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Topical Delivery of Fenoprofen Proliposomes: Preparation, Evaluation and *In Vitro* Release.

[1,]Manjula D , [2,]Shabaraya AR , [3,]Somashekar Shyale
DayanandaSagar College of Pharmacy, Bangalore, Karnataka, India.
Srinivas College of Pharmacy, Mangalore, Karnataka, India.
H.S.B.P.V.T. college of Pharmacy, Maharashtra, India.

ABSTRACT:
BACKGROUND: *Patients with rheumatic disease are usually prescribed with non-steroidal anti-inflammatory drugs which causes increased gastrointestinal complications. The aim of the current study was to formulate and characterized a vesicular type of drug carrier system such as proliposomal system for transdermal delivery of fenoprofen in order to avoid the problems associated with oral administration.*

METHODS: *Fenoprofenproliposomes were prepared by Xiao et al., method by film-deposition. So prepared proliposomes were characterized for their size, surface morphology, drug content, percent encapsulation efficiency, drug retained in skin, in vitro permeation studies and stability studies. Drug-excipient interaction was studied by FT-IR.*

RESULTS: *The results revealed no interaction between drug and the excipients, SEM photography confirmed spherical shaped proliposomes of fenoprofen with an average size of about 10µm. The maximum drug content and percent entrapment efficiency were found to be97.13±0.62, 78.75±1.92 respectively. Maximum drug permeation was seen in F10 formulation where the lecithin concentration was optimum. The prepared liposomes were found to be stable at lower temperatures.* ***Conclusion:*** *To conclude, fenoprofen loaded liposomes seem to be promising controlled delivery system.*

KEYWORDS: ***Fenoprofen, Proliposomes, in vitrorelease***

I. INTRODUCTION.

Arthritis is one of the vital syndrome affecting majority of the elderly patients and for this NSAID's are advised to reduce pain and inflammation[1]. The medications prescribed for the relief of inflammation and associated pain are available as conventional dosages like tablets and capsules. These conventional medications cause GI disturbances and drug level fluctuates, thereby making the patient to suffer with over-dosage. To minimize the GI disturbances and to improve the bioavailability of the drug, certain novel dosage forms are being investigated. Transdermal drug delivery systems (TDDS) are such delivery systems which are designed to support the passage of drug substances from the surface of skin, through its various layers, into the systemic circulation. Their advantages over conventional dosage forms include improved patient compliance, avoidance of gastric irritation and first-pass effect and controlled therapeutic responses[2]. Clinical evident suggest that topically applied non-steroidal antiinflammatory drugs are safer and at least as efficacious as oral NSAID's in the treatment of rheumatic diseases.

Drug delivery from various liposomes in transdermal formulation has been studied for many number of purposes, but unstable nature and poor skin permeation of liposomes limits their use for topical delivery. Further, problems in the sterilization and large-scale production of liposomes remain unsolved. In order to improve the stability of liposomes, the concept of proliposomes was proposed. Proliposomes offer an elegant alternative to conventional liposomal formulations and are defined as dry, free-flowing particles that immediately form a liposomal suspension when come in contact with water. Because of the solid properties, the stability problems of liposome can be solved without influencing their intrinsic characteristics. Proliposomes are composed of drug, phospholipid and a water soluble porous powder and can be stored in a dried state. Moreover, by controlling the size of the porous powder in proliposomes, relatively narrow range of reconstituted liposome size can be obtained.

Fenoprofen, is a non-steroidal drug used as antiinflammatory, analgesic and antipyretic. It is used in the treatment of osteoarthritis, rheumatoid arthritis and ankylosingspondilytis. Though it is rapidly being absorbed after oral administration, it undergoes significant first-pass metabolism. It has a very short half life of about 2-3 h and is associated with gastro-intestinal side effects like nausea, gastric irritation etc. To extend drug action, to improve delivery of drug into systemiccirculation, the present study was undertaken with the aim to develop and characterize fenoprofenproliposomes. Further *in vitro* drug release studies was carried out in Keshary-Chien diffusion cell, stability studies at various temperatures was carriedout for the best formulation.

II. MATERIALS AND METHODS

Materials

Fenoprofen was procured as gift sample from D. K. Pharma, Mumbai,India, lecithin was purchased from Himedia laboratories Ltd. (Mumbai, India.) mannitol, chloroform and all other chemicals were commercially purchased from SD fine chemicals. All the chemicals used were of analytical grade.

