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FORMULATION AND EVALUATION OF LORNOXICAM LOADED TRANSFERSOME GEL

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ABSTRACT
Objective: Transdermal drug delivery has made an important contribution to medical practice, but has yet to fully achieve its potential as an alternative to oral delivery and hypodermic injections. Various new technologies have been developed for the transdermal delivery of some important drugs. The goal of the present study was to formulate and evaluate the potential use of transfersomal vesicles as a transdermal drug delivery system for poorly soluble drug, Lornoxicam. Methods: It was investigated by encapsulating the drug in various transfersomal formulations composed of various ratios of different spans (80,60,40) and tween-80 prepared by thin film hydration method. The prepared formulations were characterized for entrapment efficiency (EE%), drug content, *In vitro* drug release studies through a cellophane membrane and stability studies. Results: The vesicles were spherical in structure as confirmed by Transmission Electron Microscopy. The EE% of Lornoxicam in the vesicles was in the range of 82.84 to 89.85 %. The result revealed that Lornoxicam in all of the formulations was successfully entrapped with uniform drug content. Transfersomal gel containing 20mg of the drug and 20% of span 80 was concluded as the optimized formulation (F6), as it showed maximum drug entrapment (89.84%) and cumulative percent drug release (88.52%). Further stability studies were carried out at 25±2℃ for a period of 4 weeks. Conclusion: It is evident from this study that transfersomes are a promising prolonged delivery system for Lornoxicam and have reasonably good stability characteristics. This research suggests that Lornoxicam loaded transfersomes can be potentially used as a transdermal drug delivery system.

KEYWORDS: Lornoxicam, Transfersomes, Transdermal drug delivery, Spans and Tweens.

INTRODUCTION
Lornoxicam is a potent nonsteroidal anti-inflammatory drug (NSAID) used for a variety of inflammatory conditions. The mechanism of action of lornoxicam is primarily due to the inhibition of prostaglandin biosynthesis through the inhibition of the cyclooxygenase (COX) enzymes COX-l and COX-2.Although oral NSAIDs are effective in the treatment of a variety of acute and chronic pain conditions, their use may be associated with serious systemic adverse effects, particularly gastrointestinal disorders.[1] Accounting these problems, drug delivery technologies should be developed which reduces drug dosing frequency along with sustained or controlled release of medicament as well as reduced systemic side-effects.

The vesicular drug-carrier system, transfersome have been reported to enhance the transdermal delivery of drugs, when applied onto the skin non-occlusively. Transfersomes have the ability to overcome the permeation difficulty by squeezing themselves along the inter-cellular sealing lipid of the stratum corneum . The resulting flexibility of transfersomes membranes minimizes the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, after application onto the skin. [2]

Transdermal delivery of NSAIDs proved to be a convenient route of administration for a variety of clinical indications. In addition, using of gel as a delivery system can increase the residence time of drugs on the skin and provide a faster release of drug substance.

Lornoxicam exhibits short plasma elimination with half-life (3–5 hrs), low oral bioavailability. It is characterized by lipophilic nature with a poor solubility in the acidic media of the stomach which gives local toxicity on the stomach. [3] Therefore lornoxicam gel formulations have been proposed as topical application.

MATERIALS AND METHODS
Lornoxicam was received as a gift sample from Hetero drugs Ltd., Hyderabad, India. Soya lecithin was obtained

from Bright laboratories. Tween 80, Span 80,60,40 were obtained from Merck specialties pvt. Limited (Mumbai).All other chemicals used in this study were of analytical grade.

