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Development of Proniosomal Drug Delivery with Different Type of Penetration Enhancers

Ashwini A. Bachhav[1]*, Anil G.Jadhav[1], Ashish Y. Pawar[2], Satish A. Ahire[3]

[1]Department of Pharmaceutics, Sandip Institute of Pharmaceutical Science, Nashik-422231, India.
[2]Department of Pharmaceutics, MGV'S Pharmacy College, Panchavati, Nashik-422003, India.
[3]Department of Chemistry, Arts, Science and Commerce College, Surgana, Tal.Surgana, Nashik- 422211, India.

Abstract : Proniosomes offer a vesicle delivery concept with the potential for drug delivery via the transdermal route.The aim of this work is to search best penetration enhancers in proniosomes as a transdermal delivery system for Piroxicam.Piroxicam is a widely used potent non-steroidal anti-inflammatory drug, with due potential for dermal delivery. This was done with the goal of optimizing the composition of proniosomes as transdermal drug delivery systems. Plain proniosomes, proniosomes containing lecithin or skin penetration enhancers were prepared by coacervation phase separation method and effect of penetration enhancers were evaluated for transdermal delivery of piroxicam. P-OA formulation which was developed with Oleic acid (OA) proved to be with high permeation and entrapment efficiency as compared to other formulations. The optimized proniosomal gel (P-OA) formulation were characterized by Scanning electron microscopy and Transmission electron microscopy, zeta potential, vesicle size determination etc. *Ex vivo* permeation studies, irritancy test, *in vivo*are also carried out for this formulation results suggest that prepared proniosomal gel of Piroxicam is a promising approach for transdermal delivery. *Ex vivo* permeation study was done by using wister rat's skin and it gives flux 8.136 μg/cm^2.h for optimized batch (P-OA) while marketed formulation showed flux 5.062 μg/cm^2.h. The investigated piroxicam loaded proniosomal formula proved to be non-irritant, with significantly higher anti-inflammatory effects compared to that of the marketed pirox gel.

Key words: Niosomes, proniosomes, piroxicam, transdermal delivery, permeation, penetration enhancer.

1. Introduction:

Vesicular drug delivery systems have distinct advantages over conventional drug delivery systems. The colloidal particulate vesicles act as drug reservoir and adjust the drug release rate by modification of the particle composition or the surface characteristics. These vesicles included traditional liposomes[1], Transfersomes and Ethosomes[2,3,4]. However, most of liposomes were reported to have stability problem and high cost. The stability

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problems of liposomes are in the form of loss of entrapped drug, change in the size upon storage as well as chemical degradation of the lipid components[5]. Accordingly, niosomes which are surfactant based vesicles that are more stable (chemically) and less expensive than liposomes were introduced[6,7]. However, although niosomes exhibit more chemical stability during storage like degradation by hydrolysis or oxidation, there may be a physical stability problem upon storage of niosome dispersion like sedimentation, aggregation, or fusion. Proniosomes were prepared as dry powder for reconstitution before use as a mean of preserving the chemical and physical integrity of vesicles[8]. For the transdermal delivery purpose proniosomes were prepared as a gel[9]. These gel like structures have the advantage of being suitable for scaling up while maintaining the skin penetration enhancing abilities and better physicochemical stability. However, the published data on proniosomes are not included many factors requiring further investigation but, study shows effect of composition and the possibility of incorporating traditional skin penetration enhancers into proniosomes.

So, the main aim of this study is to investigate the effect of incorporation of penetration enhancers in proniosomes on the transdermal delivering ability of this system.

To achieve this objective, Piroxicam (PC) is one of the most potent non-steroidal anti-inflammatory agents that also has anti-pyretic activity and has been used for the treatment of rheumatoid arthritis, osteoarthritis and traumatic contusions was selected[10]. PC has been classified in the Biopharmaceutics Drug Classification system as a Class II drug with low solubility and high permeability. It demonstrates a slow and gradual absorption via the oral route and has a long half-life of elimination, rendering a prolonged therapeutic action and a delayed onset of anti-inflammatory and analgesic effect[11]. PC is well absorbed following oral administration; however, its use has been limited by a number of sideeffects, including bleeding and ulceration[12]. Therefore, there is need of an alternative non-invasive mode of drug delivery. Transdermal administration of piroxicam can overcome these side effects and higher local concentration can be maintained at the target site, which is desirable for anti-inflammatory agents[13]. The transdermal drug delivery system appears to be an attractive route of administration to maintain the drug blood levels of PC for an extended period[14].

Thus the study encompasses the ability of lipid vesicles to deliver piroxicam across skin in order to evaluate it transdermal delivery potential. Moreover greater stability can be accorded by proniosomal formulation as compared to niosomes and access their potential towards dermal delivery. The study will thus provide dual functions as it will add a new factor in optimized transdermal delivery from provesicular systems and develop a method for enhanced piroxicam transdermal delivery.

2. Experimental

2.1 Material

Piroxicam was procured from GlaxoSmithKline Pharmaceuticals Ltd, Mumbai. India. Span 60, ethanol, cholesterol, lecithin, oleic acid, Isopropyl myristate, propylene glycol, polyethylene glycol 400, urea, dimethyl sulfoxide, ethylene diamine tetra acetate were obtained from Modern Science Apparatus Pvt.Ltd. All other chemicals used in this study were of analytical grade.

2.2 Preparation of proniosomes[15]

Proniosomal gels were prepared according to the method reported by El Maghraby et al. The surfactant mixture (Span 60 - cholesterol), ethanol and penetration enhancer (ifany) were mixed and drug were weighed in a clean and dry, wide mouth small glass tube. After mixing all the ingredients, the open end of the glass tube was covered with a lid to prevent loss of solvent and heated to 65 + 1°C for 5 minutes in a stoppered vessel. This provided clear liquid system. The aqueous phase was added and the mixture was warmedon until clarity. The mixture was allowed to cool down by continuous mixing at room temperature till formation of proniosomal gel (Table-1).

Table-1: The composition of the tested formulations (expressed in grams per formulation).

Formula-tion code	Surfactant System*(gm)	Ethanol (gm)	Aqueous Phase**(gm)	Penetration enhancer(gm)							
				Lec	OA	IPM	PG	PEG	Urea	DMSO	EDTA
Control	5	5	4	-	-	-	-	-	-	-	-
P / Lec	5	5	4	1	-	-	-	-	-	-	-
P / OA	5	5	4	-	1	-	-	-	-	-	-
P / IPM	5	5	4	-	-	1	-	-	-	-	-
P / PG	5	5	4	-	-	-	1	-	-	-	-
P / PEG	5	5	4	-	-	-	-	1	-	-	-
P / Urea	5	5	4	-	-	-	-	-	1	-	-
P/ DMSO	5	5	4	-	-	-	-	-	-	1	-
P / EDTA	5	5	4		-	-	-	-	-	-	1

*The composition of the surfactant system in all formulation was Span 60 : cholesterol (4.5:0.5).
**The aqueous phase used Phosphate buffer (pH-7.4). The amount of the drug added to each formulation was 0.075 g.

