- weight heparin (LMWH), (d) polyelectrolyte complexed nanoparticles of LMWH

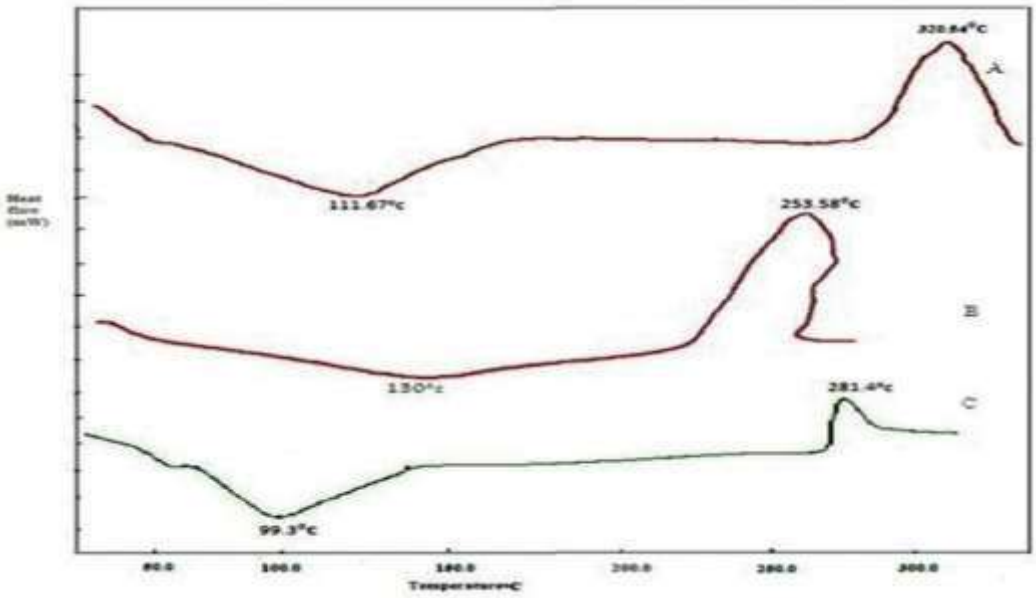


Fig. 2: Differential scanning calorimetry thermograms of (a) pure chitosan (CH),(b) pure sodium alginate, (c) polyelectrolyte complexed alginate/ CHnanoparticles

The optimized nanoparticle formulation was subjected to stability studies at differentstorage temperatures in terms of particle size and EE. The nanoparticles showed betterstability at 4°C than at 25°C in terms of increased particle size and decreased EE (Table6).

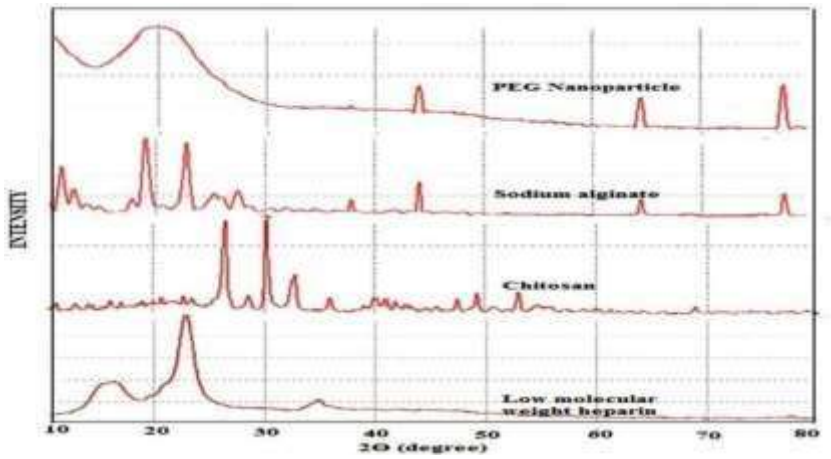


Fig. 3: X-ray diffraction patterns of polyelectrolyte complexation nanoparticlesand itsformulation ingredients