Method of Preparation

The proliposomes were prepared according to the method reported by Xiao et al.[3] In this method 5 gm of mannitol powder was placed in a 100 ml round bottom flask which was held at 60-70^0 C and the flask was rotated at 85 ± 5 rpm for 30 min and dried under vaccum drier at 10 psi for 30 min. Fenoprofen (100 mg) and lecithin (drug to lecithin ratios of 0.1:1, 0.1:2, 0.1:3, and 0.1:4, 0.1:5 and 0.1:6) were dissolved in chloroform and methanol solvent in the ratio of 8:2 v/v, and 0.5 ml aliquot of the above organic solution was introduced into the round bottom flask containing mannitol, at 37^{0}C. After drying, second aliquot (0.5 ml) of the solution was added and dried similarly. Proliposomes so generated kept in a lyophilizer (Modulyo D-230) for 1 h and subsequently fenoprofen loaded mannitol powder (proliposomes) was placed in desiccator overnight and then sieved with 100 mesh. So prepared fenoprofen loaded proliposomes were stored under freeze temperature until further use.

Table 1: Formulation codes of fenoprofen loaded proliposomes.

Ingredients	Formulation Codes					
	F7	F8	F9	F10	F11	F12
Mannitol (gms)	5	5	5	5	5	5
Fenoprofen (mg)	100	100	100	100	100	100
Lecithin (gms)	1	2	3	4	5	6
Chloroform (ml)	8	8	8	8	8	8
Methanol (ml)	2	2	2	2	2	2

The above formulae gives an average of 6.75 gms of proliposomes.

Liposomal Characterization

Compatibility study

Possible drug and polymer interaction was studied using FT-IR[4](Perkin-elmer 1600 series USA), at 400cm-1 to 4000 cm-1.

Surface Morphology[5].

In order to asses the surface morphology of fenoprofen loaded proliposomes, they were subjected for scanning electron microscopy (SEM). Gold coating was done by sputter coater (Polaron SEM coating system). Then the samples were observed under the scanning electron microscopy (JSMT330A, JEOL) at a beam voltage of 15 kV.

Determination of drug content and % entrapment efficiency[6].

The drug content in fenoprofen loaded proliposomes was assayed by an UV spectrophotometer (Shimadzu 1700). 100 mg of proliposomes were dissolved in a mixture of phosphate buffer saline pH 7.4 and methanol (1:9 v/v ratio) by shaking the mixture for 5 min. 1 ml of the resultant solution was taken and diluted with methanol suitably and then absorbance was recorded at 272.5 nm using UV spectrophotometer.

For the determination of percentage entrapment efficiency, 10 ml of distilled water was added to 100 mg proliposomal granules. This mixture was subjected for centrifugation at 10,000 rpm at 4^0 C for 45 min. the clear supernatant was collected to get unentrapped drug. The supernatant liquid was suitably diluted with methanol and absorbance was recorded at 272.5nm. Then 1 ml of sediment was resuspended in 2% Triton x-100, suitably diluted with methanol and absorbance recorded at 272.5nm. The % entrapment efficiency was calculated using following formula,

$$\% \text{ Entrapment} = \frac{\text{Amount of fenoprofen in sediment}}{\text{Total amount of fenoprofen added}} \times 100$$

Drug permeation studies[7].

Drug permeation through rat abdominal skin was carried out after getting approval from institutional animal ethical committee, bearing registration number 557/02/C/CPCSEA It was conducted as per the principles and guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Preparation of Skin[8]:

The abdominal skin of excised hairless Sprague Dawley rat skin was separated along the epidermal junction and was heated for 50 seconds with a stream of 60^oC water. The heat-treated skin was cleared of subcutaneous fatty substance and kept in normal saline solution to flatten and smooth. This step caused the layer to unwrinkle. This skin was mounted on to the donor cell of the Keshary-Chien cell.

