Development and Evaluation of Fenoprofen Calcium loaded Proniosomal Gel for Topical Anti-inflammatory activity

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ABSTRACT

Background: Proniosomal gel of Fenoprofen calcium is a novel drug delivery system designed to enhance the stability, bioavailability and controlled release of Fenoprofen calcium a non steroidal anti-inflammatory drug used for pain and inflammation management.
Aim: The aim of the work is to formulate and evaluate topical proniosomal gel of Fenoprofen calcium using coacervation-phase separation method, along with varying concentration of cholesterol and surfactant. The work was focused to sustain the release profile of Fenoprofen calcium drug along with overcoming its lower solubility profile.
Place and Duration of Study: Department of Pharmaceutics, BVVS Hanagal Shri Kumareshwar College of Pharmacy Bagalkote, Karnataka, India, between December 2023 and November 2024.
Methodology: Fenoprofen calcium loaded proniosomal gel were prepared by Coacervation and phase separation method. The prepared Fenoprofen calcium loaded proniosomal gels were further characterized for zeta potential, drug content, vesicle size, pH, viscosity, entrapment efficiency, invitro drug release and in vivo anti-inflammatory study. .
Results: All the evaluation parameters shown good results for all the formulations. Vesicle size found to be in micro meter scale, DSC studies confirms formation of double layer, SEM studies shown good powder characteristics, FTIR studies confirms there is no interaction between drug and excipients, Zeta potential confirms the charge on vesicle and its stability, all the formulations shows drug content above 90% and the entrapment efficiency of formulation F1 is been maximum among all the formulations. Diffusion studies shows maximum amount of drug release at the end of 24 hours. Kinetic studies confirms the drug release pattern is been diffusion controlled; gel evaluations shows good rheological property of formulations. In vivo anti-inflammatory studies of optimized formulations F1 shows good results.
Conclusion: Overall, the study confirms that, acute inflammation could be treated efficiently by proniosomal gel, in which nonionic surfactant vesicles are utilized competently to load the drug Fenoprofen and treated the acute inflammation topically in animals, which shows good inflammatory action.

Keywords: Proniosomes, Proniosomal gel, Fenoprofen calcium, Coacervation-phase separation method, in vitro drug release, in vivo anti-inflammatory study.

1. INTRODUCTION

Topical drug delivery can be defined as administration of drug through skin to directly treat or cure the various skin disorders. These topical drug delivery systems are generally used for treating local infections like fungus growth, inflammation, redness, itching, pimples, rashes, wounds.[1]

A novel drug delivery system is that delivers drug at particular site at predetermined rate decided as per the requirement, pharmacological aspects, drug profile and physiological conditions of subject's body.[2]

Proniosomes are vesicles made up of non-ionic surfactants, which are going to form niosomes immediately after hydration. Proniosomes are now days used to enhance drug delivery in addition to conventional niosomes. They are converted into niosomes respectively upon simple hydration or by the hydration of skin itself after application. Niosomes appears as multilamellar or unilamellar vesicles obtained when hydration of nonionic surfactants takes place with or without incorporation of cholesterol or other lipids. These vesicles can act as drug reservoirs. The nonionic surfactants used in the development of proniosomes are biodegradable, biocompatible and non-immunogenic in nature.[3-4]

The size of niosomes is microscopic and lies within nanometric and micro metric scale. Niosomes can be developed to deliver the active medicaments through various rotes such as nasal, ophthalmic, pulmonary and transdermal routes. Niosomal vesicles had the special property of encapsulating both liphophillic and hydrophilic drugs and even these vesicles provide them the protection against acidic and enzymatic effects. Niosomes delays the release of medicament from the vesicle's adherence exhibit sustained release of the formulations.[5]

Proniosomes contains surfactants, membrane stabilizers, carriers, solvents, aqueous phase and drug.

2. MATERIAL AND METHODS

Fenoprofen Calcium(Gift sample from SUVEN Pharmaceuticals, Hyderabad), Span 60(Loba chemie Pvt. Ltd. Mumbai), Cholesterol(Loba chemie Pvt. Ltd. Mumbai), Soya Lecithin(HIMEDIA laboratories Mumbai), Ethanol(Changsu Hongshong FC Jiangsu), Maltodextrin(HIMEDIA laboratories Mumbai), Carbopol 934(Loba chemie Pvt. Ltd. Mumbai), Glycerol(SDFCL Mumbai), Propylene Glycol(Loba chemie Pvt. Ltd. Mumbai), Propyl Paraben(Genuine Chemicals CO. Mumbai), Methyl Paraben(NR Chem Mumbai), Triethanolamine(SDFCL Mumbai), Chloroform(SDFCL Mumbai), Potassium Di hydrogen Orthophosphate(SDFCL Mumbai), Sodium Hydroxide(Fisher Scientific Mumbai), Carrageenan(Altrafine gums, Gujarat).

2.1 Pre-formulation studies:

Description

The Fenoprofen calcium samples physical characteristics including its color, odor, and powder texture were assessed.[6]

2.1.1 Determination of Melting Point:

Melting point of the Fenoprofen Calcium was carried out to ensure its unchanged form, for this a very little amount of about 2 mg to 3 mg of drug sample was taken into a capillary tube which was already sealed at one end. Then the API filled capillary was tied tightly to the edge of thermometer. The thermometer was immersed in a Thiele's tube containing liquid

paraffin. The tube was heated at the rate of 2 ℃ per minute. The temperature at which the powder present in capillary tube gets melted was observed and particular temperature was reported as its melting point.[7]

2.1.2 Solubility Profile:

Solubility test of Fenoprofen Calcium was carried out using various solvents. To determine its solubility, in separate test tubes 10 ml of different solvents of different polarity are been taken and to those individual solvents 10 mg of Fenoprofen Calcium API was added separately and shaken well. The solubility of Fenoprofen Calcium in individual solvents was reported. For examining its solubility ethanol, methanol, petroleum ether, distilled water and buffers are used.

2.1.3 Determination of Absorption Maxima:

2.1.3.1 Preparation of Fenoprofen standard stock solution:

In a 100 ml volumetric flask, accurately weighed 100 mg of Fenoprofen was taken and is been dissolved in little quantity of Ethanol. Then the volume was made up to 100 ml mark with same solvent and it is referred as stock I solution. Then from the above stock solution, 5 ml was pipette out and transferred into another 100 ml volumetric flask and volume was made up to the 100 ml mark with same solvent. This is referred as stock II solution and used for determination of absorption maxima.[6]

2.1.3.2 Determination of absorption maxima:

Thus, prepared stock II solution was a typical solution to determine absorption maxima since it is within the beers range. The wavelength range in UV spectrophotometer was adjusted from 200 nm to 400 nm and base line correction was carried out using methanol to eliminate any kind of absorbance. Then in sample cuvettes the Fenoprofen stock II solution was transferred and spectrum was analyzed. The maximum absorbance at particular wavelength was reported as its absorption maxima.[6]

2.1.4 Authentication of Fenoprofen using FTIR:

Fenoprofen was analyzed using Fourier transform infrared spectroscopy to authenticate its functional groups of the pure drug. Infrared spectrum of Fenoprofen was recorded in Bruker alpha instrument kept at ambient temperature of 25 + 0.5℃. The analytical procedure was simple and did not need any special sample preparation. The spectra were recorded by placing the Fenoprofen API on zinc solenoid crystal plate and screwing the anvil over the sample carefully and scanning the drug sample in the region of 4000 – 400 cm-1 and the different functional group of Fenoprofen were interpreted.[8]

2.1.5 Development of proniosomal gel formulation:

2.1.5.1 Method: Coacervation- Phase separation method:

In this method of developing proniosomal gel, firstly accurately weighed span 60, cholesterol, drug and soya lecithin were taken into a wide mouth glass bottle. To this mixture required quantity of ethanol is been added. The mouth of bottle is closed tightly and the mixture is heated on a water bath at 60℃ to 65 ℃. After 5 to 6 minutes the solid mixture present in the bottle gets melted and forms a clear solution. Remove the bottle from water bath and slowly add the required quantity of distilled water with stirring. Close the lid and

keep the bottle aside and leave for 24 hrs. The solution after cooling forms a creamish white translucent mass of proniosomes. This gel is used for further characterizations.[9 & 53]

Table 1. Formulation chart of proniosomal gel: Coacervation & phase separation method.

