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# SERRATIOPEPTIDASE LOADED NANOCOCHLEATE: NOVEL APPROACH FOR TRANSDERMAL DRUG DELIVERY SYATEM

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**Abstract:**

*Objective: Nanoochleates are tubular or cockle shaped structures derived from liposomes. Although investigated for several applications to be administered by oral and parenteral route, the potential of cochleates in topical drug delivery remains unexplored. The present study is a comprehensive report aimed at ascertaining the role of cochleates in topical delivery of drugs.Methods involved development and evaluation of lipoid S 75 based nanocochleate formulation of serratiopeptidase. A $2^3$ factorial design was utilized to optimize nanocochleate formulation and to study effect of lipoid S 75, cholesterol and calcium chloride on percent entrapment efficiency and particle size of nanocochleate. Nanocochleates were also characterized by FTIR and DSC. The anti-inflammatory activity and stability of SRP nanocochleates was investigated. The Results of Nanocochleates were prepared by using 749.98 mg of lipoid S 75, 250 mg of cholesterol and 15 mM of $CaCl_2$ having a particle size 231.4 µm and entrapment efficiency of 80.12. The variables of the $2^3$ factorial design significantly affected the percent entrapment efficiency and nanocochleate size. Carbopol 940 was chosen for gel preparation because of its bio-adhesive property and it is known to be retained for longer time at the site of administration. For determining anti-inflammatory activity SRP loaded nanocochleate gel was subjected for in vivo study. SRP loaded nanocochleate gel showed greater percent edema inhibition then the plain SRP gel and comparable with diclofenac gel (VoveranEmulgel). Nevertheless, the prepared nanocochleate formulations possessed stability profile similar to liposomal counterpart.*

**Keywords:** *Serratiopeptidase, Nanocochleate, Anti- inflammatory, Factorial design, Liposome*

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## INTRODUCTION:

Serratiopeptidase (SRP), a proteolytic enzyme, is an important anti-inflammatory agent that can bind itself to the alpha 2 macroglobulin in our plasma where it is shielded from the immune system while retaining its enzymatic activity, and in this way, it is transferred to the target sites. By hydrolyzing bradykinin, histamine and serotonin it indirectly reduces dilatation of blood capillaries and controls permeability. Serratiopeptidase blocks plasmin inhibitors thus helping the fibrinolytic activity of plasmin. Degradation of extra-fibrin to small fragment prevent clogging of microcapillaries, helps clearance of exudates, reduces swelling and improves microcirculation & expectoration of sputum etc[1]. It isassociated with a number of adverse effects such as skin rash, diarrhoea, anorexia, loss of appetite, gastrointestinal disturbance and allergic reaction. The current research documents a side effect of pneumonitis due to serratiopeptidase. Being sensitive to gastric degradation, serratiopeptidase is conventionally given orally in the form of enteric coated tablet formulation. Orally administered SRP has been reported to show systemic side effects like anticoagulant effect and ttherefore, to reduce systemic side effects and to increase local effects one of the approaches is to deliver SRP by topical route.[2]

Serratiopeptidase was formulated as a topical preparations to reduce the adverse side effects and avoid the gastric degradation. But it is difficult to maintain effective concentrations by topical delivery of serratiopeptidase. The application of nanocochleate drug delivery for transdermal drug is one of the numerous strategies for effective modulation of drug release and penetration enhancement. It act as carriers for serratiopeptidase and help to overcome the barrier properties of the skin, which in turn improves local anti-inflammatory efficacy and therapeutic index of SRP upon topical administration. Nanocochleate drug delivery system has been use as a drug carrier or reservoir due to its intrinsic skin penetration enhancing properties and increases stability of drug via encapsulation.[2] In the dermatological field, it is use only because of their restoring action and their capability of enclosing many different biological materials and of delivering them to the epidermal cells or even deeper cell layers.[3] Serratiopeptidase loaded nanocochleate prepare for imoproving delivery of drug to the skin. it require minimum concentration of drug for achieving effective therapeutic index, which Results reduction in a doses of drug, hence patient compliance will be increase. It also prepare forreducing the adverse side effects of serratiopeptidase.[2] The interior of nanocochleate is essentially free of water and resistant to penetration by oxygen which will be improve shelf-life of serratiopeptidase formulation.[4]

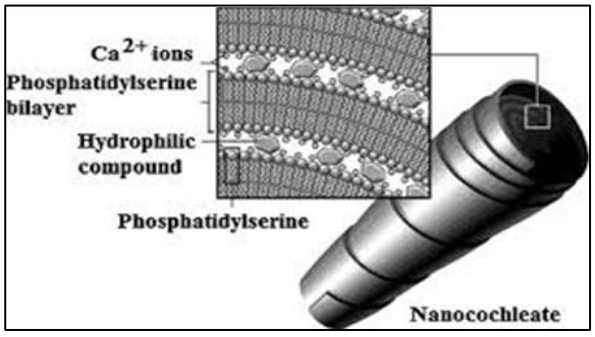



**Fig 1: Nanocochleate**

## MATERIALS AND METHODS:

**Materials**: Serratiopeptidase (SRP) was a generous gift from Advanced Enzyme Technology, Thane, India. Lipoid S 75, Lipoid PS 50x, phospholipon 90G was a gift from Lipoid (Germany). Methanol, Chloroform, Cacl2 solution and other reagents were of analytical grade. Marketed SRP gel was procured from the local market**.**

**PREFORMULATION STUDY**

**a) Standardization of Serratiopeptidase**

Serratiopeptidase was identified and confirmed by its melting point, FTIR and DSC spectra by comparing the obtained result with standard.

**b) Analytical method development**

**Double derivative spectroscopy method**

Different concentration of (100, 200, 300, 400 µg) SRP loaded nanocochleate dispersion was centrifuged and obtained pellet was treated with EDTA solution due to which structure of nanocochleates was disrupted and vesicles were obtained. These were then dissolved in chloroform: methanol (2:1) solvent system. The solutions were scanned and spectrum was converted into second order derivatives. Wavelength at which absorbance was increasing with increase in concentration was selected as a λmax for estimation of drug. It was found to be 238nm. The concentration range of 0.1-0.9mg of drug loaded nanocochleate was selected for development of standard curve. The absorbance of these solutions was measured at 238 nm by double derivative spectroscopy method. Absorbance values were plotted against concentration to obtain standard curve.[5]

**c) Drug - Excipient compatibility study**

**FTIR spectroscopy**:

The compatibility of drug and formulation components is studied by FTIR spectrometer (Shimadzu, 18400S, Japan). IR spectra of serratiopeptidase, cholesterol, lipoid S 75, SRP with cholesterol, SRP with lipoid S 75 and mixture of SRP with cholesterol and lipoid S 75 were obtained separately at room temperature in KBr pellets between the range of 400-4000cm-1 with resolution 2cm-1.[6]

**FORMULATION AND DEVELOPMENT OF NANOCOCHLEATE:**

**a) Method of preparation of nanocochleate**

For preparation of nanocochleate there are five methods reported in literature.

Hydrogel method 2) Trapping method 3)Liposome before cochleates dialysis method 4)Direct calcium dialysis method 5) Binary aqueous-aqueous emulsion method.