2.3 Evaluation of effect of penetration enhancers in proniosomal formulations

2.3.1 Determination of entrapment efficiency[16]

To determine the entrapment efficiency of the drug into the proniosomes, the formulation was hydrated to develop the corresponding niosome. This was achieved by hydrating the 0.2 g of proniosome gel, weighed in a watch glass , 10 ml of the aqueous phase (phosphate buffer pH 7.4) were added; the aqueous suspension was then sonicated. Niosomes containing piroxicam were separated from untrapped drug by centrifugation at 9000 rpm for 45min at room temperature. The supernatant was recovered and assayed spectrophotometrically by using UV-Visible double beam spectrophotometer (Shimadzu 2450) at 254 nm. The encapsulation efficiency of drug (EE) was calculated by the following equation:

$$EE(\%) = \left[\frac{C_t - C_r}{C_t}\right] \times 100$$

where Ct, concentration of total piroxicam; Cr, concentration of free piroxicam.

2.3.2 Drug content[17]

Piroxicam content in the prepared proniosomal gels was determined by dissolving 0.2 g of the gel in methanol. The contents were passed through 0.4 µm membrane filter. 1 ml of above solution was further diluted to 10 ml with phosphate buffer of pH 7.4. The resultant solution was subjected to UV spectrophotometric analysis at 254 nm and the absorbance was noted down.

2.3.3 Rate of Spontaneity[18]

Spontaneity of niosomes formation is described as number of niosomes formed after hydration of proniosomes for 15 min. Proniosomes (10mg) were transferred to the bottom of a small stoppered glass tube and spread uniformly. One ml saline (0.154 M NaCl) was added carefully along the walls of the test tube and kept aside without agitation. After 15 min a drop of aqueous layer was withdrawn and placed on Neubaur's chamber (Fein-optik, Germany). The number of niosomes eluted from proniosomes were counted.

2.3.4 *In vitro* permeation study[19]

In vitro permeation studies were carried out using modified Franz diffusion cell with the diffusional area of 1.13 cm2. Cellophane membrane was sandwiched between the lower cell reservoir and the glass cell-top which was secured in place with a pinch clamp. The receiving compartment was filled with 35 ml phosphate buffer pH 7.4, which was maintained at 37±0.5°C by using magnetic stirrer, resulting in a membrane-surface temperature was 37°C. A Teflon coated magnetic bar continuously stirred the receiving medium to avoid diffusion layer effects. 0.5 g sample was placed evenly on the surface of the membrane in the donor compartment. The 2 ml samples were removed from the receptor compartment at predetermined time intervals

at 1, 2, 3, 4, 5 and 6 hours , and replaced by the same volume of fresh buffer to maintain a constant volume. Sample was assayed spectrophotometrically. The steady state flux (Jss, µg/cm^{2}·hr) of Piroxicam was calculated from the slope of the plot using linear regression analysis.

The cumulative amount of Piroxicam permeated into the receptor compartment was plotted against time to obtain a percentage permeation profile. The steady state flux, Jss (µg/cm^{2}.h), was calculated from the slope of the linear portion of the plot of the cumulative amount permeated versus time and expressed as

$$J_{ss} = \frac{dM}{dt}$$

Where, dM is the cumulative amount of Piroxicam permeated through skin per unit area in in experimental time t in (h).

The apparent permeability coefficients (P_{app}) were calculated according to the following equation:

$$P_{app} = \frac{J_{ss}}{C_{donor}}$$

Where, P_{app} in (cm. h^{-1}) is the permeability coefficient of piroxicam through the membrane. C_{donor} is the concentration of piroxicam in the donor chamber.

Enhancement ratios were calculated according to the following expression:

$$ER = \frac{J_{enh}}{J_{ctrl}}$$

Where,J_{enh} is the enhanced flux with application of proniosomal formulation and J_{ctrl} is the flux of drug from the control formula.

2.4 Characterization of optimized formulation

2.4.1 Vesicle size determination[20]

The average size of the optimized prepared proniosomal gel (P-OA) was performed by using Mastersizer S Laser Diffraction Particle Size Analyzer (Malvern instruments Ltd., Malvern, UK). Before measurement, samples were dispersed inDistilled water.

2.4.2 Zeta potential[18]-

Zeta potential of niosomes were determined at 25°C by using Malvern Zetasizer(Malvern Instruments UK).

2.4.3 Microscopical examination

2.4.3.1 Optical microscopy[18]-

A thin layer of proniosomal gel was spread in a cavity slide and after placing the cover slip observed under microscope with and without polarized light. A drop of water was added through the side of the cover slip into the cavity slide while under micro scope again observed. Photomicrographs were taken at suitable magnifications before and after addition of water for the formulation P-OA.

2.4.3.2 Scanning Electron Microscopy[21]-

Scanning electron microscopy (SEM Make-JEOL Model JSM–6390LV,Cochin.Stic) was conducted to characterize the surface morphology of the optimized proniosomal gel at resolution - 3nm Acc V 30 KV, WD 8 mm, SEI.

2.4.3.3 Transmission Electron Microscopy[17]-

Morphology and structure of the optimized proniosomal gel was studied using transmission electron microscopy (TEM Make – Jeol /JEM 2100, Cochin, stic) operating at voltage 200 kV and capable of point-to-point resolution(Resolution-Point:0.23nm,Lattice :0.14nm).

2.4.4 *Ex vivo* permeation study[16]-

Permeation of Piroxicam, through excised rat skin, from the selected proniosomal preparations was assessed. The abdominal hair of male Wistar rats (150 ± 50 gm) was removed carefully. After the animals were sacrificed, the abdominal skin was excised and the adhering fat eliminated. The whole skin was equilibrated in phosphate buffer solution (pH 7.4, the human blood pH) for 1 h before the experiment. This membrane was mounted on a vertical Franz type diffusion cell with the dermis facing the receptor compartment. The donor side was charged with 500 mg of the investigated preparation containing 0.5% Piroxicam. The membrane surface area available for diffusion was 1.13 cm^2. The receptor compartment was filled with the buffer. Temperature was maintained at 37 ± 0.5 ◦C to simulate human blood temperature. The receptor compartment was constantly stirred at 300 rpm. Samples from the receptor fluid (2 ml) were withdrawn at various time intervals up to 24 h and replaced immediately by fresh buffer solution; to maintain the “sink” conditions constantly and a constant volume as well. The samples were then assayed spectrophotometrically at 254nm.

2.4.5 Irritation test[22]-

Irritancy test was carried out to determine possible localized reaction of the selected formula on the skin since skin safety is of prior consideration for transdermal delivery systems. A single dose of 200 mg of the selected medicated formulations was applied to the shaved back of male wistarrat(150±50gm).The development of erythema was monitored daily for 6 days. Extents of development of erythema were indicated on the basis of the following.