Preparation of Lornoxicam loaded transfersomes [4,5]

Twelve Transfersome formulations were prepared by thin film hydration method using Lornoxicam, Soya Lecithin, and different surfactants (Tween-80, Span-80,60,40). The amount of drug is kept constant (20mg) in all the formulations (Table 1). Lecithin, surfactants and the drug are dissolved in 5ml of organic solvent (ethanol) and then placed in a clean, dry bottom flask. The organic solvent was carefully evaporated by rotary evaporation under reduced pressure above the lipid transition temperature to form a lipid film on the wall of the flask and lipid film was hydrated with a phosphate buffer solution (pH 7.4) by rotation for 1hr at room temperature at 60 rpm. The resulting vesicles are swollen for 2 hrs at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated using probe sonicator (Heldolph vcx750) for 30 minutes at 40^{o}C.

Table 1: Formulation chart of formulations f1-f12

Formulation code	Lornoxicam (mg)	Soya Lecithin (mg)	Tween 80 (mg)	Span 80(mg)	Span 60(mg)	Span 40(mg)
f1	20	90	10	-	-	-
f2	20	85	15	-	-	-
f3	20	80	20	-	-	-
f4	20	90	-	10	-	-
f5	20	85	-	15	-	-
f6	20	80	-	20	-	-
f7	20	90	-	-	10	-
f8	20	85	-	-	15	-
f9	20	80	-	-	20	-
f10	20	90	-	-	-	10
f11	20	85	-	-	-	15
f12	20	80	-	-	-	20

Compatibility study of drug and polymer using FTIR[6]

The compatibility between the pure drug and excipients was detected by FTIR spectra obtained on Bruker FTIR Germany (Alpha-T-1020).The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in^2. The spectra's were recorded over the wave number of 3500 to 500cm^{-1}.

Characterization of transfersomes

Vesicle shape [7]

Transfersomes vesicles can be visualized by optical microscope. The Morphological characterization of transfersome vesicle such as shape and surface feature were projected by using optical microscope. A drop of transfersome suspension was placed over the slide and Photo micrograph was taken at 10x resolution.

Vesicle size, size distribution and zeta potential analysis [8,9]

The average diameter and size distribution profile and zeta potential analysis of Vesicles were determined by Zetasizer. Particle size and zeta potential of transfersomes in the dispersion was determined by photon correlation spectroscopy (PCS) using Malvern zeta sizer at a fixed angle of 90° at 25 °C using water as dispersant for size determination and zeta potential measurement.

Determination of pH of Transfersome gel [10]

The value of pH of topical transfersome gels was measured by using digital pH meter (Labindia Sab 5000 pH meter) at the room temperature.

Determination of percentage entrapment efficiency [11,12]

The amount of Lornoxicam entrapped in transfersome gel was estimated by centrifugation method. 1gm of Transfersome gel was taken and diluted with 10ml phosphate buffer (pH 7.4). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution was placed in centrifugation tube and centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted 20 times before going for absorbance measurement using UV spectrophotometer (UV-3200 Lab India) at 380 nm. This gives us the total amount of unentrapped drug. Entrapment efficiency is expressed as the percent of drug trapped.

$$E.E\% = [(Ct - Cf) / Ct] \times 100$$

% Drug content [13]

1gm of transfersome gel formulation was taken and the vesicles were lysed with 25 ml of ethanol by sonication for 15 min. Later this solution was placed in centrifugation tube and centrifuged at 14000 rpm for 30 minute. The clear solution was diluted to 100 ml with methanol. Then 10 ml of solution was diluted to 100 ml

with saline phosphate buffer pH7.4. Aliquots were withdrawn and drug content was calculated for Lornoxicam by using UV spectrophotometer at 380nm.

***In vitro* drug release studies through cellophane membrane [14]**

The *in vitro* permeation behaviour of Lornoxicam from all transfersomal gel formulations and were investigated using cellophane membrane (Molecular weight cut of 12000–14000, HI Media Ltd, Mumbai, India). The vertical type of the Franz Diffusion cell was used for the permeation study. The cellophane membrane was mounted on a diffusion cell assembly with an effective diffusion area of 2 cm^2. The receptor compartment consisted of a 30 ml phosphate buffer at pH 7.4 as the receptor fluid agitated at 100 rpm, and was maintained at 37 ± 0.5°C throughout the experiments. The prepared formulation was applied to the membrane in the donor compartment. An aliquot of 2 ml sample was withdrawn at suitable time intervals and replaced immediately with an equal volume of fresh diffusion medium. The cumulative amount that permeated across the cellophane membrane was calculated and plotted against time.