Amongst the above five mentioned methods, trapping method was chosen for nanocochleate preparation in current research work. This method involves the formation of liposomes followed by drop-wise addition of a solution of CaCl2. Liposomes can be generated by "Thin film hydration" method. In this method a thin film was prepared from mixture of vesicles forming ingredients that is lipoid S 75 and cholesterol by dissolving in volatile solvent like chloroform: methanol (9:1 ratio). Organic solvent was then evaporated at 450C using rotary evaporator leaving a thin layer of mixture deposited on the wall of the flask which was then hydrated by using water at 60 rpm for 20 minutes.[ 7]

**b) Preliminary selection**

**I) Selection of RPM and temperature**

Primarily the effect of rotational speed of the Rotary vacuum evaporator and temperature on lipid film formation was studied**.** The rotational speed of Rotary vacuum evaporator and drying temperature was optimized to obtain a uniform film. Blank batches were taken in order to check combined effect of rpm and drying temperature on film formation. Batches with rpm 85, 60 and 95 and drying temperature 40, 45 and 55 were carried out. The rpm and temperature which gives proper thin lipid film was selected for further study.

**II) Selection of lipid**

Batches were taken with lipids i.e. phospholipon 90 G, Lipoid S 75 and Lipoid PS 50x with cholesterol and calcium chloride. Based on the nature of films formed, particle size and polydispersity index of nanocochleate lipid was selected.

**c) Factorial Design**

23 factorial design was applied for optimization of the formulation. The design contained three variables I.e. amount of lipid, amount of cholesterol and concentration of calcium chloride which were varied at two different levels. The responses measured were particle size and entrapment efficiency. The actual values of the variables and responses of experimental design were given in Table 1. The Software Design Expert (Version 9.0.2, Stat-Ease Inc, Minneapolis, MN) was used to optimize and evaluate main effects and interaction effects of the formulation.[8,9]

**Table 1: $2^3$ factorial design layout**

| Batch No | Amount of lipid(mg) | Amount of cholesterol (mg) | Conc. Of calcium chloride (mM) |
|---|---|---|---|
| 1 | -1 | -1 | -1 |
| 2 | +1 | -1 | -1 |
| 3 | -1 | +1 | -1 |
| 4 | +1 | +1 | -1 |
| 5 | -1 | -1 | +1 |
| 6 | +1 | -1 | +1 |
| 7 | -1 | +1 | +1 |
| 8 | +1 | +1 | +1 |

**EVALUATION OF NANOCOCHLEATE:**

**a) Particle size analysis and Polydispersity index:**

Particle size and Polydispersity index was determined by using HORIBA particle size analyser.[10]

**b) Surface morphology:**

Surface Morphology of nanocochleate was determined by transmission electron microscopy and cryo- scanning electron microscopy.[11]

**c) *In vitro* diffusion study**

Franz diffusion cell was used for *in vitro* diffusion studies. The cellophane membrane was kept in between the donor and receptor compartments. Nanocochleate sediment was placed on the donor side of the membrane. The receptor compartment was filled with tris- buffer pH 7.0 and maintained at 37º C. Magnetic bar driven by a synchronous motor was placed in receptor compartment and driven at 100 rpm. At time intervals of 1, 2, 3, 4, 5, 6, 7, and 24 hr, 1ml of aliquot from receptor compartment was withdrawn, and the same volume of fresh medium was added back into the compartment. The samples were analyzed spectrophotometrically for drug content at wavelength of 278nm.[12]

**PREPARATION OF SRP LOADED NANOCOCHLEATE GEL:**

The low viscosity of nanocochleate dispersion is improved by converting to a gel matrix, more suitable for topical application. Carbopol 940 was selected as the gel matrix base. Different concentrations like 0.5%, 1% and 1.5% of carbopol 940 were screened for preparation nanocochleate gel formulations. Quantity of sediment equivalent to 1% SRP gel was dispersed in blank gel prepared with above concentrations under constant stirring with a glass rod, taking care to avoid formation of lumps and the pH values were subsequently adjusted to 7 by using triethanolamine.[13] The formulations were observed visually for appearance.

**EVALUATION OF SRP LOADED NANOCOCHLEATE GEL:**

**a) Physical examination:** The prepared gel formulations was inspected visually for colour, homogeneity, consistency and spreadability.[14]

**b) pH:**

The pH of nanocochleate gels was measured by using the digital pH meter (Universal Enterprises) at room temperature. The pH meter was standardized by using pH 4.0 and 7.0 standard buffers before use.[17]

**c) Viscosity study:**

The viscosity of the developed formulation was estimated by using Brookfield viscometer. The dial scale reading multiplied by factor (obtained from table) gave the viscosity in centipoises.[15]

**d) Spreadability:**

Spreadability is a term that denotes the extent to which the topical application spreads when applied to skin. The therapeutic efficiency of the formulation also depends upon its spreading value. Hence, determination of spreadability is very important in evaluating topical application characteristics. For the determination of spreadability, excess of sample (3gm) was applied in between two glass slides and was compressed to uniform thickness by placing 1000gm weight for 5 minute. Thereafter weight (50 gm) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves the lower plate to cover a distance of 10cm is noted. A shorter interval indicated better spreadability. The spreadability (S) can be calculated using the formula[16]

$S = M.L/T$

Where, S- spreadability

M -weight tied to upper glass slide, L-Length moved on glass slide, T - time taken

**e) Drug content analysis:**

The amount of drug contained in the SRP loaded nanocochleate gel was determined by dissolving 1gm of

the formulation in 10 ml of chloroform: methanol (2:1).it was diluted appropriately and analyzed for SRP content by double derivative spectroscopy method at wavelength of 238 nm[17]

**f) Differential scanning calorimetry (DSC)**

DSC analysis of serratiopeptidase, serratiopeptidase loaded nanocochleate and serratiopeptidase loaded nanocochleate gel was carried out for determining entrapment of drug in nanocochleate and uniform dispersion of nanocochleate in gel. The thermal analysis was performed in a nitrogen atmosphere at a heating rate of 100C/min over a temperature of 30°C to 300°C.[20]

**g) *In vitro* diffusion study on developed nanocochleate gel formulation**

Franz diffusion cell was used for *in vitro* diffusion studies. The cellophane membrane was kept in between the donor and receptor compartments. Nanocochleate gel was placed on the donor side of the membrane. The receptor compartment was filled with tris- buffer pH 7.0 and maintained at 370C. Magnetic bar driven by a synchronous motor was placed in receptor compartment and driven at 100 rpm. At time intervals of 1, 2, 3, 4, 5, 6, 7 and 24 hr, 1ml of aliquot from receptor compartment was withdrawn, and the same volume of fresh medium was added back into the compartment. The samples were analyzed spectrophotometrically for drug content at wavelength of 278nm.[12,18]

**h) *In Vivo* Efficacy Evaluation**

*In Vivo* Evaluation of Anti-Inflammatory Activity of SRP Nanocochleate Gel. The relative anti-inflammatory efficacy of the tested preparations and the commercially available reference preparation were measured and compared. Male Wistar rats weighing 160–180 g were used in this experiment. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC). The anti-inflammatory action was evaluated using the carrageenan - induced hind paw edema method. Rats were randomly selected and divided into four groups of six animals each. These groups were divided, according to the formulae administered, control (vehicle base), plain SRP gel, SRP loaded nanocochleate gel, and reference gel (Diclofenac gel) groups. The animals were housed in polypropylene cages at 25 ± 10C and 60 ± 5% relative humidity, with free access to food and water. One day prior to application of the trial formulation, the hair on the dorsal surface of the rat was shaved. Plain SRP gel, SRP loaded nanocochleate gel, reference gel, and control formulae were applied on the shaved dorsal surface by gentle rubbing for 15 seconds. After two hours, 0.1mL of 1%w/v suspension of carrageenan in normal saline was injected into the sub plantar region of the right hind paw of all control and treated rats. Edema volume, was measured in all four groups at hours 1,2,3, 4 and 24 after carrageenan injection using plethysmometer.[19]

The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. Percentage inhibition was calculated using the formula

Percentage inhibition = [ (Vc–Vt)/Vc ]× 100

Where, Vt– paw volume of treated group, Vc – paw volume of control group

**STABILITY STUDIES:**

For this study, the serratiopeptidase loaded nanocochleate gel was kept at 2-8°C for a period of 3 months. The stability of the serratiopeptidase loaded nanocochleate gel in terms of change in Drug content, spredability, viscosity, pH and In vitro diffusion was investigated.