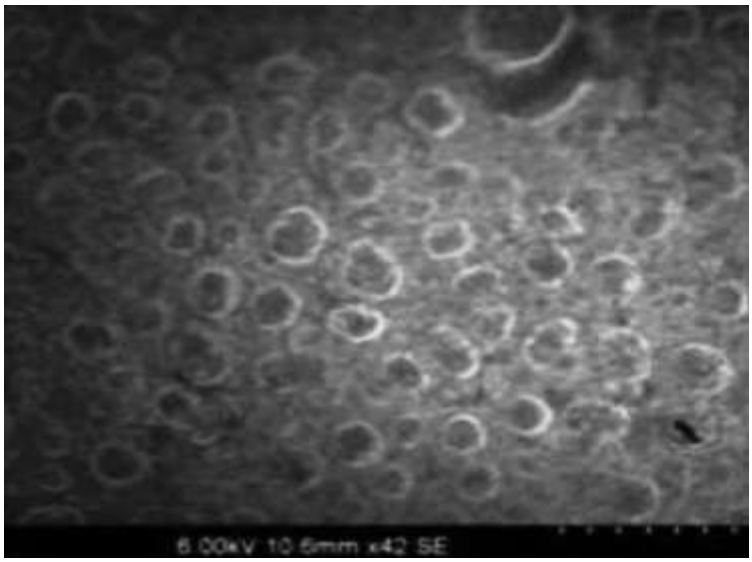
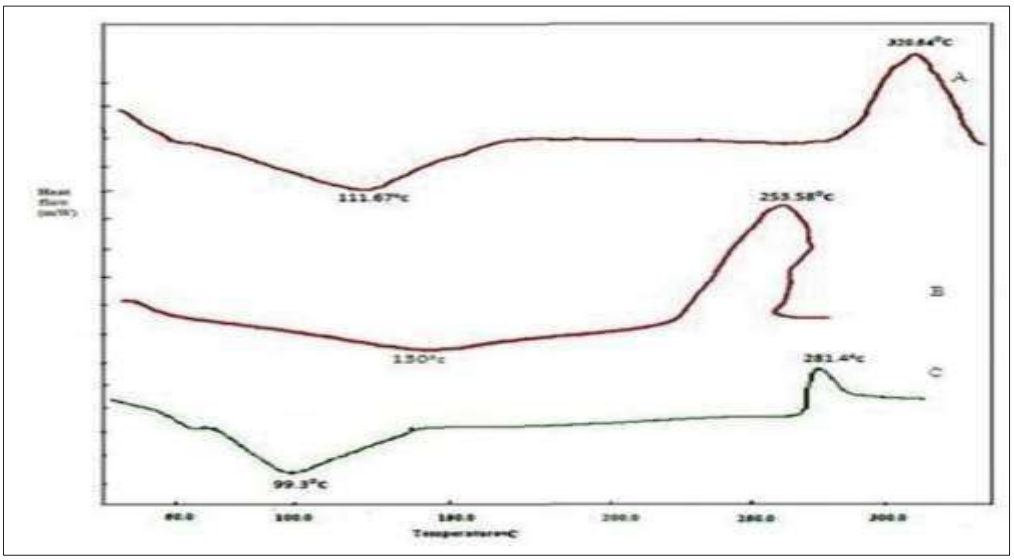


Fig. 4: Scanning electron microscopic micrographs of low- molecular-weightheparin loaded polyelectrolyte complexation nanoparticles



polyelectrolyte complexation nanoparticles in different pH environments. Data represent mean±satndard deviation (n=6)

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

Pharmacokinetic parameters were determined from the plasmaconcentration-time profile of different formulation

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,the maximum value was observed of

LMWH loaded PEC nanoparticals, which was 0.45±0.03IU/ml.