Sl	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Fenoprofen calcium	200	200	200	200	200	200	200	200	200
2	Span 60	3600	3600	3600	3000	3000	3000	2400	2400	2400
3	Cholesterol	1600	1000	400	400	1600	1000	1000	1600	400
4	Soya lecithin	800	800	800	800	800	800	800	800	800
5	Ethanol	2	2	2	2	2	2	2	2	2
6	Distilled water	10	10	10	10	10	10	10	10	10

**Quantities are in milligrams for solids & milliliters for liquid excipients*

2.1.6 Characterization of Proniosomal gel:

2.1.6.1 Compatibility Studies:

Attenuated total reflectance – Fourier transform infrared spectroscopy was carried for individual excipients and a formulation from each method. The study carried out to identify any kind of drug excipients interactions. Any changes or shift in the peaks of FTIR indicates the changes within functional groups. To ensure the drug excipients interactions ATR-FTIR study was carried out.

Excipients and formulations are kept separately in bruker alpha at ambient temperature of 25 + 0.5℃. The analytical procedure was simple and did not need any special sample preparation. The spectra were recorded by placing the individual excipients and formulation on zinc solenoid crystal plate and screwing the anvel over the sample carefully and scanning the samples in the region of 4000 – 400 cm-1 and the different functional groups of the samples were interpreted.[10]

2.1.6.2 Vesicle Size Analysis:

The vesicle size evaluation of niosomes was carried out using compound microscope with 10 x, 40 x and 100 x lens. The eye piece micrometer was calibrated using the stage micrometer. Further a small amount of about 200 mg of proniosomal gel was dispersed with 10 ml of 7.4 ph phosphate buffer and few drops of this dispersion was placed on slide, then vesicle are observed under microscope. The number of divisions covered by eyepiece micrometer was counted and actual diameter was calculated. For calculating average vesicle size, about 100 vesicles in each formulations were observed and average vesicle size is been calculated.[11-12]

Actual diameter = no. Of divisions covered by eye piece micro meter × calibration factor.

$$\text{Average vesicle size} = \frac{\Sigma \text{ actual diameter}}{\text{No. of observations}}$$

2.1.6.3 Scanning electron microscopy:

Scanning electron microscopy was carried out to study the surface morphology of proniosomes. Optimized formulations were selected for the surface morphology study. For SEM studies 0.2 gm of proniosomal gel was diluted with 7.4 pH phosphate buffer. The niosomes were mounted on an aluminum stub using double sided adhesive carbon tape. Then the vesicles were sputter coated with gold palladium (Au/Pd) using a vacuum evaporator and examined using a scanning electron microscopy (Hitachi 7500, Japan) equipped with a digital camera and morphological appearances were reported.[13-14]

2.1.6.4 Zeta Potential:

Zeta potential of the optimized formulation was carried out using Horiba SZ 100. The charge on the surface of proniosomes is characterized by zeta potential evaluation. When the proniosomal gel is been placed between two electrodes of opposite charge due to influence of applied voltage the particles move towards oppositely charged electrodes. The higher repulsive force indicates the higher net charge on vesicles. The presence of charge gives stability to vesicles, uniformity of formulation, enhance sedimentation rate and prevents agglomeration. The sample is injected into a disposable cell and measurement of the particle

electrophoretic mobility results in ultimate zeta potential. The proniosomal gel having zeta potential value more than ± 30 mv considered as stable formulation.[15]

2.1.6.5 Thermal analysis:

Thermal analysis is a technique used to analyze the time and temperature at which physical changes occurs on a substance when it is heated or cooled. DSC is a fast and reliable method to screen drug excipient interactions as indicated by appearance of a new peak, change in the peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy***.***

The thermotropic properties of the samples were explored to assess the degree of crystallinity and the presence of possible interactions between Fenoprofen calcium, span 60, cholesterol. DSC analysis was performed with a DSC Q2000 V24.4 build 116. Previously DSC instrument is calibrated for heat flow and temperature with help of pure indium [M.P 156 ℃ & Δh of 25.45 J gm^{-1}]. The temperature range was from 0 to 300°C with a heating rate of 10°C/min. The gas used was nitrogen with a purging rate of 50 ml/min. The weight of each sample taken was 10 mg. Thus obtained graphs were interpreted.[16]

2.1.7 Evaluation of Proniosomal gel:

2.1.7.1 pH:

pH of the gel formulation was determined using the digital pH meter, Hanna, HI 98107 model. About 0.5 grams of gel was diluted with 50 ml of distilled water and electrode was dipped into the beaker and wait for 5 minutes. The observed pH was recorded and procedure is been repeated thrice to get values in triplicates.[17-18]

2.1.7.2 Viscosity:

Viscosity of the gel formulations were determined using Brookfield viscometer. In a beaker sufficient quantity of gel was taken then the Brookfield viscometer settings were adjusted, spindle no. 64 was selected and rotation was set to 20 rpm. Then the rotating spindle is dipped into gel and the dial readings were noted. The process is repeated thrice to get triplicates of dial readings.[19]

2.1.7.3 Spreadability:

Spreadability of the proniosomal gel was determined by placing 0.5 gm of gel on center of the glass slide which was fitted to broad glass plate and place another slide on it, put the weight of 50 gm on upper slide and leave for 5 minutes to settle. Then apply 20 gm of load towards the gravity, so that 2 slides get separated. Note the time taken for separation of 2 slides and calculate spreadability using below formula.[20-21]

$$S = \frac{m \times L}{t}$$

Where, S: Spreadability (gm. cm/ sec).
m: load applied (grams).
L: length travelled by slide (cm).
t: time (sec).

Repeat the procedure thrice to get triplicates of the results.

2.1.7.4 Extrudability:

Extrudability of the gel formulations were determined by placing about 5 grams of the gel into collapsible aluminum tubes and sealing it at open end. The entire amount of gel was extruded out on applying gentle force. Then the extruded gel was weighed again and noted. Then using both the weights the extrudability was calculated using below formula.[22]

$$E = \frac{W2 \times 100}{W1}$$

Where, E = Percentage Extrudability.
W1 = weight of gel taken (gm).
W2 = weight of gel extruded out from tubes (gm).