0: No erythema development;
2: barely visible few blood vessels and light erythema development;
4: main blood vessels visible and slight erythema development;
6: main blood vessels more obvious and slight erythema development.
Irritation potential was calculated using the following equation:

$$\text{Resultant index} = \frac{A.B}{\text{number of observation days}}$$

Where, A and B represent erythema value and corresponding day, respectively

2.4.6 *In vivo*anti-inflammatorystudy[16]

The experiment was performed in accordance with the guidelines of committee for the purpose of control and supervision of Experiments on Animal (CPCSEA) and approved by Institutional Animal Ethical Committee (IAEC) of MGV’s pharmacy college.Experimental inflammation was induced in male Wistar rats (200 ± 50 gm) according to the method described by Selye (1949).The animals were divided into four groups, each containing 5 rats. One group received no medication (negative control group) and the second received the transdermal Piroxicam gel (Pirox 0.5% by Cipla) (standard group) third group served as placebo (positive control group), while the other received the Piroxicam transdermal formulation. The anti-inflammatory effect of the four groups was simultaneously monitored. Localized inflammation was induced by subplantar injection of 0.1 ml of 4% formaldehyde solution into the left footpad of the rat’s hind paw, 30 min before drug administration where maximum oedema was reached. The initial paw size was then determined using plethysmometer. Each group received its medication and the oedema volume was then assessed at different time intervals using plethysmometer. Readings were taken every hour for 6 h in the first day and then at 24, 48 and 72 h.

2.4.7 Comparison of plain piroxicam,control and optimized proniosomal gel (P-OA) formulation by using different analytical technique:-

1) **FTIR study[17]**-FTIR study was carried by using Fourier transform infrared spectrophotometer (Shimadzu)on plain piroxicam drug, proniosomal gel formulation P-OA and P-control. The IR spectrum of the

formulations was compared with that of the pure drug to check for any possible change in drug properties during formulation. The infrared spectra of mixture were recorded.

2) **UV visible study-** 20 mg of equivalent of drug containing proniosomal gel (P-OA and P-control) was accurately weighed, transferred into 100ml volumetric flask and dissolved in 15 ml of methanol. The volume was made up to 100 ml using PBS pH 7.4 to get a concentration of 200 µg/ml. From the prepared stock solution 10 ml solution was withdrawn and transferred to another 100 ml volumetric flask and volume was make up to 100 ml to get a concentration of 20µg/ml. The UV spectrums were recorded in the range 200-400 nm by using UV-Visible double beam spectrophotometer (Shimadzu 2450). The wavelength of maximum absorption (λ max) was determined, compare it with plain piroxicam drug by doing its overlay.
3) **DSC study[21]**- To study the possible interactions between Piroxicam and vesicle ingredients, P-OA of highest EE% was chosen and samples of 4 mg of each of piroxicam, span 60 ,empty and drug loaded proniosomal gel were submitted to DSC analysis using differential scanning calorimeter (Shimadzu DSC-60, Kyoto, Japan). Each sample was sealed in a standard aluminum pan and scanned between different temperature ranges while another empty pan was used as a reference. The Thermograms were obtained at a scanning rate of 10° C/min.

2.4.8 Stability study[23]- Theoptimized proniosomal gel (P-OA) were stored at 4, 25 and 40^oCand 75% relative humidity. The physical stability was evaluated by determining % E.E. Over 3-months periods.

2.4.9 Statistical analysis

The Students` t-test was used to test for significance.

3 Results and Discussion

3.1 Evaluation of effect of penetration enhancers in proniosomal formulations

3.1.1 Entraptment Efficiency(%)

The entrapment efficiency is one of the important parameters in the design of vesicular formulations. Vesicle entrapment efficiency relies on the stability of the vesicle which is highly dependent on the type and amount of surfactant forming the bilayers, the amount of both cholesterol and lecithin and the drug load. The plain proniosomal gel was shows highest entraptment efficiency with incorporation of penetration enhancers into the proniosomal gel reducing the entraptment efficiency. In all formulation P-OA shows highest entraptment efficiency i.e.87.80% and P-DMSO shows lowest entraptment efficiency the i.e. 76.53 % and the recorded entrapment efficiency values are presented in Table-2.

Table-2: Optimization of Proniosomal gel

Formula-tion code	Entraptment Efficiency (%)	Drug content (Mean±SD)	Spontaneity
Control	90.89	98.52± 0.04	1.33×10^4
P / Lec	83.01	98.99± 0.01	2.66×10^4
P / OA	84.14	99.84± 0.01	6.33×10^4
P / IPM	83.29	101.78± 0.02	8.66×10^4
P / PG	76.53	96.74± 0.02	2.83×10^3
P / PEG	87.80	99.70± 0.02	5.33×10^4
P / Urea	77.66	101.5± 0.03	3.33×10^4
P/ DMSO	78.78	98.29± 0.04	6.66×10^4
P / EDTA	84.98	98.85± 0.01	$9.33 \quad 10^4$

3.1.2 Drug content

Drug content determines the amount of piroxicamin the prepared proniosomal gels. Results showed the more uniformity of the drug in the proniosomal gels and indicated the less drug loss in formulations as shown in Table 2. The percent drug content of most of the formulation is within the range of 98 to 101%. The drug content was highest in P-IPM and lowest in case of formulation P-PG.

3.1.3 Rate of Spontaneity-

Spontaneity is defined as rate of hydration that is number of proniosomes converted into niosomes after hydration of proniosomes for 15 minutes. Spontaneity studies show that niosome formed from all formulation was more spontaneously except formulation P-DMSO and results are shown in Table 2.

3.1.4 *In vitro* permeation study

Using cellophone membrane

The permeation profiles were constructed by plotting the Cumulative amount of Piroxicam permeated per unit membrane surface area (Q,µg/cm^2) v/s time (hr).

The following Table 3 shows that control formulation permeated drug 178.4µg/cm^2 after 6 hrs and maximum drug permeation seen in case of P-OA formulation it was 301.9µg/cm^2. All proniosomal gel revealed significantly higher permeation rates compared with the control formulation. With respect to the effect of composition of proniosomal gel on the drug permeation, incorporation of various penetration enhancers resulted in a significant increase in the rate of piroxicam permeation compared with the plain (control) formulation. Other formulations P-Lec, P-PG, P-IPM, P-DMSO, P-Urea, P-EDTA and P-PEG shows 247.9µg/cm^2, 287.3µg/cm^2, 251.7 µg/cm^2, 238.7µg/cm^2, 236.7 µg/cm^2,191.0 µg/cm^{2}and 265.4 µg/cm^2 shows drug permeation after 6 hrs respectively.

The following Figure 1 shows graphical representation of drug permeation after 6hrs through cellophone membrane.

Figure (a) - shows drug permeation through control, P-Lec and P-PG formulation incorporation of lecithin and propylene glycol shows significantly increase in drug permeation.
Figure (b) -shows P-IPM and P-DMSO gives satisfactory drug permeation as compared to control formulation.
Figure (c) -P- OA shows highest drug permeation as compared to P-urea and control formulation.
Figure (d) shows P-PEG and P-EDTA have higher drug permeation after six hours as compared to control.

