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Design and Development of A Proniosomal Transdermal Drug Delivery System of Lornoxicam

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ABSTRACT

The aim of present study was to design and development of a proniosomal transdermal drug delivery system of lornoxicam for the treatment of rheumatoid arthritis and enhanced skin targeting effect, sustained & prolonged drug release, enhanced skin bioavailability by using different type of non ionic surfactant & cholesterol. Proniosomes of Lornoxicam were prepared by coacervation-phase separation method. The formulation systems were characterized in vitro for size, vesicle count, drug entrapment, drug release profile and vesicular stability. The method used for preparing proniosome resulted in an encapsulation yield of 67.71-87.64%. Proniosomes were characterized by transmission electron microscopy. In vitro studies showed prolonged release of entrapped lornoxicam. A successful attempt was made to develop proniosomal gel for transdermal delivery of lornoxicam using different grades of nonionic surfactant.

Keywords: Proniosomes, Niosomes, skin penetration, stability, transdermal, coacervation.

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INTRODUCTION

One of the major advances in vesicle in research was the finding that modified vesicles possessed properties that allowed them to successfully deliver in deeper layers of skin. Transdermal delivery is important because it is a noninvasive. Further, problem of the drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided. It is the most preferred route for system delivery of the drug for several diseases. Hence, transdermal dosage form enjoy being the most patient compliant mode of drug delivery.[1-5]

The principle of transdermal drug delivery system is that they could provided sustained drug delivery (and hence constant drug concentration in plasma), over a prolonged period of time.

The latest approach in the field of vesicular delivery is to combine the two previously mentioned techniques by extending the pro-vesicular approach to niosomes through the formation of "proniosomes" which are converted to niosomes upon hydration Proniosomes are non-ionic based surfactant vesicles, which may be hydrated immediately before use to yield aqueous noisome dispersions. Proniosomes are now days used to enhance drug delivery in addition to conventional niosomes. Proniosomal system serves as a rate limiting barrier for absorption of drugs. These systems can overcome the permeation barrier of the skin and act as a penetration enhancers for the drugs. The vesicles may serve as non toxic penetration enhancer for drug because of the amphiphilic nature of the vesicles; they are more stable and compatible with the skin. Provesicular system can be simply converted into vesicular system, which presents a useful vesicular delivery concept with potential to deliver drugs via transdermal route [6].

Stability is a prime concern in the development of any formulation. Niosomes have shown advantages as drug carriers, such as being cheap and chemically stable alternatives to liposomes**,** but they are associated with problems related to physical stability, such as fusion, aggregation, sedimentation, and leakage on storage**.** The proniosome approach minimizes these problems by using dry, free-flowing product, which is more stable during sterilization and storage. Ease of transfer, distribution, measuring, and storage make proniosomes a versatile delivery system. They are water-soluble carrier particles that are coated with surfactant and can be hydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media.

Lornoxicam is absorbed rapidly and almost completely from the gastro-intestinal tract. Peak plasma concentration is attained with in 2.5 hrs. On repeated administration, Cmax is increased in dose related manner. No evidence of drug accumulation on repeated drug administration has

been reported. Food reduces the absorption of the drug. The absolute bioavailability of lornoxicam is 90-100%. Almost 99% is protein bound exclusively to albumin. No first-pass effect has been observed. Lornoxicam is found in the plasma in unchanged form and as its hydroxylated metabolite. The hydroxylated metabolite exhibits no pharmacological activity. CYP2C9 has been shown to be the primary enzyme responsible for the biotransformation of the lornoxicam to its major metabolite, 5'-hydroxylor noxicam.11 approximately 2/3 is eliminated via the liver and 1/3 via the kidneys as inactive substance. Unlike other oxicams, it has a relatively short plasma half-life (3 to 5 hours).

Thus in current research work an attempt was made to develop transdermal proniosomal gel of Lornoxicam in order to supply local medication to the affected tissues (painful jouints), avoids its gastrointestinal side effect and to improve patient compliance by supplying sustained release medication.

MATERIALS AND METHOD

Material:

Lornoxicam was obtained as a gift sample from Aristo Pharmaceuticals Pvt. Ltd Mumbai. Span 40, 60 and Tween 60, 80 was procured from S. D. fine Chemical, Mumbai. Lecithin was obtained from Himedia, Mumbai. All other ingredients were used of pharmaceutical grade.