***In vitro* permeation studies across hairless rat abdominal skin[9]:**

Permeation studies were carried out in a keshary-chien diffusion cell of 50 mL capacity with 3.14 cm^2 diffusion area, with a teflon coated magnetic bead inside receptor compartment kept over a magnetic stirrer. Initially, the cell was calibrated and validated for surface area of the donor compartment, capacity of the receptor compartment, temperature 37^0 Cof the receptor fluid and and also for rpm of the bead (100 rpm). 100 mg of proliposomes (fenoprofen) were applied on the rat abdominal skin evenly and the skin was mounted between the donar and receptor compartment. The receptor compartment was filled with freshly prepared phosphate buffer saline pH 7.4 (PBS). The permeation studies was carried out for a period of 24 hrs. 1 ml Sample was withdrawn at regular intervals of time and sink condition was maintained by replacing 1 ml of blank phosphate buffer saline solution.

Determination of fenoprofen retention in skin[10].

The ability of proliposomes to retain drug within the skin was investigated by determining the amount of drug retained in skin samples employed in permeation studies. After completion of the permeation experiment, the skin mounted on the diffusion cell was removed, the skin was cleaned with cotton dipped in saline solution and blotted with tissue paper to remove any adhering formulation. Subsequently, the skin sample was homogenized with 10 ml of chloroform:methanol mixture (2:1, v/v), for the extraction of fenoprofen. Homogenate suspension thus obtained was filtered using membrane filter (0.45m) and quantified for the percentage of drug content.

Stability studies of fenoprofen loaded proliposomes[11].

Stability testing was carriedout for optimized fenoprofenproliposomes (F8). The proliposomes were placed in vials, sealed and kept at 4 different temperature of 8^0C (refrigeration),25^0 C (room temperature), 37^0 C and 45^0C (in oven) for a period of 5 weeks. The proliposomes were sampled at regular intervals of 7, 15, 30, 45, 60, 75 and 90 days and tested for surface morphology, colour changes and residual drug content.

III. RESULTS AND DISCUSSION:

Compatibility study:

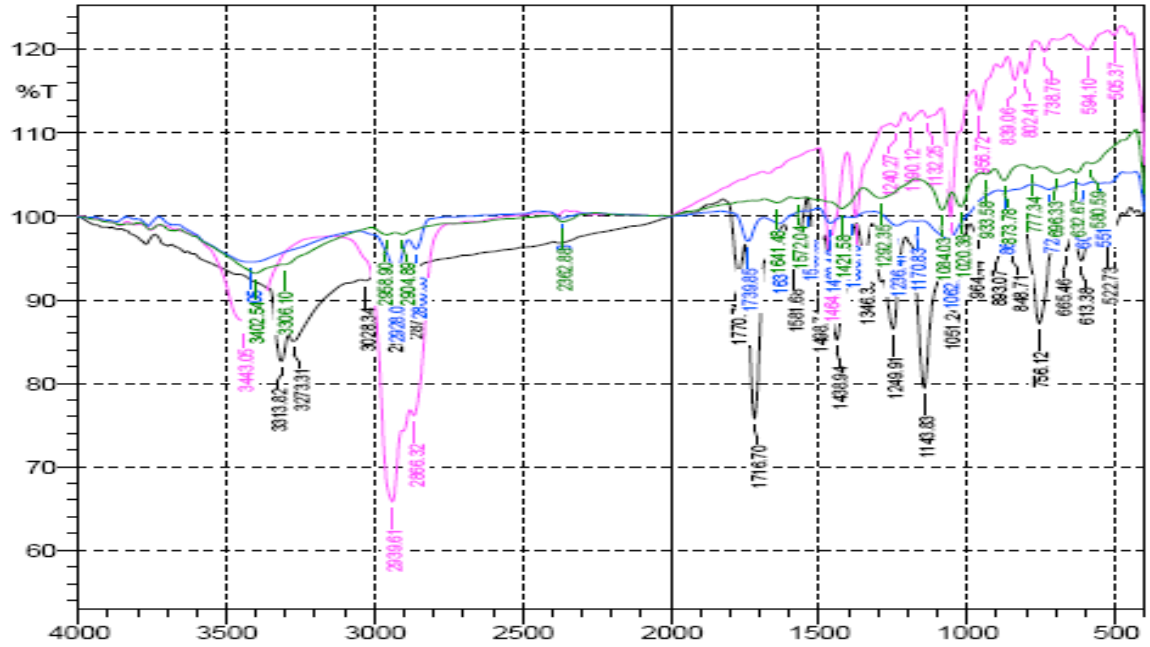

Fig 6: The merged FT-IR spectra of mannitol, cholesterol, lecithin and fenoprofen.