2.1.7.5 Drug content:

The practical yield was noted down for each of the formulations. According to the practical yield exactly 10 mg of Fenoprofen Calcium equivalent gel was taken in 100 ml volumetric flask and about 50 ml of phosphate buffer pH 7.4 was added to it. By using the bath sonicator the solution was sonicated at 30 ℃ for 5 minutes. Then make up the volume to 100 ml mark with same buffer. Filter the solution and take 1 ml from filtrate and dilute with 10 ml buffer. Then take the UV absorbance at 271 nm further calculate % drug content using following formula.[23-24]

$$\% \text{ drug content} = \frac{\text{Concentration} \times \text{Diluation factor} \times 100}{1000}$$

2.1.7.6 Entrapment Efficiency:

Entrapment efficiency of proniosomes was determined by separation of unentrapped drug by centrifugation method. In 15 ml capacity centrifuge tubes 10 mg Fenoprofen Calcium equivalent gel was taken and 10 ml of 7.4 pH phosphate buffer was added. This mixture was shaken well and centrifuged at 15000 rpm for 30 minutes. After some while unentrapped drugs was separated and forms supernatant. From the supernatant 1 ml of solution was pipette out and suitable dilution was made to take absorbance in UV. Further calculations gives unentrapped drug content and difference in total drug content gives actual entrapment efficiency of proniosomal gel.[25]

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug content} - \text{Drug in supernatant} \times 100}{\text{Total drug content}}$$

2.1.7.7 In vitro Diffusion Studies:

In vitro diffusion studies were carried to ensure the drug release from the gel. Franz diffusion cell was used to develop the drug release profile. The dialysis membrane was soaked overnight in phosphate buffer 7.4 pH. The dialysis membrane was clamped between donor compartment and receiver compartment, in the donor compartment 10 mg of Fenoprofen Calcium equivalent gel was placed and in the receiver compartment 100 ml of phosphate buffer 7.4 pH was added. It was stirred continuously at 50 rpm using a magnetic bead and temperature was maintained at 37ºC±0.5 ºC throughout the experiment. 1ml of the samples were withdrawn from the receiver compartment at the interval of 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 8hr, 12 hr, 16hr, 24 hr and replaced with 1 ml buffer to maintain sink condition. Then suitable dilution was made to take UV absorbance and then the absorbance was taken and % cumulative drug release was calculated.[26-27]

$$\% \text{ cumulative drug release} = \frac{\text{Cumulative drug release} \times 100}{\text{Dose}}$$

2.1.7.8 Drug Release Kinetic Modeling:

The results of in vitro drug release profile obtained for all the formulations were plotted in models of data treatment as follows:

Zero order kinetics:

Data: Cumulative % drug released v/s time.
Zero order release would be predicted by the following equation

$$A_t = A_0 - k_0 t$$

Where, A_t = Drug release at time 't'.
A_0 = Initial drug concentration.
K_0 = Zero-order rate constant (hr^{-1}).
When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero-order kinetics and its slope is equal to Zero order release constant k_0.[28]

First Order Kinetics:

Data: Log cumulative percent drug remaining v/s time.
First-order release could be predicted by the following equation:

$$\text{Log } C = \log C_o - Kt/2.303$$

Where, C = Amount of drug remained at time 't'.
C_0 = Initial amount of drug.
K = First-order rate constant (hr^{-1}).
When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K1' can be obtained by multiplying 2.303 with the slope value.[28]

Higuchi's Model :

Data: Cumulative percent drug released v/s square root of time.
Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q = [D\epsilon/t\,(2A - \epsilon Cs)]\ ½$$

Where, Q = Amount of drug release at time 't'.
D = Diffusion coefficient of the drug in the matrix.
A = Total amount of drug in unit volume of matrix.
CS = Solubility of drug in the matrix.
ϵ = Porosity of the matrix. ι= Tortuosity.
T = Time (hrs at which q amount of drug is released).
Above equation can be simplified as if we assume that 'D', 'Cs' and 'A' are constant. Then equation becomes

$$Q = ½\ kt$$

When the data is spited according to equation i.e. cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.[28]

Korsmeyer Peppas Model:

Data: Log cumulative percent drug released v/s log time.

To study the mechanism of drug release from the liposomal solution, the release data was also fitted to the well-known exponential equation which is often used to describe the drug release behavior from polymeric systems.

$$Mt / M\alpha = Ktn$$

Where, Mt / Mα = Fraction of drug released at time 't'.

K = Constant incorporating the structural and geometric characteristics of the drug/polymer system.

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides

$$\text{Log } Mt / M\alpha = \text{Log } K + n \text{ Log } t$$

Hixson-Crowell cube root model:

Data: Time v/s Cube root of % drug remaining.

The Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles or tablets. For a drug powder consisting of uniformly sized particles, it is possible to derive an equation that expresses the rate of dissolution based on the cube root of the particles.[28]

$$Q_o\ 1/3 - Q_t\ 1/3 = Kt$$

Where, Q_0 = Initial amount of drug in the tablet.

Q_t = Amount of drug released in time 't'.

K = Rate constant for Hixson-Crowell rate equation

2.1.7.9 Stability Studies:

Stability study was carried out for ensuring the vesicles stability during the storage, for this one optimized formulation from each method was selected and kept for stability studies for 3 months at room temperature (30 ± 2°C) and at refrigerator temperature (4 ± 2°C) to determine any kind of physical and chemical changes. The major parameters like entrapment efficiency and vesicle size determination were carried out at the interval of 1st day, 30 days, 60 days and 90 days. The changes within the study period were reported.[29]

2.1.8 In vivo anti-inflammatory study:

2.1.8.1 Anti Inflammatory Study:

The animals used in the anti-inflammatory study are been caged in polycarbonate trays prior to the experimentation and they were given with normal diet and water. The animals should be stored in air-conditioned room.[54]

2.1.8.2 Animal information:

Table 2. Information of animals used for In vivo studies

SL NO.	PARAMETER	SPECIFICATION
1	Animals	Rats
2	Strain	Wister Albino
3	Sex	Either Sex
4	No. of animals	15 + 15 = 30
5	Body weight	250 to 300 gms
6	Source	**ANIMAL HOUSE** Hanagal shri kumareshwar college of pharmacy Bagalkote.

2.1.8.3 Carrageenan induced rat paw edema method:

In rats firstly edema is been induced Then the proniosomal gel been applied topically further the decrease in edema was calculated. The wister albino rats were divided into five groups.[30]

Table 3. Grouping of animals for In vivo studies

Sl. NO	GROUP	NO. OF ANIMALS
1	Controlled group	06
2	Standard group	06
3	Treatment group: **F1**	06
4	Treatment group: **FCG**	06

The inflammation (edema) is been induced to all the animals by injecting 0.1 ml of 1% Carrageenan solution to the left hind paw. Within half an hour the paw will be gets inflamed due to Carrageenan. After half an hour the controlled group left untreated, standard group treated with marketed gel, first treatment group given with proniosomal gel F1, second group treated with Fenoprofen calcium plain gel. The paw volume was measured with help of Plethysmometer at the intervals of 1hr, 2hr, 3hr, 4hr and 5th hour after treatment. The results are summarized in the table. The percentage inhibition of paw edema volume of each treated groups is calculated by the equation.[31-32]

$$\text{Percentage of inhibition}(\%) = \frac{\text{Volume of control} - \text{Volume of test} \times 100}{\text{Volume of control}}$$

3. RESULTS AND DISCUSSION

3.1 Assessment of Fenoprofen Calcium API:

3.1.1 Physical Characterization:

Table 4. Physical characteristics of Fenoprofen Calcium

SL NO	PHYSICAL CHARACTER	OBSERVATION
1	Physical state	Powder
2	Appearance	Amorphous
3	Colour	White
4	Odour	None
5	Texture	Smooth

The Fenoprofen Calcium drug was investigated for various physical parameters and the results were expressed in table no 04. Physically the drug was in powder form, having amorphous and smooth texture, which is a white coloured, odorless powder.