Drug release kinetics [15]

To understand the drug release kinetics of the Lornoxicam gel formulation, the drug release data were treated with zero order, first order kinetics and Higuchi equation. The release mechanism was understood by fitting the data to Korsmeyer-Peppas equation .If the value of 'n' is less than 0.45 then it is considered as Fickian release, values more than 0.45 and less than 0.89 is considered as anomalous (non- Fickian) transport and finally 'n' value greater than 0.89 follows super case-II release mechanism.

Stability Studies

The stability studies were carried out according for % entrapment efficiency and drug content at 25±2°C for period of 30 days.

RESULTS AND DISCUSSION

Formulation of transfersomes

Span 80 was selected as the edge activator surfactant for the transfersomal formulation as it is biocompatible and pharmaceutically acceptable. Phospholipid was used as the bilayer-forming agent and ethanol was used as the hydrating agent because ethanol is known to extract stratum corneum lipids and alter the barrier property of the intracellular lipoidal route, thereby allowing higher drug permeation.

Drug Excipient Compatibility Study

Drug Excipient Compatibility studies were performed using FTIR spectrophotometer. The IR spectrum of pure Lornoxicam and physical mixture of drug and other excipients i.e., optimized formulation were studied. The correlation between the pure drug and excipients indicated that the drug was compatible with the formulation excipients.

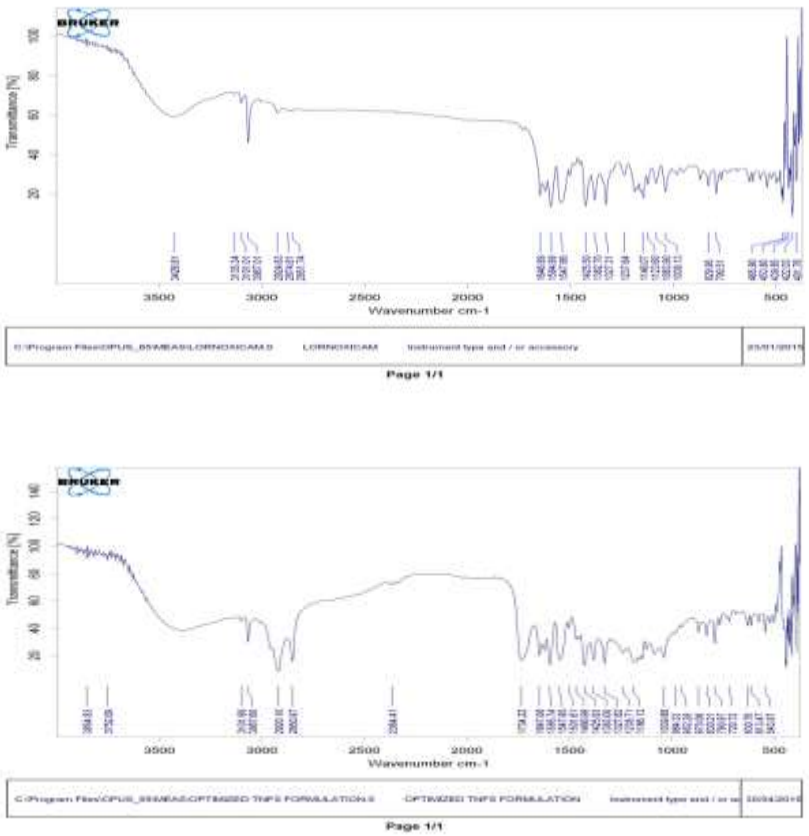

Figure 1: FTIR spectrum of pure drug and optimized formulation

FTIR data of the pure drug and the drug with excipients (span 80 and soya lecithin) suggested that there was no interaction between the drug and the excipients used.