The merged FT-IR spectra ofmannitol, cholesterol, lecithin and fenoprofen has been illustrated in figure 6. As shown in the figure, all the peaks remain the same with small modifications which may be due to the other excipients. This indicates that there was no interaction between the ingredients used in the formulation.

Surface morphology: The morphology and surface appearance of fenoprofen loaded proliposomes were examined using SEM (fig 7). The SEM image of F4 formulation showed that fenoprofen loaded proliposomes were spherical in shape with particle size 10 µm.

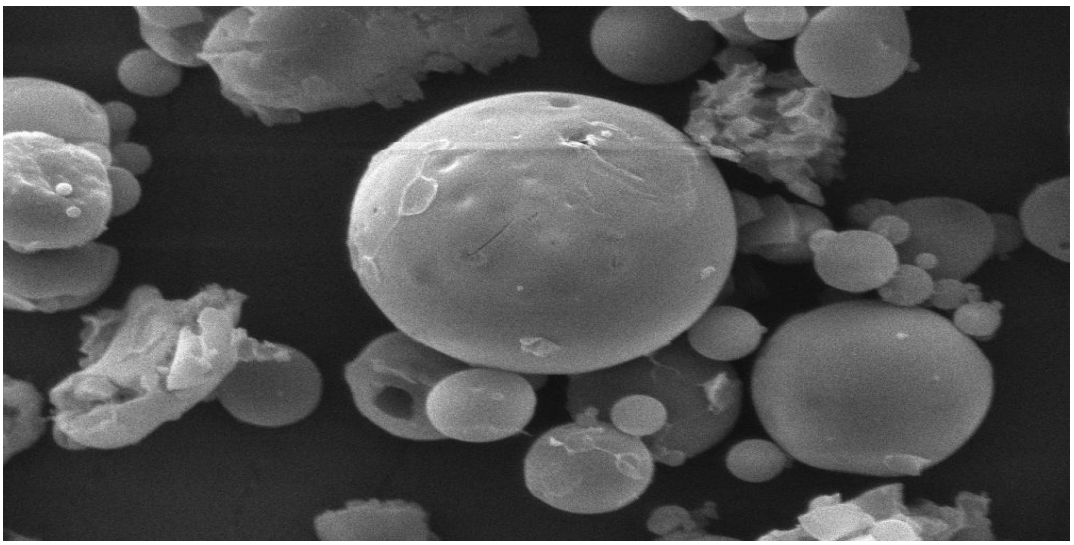

Fig 7: SEM photograph of fenoprofen loaded proliposomes.

Drug content and% entrapment efficiency: The fenoprofen contents (table 2) in proliposomes from F7, F8, F9, F10, F11 and F12 was found to be 94.26%, 95.83%, 96.15%, 97.13%, 96.25% and 95.82% respectively. The percentage entrapment efficiency (table 2) was determined to be 48.25%. 55.36%, 65.33%, 78.75%, 76.95% and 75.13% for a fenoprofenproliposomes from F7 to F12 formulations respectively. From the results obtained it could be concluded that as lecithin concentration increased, both the drug content and percentage

entrapment efficiency in fenoprofen loaded proliposomes also increased, which may be attributed to their dependency on lipid concentration upto 1:4 concentration, but as the lipid concentration still increased further there was decrease in the drug content and percentage entrapment efficiency which may be due to sustained release of drug for longer period of time. The results are shown in table 2.

Table 2: Drug content and % encapsulation efficiency infenoprofen loaded proliposomes.

Formulation Codes	* Drug Content	% encapsulation efficiency
F7	94.26 ± 0.63	48.25±1.35
F8	95.83 ± 0.56	55.36±1.63
F9	96.15 ± 0.74	65.33±1.75
F10	97.13 ± 0.62	78.75±1.92
F11	96.25 ± 0.55	76.95±1.53
F12	95.82 ± 0.65	75.13±1.25

* →Average of triplicate readings.