Method:

Reagents preparation (Indian pharmacopoeia., 2007)

- Phosphate buffer: Place 250 ml of 0.2M KH2 PO4 in 1000 ml volume metric flask. Add 195.5 ml of 0.2 ml NaoH and make up the volume.
- 0.2M potassium di hydrogen phosphate: 27.218 g of KH2 PO4 in water and dilute with water to 1000 ml.
- 0.2M NaoH: 8 g of NaoH in 1000 ml.
- Normal Saline: Dissolve 0.9% w/v NaCL in 100 ml purified water.

Preparation of standard curve of lornoxicam in phosphate buffer saline pH (7.4)

Lornoxicam (10 mg) was dissolved in phosphate buffer saline and volume was made to 100 ml. 1 ml of stock solution (100 µg/ml) was further diluted with phosphate buffer saline to obtained solution of 5 µg/ml, 10 µg/ml, 15 µg/m and, 20 µg/ml. Absorbance of each standard solution was measured at 274 nm using UV spectrophotometer (Shimadzu 1601A). The phosphate buffer saline (pH 7.4) was used as a reference standard and the standard calibration curve was generated.[7]

Formulation of lornoxicam Proniosomal gel

Lornoxicam loaded proniosomes were formulated by coacervation-phase separation method. Clean, dry wide mouth vials were taken and in that accurately weighted amount of lornoxicam was added, to it, 1.6 ml of ethanol (95%) was added and sonicated in ultrasonicator for about 10 min. Some particles were remained undissolved and their solubility was increased by adding ammonia solution. Accurately weighted surfactants, lecithin and cholesterol were added and covered with the lid to prevent loss of the solvent. These prepared vials were heated on water bath for about 5 min to dissolve the contents and then aqueous phase was added and again the vial was covered and heated for 2 minutes to make the dispersion. On cooling this dispersion is converted to the gel. These prepared gels were mixed with carbopol gel as base in 1:1 proportion and stored in the same containers in clean, dry and dry place. Composition of all the batches is given in table 1.[8]

Table 1: Composition of Proniosome of Lornoxicam

Formulation	Drug(mg)	Span 40(mg)	Span 60(mg)	Tween 60(mg)	Tween 80(mg)	Lecithin (mg)	Cholesterol (mg)
F1 PNG	80	1000				500	100
F2 PNG	80	1000				500	200
F3 PNG	80		1000			500	100
F4 PNG	80		1000			500	200
F5 PNG	80			1000		500	100
F6 PNG	80			1000		500	200
F7 PNG	80				1000	500	100
F8 PNG	80				1000	500	200
F9 PNG	80		1000				400

EVALUATION OF PRONIOSOMAL GEL

1. pH

The pH of the various gel formulations was determined by using digital pH meter.

2. Vesicle size analysis

Particle size of different batches of proniosomal gel was determined by optical microscope 100X magnification. Hydration of proniosomal gel (100mg) is done by adding saline solution (0.9% NaCl solution) in a small glass vial with occasional shaking for 10 mins. The dispersion is observed under optical microscope. The size of 50 vesicles was measured using stage micrometer.

3. Entrapment efficiency

To 0.5 g of proniosomal gel, weighed in a glass tube, 10 ml of the aqueous phase (phosphate

buffer pH 7.4) were added; the aqueous suspension was then sonicated. Niosomes containing lornoxicam were separated from untrapped drug by centrifugation at 9000 rpm for 45 min at 4^{o}C. The supernatant was recovered and assayed spectrophotometrically using Shimadzu UV spectrophotometer (Japan), at 261 nm. The encapsulation efficiency was calculated by the following equation:[9]

$$\% \textbf{ Drug entrapment efficiency} = \frac{\textbf{Actual drug content}}{\textbf{Total weight of drug and polymer}}$$

4. Spreadability

It was determined by wooden block and glass slide apparatus. Weights of about 10g were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slide.

Spreadability was then calculated by using the formula;

$$S=ML/T$$

Where,

S= Spreadability

M=Weight tied to upper slide

L=Length of glass slide

T=Time taken to separate the slide completely form each other.

5. *In Vitro* Drug permeation Study

In vitro drug release study was carried out using Keshary-Chien (K-C) cell of 25 ml capacity using egg membrane, in phosphate buffer saline (PBS) pH 6.8. The receptor compartment was filled with phosphate buffer saline pH 6.8 while a 2ml volume of formulation was taken in the donor compartment. The temperature of the receptor compartment was maintained at 37 ± 0.5^{0}C with the help of a circulating water bath. Samples (1 ml) were withdrawn at regular interval and replaced with equal volume of PBS pH 7.4 to maintain the sink conditions. Samples were filtered through Whatman filter, diluted buffer and analyzed spectrophotometrically at 374 nm against reagent blank.