Vesicle shape and type

The surface morphology was studied by Optical Microscopy and transmission electron microscopy. The shapes of most of the Lornoxicam containing transfersomes were found to be spherical, as shown in Figure 2.

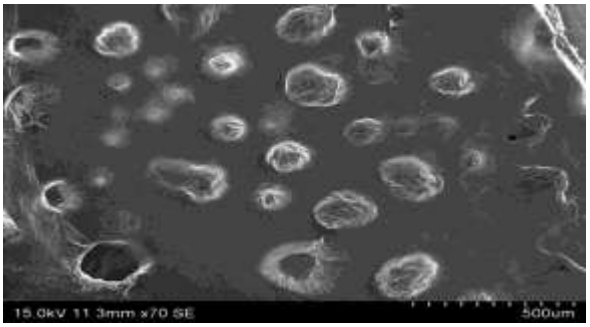

Figure 2: Photomicrograph of Lornoxicam loaded transfersome (f6) at 10X

Vesicle size, size distribution and zeta potential analysis

The vesicle size, size distribution and zeta potential of the optimized formulation (f6) were determined by light scattering method using Zetasizer (DTS version 5.03, Malvern). The mean vesicle diameter was found to be 195.3 nm and zeta potential was found to be -14.3mV. Size distribution curve confirms the normal size distribution of the vesicles.

Table 2: Z-Average size, zeta potential and PDI of Optimized formulation f6

Particle size & Z-Potential analysis			
Formulation code	**Z-Average size (d.nm)**	**PDI**	**Z-Potential (mv)**
F6	195.3	0.251	-14.3

pH value of topical transfersome gel

The value of pH of topical transfersome gels was measured by using digital pH meter (LabindiaSab 5000 pH meter) at the room temperature. The pH of all topical transfersomal gels were found to be in the range of 7.4±0.02 to 7.4±0.08.

% Entrapment efficiency

The percentage entrapment efficiency of Lornoxicam was found to be maximum with formulation f6 (maximum 89.84 ± 0.049) because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume (Figure 3). The entrapment efficiency of drug decreased when molar ratio of lipid to surfactant was decreased from 90:10 to 80:20. The effect of phospholipids and edge activator ratio in the lipid components of vesicles on the entrapment efficiency of lipophilic drug, Lornoxicam, the efficiency decreased with increasing surfactant concentration and thus increased with increasing lipid concentration.

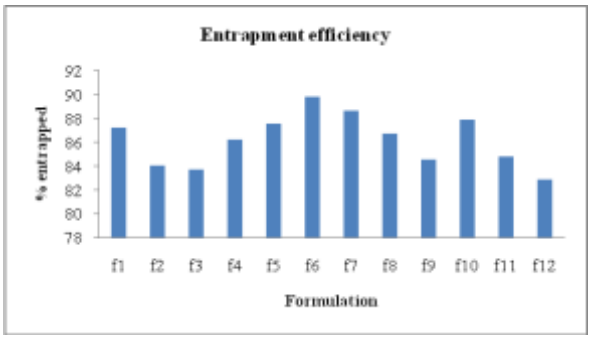

Figure 3: % Entrapment efficiency of formulations f1-f12

Percentage (%) Drug content: The results obtained shows 83.2-91.8% drug content in all the formulations (Figure 4), which shows that there is no degradation of the drug in the process.