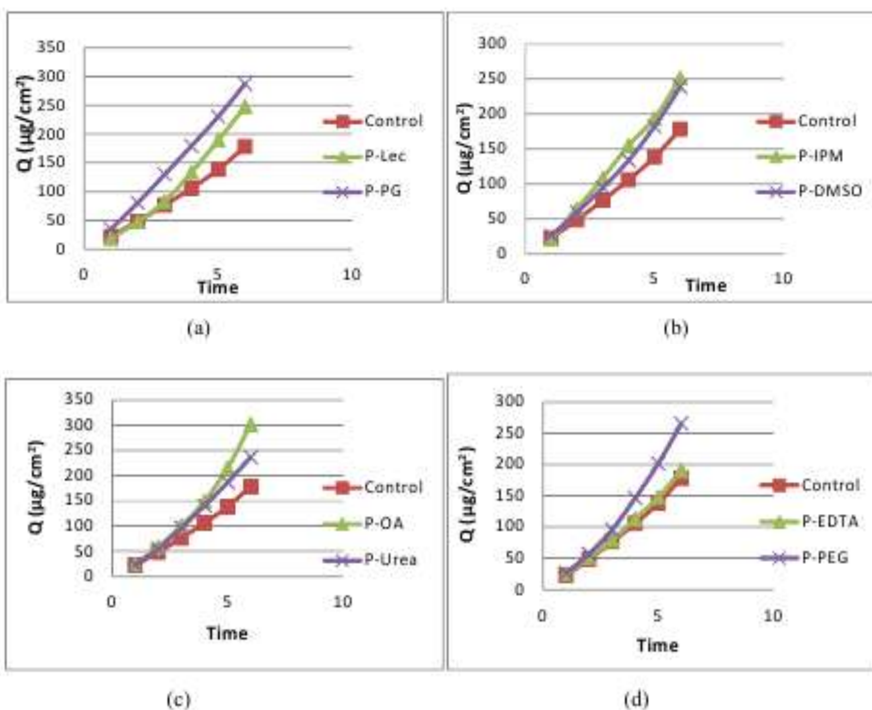


Figure-1: Cumulative amount of drug permeated v/s time for different formulation of Piroxicam proniosomal gel across cellophone membrane.

The following Table-3 shows that all proniosomal gel shows increase in flux after a addition of different types of penetration enhancers. Incorporation of oleic acid shows highest flux as compared to all other formulations and with respect to this it shows apparent permeability coefficient 21.3 $\times 10^{-3}$ cm h^{-1}.

Table-3:Permeation parameters for piroxicam across cellophone membrane

Formulation code	**Q6** (µg/cm^2)	**Jss** (µg/cm^2.h)	**K P_{app} (cm h^{-1})**	**ER**
Control	178.4	28.44	11.4 $\times 10^{-3}$	1
P-Lec	247.9	44.78	17.9 $\times 10^{-3}$	1.58
P-PG	287.3	45.47	18.2 $\times 10^{-3}$	1.60
P-IPM	251.7	40.70	16.3 $\times 10^{-3}$	1.43
P-DMSO	238.7	39.50	15.8 $\times 10^{-3}$	1.39
P-OA	301.9	53.17	21.3 $\times 10^{-3}$	1.87
P-Urea	236.7	40.18	16.1 $\times 10^{-3}$	1.41
P-EDTA	191.0	30.84	12.3 $\times 10^{-3}$	1.08
P-PEG	265.4	46.28	18.5 $\times 10^{-3}$	1.63

Q6=cumulative amount permitted at 6 hours
Jss= steady state flux
KP_{app}=Apparent Permeability coefficient
ER=Enhancement ratio.

As the P-OA have maximum flux therefore, it has maximum enhancement ratio i.e. 1.87 as compared to other formulations. Dunnett's Multiple Comparison Test was applied for comparing the prepared formulations with control formulations which showed P value 0.0001 which is extremely significant.

Comparing the in vitro permeation profile of formulation P-OA to that of other proniosomal gels; higher permeation rate and efficiency were observed, revealing that this particular proniosomal structure improved piroxicam release properties.

Among various formulations developed P-OA formulation containing oleic acid as penetration enhancer shows excellent entrapment efficiency (%), permeation property and also satisfactory rate of spontaineity and drug content therefore this formulation was subjected to further investigations.

3.2Characterization of optimized formulation

3.2.1 Vesicle size determination

The graph for vesicle size determination for optimized batch represents that vesicle which was formed after dispersion of proniosomal gel having size 3,631nm. Result obtained for vesicle size determination is shown in Figure.2.

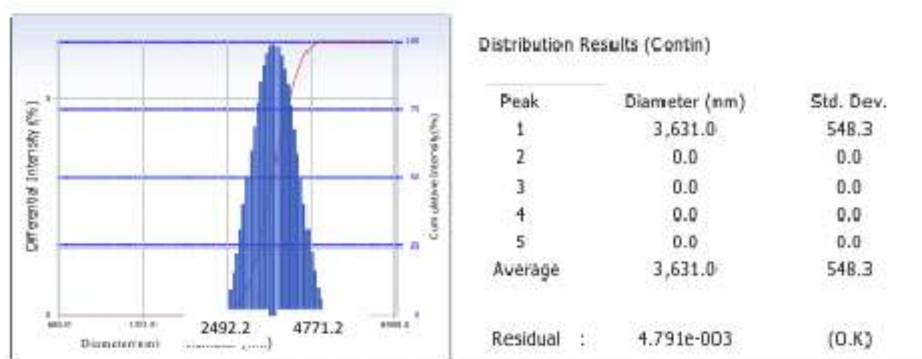


Figure-2:Vesicle size of formulation code P-OA

3.2.2 Zeta potential

Zeta potential is defined as the difference in potential between the surface of the tightly bound layer, and the electro neutral region of the solution. It is also known as the electro kinetic.

Zeta potential analysis was done for determining the colloidal properties and stability of the prepared formulations. For stability of semi solid formulation, zeta potential should be minimum for better stability. Zeta potential of P-OA formulation shown in Figure.3, it shows zeta potential **-49.1** with remark good.

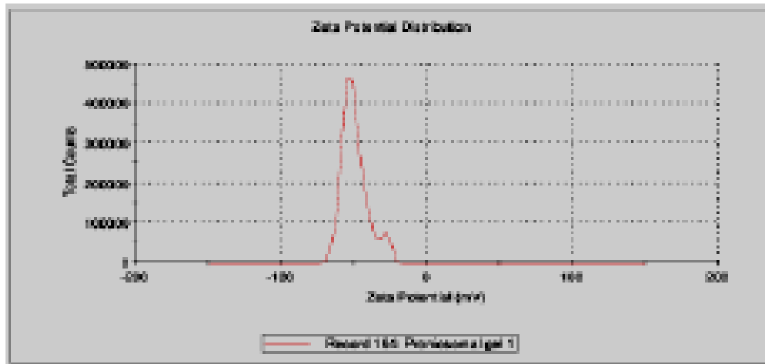


Figure-3:Zeta potential of formulation code P-OA

3.2.3 Microscopical examination

3.2.3.1 Optical microscopy

The diagrammatic representation of the sequence/mechanism of niosomes formation from proniosomal gel upon hydration shown in Figure. 4. The transformation of lamellar liquid crystalline proniosomes to niosomes may be explained by different degree of hydration of span and phospholipid molecules and simultaneously by change in shape of the hydrated molecules characterized by their packing parameter. Due to the limited solvent system present, the proniosomes formed were the mixture of lamellar liquid crystals resembled palisades and vesiculating lamellas stacked together which may be termed as compact niosomes. Further addition of water leads to swelling of bilayers as well as vesicles due to interaction of water with polar groups of the surfactant. Above a limiting concentration of solvent, the bilayers tend to form spherical structures randomly giving rise to multilamellar, multivesicular structures. When shaken with water i.e. with excess aqueous phase, complete hydration takes place leading to the formation of niosomes.