6. Release kinetics

To analyze the mechanism for the release and release rate kinetic of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix, and Peppas model. By comparing the r-values obtained, the model was selected.

❖ **Zero Order Kinetic**

Drug dissolution from dosage form that do not disaggregate and release and the drug slowly,

assuming that the area does not change and no equilibrium condition are obtained can be represented by the following equation

$$\mathbf{Q_t = Q_o + K_o\, t}$$

Where,

Q_t = Amount of drug dissolved in time t,

Q_o = Initial amount of drug in the solution and

K_o = Zero order release constant.

❖ **First Order Kinetic**

To study the first order release rate kinetic the release rate data were fitted to the following equation.

$$\mathbf{\log Q_t = \log Q_o + K_1 t / 2.303}$$

Where,

Q_t = Amount of drug dissolved in time t,

Q_o = Initial amount of drug in the solution and

K_1 = First order release constant.

❖ **Higuchi Model**

Higuchi developed several theoretical models to study the release of water soluble and low-soluble drug incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The Higuchi equation is

$$\mathbf{Q_t = K_H \times t_{1/2}}$$

Where,

Q_t = Amount of drug released in time t and

K_H = Higuchi dissolution constant.

❖ **Peppas Release Model**

To study this model the release rate data is fitted to the following equation

$$\mathbf{Mt / M_\infty = K.\, t^n}$$

Where,

Mt / M_∞ = Fraction of drug release,

K = Release constant,

T = Drug release time and

n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

The result obtained from *in vitro* drug release studies were plotted adopting four different mathematical models of data treatment as follows:

- % Cum. Drug Release vs. Time (Zero order rate kinetic).
- Log % Cum. Drug Retained vs. Time ((First order rate kinetics).
- % Cum. Drug Release was plotted against √T (root time). (Higuchi model)
- Log % Cum. Drug Release vs. Log Time (Peppas exponential equation).

7. Short-term Stability Study

If long-term studies are conducted at 25°C ± 2°C/60% RH ± 5% RH and "significant change" occurs at any time during 6 months testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. Testing at the intermediate storage condition should include all tests, unless otherwise justified. The initial application should include a minimum of 6 months data from a 12-month study at the intermediate storage condition. "Significant change" for a drug substance is defined as failure to meet its specification.[10]

RESULTS AND DISCUSSION

Preformulation studies showed the absorption maxima for Lornoxicam at 374 nm and the developed Spectrophotometric method obeyed beer's law with linearity range 5-20 µg/ml.