3.1.2 Determination of Melting Point:

The melting point of the Fenoprofen Calcium was carried out using Thiele 's tube which was found to be 118 ℃. According to literature references the melting point of pure Fenoprofen Calcium API was found in between the range of 118 – 123 ℃.

3.1.3 Solubility Profile:

Table 5. Solubility profile of Fenoprofen Calcium.

SL NO	SOLVENT	SOLUBILITY
1	Acetone	Freely Soluble
2	Methanol	Freely Soluble
3	Ethanol	Freely Soluble
4	Chloroform	Freely Soluble
5	Water	Insoluble
6	1.2 pH Buffer	Insoluble
7	6.8 pH Buffer	Partially soluble
8	7.4 pH Buffer	Freely soluble

Fenoprofen Calcium was evaluated for its solubility using various solvents including organic solvents and buffers. The results were depicted in table no 05. Fenoprofen Calcium was completely soluble in Acetone, Ethanol, Methanol, chloroform and 7.4 pH buffer. It is practically insoluble in 1.2 pH buffer and Distilled water and partially soluble in 6.8 pH buffer.

3.2 Development of UV Spectroscopic Methods.

3.2.1 Determination of Absorption Maxima:

Figure 1. Absorption maxima of Fenoprofen Calcium

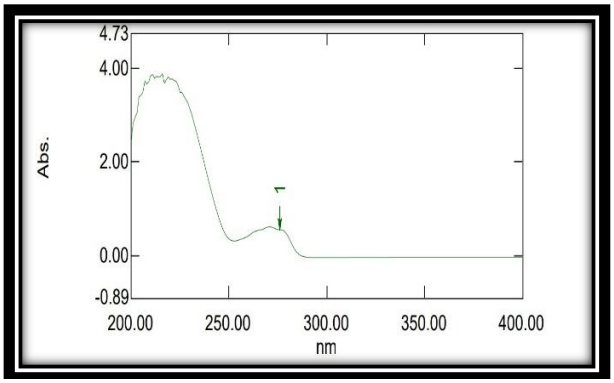


Table 6. Absorption maxima of Fenoprofen Calcium in buffer

SL NO	SOLVENT	λ_{max}
1	7.4 pH phosphate Buffer	272nm

The UV spectroscopic peaks were shown in Figure 01 and peaks were given in table no 06. Fenoprofen Calcium in 7.4 pH buffer was shown peak at 272 nm.

3.2.2 Development of Standard Calibration Curve:

Table 7. Absorbance data of Standard calibration of Fenoprofen Calcium:

SL NO	CONCENTRATION (µg/ml)	ABSORBANCE MEAN ± SD
1	20	0.136 ± 0.001
2	40	0.262 ± 0.030
3	60	0.394 ± 0.002
4	80	0.541 ± 0.006
5	100	0.692 ± 0.029
6	120	0.812 ± 0.012
7	140	0.965 ± 0.005

**Data expressed are average of Triplicate (n=3 ± SD)*

Fig 2. Standard calibration curve of Fenoprofen Calcium using 7.4 pH phosphate buffer.

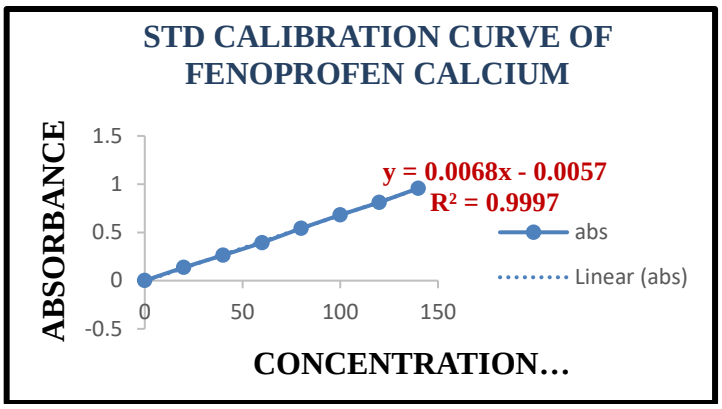


Table 8. Statistical data for standard calibration curve.

SL NO	PARAMETERS	VALUES
1	λ_{max} (nm)	272 nm
2	Beer's range	20- 150 µg/ml
3	Slope	0.0689
4	Intercept	0.0066
5	R2	0.999

Standard calibration curve of Fenoprofen Calcium was prepared by developing the graph of concentration v/s absorbance. For this average value of 3 absorbances were taken. From the graph the slope obtained was 0.0689 and intercept obtained was 0.0066. The regression coefficient was found to be 0.999. Standard calibration graph was shown in figure no 02 and absorbance data was expressed in table no 07.

3.2.3 Authentication of Fenoprofen Calcium Using ATR-FTIR:

Fig 3. FTIR spectroscopy of Fenoprofen Calcium:

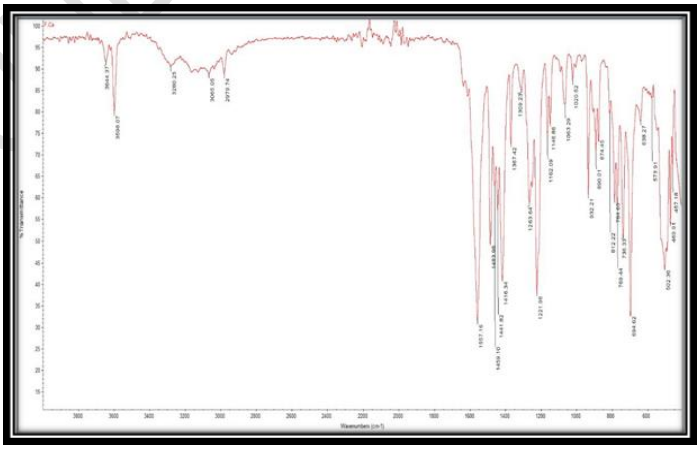


Table 9. ATR-FTIR Interpretation of Fenoprofen Calcium:

Sl no	Compound	Functional groups	Frequency range cm^{-1}	Peak cm^{-1}	Vibration
1	Fenoprofen Calcium	OH	3600 – 3200	3598	Stretching
		Aromatic – CH	3080 – 3030	3065	Stretching
		Aliphatic - CH	2980 – 2850	2979	Stretching
		C=O	1675 – 1500	1557	Stretching
		C-O	1230 – 1160	1162	Stretching

Qualitative estimation of Fenoprofen Calcium was carried out using ATR- FTIR studies. Whereas the functional groups present in the Fenoprofen Calcium structure were identified using the IR peaks obtained from interpretation. The FTIR spectrum of Fenoprofen Calcium was represented in figure no 03 and all the peaks were illustrated in table no 09. The peak wavelength of all the functional groups were within the frequency range and thus the drug is been in pure form and there will not be any kind of contaminations are been observed.

3.3 Characterization of Proniosomal gel:

3.3.1 Compatibility Studies:

Fig 4. FTIR spectrum of Fenoprofen Calcium

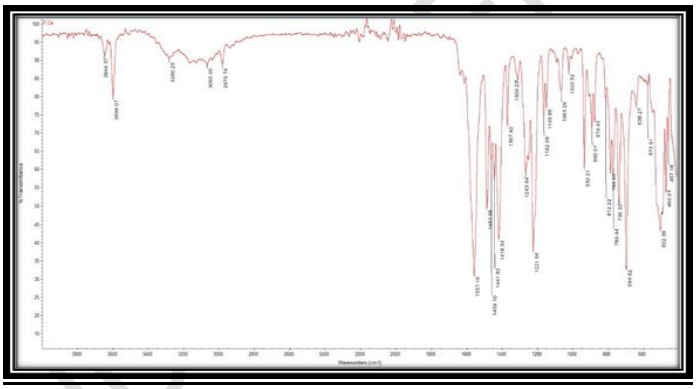


Fig 5. FTIR spectrum of Span 60.