**RESULTS AND DISCUSSION**

**1) PREFORMULATION STUDY**

**a) Standardization of Serratiopeptidase**

**I) Fourier Transformation Infrared Spectroscopy (FTIR):** Characteristic peak of pure SRP were observed in below mentioned spectra which confirms the identification of drug i.e. serratiopeptidase. FTIR of SRP is given in fig no.2

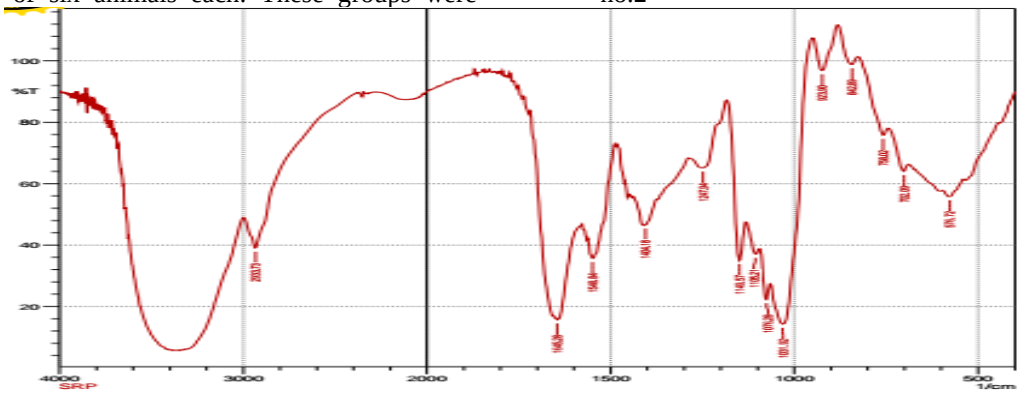



Fig 2: FTIR of serratiopeptidase

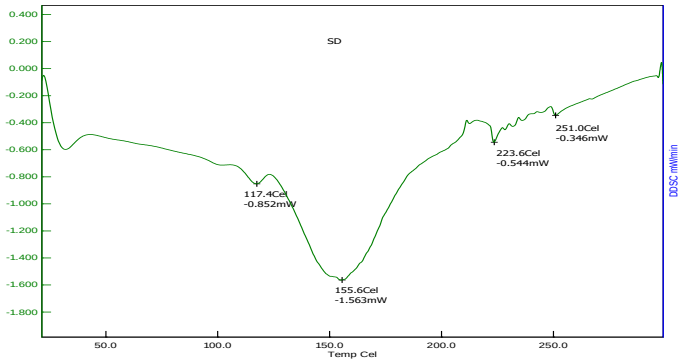
**Table 2: The corresponding important characteristic peaks assigned for SRP**

| Peak Wave Number | Characteristic peak |
|---|---|
| 1404 | C-O stretching amide III bond |
| 1544 | C=O amide II stretching vibration characteristic of protein. |
| 1646 | NH-C-O stretching amide I |
| 2933 | NH,OH stretching (amide A bond) |
| 3287 | Strong and broad O-H stretching. |

**Differential Scanning colarimetry (DSC)**

The characteristic endothermic peak for serratiopeptidase was obtained at 155.6$^0$C indicating melting temperature of serratiopeptidase. DSC of serratiopeptidase represented in fig. no.3

Fig 3: Differential scanning calorimetry of SRP




**III) Melting point of SRP**

According to differential scanning calorimetry Melting point of Serratiopeptidase was found to be at 155$^0$C.

**b)Analytical method development**

**Double derivative spectroscopy method:**

The concentration range of 0.1-0.9mg/ml of drug-loaded nanocochleate was selected for development of standard curve. The value of $R^2$ was found to be 0.9911 indicating that the relation of drug concentration and absorbance was linear. The standard curve is represented in figure no.23.The equation obtained was y= -0.7237x-0.1322, where y is absorbance and x is concentration.

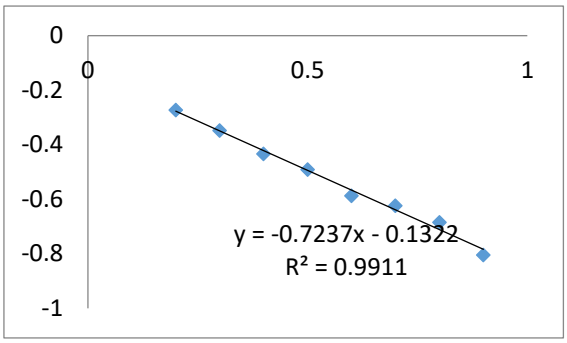



Fig 4: Calibration curve of SRP loaded nanocohleate

**c) Drug - Excipients compatibility study**

**Fourier Transformation Infrared Spectroscopy**

All the characteristic peak of SRP were observed in physical mixture of SRP with lipoid S 75 and cholesterol. no extra new peaks was observed suggesting no chemical interaction between SRP and excipients chosen for the production of SRP loaded nanocochleate. These results suggest that all nanocochleate excipients used in formulation are compatible with SRP. IR spectra of serratiopeptidase, cholesterol, lipoid S 75, SRP with cholesterol, SRP with lipoid S 75 and mixture of SRP with cholesterol and lipoid S 75 is represented in fig no: 5, 6, 7, 8, 9, 10 respectively.