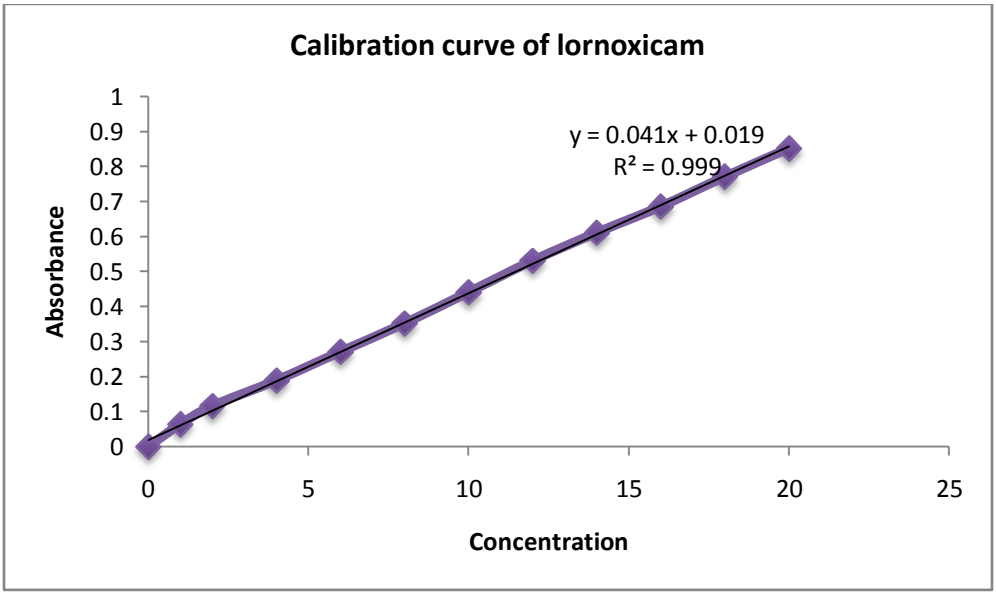

Figure 1: Calibration curve of Lornoxicam

Nine formulation of Lornoxicam proniosomal gels; were formulated using different non-ionic surfactants and cholesterol ratios, the composition of which is shown in the Table 2. The formulations are subjected to evaluation parameters such as particle size analysis, entrapment

efficiency, vesicle morphology, differential scanning calorimetry, analysis of physical parameter of gels, *in vitro* diffusion.

Table- 2 Characterization of Proniosome Gel

Formulation	pH*	Entrapment efficiency (%)*	Particle Size (µm)*	Zeta Potential (mV)*
F1 PNG	7.02	77.78	7.57	-28.72
F2 PNG	7.24	84.15	5.30	-25.11
F3 PNG	7.47	83.11	6.21	-25.30
F4 PNG	7.05	87.64	4.50	-26.30
F5 PNG	6.77	67.71	13.17	-28.11
F6 PNG	6.93	72.16	11.24	-29.56
F7 PNG	7.02	69.43	15.24	-26.22
F8 PNG	7.31	75.52	12.01	-24.22
F9 PNG	7.47	72.45	9.15	-26.22

*Value expressed are mean of triplicate.

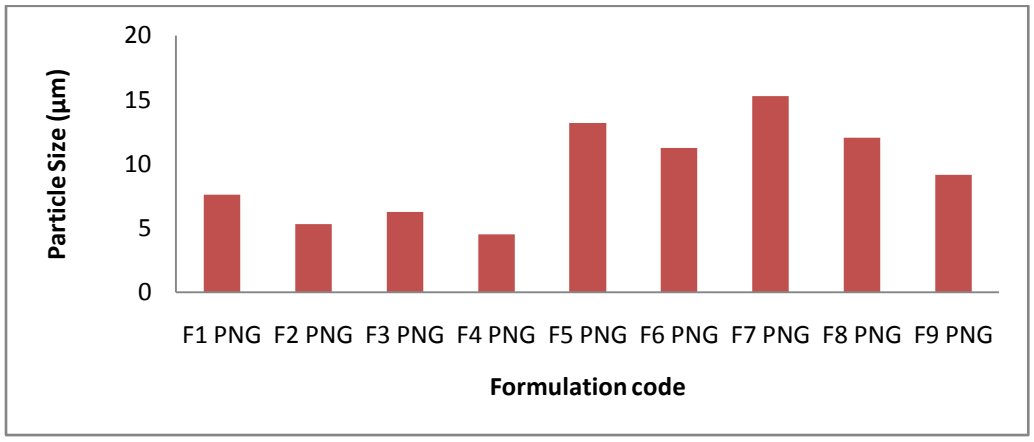

Figure 2: Composition of proniosomal vesicle size of formulation.

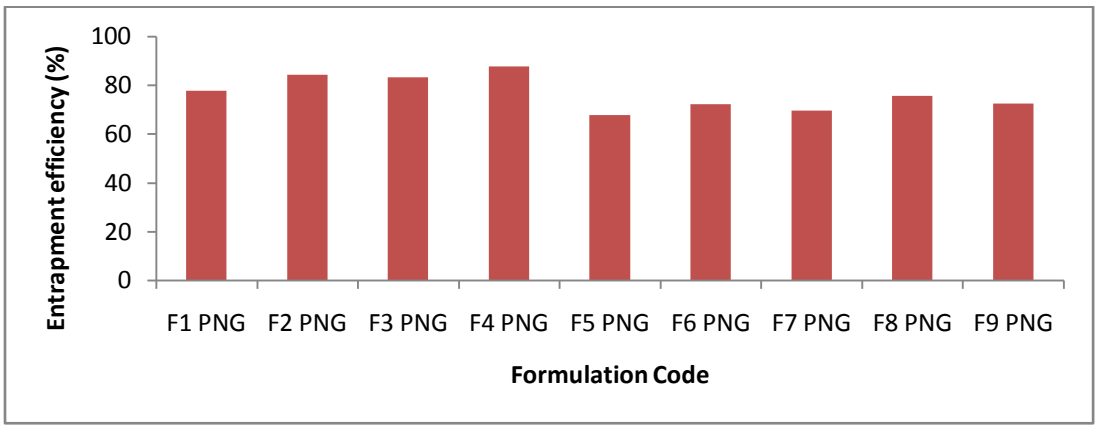

Figure 3: Composition of entrapment efficiency of formulations.