Table 6: 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 7: Comparison of estimated parameters deduced from *in vitro* LMWHrelease 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	
	r2	r2	r2	r2	r2	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 8: Particle size, zeta potential, and polydispersity index of LMWH loadedPEC nanoparticles

Formulation	Particle size	Zetapotential	Polydispersityindex
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. Data represent mean±standard deviation (n=6)

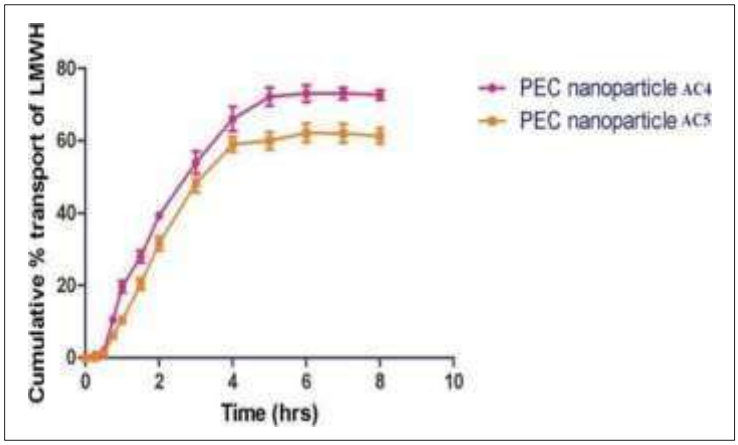


Fig. 6: Cumulative percent transport of low molecular weight heparin frompolyelectrolytecomplexation nanoparticles AC4 and AC5. Data represent

mean±standard deviation (n=6)

Table 9: Effect of storage temperature on particle size and EE of LMWH loadedPEC nanoparticles

Formulation	Particle size (nm)			EE (%)		
	0 day	fter 60days		0 day	after 60days	
	Room temperature	4±1°C	25±1°C	Room temperature	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.

DISCUSSION

- FTIR and DSC studies confirmed that the PEC was formed between AG andCH. DSCand XRD studies indicated that AG and CH were transformed to amorphousforms on complexation and LMWH was entrapped.
- SEM studies revealed that theformed nanoparticles were of spherical shape and the micrographs did not show theappearance of aggregated particles. Based on % EE and
- % transmittance studies, the formulations AC4, AC5, AC6, AC7, and AC8 wereoptimized.
- *In vitro* release studies indicate that PEC nanoparticles were successful in retarding therelease of drug in acidicpH 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 becauseof the interaction of AG with alkaline media and an increasein the solubility of AG .
- The possible mechanism of drug release from LMWH PEC nanoparticles was found tobe super case-II transport, which was considered as swelling-controlledrelease. 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 inconcealing CH from degradation in the gastric environment.

- Pharmacokinetic studies of the present study have shown an enhancement in oral 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. These amino groups get attached to the negative charge of epithelial lining and help with paracellular transportacross 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 nanoparticulate system can be considered as a useful oral deliverysystem for enhancing the bioavailability of LMWH.

CONCLUSION

- In this study, a novel formulation of oral nanoparticles for Low Molecular Weight Heparin (LMWH) was meticulously developed, with the goal of enhancing therapeuticefficacy through improved oral bioavailability. LMWH, a potent anticoagulant traditionally administered via injection, poses significant challenges for oral delivery due to its poor bioavailability when taken by mouth. This study aimed to address thesechallenges bycreating a nanoparticle system that could effectivelydeliverLMWH orally.
- The innovative nanoparticle formulation utilized a combination of chitosan andalginate,both of which are biocompatible and widely used in drug delivery systems. Chitosan, derived from chitin, is known for its ability to form stable nanoparticles andits favorable interaction with biological tissues. Alginate, extracted from brown seaweed, is a naturalpolysaccharide that also forms stable and biocompatible nanoparticles. The combination of these two materials aimed to create a nanoparticle system that could enhance the stability and absorption of LMWH in the gastrointestinaltract.
- The formulation process was finely tuned to achieve optimal particle size and encapsulation efficiency. Nanoparticles were carefully engineered to ensure they were small enough to be absorbed effectively through the intestinal epithelium while maintaining a high encapsulation efficiency. The encapsulation efficiency, measured at 81.5%, indicates that a substantial proportion of LMWH was successfully incorporatedinto the nanoparticles. This high encapsulation efficiency is crucial for ensuring that a significant amount of the drug reaches systemic circulation, thereby enhancing its therapeutic effectiveness.
- In vitro release studies were conducted to evaluate the performance of the nanoparticlesin releasing LMWH over time. These studies demonstrated that the nanoparticles were capable of releasing approximately 93% of the encapsulated LMWH over a 24-hour period. This controlled and sustained release profile is essential for maintaining therapeutic levels of LMWH in the

bloodstream. A prolonged release ensures that the anticoagulant activity is sustained over an extended period, which is beneficial for managing conditions that require continuous anticoagulation, such as thrombosis and pulmonary embolism.