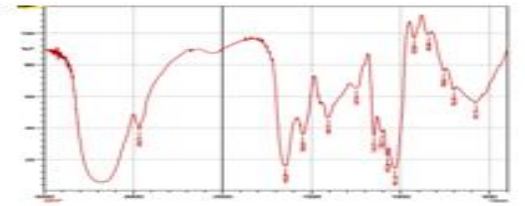


Fig 5: FTIR OF SRP

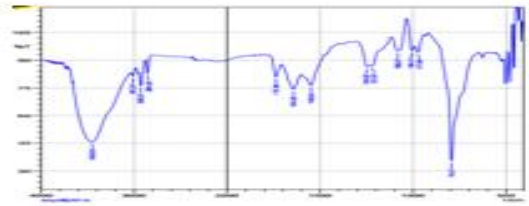


Fig 6: FTIR OF LIPOID S 75

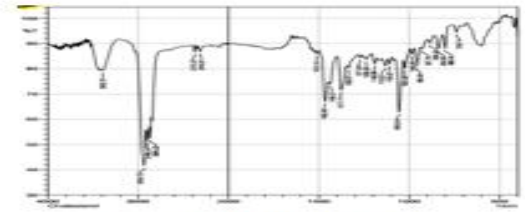


Fig 7: FTIR OF CHOLESTEROL

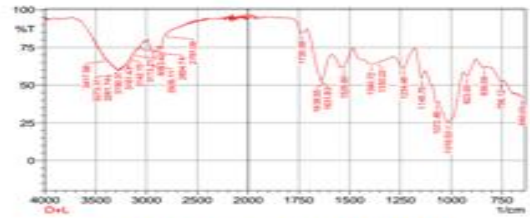


Fig 8: FTIR OF SRP AND LIPOID S 75

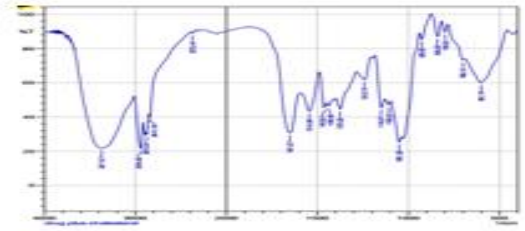


Fig 9: FTIR OF SRP AND CHOLESTEROL

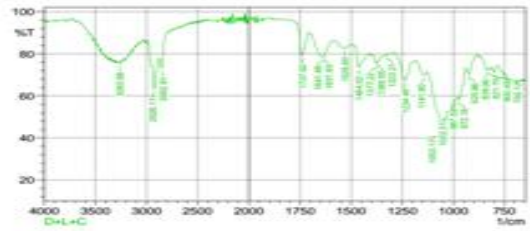


Fig 10: FTIR OF SRP, LIPOID S 75 AND CHOLESTEROL

**2) FORMULATION AND DEVELOPMENT OF NANOCOCHLEATE:**

**a) Method of preparation of nanocochleate**

Nanocochleates were prepared from liposomes by the folding action of Ca++. In the preliminary study, a 15mM concentration of calcium was found optimum to convert liposomes into cochleates and was kept constant throughout the study.

**b) Preliminary selection**

**I) Selection of RPM and temperature**

Batch with rpm 85 and 95 and temperature $40^0$ C and $55^0$ C gave uneven lipid film formation. Batch with rpm 60 and temperature $45^0$ C gave thin layer of lipid and film was efficiently formed. Hence, RPM 60 and temperature $45^C$C was used in further formulation trials.

**Table 3: Selection of RPM and temperature on basis of physical properties**

| RPM | TEMPERATURE | NATURE OF LIPID FILM FORM |
|---|---|---|
| 85 | 0 | Uneven layer of lipid |
| 60 | 45 | Efficiently formed uniform film |
| 95 | 55 | Uneven layer of lipid |

**II) Selection of lipids**

Batch with lipoid S75 gave uniform film and particle size of nanocochleate was found to be 268.7nm with uniform distribution. Hence lipoid S75 was used in further formulation trials.

Table 4: Selection of lipid on basis of particle size and PI

| Batch no. | Lipid | Cholesterol | Drug | Nature of lipid film form | Particle size | PI |
|---|---|---|---|---|---|---|
| $B_1$ | Lipoid S 75 (750mg) | 250mg | 100mg | Efficiently formed uniform film | 231.4nm | 0.368 |
| $B_2$ | Lipoid ps 50x (750mg) | 250mg | 100mg | Uneven layer Of Lipid | 264.5nm | ----- |
| $B_3$ | Phospholipon 90G (750mg) | 250mg | 100mg | Efficiently Formed uniform film | 140nm | 5.20 |

**c) $2^3$ factorial design:**

**I) $2^3$ factorial design:**

A $2^3$factorial design was employed for further study. Percent entrapment efficiency and determination of vesicular size was performed and the results were fed into the design expert software. The data was analyzed by ANOVA and was reported to be significant.

**Table 5: Responses for $2^3$ factorial design batches**

| Batch No | Amount of lipid (mg) | Amount of cholesterol (mg) | Concentration ofcalcium chloride (mM) | % EE | Particle size (nm) |
|---|---|---|---|---|---|
| 1 | 500 | 250 | 5 | 50.62 | 780.6 |
| 2 | 750 | 250 | 5 | 81.92 | 264.5 |
| 3 | 500 | 500 | 5 | 57.75 | 330.7 |
| 4 | 750 | 500 | 5 | 70.82 | 509.2 |
| 5 | 500 | 250 | 15 | 53.68 | 739.5 |
| **6** | **750** | **250** | **15** | **85.38** | **231.4** |
| 7 | 500 | 500 | 15 | 63.78 | 310.7 |
| 8 | 750 | 500 | 15 | 72.66 | 345.3 |

**II) Optimization of factorial design batches**

**i) The effect of formulation variable on the % EE**

In percent entrapment efficiency polynomial equation in terms of coded factor was obtained as percent entrapment efficiency =+67.08+10.62*A-0.82*B+1.80*C-5.13*A*B. $R^2$ was found to be 0.9959 which implies that 99.59 of the

variation in the responses was attributed to independant variables. There is only 0.06% chances that an F value this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant.

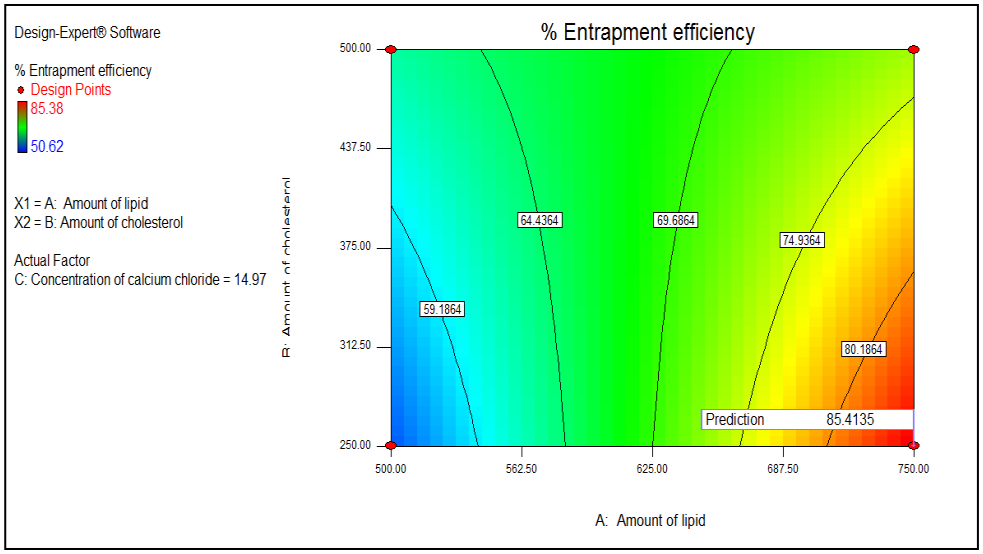



Fig 11: Contour Plot of percentage entrapment efficiency.

**ii) The effect of formulation variable on the particle size:**

In case of particle size polynomial equation in terms of coded factor was obtained as particle size = +438.99– 101.39* A - 65.01*B – 32.26*C + 154.66*A*B. $R^2$ was found to be 0.9792 which implies that 97.92 of the variation in the responses was attributed to independant variables. There is only 0.74 % chances that an F value this large could occur due to noise. Values of "Prob>F" less than 0.0500 indicate model terms are significant.