1. %Entrapment efficiency

Entrapment efficiency of proniosomes formulation ranged from 67.71% to 87.64%. The drug encapsulation efficiency of all nine formulations shown in table 2 and figure 3. As shown in

result, proniosomes formed from Span 40, Span 80 and Tween 60, Tween 80 exhibited good encapsulation efficiency. Most of the surfactant used to make nonionic surfactant vesicles have a low aqueous solubility. However , freely soluble nonionic surfactant such as Tween can form micelles on hydration in the presence of cholesterol. The Tween 80 formulations in the present study were also able to entrap lornoxicam efficiency. However, the encapsulation efficiency was relatively low as compared to those composed of Span. This is because the vesicles can be successfully formed by Tween only in the presence of cholesterol. As the cholesterol content of the formulation decreased, the encapsulation of drug also decreased.

2. Vesicle morphology

The mean vesicle size of the lornoxicam proniosome formulation ranged from 4.50 to 15.24µm shown in Table 2. The differences in vesicle size among the proniosomes with span were not significant.

3. pH

Skin compatibility is the primary requirement for a good topical formulation, it was found that the pH of all the formulations were in the range of 6.77 to 7.47 that the skin pH, indicating skin compatibility. The results of pH determination are reported in Table 2.

4. Spreadability

The value of spreadabilty of all proniosomal gel formulation ranged from 13.4 to 14.3 (g.cm/sec). The value of spreadailty indicate that the gel is easily spreadable with minimal of shear.

***5.* Release kinetics**

In order to describe the kinetics of the release process of drug in all formulations, various equations were used, such as order rate equation, which describe the system where release rate is independent of the concentration of the dissolved drug. The first order equations describe the release form the system where dissolution rate is dependent on the concentration of the dissolving drug. Higuchi equation describes the release form system where solid drug is dispersed insoluble matrix, and the rate of drug release is related to the rate diffusion. The Korsmeyer Peppar equation is used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

The applicability of all these equations was tested [Table 3]. Drug release process from the formulations, which were prepared with span, was not zero order or first order in nature. To find out exact mechanism, dissolution data of these formulations were fitted in Higuchi equation &

Korsmeyer – Peppas equation. All the formulation in this study were best expressed by Higuchi's classical diffusion equation. The linearity of the plot indicated that the release process was diffusion-controlled. Thus amount of the drug released was dependent on the matrix drug load. To confirm the diffusion mechanism, the data were fitted to Korsmeyer-Peppas model. All formulation showed good linearity.

On the other hand formulations which were prepared with Tween showed first order as well as Higuchi release process. F5, F7, F8 showed first order and F6 showed Highuchi equation. Since Tween being hydrophilic the addition of cholesterol result in increasing the hydrophobicity of these formulation and showed fluctuations in the drug release nature **shown in Figure 4-7.**

Table 3: Drug Release Mechanism

Formulation Code	Zero order (r value)	First order (r Value)	higuchi	Peppas		Best Fit model
			r Value	r Value	n Value	
F2 PNG	0.9574	0.9492	0.9818	0.9939	0.6086	0.6086
F1 PNG	0.9562	0.9794	0.9798	0.9952	0.6368	0.6368
F4 PNG	0.9603	0.9166	0.9789	0.9887	0.5779	0.5779
F3 PNG	0.9514	0.9761	0.9856	0.9981	0.6375	0.6375
F6 PNG	0.9551	0.9934	0.9727	0.9848	0.6446	0.6446
F5 PNG	0.9477	0.9942	0.9826	0.9968	0.6423	0.6423
F8 PNG	0.9447	0.9447	0.9878	0.9919	0.5298	0.5298
F7 PNG	0.9508	0.9951	0.9851	0.9948	0.6227	0.6227
F9 PNG	0.9787	0.6876	0.9544	0.9858	0.6311	0.6311