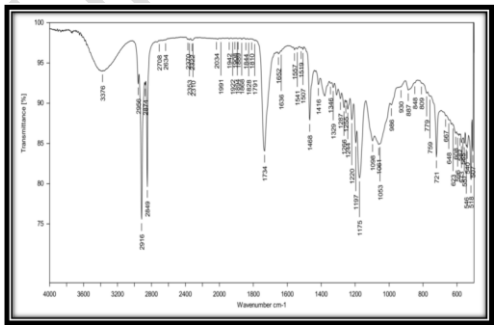


Fig 6. FTIR spectrum of Cholesterol

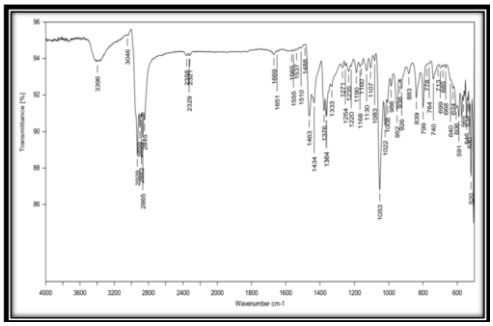


Fig 7. FTIR spectrum of Soya Lecithin

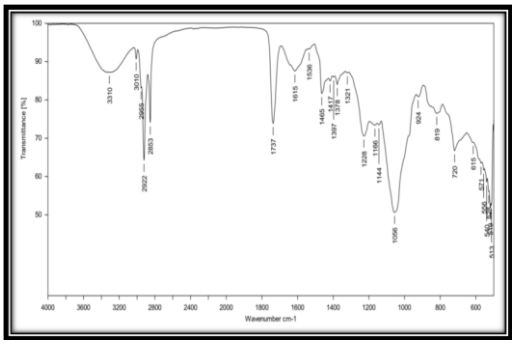


Fig 8. FTIR spectrum of Maltodextrin

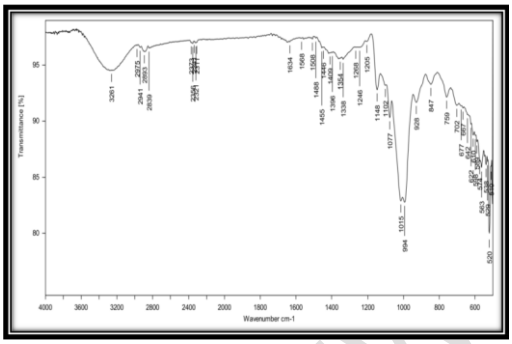


Fig 9. FTIR spectrum of F1

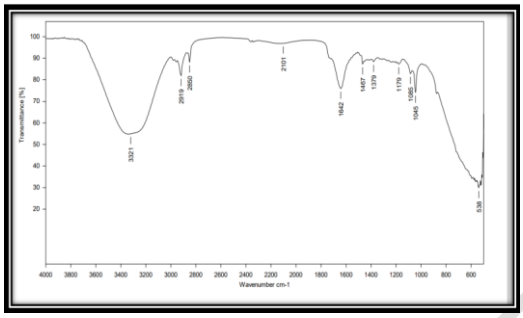


Table 10. FTIR peaks of Fenoprofen calcium, excipients and Formulations

Sl no	Compound	Functional groups	Frequency range cm^{-1}	Peak cm^{-1}	Vibration
1	Fenoprofen Calcium	OH	3550 – 3200	3340	Stretching
		Aromatic – CH	3080 – 3030	3065	Stretching
		Aliphatic – CH	2960 – 2850	2942	Stretching
		C=O	1675 – 1665	1669	Stretching
		C-O-C	1310 – 1250	1278	Stretching
2	Span - 60	OH	3550 – 3200	3376	Stretching
		R- O – C = O | R	1740 – 1720	1734	Stretching
		C = C	1662 – 1626	1636	Stretching
		C- O –C	1225 – 1200	1220	Stretching
3	Cholesterol	OH	3550 – 3200	3396	Stretching
		Aliphatic –CH	3000 – 2840	2845	Stretching
		C = C	1675 – 1665	1669	Stretching
4	Soya Lecithin	C = O	1750 – 1735	1737	Stretching
		CH	3000 – 2840	2853	Stretching
		NH	1650 – 1580	1615	Bending
		CF	1400 - 1000	1378	Stretching

Sl no	Compound	Functional groups	Frequency range cm^{-1}	Peak cm^{-1}	Vibration
5	Maltodextrin	OH	3550 – 3200	3261	Stretching
		Aliphatic – CH	3000 – 2840	2893	Stretching
		C=C	1630 – 1556	1634	Stretching
		C- O – C	1275 – 1200	1246	Stretching
6	Formulation F1	OH	3550 – 3200	3321	Stretching
		CH	3000 – 2840	2919	Stretching
		C = C	1675 – 1665	1642	Stretching
		CF	1400 – 1000	1379	Stretching

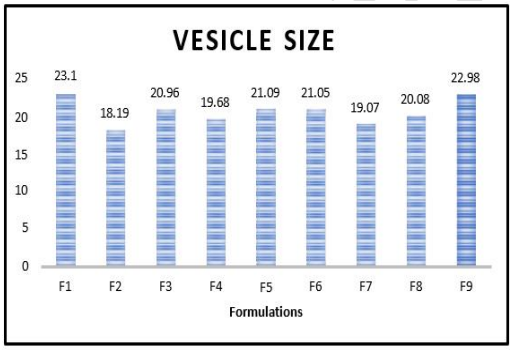
The drug and excipient compatibility study was carried out using ATR – FTIR studies. The presence of IR peaks associated with functional groups of the drug was interpreted and those compared with the IR peaks associated with formulations. The FTIR spectrum of drug and excipients were represented from figure no.04 to figure no 09. Functional groups and the IR peaks were tabulated in table no 10. By carrying out ATR - FTIR found that there is no cross interactions between drug and excipients.

3.3.2 Vesicle Size Analysis:

Table 11. Average vesicle size of formulations (F1-F9).