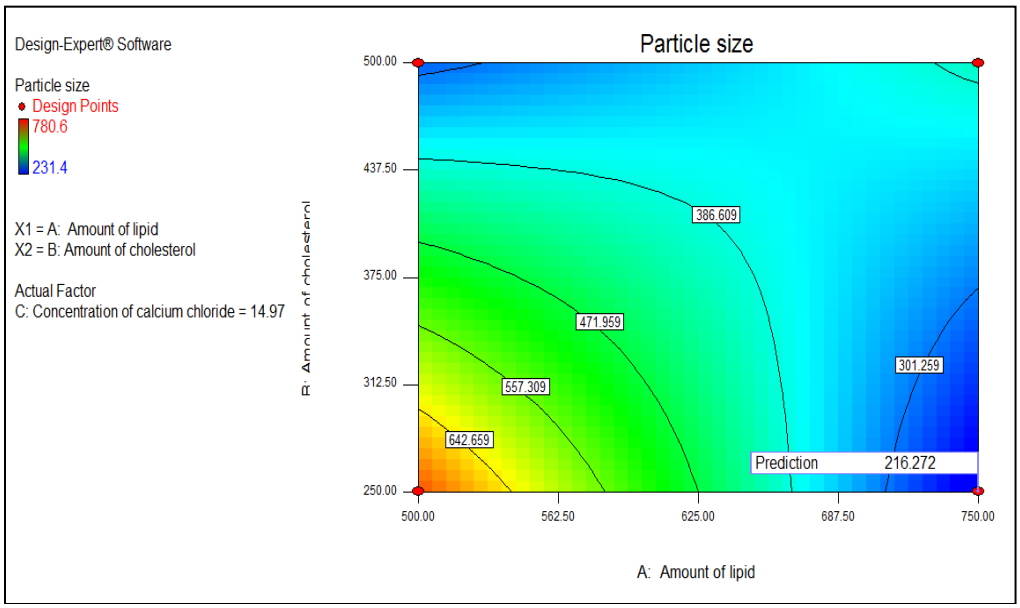



Fig 12: Contour Plot of particle size

**III) Selection Of Optimised Batch**

Software provides 7 new batches, batch with highest desirability value was selected and performed experimentally.

**Table 6: Selection of Optimised Batch**

| Batch No | Amount of lipid | Amount of cholesterol | Conc. of $CaCl_2$ | % EE | Particle size (nm) | Desirability |
|---|---|---|---|---|---|---|
| 1 | **749.98** | **250.00** | **15** | **83.64** | **2 15.72** | **0.987** |
| 2 | 750.00 | 250.00 | 14.93 | 83.64 | 2 16.16 | 0.986 |
| 3 | 750.00 | 250.00 | 14.88 | 83.65 | 2 16.45 | 0.984 |
| 4 | 750.00 | 250.00 | 14.77 | 83.64 | 2 17.15 | 0.982 |
| 5 | 749.18 | 250.00 | 14.52 | 83.54 | 220.46 | 0.974 |
| 6 | 750.00 | 342.15 | 15 | 79.26 | 281.75 | 0.930 |
| 7 | 724.16 | 500.00 | 15 | 70.60 | 383.97 | 0.781 |

| Batch | Amount of lipid | Amount of Cholesterol | Conc. of $CaCl_2$ | % EE | Particle size (nm) |
|---|---|---|---|---|---|
| Predicted | 749.98 | 250.00 | 15 | 83.64 | 215.72 |
| Actual | 749.98 | 250.00 | 15 | 85.38 | 231.4 |

There is no significant difference in Percent entrapment efficiency and particle size of predicted batch and actual batch. This indicates that the concentration of lipid, cholesterol, and calcium chloride are optimised. This optimised batch was further selected and studied for drug release profile.

**EVALUATION OF NANOCOCHLEATE:**

**a) Particle size and polydispersity index**

The most important parameter, which needs to be monitored during nanocochleate formulation is the particle size and size distribution of nanocochleate. The size and size distribution determines there *in vivo* or *ex vivo* performance. The mean particle size was found to be 231.4nm for optimized batch and polydispersity index was found to be 0.368. Low polydispersity index indicates particle size uniformity within the formulation.

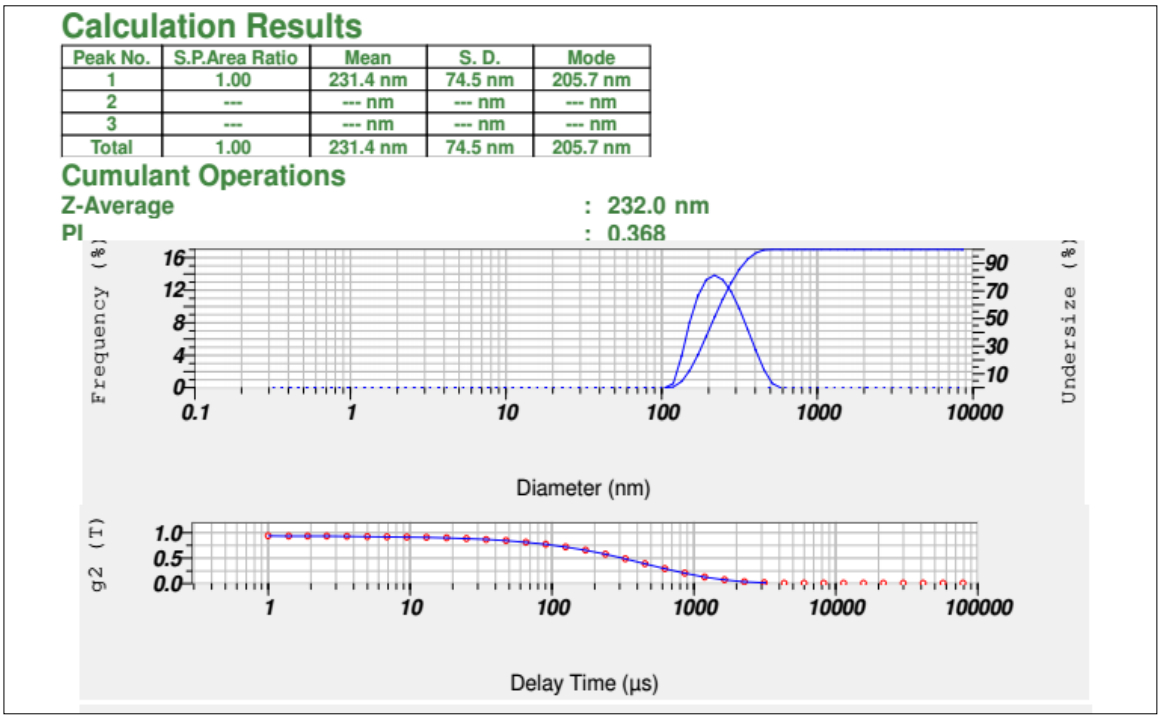



**Fig 13: The particle size and size distribution of nanocochleate**

**b) Surface Morphology:**

**I) Cryo- scanning electron microscopy (Cryo-SEM)**

Cryo scanning electron microscopy was performed on the optimized SRP laded nanocochleate formulation after 1000 fold dilution by distilled water, The cryo-SEM confirmed the presence of few rolled-up structures as well as elongated tubular structures of cochleates.

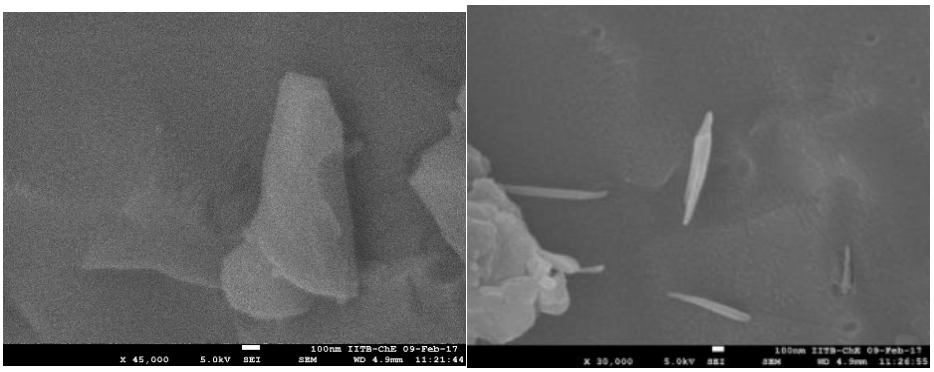


Fig 14:Cryo- scanning electron microscopy

**II) Transmission scanning electron microscopy (TEM)**

Transmission scanning electron microscopy was performed on the optimized SRP loaded nanocochleate, The TEM confirmed the presence of few rolled-up structures as well as elongated tubular structures of cochleates.