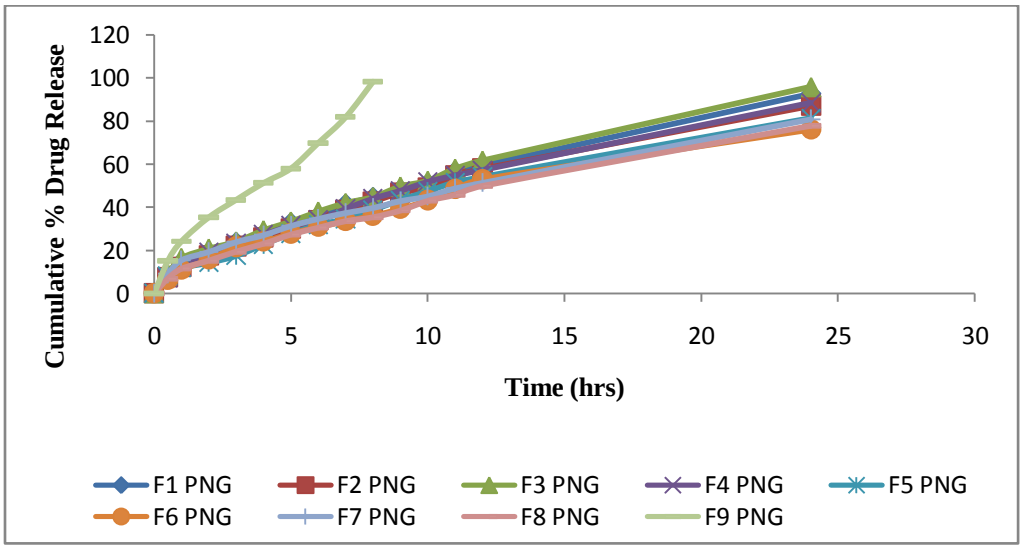

Figure 4: Comparative zero order plot of formulation.

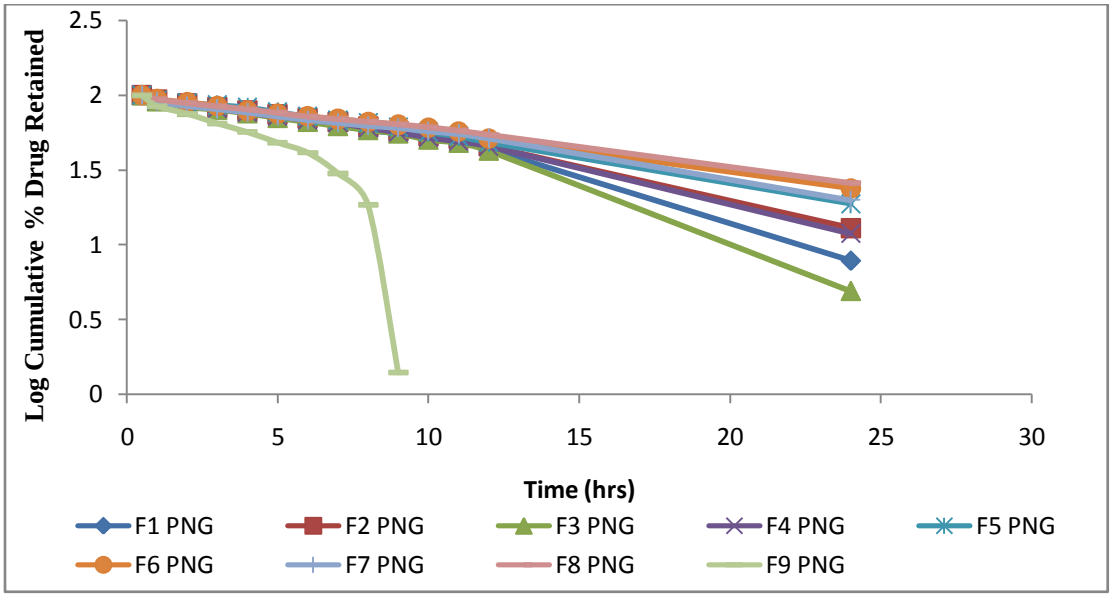

Figure 5: Comparative first order plots of formulations.