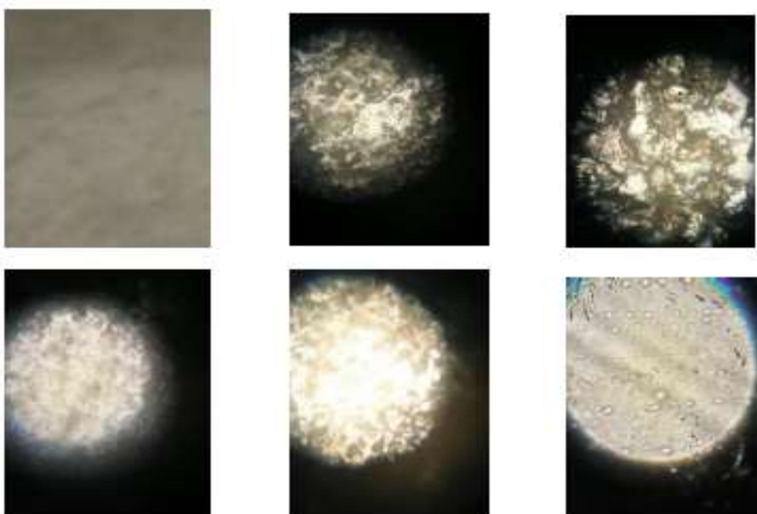


Figure-4:Different stages of conversion of proniosomal gel to niosomes.

3.2.3.2 Scanning Electron Microscopy

Scanning Electron Microscopy done on optimized batch before and after dilution. Before dilution micrographs shows smooth surface morphology (Figure.5).Scanning electron micrographs revealed the formation of well identified spherical niosomal vesicles with sharp boundaries after hydration of proniosomes shown in Figure.6 and it shows vesicle having sizes 145.60nm,188.68nm and 189.74nm was observed.

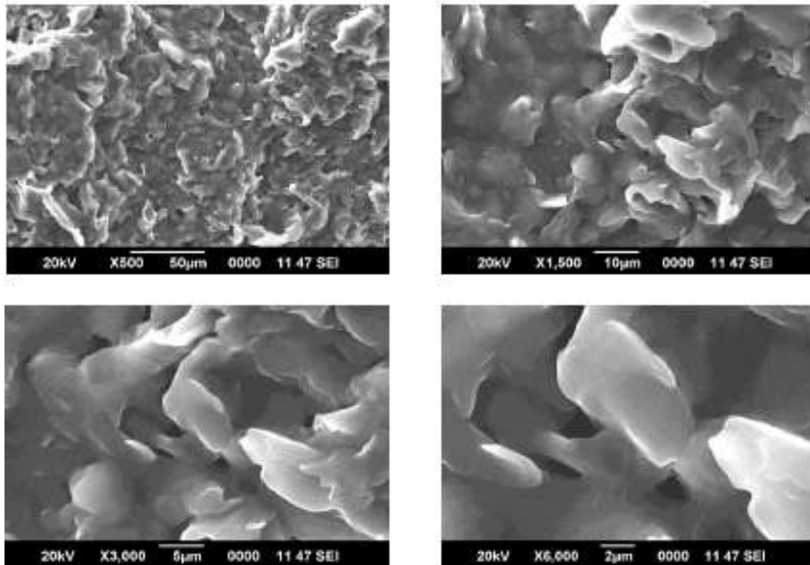


Figure-5:Scanned electron microscopy (SEM) images of proniosomal gel formulations: P-OA before dilution.

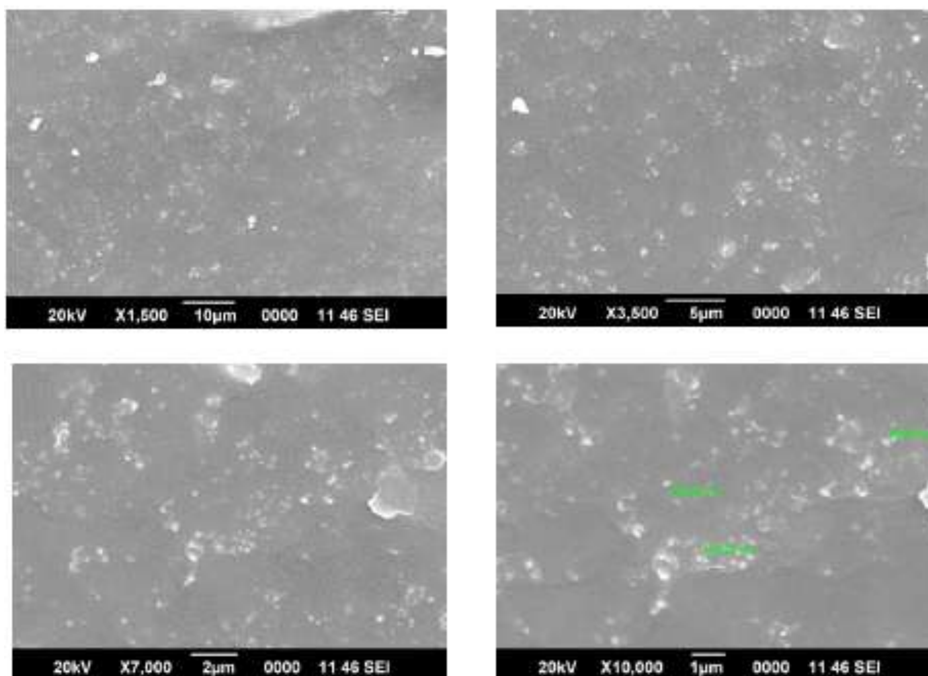


Figure-6:Scanned electron microscopy (SEM) images of proniosomal gel formulations: P-OA after dilution.

3.2.3.3 Transmission Electron Microscopy

The optimized proniosomal gel (P-OA) was examined microscopically using Transmission Electron Microscopy (TEM). TEM provides details about internal composition. Results of transmission electron microscopic study of proniosomal formulations are shown in Figure. 7. Most of the vesicles are well identified,

spherical and discreet with sharp boundaries having large internal aqueous space. TEM showed that the drug particles are finely embedded in formulation.