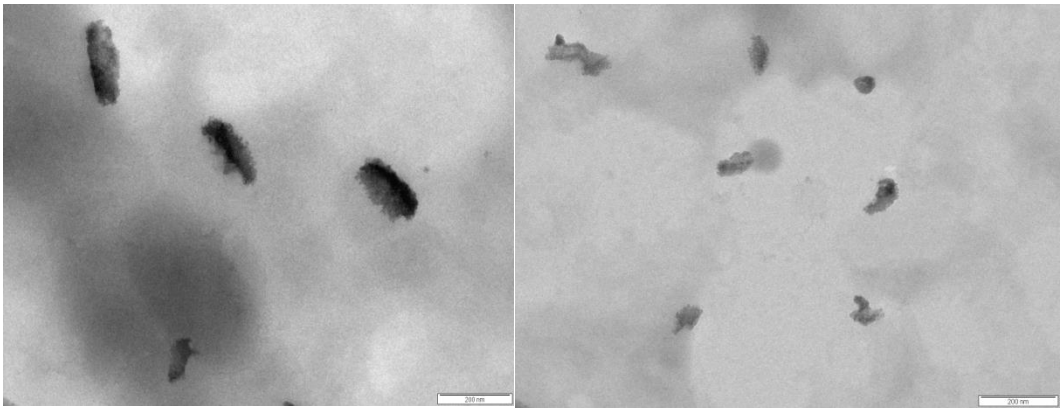


Fig 15: Transmission scanning electron microscopy

***c) In vitro* release kinetics study**

From the observation it could be concluded that rapid release was observed during initial hours due to release of surface absorbed drug, followed by sustained release profile upto 24 hrs.

Table 7: *In vitro* drug release of optimized batch of SRP nanocochleate

| **Time (hrs)** | **% cumulative release** |
|---|---|
| 1 | 21.46 |
| 2 | 29.04 |
| 3 | 34.24 |
| 4 | 50.22 |
| 5 | 55.87 |
| 6 | 60.59 |
| 7 | 66.83 |
| 24 | 80.12 |

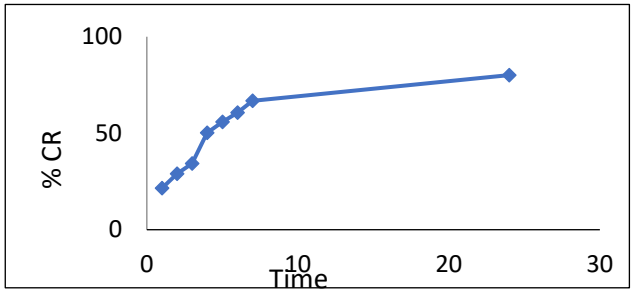



Fig 16: Graph of in vitro drug release of optimised batch

**PREPARATION OF SRP LOADED NANOCOCHLEATE GEL:**

Nanocochleate sediment of optimized batch weighing 1.176gm which was equivalent to 100mg in order to yield 1% gel of SRP that is comparable with marketed formulation were converted into gel using carbopol 940 as gelling agent and further evaluation was done.

Table 8: Optimised Formula for nanocochleate gel

| **Ingredients** | **Amount Added** |
|---|---|
| **Sediment of SRP loaded nanocochleate equivalent to100mg SRP** | **1.176gm** |
| **Carbopol 940** | **1%** |
| **Propylene glycol** | **10gm** |
| **Triethanolamine** | **q.s. pH 7** |
| **Distilled water** | **q.s. to 100** |

**EVALUATION OF SRP LOADED NANOCOCHLEATE GEL:**

**a) Physical examination:**

Appearance, homogeneity and consistency of optimised formulation was observed and it was found to be translucent.

**b) pH:** It was found that optimised formulation had a pH 7.

**c) Viscosity:** The viscosity of nanocochleate gel was found to be 52000cps.

**d) Spreadability**: Spreadability of nanocochleate gel was found to be 17.24mg.cm/sec.

**e) Drug content**: The drug content was found to be in acceptable range for optimized gel formulation i.e. 98%.

**f) Differential scanning calorimetry (DSC)**

When the DSC thermogram of plain serratiopeptidase showing endothermic peak at 155.6$^0$C was compared to that obtained with SRP loaded nanocochleate and SRP loaded nanocochleate gel, it was observed that characteristic, endothermic peak for SRP was slightly shifted i.e. at 141.5$^0$C in SRP loaded nanocochleate and at 133$^0$C in case of SRP loaded nanocochleate gel. Presence of endothermic peak in thermograms of developed formulations indicate that the drug is entrapped in nanocochleate and nanocochleate uniformly dispersed in gel. DSC of serratiopeptidase, SRP loaded nanocochleate and SRP loaded nanocochleate gel is represented in fig no: 16, 17 and 18 respectively.

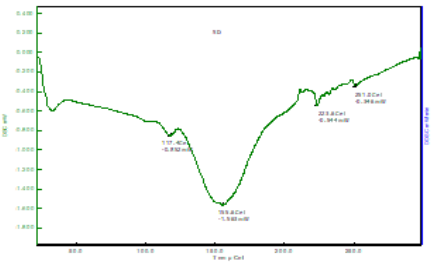


Fig 16: DSC of serratiopeptidase

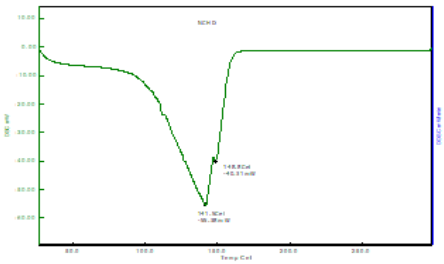


Fig 17: DSC of SRP loaded nanocochleate

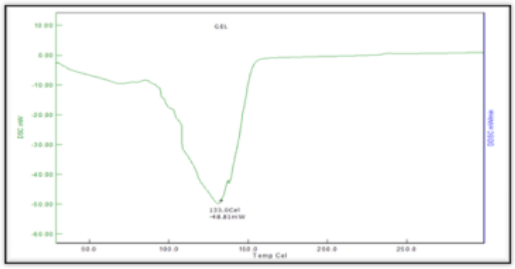


Fig 18: DSC of SRP Loaded Nanocochleate Gel

**g*) In vitro* diffusion study of SRP loaded nanocochleate gel**

*In vitro* diffusion was performed for SRP loaded nanocochleate gel. The results of diffusion study are shown in table 9. Percent cumulative release was found to be 73.93% after 24 hrs.