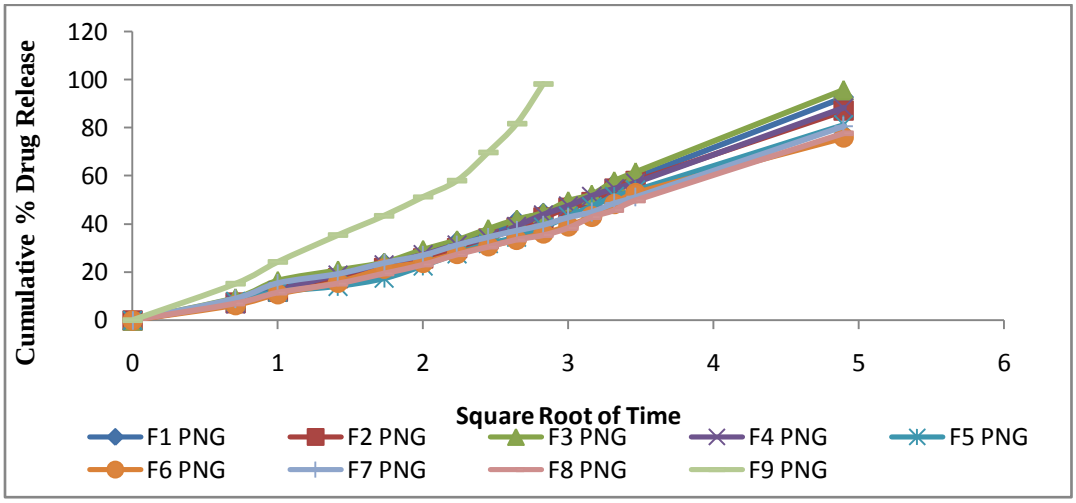

Figure 6: Comparative higuchi matrix plots of formulation.

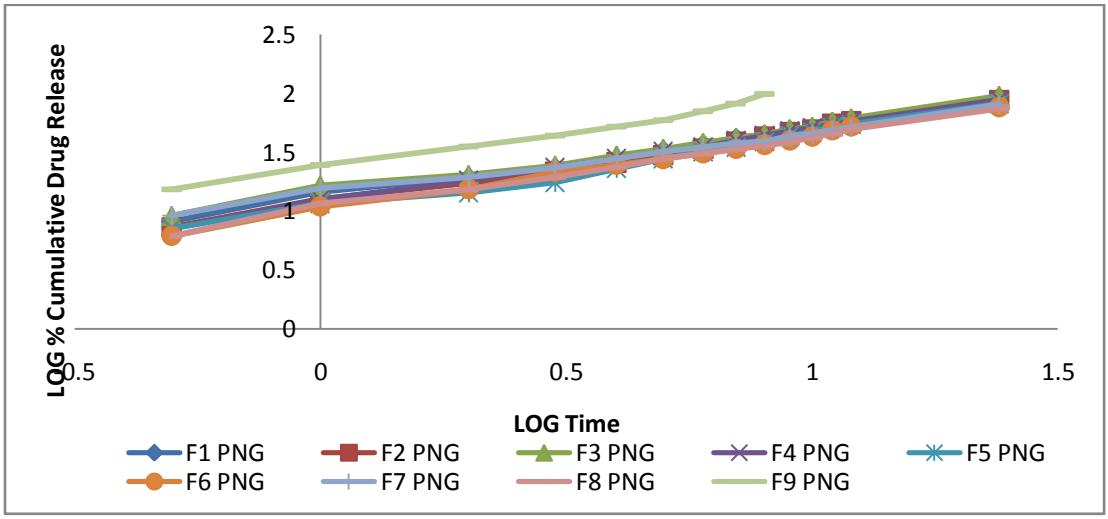

Figure 7: Comparative korsmeyer peppas plots of formulation

6. Short-term stability studies:

Studies were carried out after stroring the promising formulation F4 PNG at three different temperatures 5˚C± 3˚C, 25˚C±2˚C temperature with 65% RH ± 5% RH and 40˚C ± 2˚C with 75% RH ± 5% RH for 3 month. Table 4, 5 and 6 shows the data for vesicle size, drug retained, *in vitro* drug diffusion after every one month till three months.

Result showed that there were no significant changes observed in the vesicle size, drug retained, *in vitro* drug diffusion of formulation at 5±3 ˚C shown in Table 4. It confirms that formulation F4 PNG was stable at the end of 90 days. On the other hand, significant change was observed in the vesicle size, drug retained, *in vitro* drug diffusion after 3 months at 25±2˚C and 40±2˚C temperature.