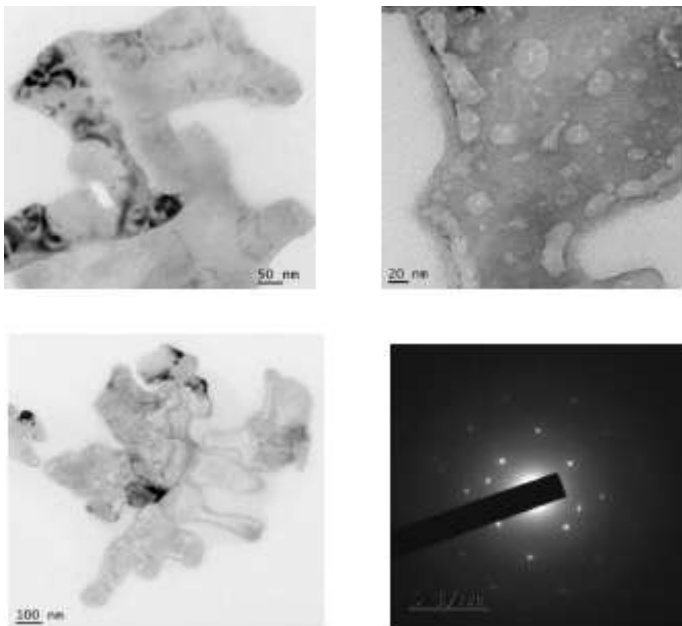


Figure -7:Transmission electron microscopicimages of proniosomal gel

3.2.4 *Ex vivo* permeation study

The following Table 4 shows steady state flux 4.746, 8.136 and 5.062 (µg/cm^2.h) whereas apparent permeability coefficient 1.9 $\times10^{-3}$cm/h, 3.3 $\times10^{-3}$cm/h and 2.0 $\times10^{-3}$ cm/h for control, P-OA and Marketed formulations respectively. The rank order of the permeability coefficients was found to be:P-OA>Marketed > control formulation.

Table-4:Permeation parameters for piroxicam across excised rat skin

Formulation code	Q24 (µg/cm^2)	Jss (µg/cm^2.h)	K P_{app} (cm/h)	ER
Control	219.2	4.746	1.9 $\times10^{-3}$	1
P-OA	398.6	8.136	3.3 $\times10^{-3}$	1.714
Marketed	243.5	5.062	2.0 $\times10^{-3}$	-

Q6=cumulative amount permitted at 6 hours: Jss= steady state flux; KP_{app}=Apparent Permeability coefficient; ER=Enhancement ratio.

The following Figure 8 shows comparison between control P-OA and marketed formulation by plotting graph of cumulative amount of drug permeation v/s time. Figure 8 shows the permeation profile of PC across excised rat skin participated with surfactants. Control PC passed through the skin is significantly lower than those proniosomal formulations prepared using oleic acid, suggesting that the lipid bilayers of niosomes act as a permeation enhancer for PC across rat skin. The mechanisms by which proniosomes permeate across skin such as permeation enhancers effect and vesicle skin interactions may justify this finding[24]. It is concluded from Tables-3 and 5 that the release rate of piroxicam across the cellophone membrane is significantly higher than its flux across the skin ($p < 0.05$), indicating the barrier properties of skin for the drug. Since there were great variance between the permeation profiles of the drug proniosomal formulations across skin and across cellophone membrane, interaction between skin and proniosomes may be an important contribution to the improvement of piroxicam transdermal drug delivery. One of the possible reasons for niosomes to enhance the permeability of drugs is structure modification of the stratum corneum[24]. It has been reported that the intercellular lipid barrier in the stratum corneum would be dramatically looser and more permeable following treatment with liposomes and niosomes[25,26]. Both phospholipids and non-ionic surfactants in the proniosomes always act as penetration enhancers, which are useful for increasing the permeation of many drugs, addition of

penetration enhancer also increases the permeation. Another explanation was that the niosomes vesicles in contact with stratum corneum aggregated and fused at the interface of the stratum corneum and a high local drug concentration in the bilayers generated a high thermodynamic activity of PC in the upper part of the stratum corneum to the surface of skin, demonstrated in a previous report, results in higher flux of the drug due to direct transfer of drug from vesicles to the skin.

Dunnett's Multiple Comparison Test was applied for comparing the control, P-OA and Marketed formulations which showed P value 0.0001 which is extremely significant.

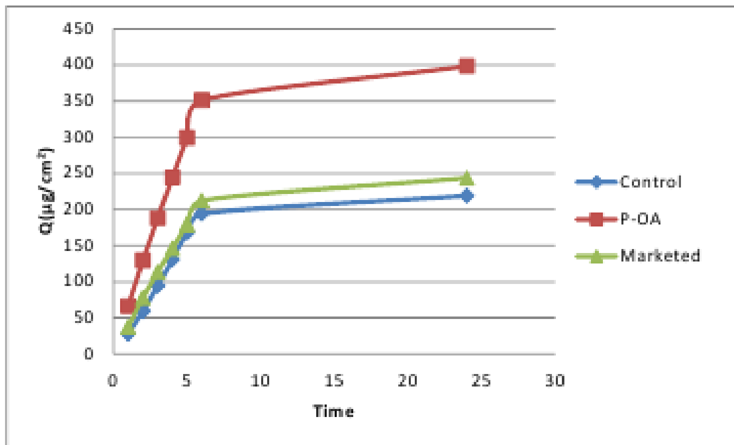


Figure-8: Cumulative amount of drug permeation v/s time for control, P-OA and marketed formulation of Piroxicam across excised rat skin.

3.2.5 Irritation test

The selected proniosome formulation (P-OA) showed an irritation potential of 0.83 shown in Table 5, thus proving to be non-irritant as it was mentioned by Van-Abbé et al., that a value between 0 and 9 in an irritancy test indicates that the applied formulation is generally non-irritant to human skin. No obvious erythema, was observed on rat's skin after one week of application of the selected formulation.

Table 5: Skin irritation score of the optimized proniosomal gel formulation

Formulation Code	Score after (days)						Mean score
	1	2	3	4	5	6	
P-OA	0.33	0.66	1	1.33	1.66	0	0.83

3.2.6 *In vivo* anti-inflammatory studies

Following Figure. 9reveals maximum increase in oedema in rats receiving no treatment (negative control) after 6 h with mean increase value equivalent to 75.92 %, and then a sharp decrease followed reaching 53.61 % after 72 h. Oedema size in rats receiving marketed piroxicam gel (Standard) increased rapidly till the rat paw reached a maximum size with mean increase in oedema equivalent to 72.30% at 6 h, followed by a slightly sharp decrease reporting a mean increase in oedema size 40.20% after 72 h. Rats receiving the non-medicated transdermal proniosome preparation (positive control) possessed an increase in oedema with maximum value after 6 h (82.35%) and then it decreased to 59.30% after 72 h. Rats receiving the medicated transdermal proniosome preparation showed maximum increase in oedema size after 6 h with a mean increase of 46.59%, which then started to decrease till 25.84% after 72 h. On comparing the anti-inflammatory efficiency of the selected transdermal piroxicam formulation to the efficiency of the marked piroxicam gel of the same dose, it was found that the medicated proniosome gel formula significantly inhibited the induced oedema .This might indicate that the non-medicated preparation has no effect on its own.