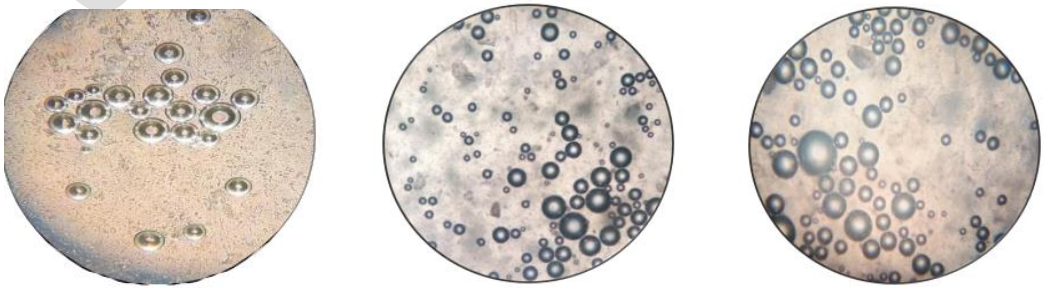
SL NO	FORMULATION CODE	VESICLE SIZE (µm)
1	F1	22.8 ± 0.36
2	F2	18.09 ± 0.34
3	F3	20.69 ± 0.30
4	F4	19.68 ± 0.31
5	F5	21.09 ± 0.44
6	F6	21.05 ± 0.09
7	F7	19.07 ± 0.27
8	F8	20.08 ± 0.30
9	F9	22.98 ± 0.19

Fig 10. Graphical representation of average vesicle size (F1- F9



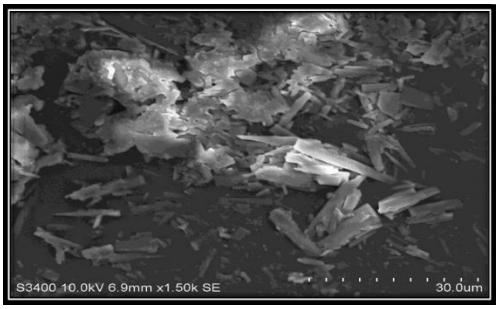
Determination of vesicle size for all the formulations were carried out using microscopic method. The average vesicle size of formulations F1 to F9 were in between 18.19 to 23.1 µm. Among the formulations F9 shows maximum vesicle size. Formulation F2 shows least average vesicle size. The average vesicle size of all the formulations were expressed in table no 11. Average vesicle size of all the formulations were graphically represented in figure no 10.

Fig 11. Micro photographs of Formulation F series.



3.3.3 Scanning Electron Microscopy:

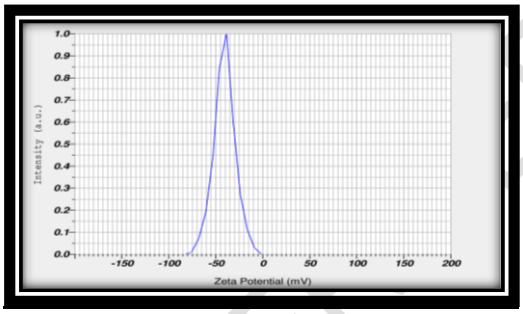
Fig 12. Scanning electron microscopy of Formulation F1.



Scanning electron microscopy evaluation was carried out to observe the morphological appearance of proniosome powder. The optimized formulations F1 was evaluated for SEM studies. The SEM morphology of formulations F1 was depicted in figure no 12. Formulation F1 shows fiber like, irregular powder structures.

3.3.4 Zeta Potential:

Fig 13. Zeta potential plot of Formulation of F1.



Zeta potential of the optimized formulations were carried out to determine the presence of charge on the vesicles. Formulation F1 was evaluated for Zeta potential analysis. F1 shows zeta potential of -40.8 mV. The results were depicted in table no 13 and graphs were shown in figure no 13. The charge on vesicle directly implies on its stability whereas the formulations having charge more than ± 30 mV are considered as formulations with good stability. Hence both formulations are stable on long term storage.

3.3.4.1 Zeta potential parameters:

Table 12. Results of Zeta Potential of F1.

PARAMETERS	FORMULATION
	F1
Temperature	25.2 °C
Voltage	3.4 V

Conductivity	**0.101 mS/cm**
Zeta potential	**-40.8 mV**

3.3.5 Thermal Analysis:

Fig 14. DSC Thermogram of Fenoprofen Calcium.

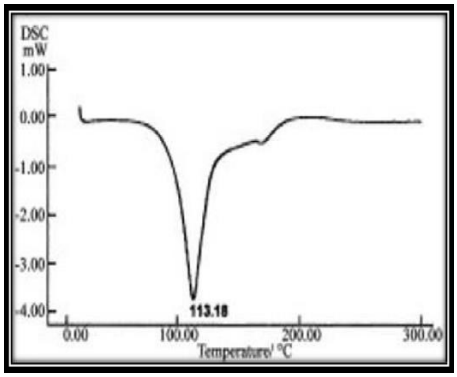
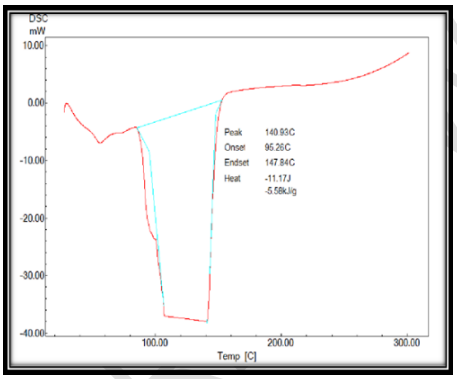


Fig 15. DSC Thermogram of Formulation F1.



Thermal analysis was carried using Differential Scanning Colorimetric method. Using DSC peaks the possible interaction between span 60, cholesterol, lecithin is been predicted. DSC analysis of Fenoprofen Calcium, proniosomal gel F1 is used for interpreting the amorphous nature of drug. In the DSC thermogram of Fenoprofen Calcium, endothermic peak was observed at 113.18℃ where as it resembles melting point of Fenoprofen Calcium. This endothermic peak was disappeared in DSC thermograms of formulation F1, this confirms the entrapment of drug into the vesicles. The DSC thermograms of Fenoprofen Calcium, formulation F1 is represented in figure no 15.

3.4 Evaluation of Proniosomal gel:

Table 13. Proniosomal gel physical evaluations (F1- F9).

Formulation code	pH	Viscosity (cps)	Spreadability (gm.cm/sec)	Extrudability (%)
F1	6.9 ± 0.05	29585 ± 55	18.3 ± 11.7	94.18 ± 0.81
F2	6.8 ± 00	29029 ± 87	18.98 ± 0.81	92.31 ± 0.94
F3	7.2 ± 00	29595 ± 220	17.61 ± 1.66	93.38 ± 1.12
F4	6.8 ±0.05	29413 ± 402	18.26 ± 1.10	93.52 ± 1.20
F5	6.6 ± 00	29283 ± 389	19.15 ± 7.97	92.98 ± 0.38
F6	6.5± 0.05	29146 ± 76	18.35 ± 0.54	94.02 ± 0.61
F7	7.1± 0.11	29773 ± 55	18.59 ± 1.46	93.68 ± 1.31
F8	6.8 ± 00	29796 ± 70	17.35 ± 0.63	93.93 ± 1.13
F9	6.6 ± 00	29653 ± 190	19.79 ± 1.75	94.61 ± 1.01
FPCa	6.8 ± 0.05	29676 ± 181	27.9 ± 2.38	93.89 ± 1.50

**FPCa Fenoprofen Calcium plain gel*

3.4.1 pH:

pH is the major parameter for the topical preparations. pH of all the formulations from F1 to F9 were evaluated. pH of all the formulations were in the range of 6.5 to 7.1 and all the formulations were having pH near to skin hence there will not be any kind of irritation upon topical application. The results were depicted in table no 13.

3.4.2 Viscosity:

Viscosity of the prepared formulations F1 to F9 were ranging from 29029 to 29796 cps. The results were depicted in table no 13. There is a slight and acceptable difference in the viscosity is been observed.

3.4.3 Spreadability:

All the formulations shown good spreadability property where as the spreadability of formulations from F1 to F9 were ranging from 17.35 to 19.79 gm.cm/sec. Spreadability of all the formulations were depicted in table no 13.