- Pharmacokinetic analysis was performed to compare the bioavailability of the optimized chitosan-alginate LMWH nanoparticles with that of orally administered Clexane, a commercially available LMWH product. The results revealed a remarkable4.9-fold increase in the area under the curve (AUC) for the nanoparticle formulation compared to Clexane. The AUC represents the total drug exposure over time, and a higher AUC indicates improved bioavailability and systemic exposure of the drug. Thissignificant increase suggests that the nanoparticle formulation markedly enhances the absorption and efficacy of LMWH when administered orally.
- These promising results underscore the potential of chitosan-alginate LMWH nanoparticles as a transformative approach in drug delivery systems. By improving theoral bioavailability of LMWH, this novel formulation could provide a patient-friendly alternative to injectable anticoagulants. Such an advancement would reduce the need for frequent injections, thereby improving patient compliance and overall treatment experience. Moreover, the ability to deliver LMWH orally could expand its use in various therapeutic contexts, including the management of thrombotic disorders and other conditions requiring effective anticoagulation.
- The development of these specialized nanoparticles represents a significant advancement in the field of drug delivery. It opens new avenues for patient-friendly methods of administering potent anticoagulants, with the potential to positively impactclinical practice and patient outcomes. However, while these initial results are promising, further studies and clinical trials are necessary to validate the findings and establish the safety and efficacy of this novel oral nanoparticle formulation in diverse patient populations. These future studies will be critical in confirming the clinical benefits and practical applications of the chitosan-alginate LMWH nanoparticles.

SUMMARY

- The primary challenge with low molecular wight heparin is its parenteral administration, which is painful and inconvenient for patients, leading to a needfor effective oral formulations. The poor intestinal absorption of low molecularwight heparin is attributed to its hydrophilicity, anionic surface charges, and instability in acidic stomach conditions.
- Various delivery systems have been explored to enhance the oral bioavailabilityof low molecular wight heparin, including liposomes and microparticles, but none have successfully reached the market. The study aims to create a viable oral formulation by utilizing chitosan and alginate, which can enhance drug permeability in the lower smallintestine and colon.
- Chitosan, a natural polysaccharide, offers advantages such as biodegradability and biocompatibility, while alginate, an anionic polysaccharide, can formpolyelectrolyte complexes with chitosan, potentially improving drug delivery.
- The preparation of chitosan nanoparticles involved adding sodium tripolyphosphate tochitosan solution, followed by centrifugation to isolate the nanoparticles.
- For the chitosan-alginate low molecular wight heparin nanoparticles, low molecular wight heparin was added to sodium alginate, and the mixture was adjusted to specific pH levels before homogenization and centrifugation.
- SEM was employed to analyse the surface morphology and shape of thenanoparticles,confirming their formation and structural characteristics.
- The zeta potential was measured to assess the electric charge on the particle surfaces, which is indicative of the physical sta bility of the colloidal systems.
- The encapsulation efficiency of the nanoparticles was determined using a turbidimetric assay, measuring the amount of non-entrapped drug in the supernatant after centrifugation.
- The in vitro release of low molecular wight heparin from the nanoparticles wasstudied using a dialysis membrane method over a 24-hour period, with samplestaken at regularintervals for analysis.
- FTIR was used to confirm the identity of the drug and assess interactions between the drug and the carriers, indicating successful nanoparticle formationwithout chemical interactions.
- DSC analysis was performed to evaluate the thermal properties of the nanoparticles, confirming interactions between chitosan and alginate.

- The optimized chitosan-alginate low molecular wight heparin nanoparticles exhibited a significant enhancement in bioavailability compared to the marketedproduct, Clexane.This innovative formulation may hold capacity for improving the therapeutic delivery oflow molecular wight heparin.

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