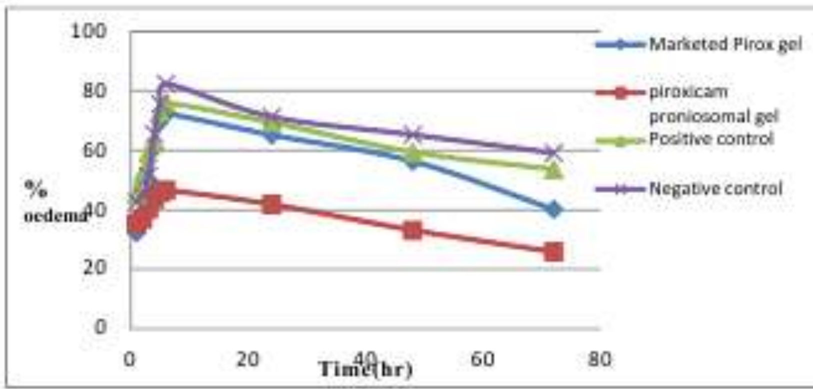
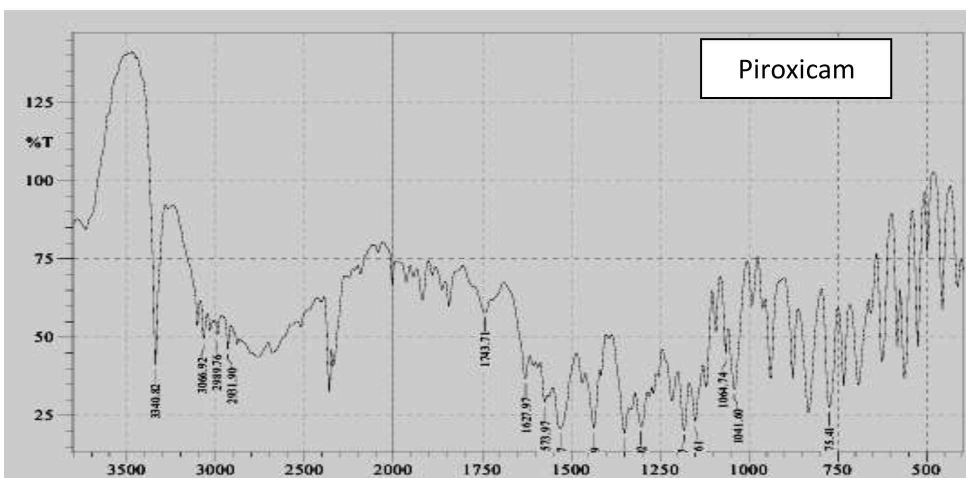


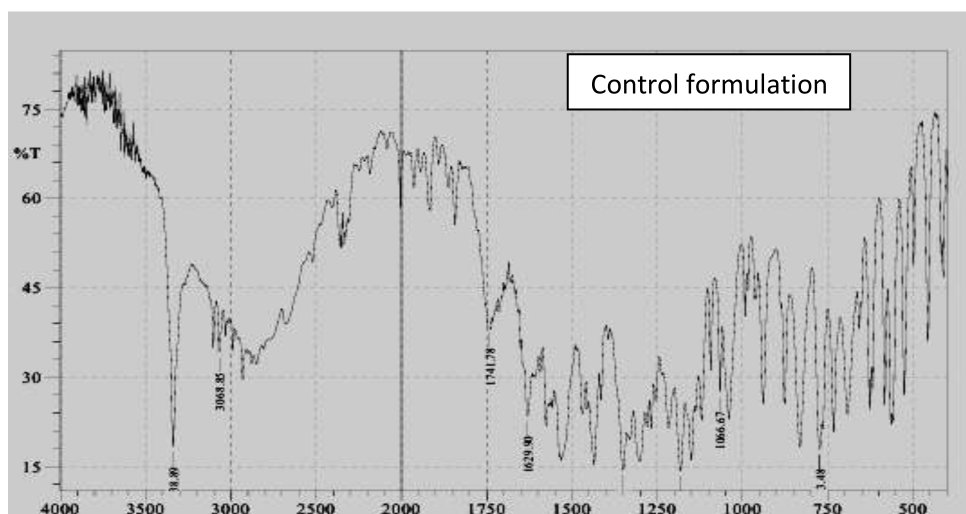
Figure-9:% oedema observed in standard, test, positive and negative control group

3.3 Comparison of piroxicam, control and P-OA formulation by using different analytical technique:-

1. FTIR study

FTIR peak of plain piroxicam, control and P-OA formulation shown in Figure.10.characteristics peaks of piroxicam are present in both formulation-control and P-OA. Some extra peaks also present in both formulations which is due to other excipient present in it.





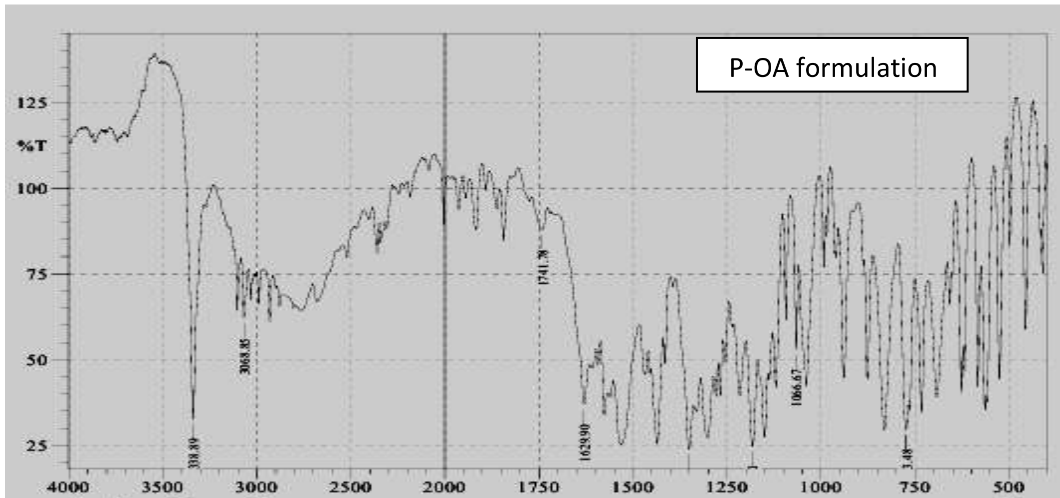


Figure -10: FTIR spectra of plain piroxicam, control and P-OA formulation

2. UV Study

The spectra of Piroxicam, control and P-OA formulation in phosphate buffer (pH-7.4)observed in the range of 200nm to 400 nm for phosphate buffer (pH-7.4)–this spectra shows four peak at same nm absorption maxima at about 221nm,250nm and 354nm and minima at about 279nm which is shown in Figure.11. It indicates that there was no change in properties of drug. Only for fourth peak there was slightly shifting was observed. Peak at 221nm shifted at 215nm in case of control formulation and peak at 204nm in case of P-OA formulation. This shifting may be due to the formulation formed. The difference between the fourth peak of control and P-OA formulation Shows that the effect may be due to the oleic acid present in formulation.