Table 9*: In vitro* Drug release of SRP loaded nanocochleate gel

| **Time** | **% Cumulative release** |
|---|---|
| **1** | 12.56 |
| **2** | 24.07 |
| **3** | 30.46 |
| 4 | 44.7 |
| 5 | 53.74 |
| 6 | 59.03 |
| 7 | 64.6 |
| 24 | 73.93 |

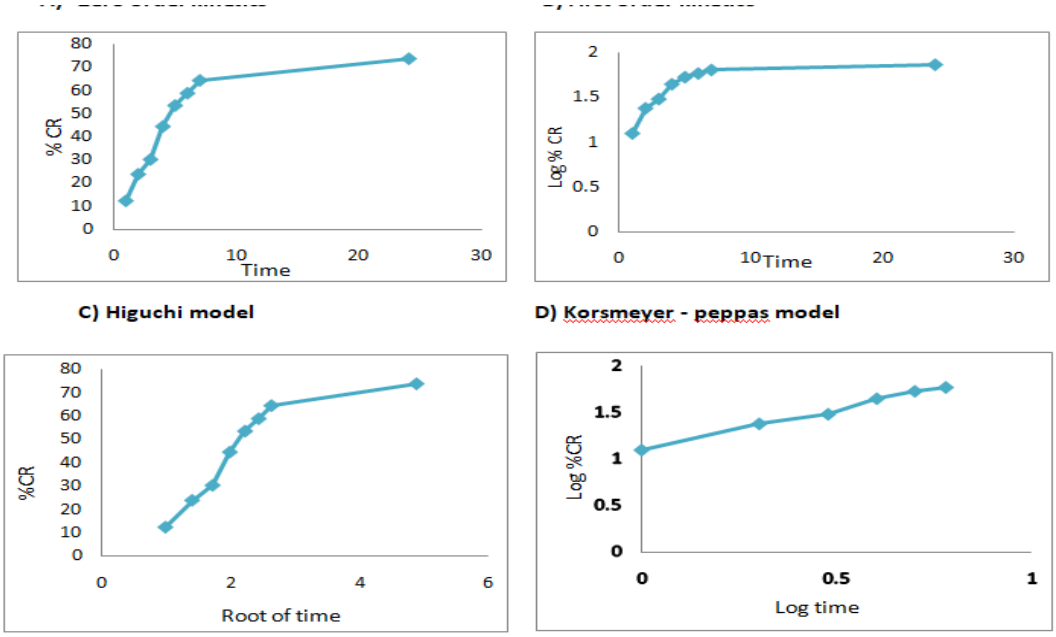



Fig 19: *In vitro* Drug release kinetics of batch B5 loaded gel

**Table 10: Release kinetics of different model**

| Model | $R^2$ | Equation |
|---|---|---|
| Zero order | 0.55 | Y = 2.169x+31.28 |
| First order | 0.40 | Y = 0.022x+1.452 |
| Higuchi | 0.74 | Y = 15.57x+9.619 |
| Koremeyer-peppas | 0.992 | y = 0.878x + 1.100 |

The first 60% drug release data were fitted in korsmeyer-peppas model. "n" value was found to be 0.878 that means it follow non-fickian transport. This indicates the drug release follows both diffusion and erosion controlled mechanism.

**g) *In Vivo* anti-inflammatory activity**

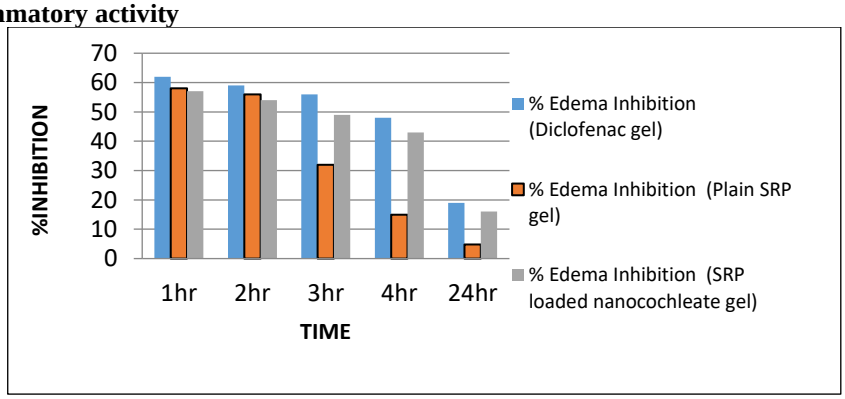



Fig 20: Percent edema inhibition by SRP loaded nanocochleate gel, diclofenac gel (VoveranEmulgel) and plain SRP gel

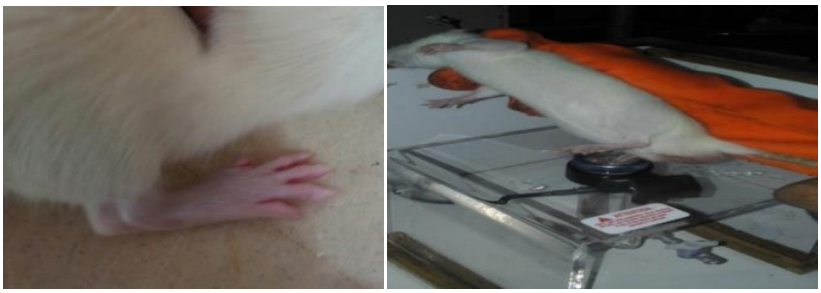


Fig: 21 Inflammation of right hind paw after carrageenan injection

**Table 11: Difference in paw edema volume (ml)**

| Group Dose: 1mg/kg | After 1 hr | After 2hr | After 3hr | After 4hh | After 24hr |
|---|---|---|---|---|---|
| | Mean ± SEM (% Inhibition | Mean ± SEM (% Inhibition) | Mean ± SEM (% Inhibition) | Mean ± SEM (% Inhibition) | Mean ± SEM (% Inhibition) |
| **Control** | 1.348±0.0754 | 1.613±0.0717 | 1.79±0.0696 | 1.893±0.0422 | 2.16±0.0504 |
| **Diclofenac gel** | 0.503±0.0717 | 0.66±0.0577 | 0.785±0.0561 | 0.983±0.0717 | 1.73±0.1197 |
| **SRP loaded NCL gel** | 0.575±0.0713 | 0.726±0.0553 | 0.908±0.0508** | 1.061±0.0672** | 1.8±0.0827* |
| **Plain SRP gel** | 0.56±0.0647 | 0.701±0.0725 | 1.205±0.0495 | 1.603±0.0422 | 2.055±0.0434 |

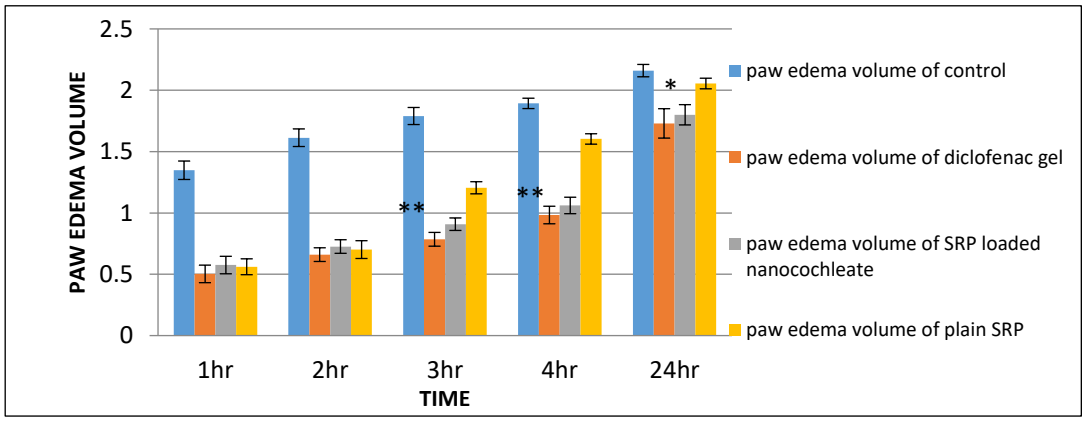



Fig 22: Effects of SRP loaded nanocochleate gel, diclofenac gel (VoveranEmulgel) and plain SRP gel on carrageenan induced paw edema

SRP loaded nanocochleate at 1hr, 2hr, 3hr, 4hr and 24 hr showed P<0.01 that is significance in comparison to control. SRP loaded nanocochleate showing P> 0.05 that is non significant in comprasion to diclofenac gel (VoveranEmulgel). SRP loaded nanocochleate at 1hr and 2hr showed no significance in comparisons to plain SRP gel, but at 3hr and 4hr showing P<0.01 and at 24hr showing P<0.05 significance.