Drug permeation studies: Permeation studies was carried out by spreading the fenoprofenproliposomes on the treated rat abdominal skin upto 24 hrs using Keshary-Chien diffusion cell containing phosphate buffer pH 7.4. The results are shown in figure 8.The results gave the opinion that as the lecithin concentration was increased the permeation of drug from proliposomes across rat abdominal skin also increased which may be due to the reason that lecithin temporarily disrupts the lipid matrix of stratum corneum and helped in drug permeation. But this increase was seen only upto 1:4 concentration of drug to lecithin ratio. As the ratio further increased, that is in 1:5 and 1:6 ratio there was retardation in permeation of drug in fenoprofenproliposomes which may be due to the reason that lecithin rich domain vesicles might be due to depot effect for drug molecules (concentration of drug in skin).

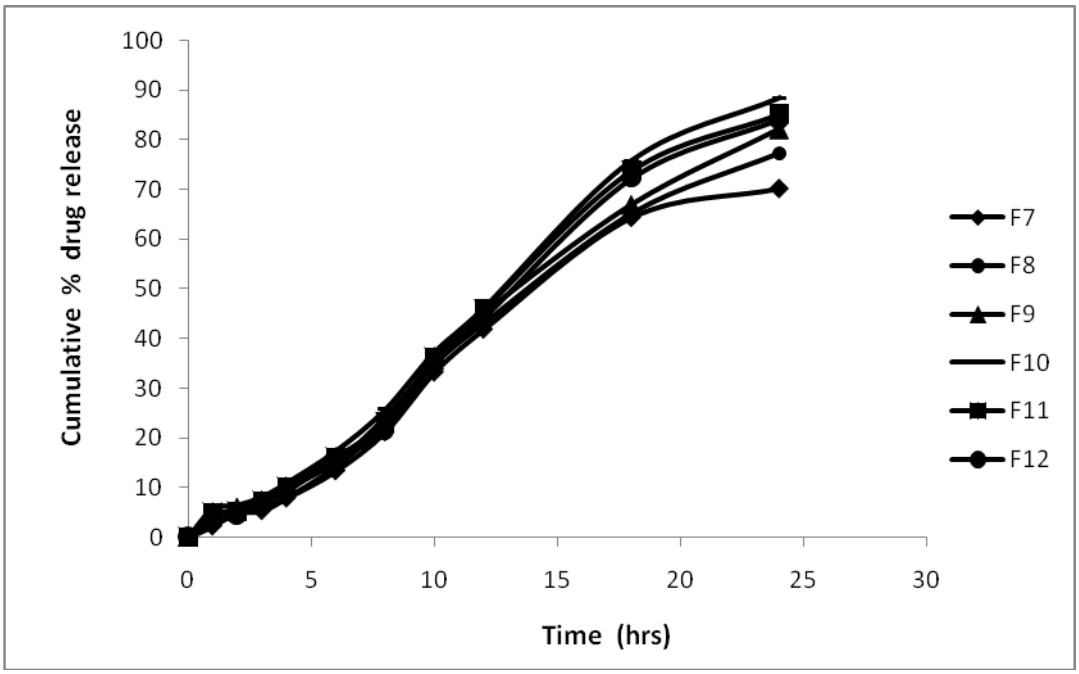

Figure 8: *In vitro* release of fenoprofen loaded proliposomes from F7 – F12.

The mechanism of drug release was confirmed by fitting the *in vitro* drug release data to various kinetic models and the results of n and r values of zero order kinetics, Higuchi's equation and Korsemayers-Peppas equation are tabulated in table 3.The n and r values indicates that the mechanism of drug release is by diffusion controlled which follows zero order kinetics as the n values are nearing 1 and follows non-fickian release.

Table 3: Comparative kinetic values from various formulations.

S.No.	Formulation Codes	Zero order equation		Higuchi's equation		Korsemayers-Peppas equation	
		n	r	N	r	n	r
1	F7	3.27	0.9862	16.37	0.9389	47.73	0.9000
2	F8	3.48	0.9923	17.33	0.9403	51.11	0.8984
3	F9	3.62	0.9943	19.99	0.9414	52.10	0.8968
4	F10	3.91	0.9928	19.41	0.9376	55.95	0.8894
5	F11	3.82	0.9911	18.95	0.9359	54.70	0.8883
6	F12	3.79	0.9913	18.72	0.9327	54.09	0.8864

Fenoprofen retention in skin.