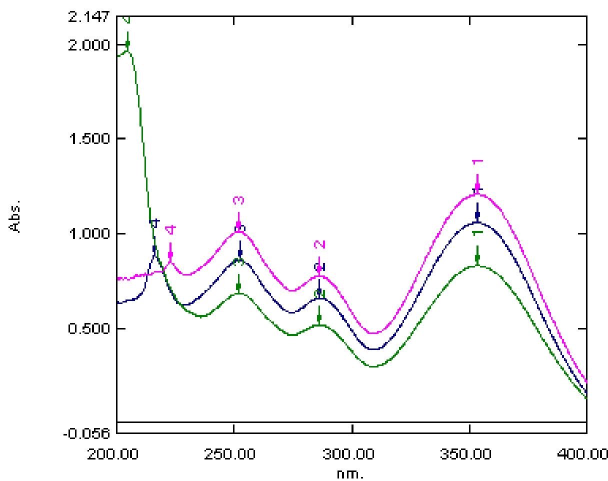


Figure-11: Overlay of λ_{max} for piroxicam, control, P-OA formulation in phosphate buffer(pH – 7.4)

3. DSC Study

DSC thermograms of piroxicam, span 60 empty and loaded proniosomes gel are illustrated in Figure.12. Piroxicam, span 60 showed endotherms at 201.20 and 55.03° C, respectively, corresponding to their melting temperatures. DSC thermogram of drug-free proniosomal gel showed the appearance of a new sharp endothermic peak at 135.55° C indicating the interaction between the molecules of span 60, cholesterol However, thermogram of piroxicam loaded proniosomal gel revealed a disappearance of the characteristic endothermic piroxicam peak, and the endotherm of the niosomal bilayer was shifted from 135.55 to a peak at 103.17°C. These results suggest the dispersion and entrapment of piroxicam .

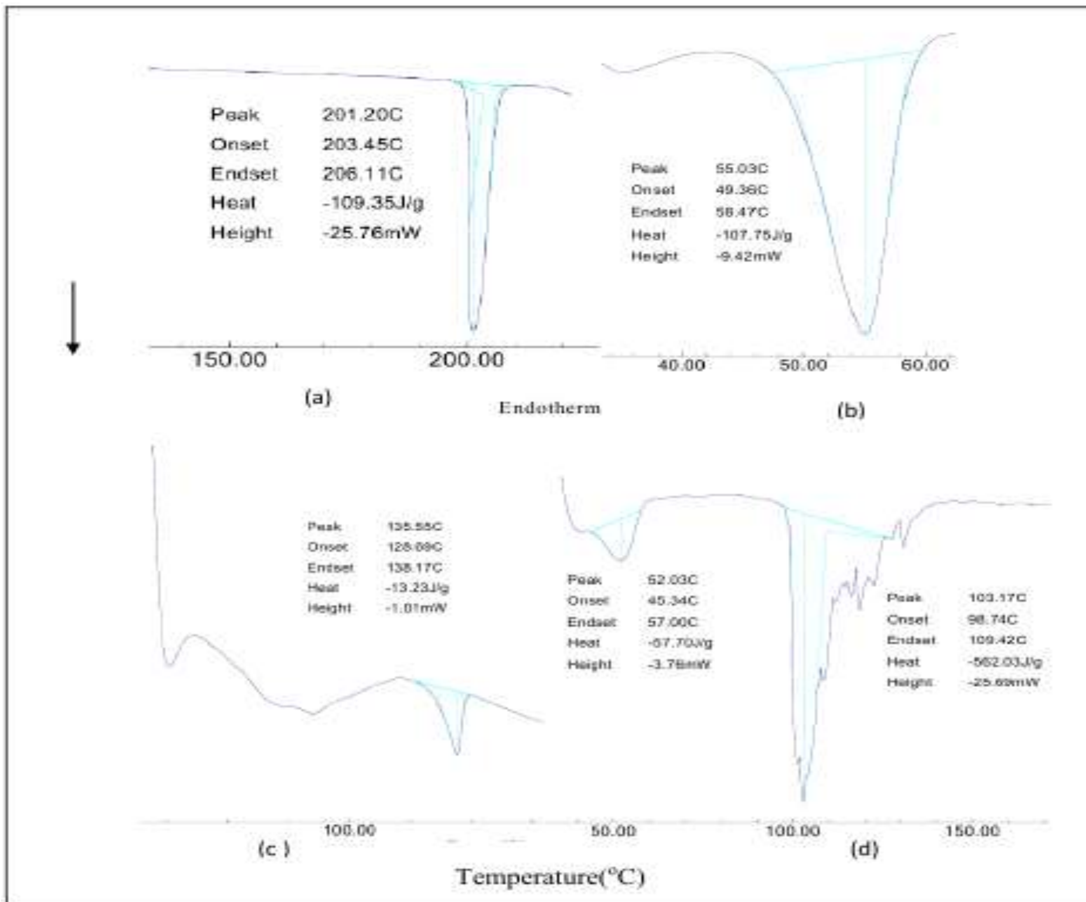


Figure -12: DSC peak of (a)piroxicam,(b)span 60,(c)empty and (d)piroxicam loaded proniosomal gel

3.4 Stability study

Physical stability of proniosomal formulations were studied for a period of three months. The EE were determined for P-OA proniosomal formulations stored at 4-8°C, room temperature and accelerated storage condition(40°c and 75% RH) as shown in Figure 13, which indicates that formulation store at 4-8°C and room temperature shows less decrease in entrapment efficiency. The formulation store at 40°C shows decrease in entrapment efficiency up to 69.77% after three months. But, it is not less than 60% therefore, it passes the stability study.

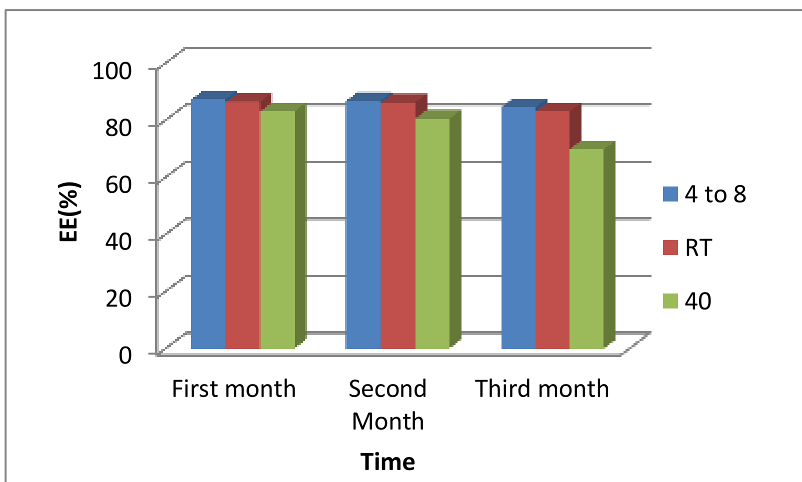


Figure-13:Entrapment efficiency for Stability study of optimized formulation

Conclusion:

From the presented study, it is clear that the penetration enhancer containing proniosomes were better than the corresponding plain and lecithin containing formulations. Successful transdermal delivery from proniosomal gel has been reported in some previous literature but, it have very few experiments. So, proper selection of penetration enhancers may lead to formation of more economic drug delivery system because lecithin is costly and it required proper storage condition as compared to other penetration enhancers. The article thus provided a new composition for transdermal proniosomes with more evaluation parameter and opened the way for researcher on optimization of the composition together with mechanistici nvestigations.

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