Table 4: data showing stability studies of promising formulation (F4 PNG) at 5±3˚C

Time(days)	Vesicle size in µm*	Drug retained (%)*	*In vitro* drug diffusion (%)*
0	6.21	83.11	95.84
15	6.28	82.92	95.16
30	6.44	82.57	95.81
60	7.08	81.31	94.88
90	7.87	81.01	93.91

*Values expressed are Mean (n=3)

Table 5: data showing stability studies of promising formulation (F4 PNG) AT 25±2˚C, 60±5˚%RH

Time(days)	Vesicle size in µm*	Drug retained (%)*	*In vitro* drug diffusion (%)*
0	6.21	83.11	95.84
15	6.47	82.42	94.94
30	6.96	81.90	93.67
60	7.82	80.75	92.42
90	8.71	79.64	91.12

*Values expressed are Mean (n=3)

Table 6: data showing stability studies of promising formulation (F4 PNG) AT 40±2˚C, 75±5˚%RH

Time(days)	Vesicle size in µm*	Drug retained (%)*	*In vitro* drug diffusion (%)*
0	6.21	83.11	95.84
15	6.85	82.02	94.16
30	7.24	81.16	93.61
60	8.28	78.01	91.88
90	9.67	74.01	88.19

*Values expressed are Mean (n=3)

CONCLUSION

A successful attempt was made to develop proniosomal gel for transdermal delivery of lornoxicam using different grades of nonionic surfactant (span 40, span 60 and tween 60, tween 80) and evaluated for different *in vitro* characterization.

From the result obtained it can be concluded that-

FTIR and DSC of lornoxicam, lecithin, cholesterol and drug excipient mixture showed no significant interaction. So it can be concluded that drug and other excipient were compatible with each other. The drug entrapment was increased with increase with increase in concentration of cholesterol. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be housed almost completely within the lipid bilayer of the proniosomes. Vesicle size was decrease with respect to increase concentration of cholesterol. Increasing the cholesterol content also contributed in increasing the hydrophobicity.

Hydrophilic surfactant like tween in the presence of cholesterol from vesicles with better entrapment efficiency and uniform size.

Lornoxicam release was dependent upon the concentration of cholesterol. Increasing the cholesterol content resulted in a more intact lipid bilayer as a barrier for drug release and decreases its leakage by improving the fluidity of the bilayer membrane and reducing its permeability, which led to lower drug elution from the vesicles. The formulation F9 PNG gave burst release because of absence of lecithin made ruptured surface of vesicle. The formulation showed good stability at the end of 90 days at 5±3˚C. Span 60 was found to show more efficiency entrapment with smaller vesicle size. *In vitro* drug diffusion was retarded with increase in concentration of cholesterol increase, more stable vesicles are formed and sustained effect is obtained. *In vitro* drug release data was fitted in higuchi and korsmeyer-peppas equation showing that drug release was controlled mainly by diffusion mechanism.

REFERENCE

1. Alber WJ, Hadgraft. Percutaneous absorption in vivo experiments. J Pharm Pharmacol. 1979: 31(1): 129-39.
2. Kanitakis J. Anatomy, histology and immune histochemistry of normal human skin. Eur J Dermatol. 2002; 12(4): 390-9.
3. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci. 2001; 14(1): 101-14.
4. Hadgraft J. Skin, the final frontier. Int J Pharm, 2001; 224(1): 1-18.

5. Hadgraft J. Modulation of the barrier Function of the Skin. Skin Pharmacol appl Skin physio. 2001; 14(1): 72-81
6. Solanki A, Parikh J, Parikh R. Preparation, Characterization, Optimization, and Stability Studies of Aceclofenac Proniosomes. Iranian J Pharma Res 2008;7(4): 237-246.
7. Shamsheer A S, Subhareesh M, Khan P R, (Formulation of Evaluation of Lisinopril Dihydrate) Transdermal Proniosomal Gels. J Applied Pharmaceutical Science. 1 2011; 8: 181-185.
8. Gamal M. Mahrous, (Proniosomes As A Drug Carrier For Transdermal Delivery of Meloxicam) Bull Pharm Sci. 2010; 33: 131-140.
9. Baboota S, Alam M I, Kohli K, (Pharmacodynamic evaluation of proniosomal transdermal therapeutic gel containing celecoxi) ScienceAsia, 2010;33:305-311.
10. Gupta A, Prajapati S K, Balamurugan M, (Design and Development of a Proniosomal Transdermal Drug Delivery System for Captopril) Tropical J Pharma Res 2007, 6: 687-693.