3.4.4 Extrudability:

Extrudability of all the formulations were optimum and for the formulations from F1 to F9 it is been ranging from 92.31 to 94.61%. The extrudability results were depicted in the table no 13.

3.4.5 Drug Content and Entrapment Efficiency:

Table 14. Drug content and Entrapment efficiency of formulations (F1 - F9).

FORMULATION CODE	DRUG CONTENT (%)	ENTRAPMENT EFFICIENCY (%)
F1	97.43 ± 0.62	88.41 ± 0.36
F2	95.29 ± 0.50	87.05 ± 0.51
F3	95.42 ± 0.65	85.03 ± 1.09
F4	96.01 ± 0.57	75.89 ± 1.52
F5	96.25 ± 0.70	82.12 ± 0.92
F6	93.92 ± 0.85	78.35 ± 0.56
F7	92.15 ± 0.55	76.67 ± 0.49
F8	95.08 ± 1.05	77.92 ± 1.12
F9	91.19 ± 0.60	74.02 ± 0.48
FPCa	98.1 ± 0.52	-

**FPCa Fenoprofen Calcium plain gel*

Drug content evaluation was carried for all the formulations. The drug content for formulations F1 to F9 were ranging from 91.19 to 97.43%. All the formulations shown good results on drug content evaluation and results were depicted in table no 14. Entrapment efficiency was carried to determine the total amount drug entrapped into the vesicles. The entrapment efficiency of all formulations from F1 to F9 were ranging from 74.02 to 88.41%. The results of entrapment efficiency of all the formulations were depicted in table no 14.

3.4.6 In vitro Diffusion Studies:

Table 15. *In vitro* release profile of proniosomal gel formulations F1– F4.

SL NO	TIME (HOURS)	DRUG SOLUTION (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
1	1	26.4 ± 0.98	21.73± 0.49	19.56 ± 0.26	22.46 ± 0.20	18.11 ± 0.62
2	2	42.44 ± 0.80	25.21 ± 0.58	29.18 ± 0.45	27.76 ± 0.25	27.2 ± 0.61
3	3	63.02 ± 1.01	30.16 ± 0.61	34.54 ± 1.04	32.8 ± 0.80	31.09 ± 0.80
4	4	79.04 ± 0.45	34.25± 1.21	42.85 ± 0.30	36.1 ± 0.47	35.9 ± 0.30
5	6	94.6 ± 0.85	42.7 ± 1.00	49.91± 0.47	41.6 ± 0.70	39.46 ± 0.64
6	8	99.4 ± 0.51	47.5 ±0.30	56.4 ± 1.10	47.6 ± 0.76	44.89 ± 0.47
7	12		68.9 ± 1.13	66.55 ± 0.25	69.52 ± 0.62	67.86 ± 0.68
8	24		94.23± 0.62	91.82 ± 0.01	90.47 ± 0.76	87.36± 0.85

Table 16. *In vitro* release profile of proniosomal gel formulations F5– F9.

SL NO	TIME (HOURS)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
1	1	15.94 ± 0.90	25.07 ± 0.35	26.01 ± 0.81	26.73 ± 1.13	21.23 ± 0.14
2	2	24.07 ± 0.50	28.9 ± 0.45	29.24 ± 1.05	29.03 ± 0.40	26.95 ± 0.54
3	3	30.2 ± 0.20	33.41 ± 0.20	35.41± 0.92	35.7 ± 0.62	29.32± 0.56
4	4	34.12 ± 0.58	35.79 ± 0.46	39.32 ± 1.25	38.9 ± 1.05	33.7 ± 0.76
5	6	40.16 ± 0.77	41.9 ± 0.77	44.2 ± 0.26	47.7 ± 0.90	39.5 ± 1.04
6	8	53.24± 0.90	48.89 ± 0.64	51.7 ± 1.00	54.3 ± 1.30	46.5 ± 0.94
7	12	66.34 ± 0.65	70.24 ± 0.98	65.93 ± 1.26	68.07 ± 0.95	65.37 ± 0.94
8	24	76.40 ± 0.25	81.81 ± 0.85	73.15 ± 1.25	86.84 ± 0.16	79.61 ± 0.53

Fig 16. % CDR v/s Time profile of F1, F2, F3, F4.

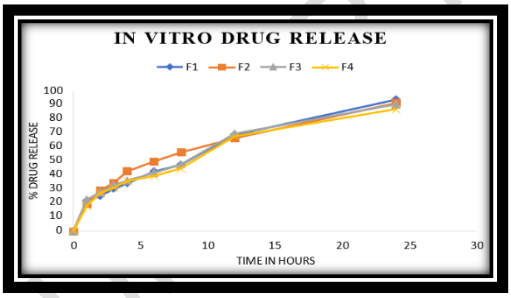
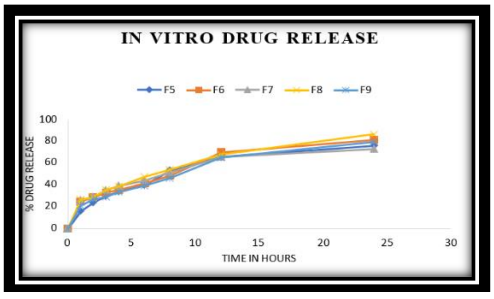


Fig 17. % CDR v/s Time profile of F5, F6, F7, F8, F9



The drug release pattern from the proniosomal gel formulation was examined using Franz Diffusion cell method. Formulations from both the methods were evaluated for Diffusion studies for the period of 24 hours. At the end of 24 hours percentage cumulative drug release for the formulations from F1 to F9 were ranging from 76.40 to 94.23 %. Whereas at the end of the diffusion study for 24 hours F1, F2 and F3 shows 94.23 %, 91.82% and 90.47% of cumulative drug release. Then F4, F5 and F6 shows 87.36 %, 76.40% and 81.81 % of cumulative drug release and formulations F7, F8 and F9 shows 73.15 %, 86.84 % and 76.61 % respectively. The percentage cumulative drug release of all the formulations were depicted in the table no 15 to table no 16. The graphical representations were shown in figure no 16 to 17.

3.4.7 Drug release kinetic modeling

Table 17. Kinetic model fitting values of proniosomal gel formulations F1-F9

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI MODEL	KORSMEYER PEPPAS	HIXON CROWEL	BEST FIT MODEL
	R^2	R^2	R^2	R^2	R^2	R^2
F1	0.8846	0.7584	0.8596	0.9943	0.7124	KORSMEYER PEPPAS
F2	0.8328	0.8241	0.7958	0.9947	0.7541	KORSMEYER PEPPAS
F3	0.8452	0.8124	0.7542	0.9925	0.6576	KORSMEYER PEPPAS
F4	0.8785	0.7654	0.8972	0.9936	0.7368	KORSMEYER PEPPAS
F5	0.8612	0.7220	0.9570	0.9814	0.7765	KORSMEYER PEPPAS
F6	0.9185	0.8553	0.9134	0.9542	0.8808	KORSMEYER PEPPAS
F7	0.8717	0.7917	0.7219	0.9745	0.8218	KORSMEYER PEPPAS
F8	0.8954	0.8541	0.7954	0.9843	0.7896	KORSMEYER PEPPAS
F9	0.9024	0.8451	0.7654	0.9834	0.7654	KORSMEYER PEPPAS

Kinetic studies of all the formulations were evaluated using the Kinet DS software. For the evaluation, the cumulative drug release data at each intervals is been used. R2 value of all the formulations were calculated using Zero order, First order, Hixon Crowell, Korsmeyer peppas and Higuchi equations. R2 closer to 1.00 is been considered as linear graph and it is the best fit model of respective formulations. The R2 value of all the formulations were depicted in table no 17. Formulations from F1 to F9 has R2 value in the range of 0.9542 to 0.9947 for Korsmeyer peppas model. All the formulations fits best in Korsmeyer peppas model.