Results of drug retained in skin is depicted in table 4. The % drug retained decreased with increase in lecithin concentration upto 1:4 ratio which may be due to the reason that lecithin temporarily disrupts the stratum corneum and increases the drug permeation. But further increase in lecithin concentration decreased the release rate which gave an understanding that liposomes could not only enhance the penetration of drug molecules but also helped localize the drug in the skin and form the drug depot for controlled and prolonged release.

Table4: Data for percentage drug retained in skin

for formulations F7- F12

Formulation Codes	**% drug retained in skin**
F7	3.78 ± 1.2
F8	3.19 ± 1.5
F9	2.88 ± 2.1
F10	2.13 ± 1.8
F11	7.98 ± 1.2
F12	9.55 ± 1.2

Stability studies: Stability studies of the optimized formulation (F10) was carried out at four different temperatures for a period of 90 days. At regular intervals of time (15, 30, 45, 60, 75 and 90 days) the formulation was observed for surface morphology, colour change and residual drug content. At the end of 90 days the proliposomes were free flowing, no colour change was seen and drug content was retained to its maximum at lower temperatures of 8^0 C, 25^0 C. But at higher temperatures they were found to be not so free flowing, there was change in colour seen, drug content was reduced to 85.54% from 97.25% (Fig 9),with considerable drug loss at elevated temperatures which may be attributed to the effect of temperature on the powder to liquid transition of lipid bilayers together with possible chemical degradation of the lecithin, leading to defects in the membrane packing. The drug leakage of less than 4 to 5% of the initial load at lower temperatures are well within the limits, when vesicles are to be advocated for topical applications.The results are depicted in figure 9.

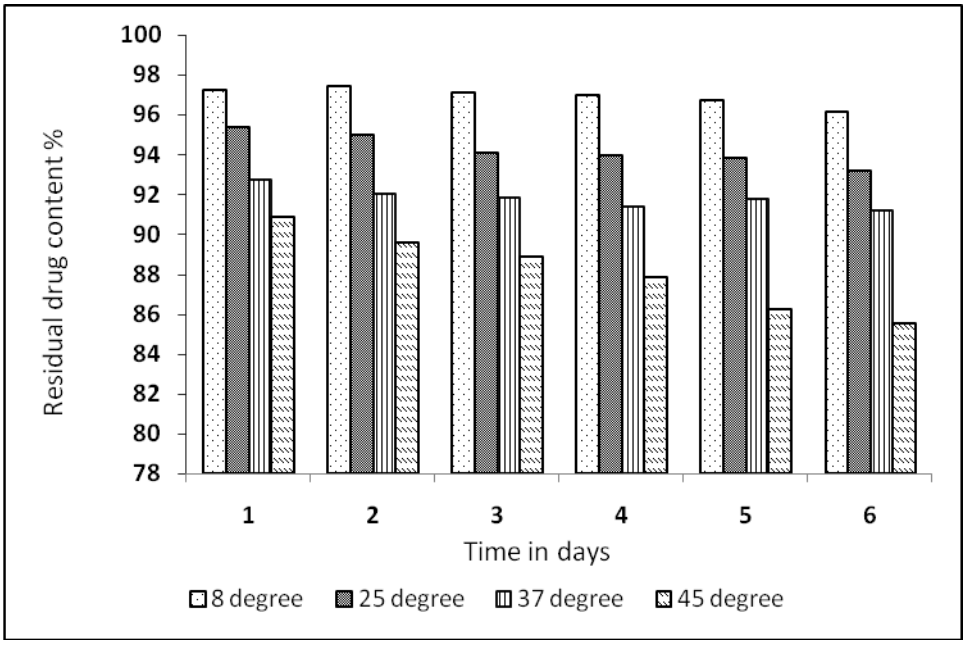

Fig 9: Stability studies of optimized formulation (F8) at ambient temperatures.

IV. CONCLUSION:

On the whole it can be concluded that the drug content and percentage entrapment efficiency increased with increase in lipid concentration of fenoprofen loaded proliposomesupto 1:4 ratio of drug to lecithin ratio, but decreased with further increase in lecithin concentration. Permeation studies indicated that a controlled release of fenoprofen can be achieved by proliposomal preparation and that the proliposomes showed a more prominent future in designing the transdermal therapeutic systems of variousdrugs. The formulated fenoprofenloaded proliposomes were more stable at lower temperatures.

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