**STABILITY STUDY:**

Stability study was carried out for SRP loaded nanocochleate gel. The parameters that was studied on $0^{th}$, $30^{th}$, $60^{th}$ and $90^{th}$ day are mentioned in table no 25. All the parameters were within the acceptable limits which showed that formulation was stable over the period of 90 days.

**TABLE 12: RESULTS OF THE PARAMETERS STUDIED DURING STABILITY STUDY**

| Parameters | 0th day | 30th day | 60th day | 90th day |
|---|---|---|---|---|
| Ph | 7 | 7 | 6.9 | 6.7 |
| Spreadability (mg.cm/sec) | 17.24 | 16.96 | 17.18 | 18.03 |
| Viscosity (cps) | 52000 | 50000 | 51000 | 50000 |
| *In vitro* Release (%) | 73.93 | 73.06 | 73.58 | 71.86 |

## CONCLUSION:

The aim of the current research work was to develop SRP -loaded nanocochleate gel which is proposed to show distinctive advantages over existing marketed conventional topical formulations like sustaining release of drug, increasing permeation across skin, improving local availability of drug to site of action, reducing the dosing frequency, improving patient compliance, reducing local side effects of drug as drug is in encapsulated form.

For preparation of SRP loaded nanocochleates trapping method was used because this method is reported to give more stable nanocochleate formulation than other system. Lipoid s 75 and cholesterol gave efficient thin lipid layer formation and gave good drug entrapment efficiency at $45^0$ C and at 60 RPM. Hence, Lipoid s 75 and cholesterol were used for further study.

$2^3$ factorial design was applied for optimisation of nanocochleate formulations. Variables such as concentration of lipid, cholesterol and calcium chloride have a profound effect on entrapment efficiency and particle size distribution. ANOVA was reported to be significant.

For percent entrapment efficiency, $R^2$ was found to be 0.9959, which implies that 99.59 of the variation in the responses were attributed to independant variables. The model was significant as model P value was found to be less than 0.05. Grid survey indicated batch no 6 was optimized as it showed good entrapment efficiency (85.38) and particle size (231.4) with good drug release (80.12% at the end of 24 hours).

Carbopol 940 was chosen for gel preparation because of its bio adhesive property and it is known to be retained for longer time at the site of administration. Different concentrations like 0.5%, 1% and 1.5% of Carbopol 940 were tried for development of nanocochleate loaded gel formulations. Depending upon appearance and consistency of gel, the concentration of 1% was finalised for incorporation in the nanocochleate gel formulations. Nanocochleate sediment of optimized batch weighing 1.176gm equivalent to 100mg of SRP was incorporated in 1% Carbopol gel in order to yield 1% gel of SRP comparable with marketed formulation.

Prepared SRP loaded nanocochleate gel was subjected for *in vitro* study. The first 60% drug release data were fitted in korsmeyer-peppasmodel."n" value was found to be 0.878 that means it follow non-fickian transport .This indicates the drug release follows both diffusion and erosion controlled mechanism.

Markated diclofenac gel (VoveranEmulgel), plain SRP gel and SRP loaded nanocochleate gel was subjected for *in vivo* study. The injection of carrageenan caused localized edema starting at 1hr after injection. The swelling increased progressively to a maximum 2.16± 0.0504ml at 24 hr after the carrageenan injection. Pre-treated, SRP loaded nanocochleate group showed a significant reduction of rat paw edema after 1 hr and continued upto 24hr. The percentage inhibition of rat paw edema by SRP loaded nanocochleate group was 57, 54, 49, 43 and 16.6 while as for diclofenac gel (VoveranEmulgel) it was 62, 59, 56, 48 and 19 and for plain SRP gel it was 58, 56, 32, 15 and 5 at 1hr, 2hr, 3hr, 4hr and 24 hr respectively. SRP loaded nanocochleate showed greater percent edema inhibition then the plain SRP gel and comparable with diclofenac gel (VoveranEmulgel).

The result of present study indicate that the nanocochleate gel formulated by using lipoid s 75, cholesterol, calcium chloride and carbopol 940 can be used to enhance skin delivery of serratiopeptidase because of excellent release and permeation of the drug.

Developed nanocochleate gel formulation possesses better skin permeation efficiency and stability. SRP loaded nanocochleate gel enhanced therapeutic efficacy and showed greater potential for the treatment of inflammation.

## REFERENCES:

1. Asadujjaman et al. A review on novel approaches in lipid based drug delivery system, 2013, 3(4), 124-130.

2. Hina, Rajani et al. Review article of lipid based drug delivery system. Journal of pharmaceutics volume 2014, 5(7). Article id 801820.
3. Salome et al. Review article of lipid based drug delivery system: recent advances and applications of lipids in drug delivery. African journal of pharmacy and pharmacology 2013, 7(48).
4. Paul A. J et al. Review article on anatomy and physiology of skin. Indian journal of pharmaceutics 2013, 5(3).
5. Khutle Nilesh et al. "Zero-crossing" first-order derivative spectroscopy method for analysis of sertraline hydrochloride in novel self micro emulsifying drug delivery system, austin journal of analytical and pharmaceutical chemistry, september11, 2014.
6. Amit Singh et al. Design, development and characterization of serratiopeptidase loaded albumin nanoparticles, Journal of Applied Pharmaceutical Science, Vol. 5 (02), February, 2015 pp. 103-109.
7. Gayatri et al. Novel vesicular carriers for topical drug delivery system and their applications. International journal of pharmaceutical sciences Review and research, 2013, 21(1), 77-86
8. Suhas et al. Optimization techniques: A future of pharmaceutical product development. International journal of current trends in pharmaceutical research 2014, 2(3) 491-493.
9. Ravi L et al. Research journal of pharmaceutical, biological and chemical sciences. Modern optimization techniques in field of pharmacy 2010, 1(2), 148 -157.
10. Landge A, Pawar A, Shaikh K. Investigation of cochleates as carriers for topical drug delivery. Int J Pharm PharmSci 2013; 5:314-25.
11. Jadhav S. M et al. Novel vesicular system: an overview, journal of pharmaceutical science 2012, 2(1), 193-202.
12. Bhosale RR et al. Nanocochleates: A novel carrier for drug transfer. J SciInnov Res 2013; 2:964-9.
13. Patel M, Patel J et al. formulation and evaluation of topical aceclofenac gel using different gelling agent, International journal of drug delivery 2011, 3, 156-164.
14. Montazam H et al. evaluation of mechanical and rheological properties of metronidazole gel as local delivery system. Journal of arch pharma 2011, 34, 931-940.
15. Manish Maheshwari el al. Development of tetracycline-serratiopeptidase-containing periodontal gel: formulation and preliminary clinical study, aapspharmscitech 2006; 7 (3) article 76.
16. Marwa H Shukr et al. Evaluation of topical gel bases formulated with various essential oils for antibacterial activity against methicillin resistant staphylococcus aureus, Tropical journal of pharmaceutical research december 2013; 12 (6): 877-884.
17. Rohit R. et al. Nanocochleate: A novel carrier for drug transfer, journal of scientific and Innovation Research 2013; 2(5): 964 – 969.
18. Sankar R.V.et al. Nanocochleate-A new approach in lipid drug delivery. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 4:220-223.
19. Ujwala S. et al. SerratiopeptidaseNiosomal Gel with Potential in Topical Delivery, Journal of Pharmaceutics, Volume 2014,Bombay College of Pharmacy, Mumbai, India 20 March 2014.
20. Pravin K. Shende et al. Modulation of serratiopeptidase transdermal patch by lipid-based transfersomes, Journal of Adhesion Science and Technology Volume 29, 2015.