3.4.8 Stability studies

Table 18. Stability studies of formulation F1

Sl no	Parameter	Formulations	Temp	1st day	30 days	60 days	90 days
1	Vesicle size	F1	4± 2℃	22.14±0.6	22.14±0.3	24.89±0.2	23.55±0.5
			30±2℃	22.8±0.7	23.8±0.3	22.62±0.6	22.09±0.2
2	Entrapment efficiency	F1	4± 2℃	88.9±1.2	88.52±1.5	87.85±1.6	89.50±1.2
			30±2℃	88.3±1.9	88.9±1.2	86.11± 1.1	88.51±0.7

Stability study was carried out to verify any kind of changes in properties of proniosomal vesicles during the storage. For this entrapment efficiency and average vesicle size is been evaluated periodically. The optimized formulations F1 was kept at two temperature zones, one is at 4 ± 2℃ (Refrigerator) and another one at 30 ± 2℃ (Room temperature). There is no

significant changes were found when it is carried out for optimized formulation. For formulation F1 average vesicle size was found around 22 to 24 µm and entrapment efficiency was found around 87 to 89 %. The stability study data was expressed in detail in table no 18.

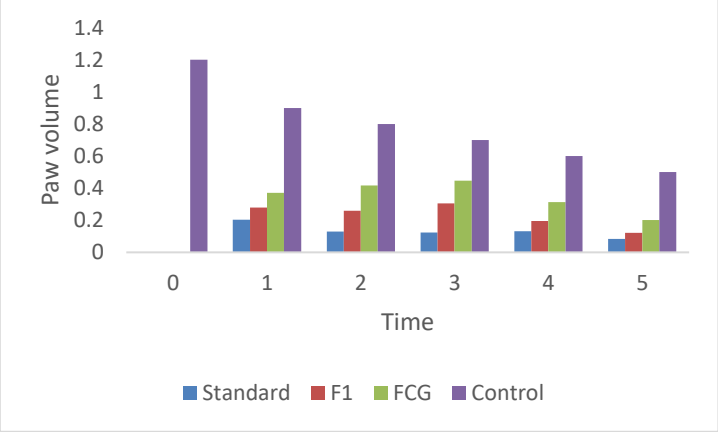
3.5 In vivo anti-inflammatory activity

3.5.1 Anti-inflammatory study

Table 19. Percentage inhibition of paw volume

HOURS	INHIBITION OF PAW VOLUME (ml)		
	STANDARD	F1	FCG
1	0.2033±0.014 (47%)	0.2783± 0.021 (28.03%)	0.3700±0.065 (4.31%)
2	0.1300±0.032 (70.25%)	0.2583±0.042 (40.85%)	0.4167±0.01 (4.57%)
3	0.1233±0.009 (75.66%)	0.3050±0.017 (39.80%)	0.4467±0.08 (11.84%)
4	0.1317±0.074 (76.12%)	0.1950±0.041 (64.65%)	0.3133±0.013 (43.21%)
5	0.0833±0.039 (85.34%)	0.1217±0.084 (78.58%)	0.2011±0.003 (49.71%)

Fig 18. Time v/s paw volume profile of control, standard, F1, S5 and FCG formulations



Acute anti-inflammatory study was carried for evaluating the in vivo assessment of the proniosomal gel. The study was carried out for 5 hours and the percentage inhibition of paw volume was reported in table no 19. For the study, standard marketed gel, proniosomal gel F1 and Fenoprofen Calcium plain gel are been used. Among these, standard marketed gel comes up with good results by showing 88.4% paw volume inhibition at the end of 5 hours, followed by proniosomal gel F1. Fenoprofen Calcium plain gel was shown 49.71% inhibition which is slightly lesser than all formulations.

Proniosomal gel formulations F1 shows good results for paw edema inhibition in animals. All the data of paw volume was introduced in the graphical representation shown in figure no 18.

4. CONCLUSION

On the basis of above studies, including Drug profile, Excipient profile, Formulation design, Methods and Evaluation results following conclusions are made:

- The present work was designed to develop a proniosomal topical gel to get sustained therapeutic action for treating acute inflammation.
- A successful effort has been made in the development of proniosomal gel loaded with Fenoprofen calcium.
- The variations in the excipient concentrations and their impact on the formulation is been assessed through various in vitro and in vivo evaluations.
- Initially the proniosomal gel of Fenoprofen calcium is been developed using coacervation and phase separation method.
- Then developed formulations were examined for various in vitro and in vivo parameters.
- Physical characterization confirms the physical state, appearance, colour, texture, odour, that matches with literature values of pure Fenoprofen calcium.
- From solubility test it concludes that the drug was insoluble in water and soluble in organic solvents.
- Standard calibration curve is been also developed and used for quantitative estimation of drug.
- Authentication of Fenoprofen calcium was carried out using ATR-FTIR studies confirms the functional groups of Fenoprofen calcium.
- Drug excipient compatibility studies were carried out using ATR-FTIR studies and spectra confirms there is no unwanted interaction between drug and excipients.
- From the data obtained from vesicle size analysis, concluded that F2 shows least vesicle size and F9 shows larger vesicle size.
- SEM images confirms that, in Coacervation and phase separation method the powder characteristics shows spikes and fibers.
- Zeta potential report of both formulations confirms charge on vesicles, which indicated the vesicles has good stability properties.
- Thermal analysis was carried out using DSC. Which confirms the surfactant, cholesterol, lecithin reaction during formulation and formed double layer on vesicles.
- pH of the formulations were in the range of skin pH, that implies the formulations are safe to apply topically. There will not any kind of irritation on skin upon application of proniosomal gel.
- Viscosity of all the formulations were moderate. Formulation F8 shows maximum viscosity where as F2 shows least viscosity.
- Spreadability of all the formulations were excellent, F9 shows maximum spreadability and F8 shows least spreadability.
- Extrudability of all the formulations were good. Formulation F9 shows maximum spreadability and F2 shows least extrudability.
- Drug content evaluation of all the formulations shows best results where as F1 shows maximum drug content and F4 shows slightly lesser drug content.
- Entrapment efficiency of all the formulations from Coacervation and phase separation method are best. Among these F1 shows highest entrapment efficiency and F9 shows lesser entrapment efficiency.
- From the results obtained from In vitro diffusion studies it is concluded that all the formulations shows sustained release of drug up to 24 hrs. Among all the formulations

within 24 hours F1 shows maximum release of drug and F7 shows some more sustained effect.

- Kinetic studies of all the formulations fits best in Korsmeyer peppas model which indicates the drug release from formulation is been diffusion controlled.
- Stability studies confirms there is no physical and chemical changes in proniosomal gel formulations. Vesicle size and entrapment efficiency is within acceptable variation throughout the studies.
- In vivo anti-inflammatory studies confirms the inhibition of inflammatory mediated receptors within the animals and the prepared formulations shows good anti-inflammatory action. Compared to marketed product, Formulation F1 shows very close agreement of result.
- Final conclusion is, totally the objectives set prior to the work were satisfied completely. A successful effort was made in development of proniosomal gel loaded with Fenoprofen calcium.

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee". (Ref. No: IAEC/HSKCOP/July2023/PG17).

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