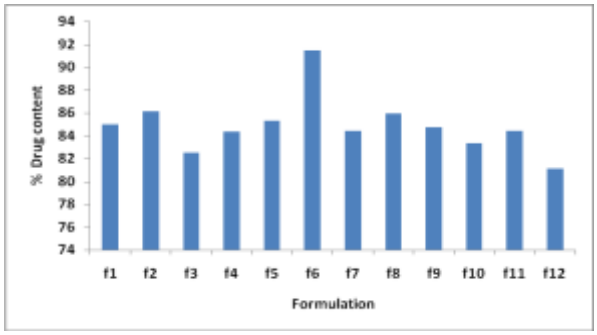

Figure 4: % Drug content of formulations f1-f12

***In vitro* drug release studies through a cellophane membrane**

Each transfersomal gel formulation was subjected to *in vitro* drug release studies using a cellophane membrane. The cumulative amount of drug release was calculated for each formulation. Results revealed that the f6 (formulation with 20% span 80) had the highest cumulative amount of drug release (88%) up to 4.5 hrs as compared to other transfersomal gel formulations (80% to 86%). The release rate of Lornoxicam from f6 was significantly higher than the other formulation (Figure 5). The release experiments clearly indicated controlled-release of Lornoxicam from the transfersomal gel formulation. The maximum release was observed in f6, because of the higher drug content and entrapment efficiency of the formulation. The maximum release was also due to optimum surfactant concentration (20%), because at this concentration the surfactant molecule gets associated with the phospholipid bilayer resulting in better partitioning of the drug, and resulted in higher drug release from the vesicles.

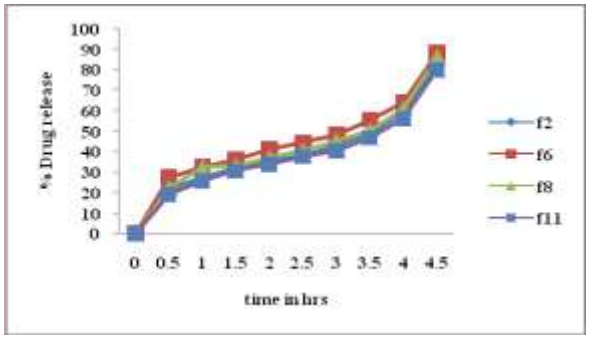

Figure 5: In-vitro drug release study for transfersome gel formulation f2, f6, f8, f11

In- vitro drug release study for pure drug and transfersome gel and marketed gel

In-vitro drug release is done by comparing pure drug, formulated transfersome gel and marketed gel both containing 0.5% Lornoxicam. It was found that transfersome gel showed prolonged release of drug than pure drug and marketed gel (Figure 6).

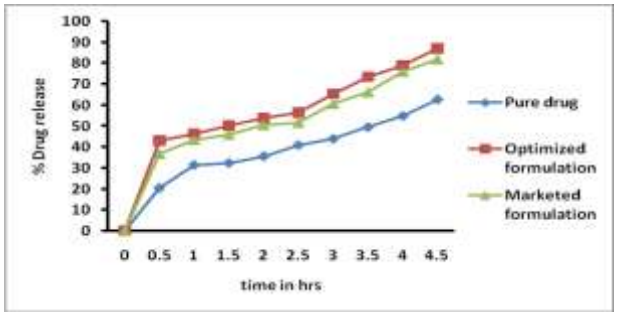

Figure 6: *In-vitro* drug release study for pure drug and transfersome gel and marketed gel

Drug release kinetics

The drug release data were explored for the type of release mechanism followed. Release kinetic study of all formulation (f1 to f12) was studied for different kinetic equations (zero order, first order and Higuchi equation). The best fit with higher correlation (r^2> 0.89) was found with the Higuchi's equation for all the formulations, which means that release of Lornoxicam from the lipid bilayer vesicles was due to diffusion (Figure 7). On the basis of the Korsmeyer-Peppas model, the best fitting was obtained with n > 0.89, indicating super case II. It is well known that when the chain relaxation process is very slow compared with diffusion, the case II transport occurs, which again confirms that the drug release is controlled mainly by diffusion (Table 3).

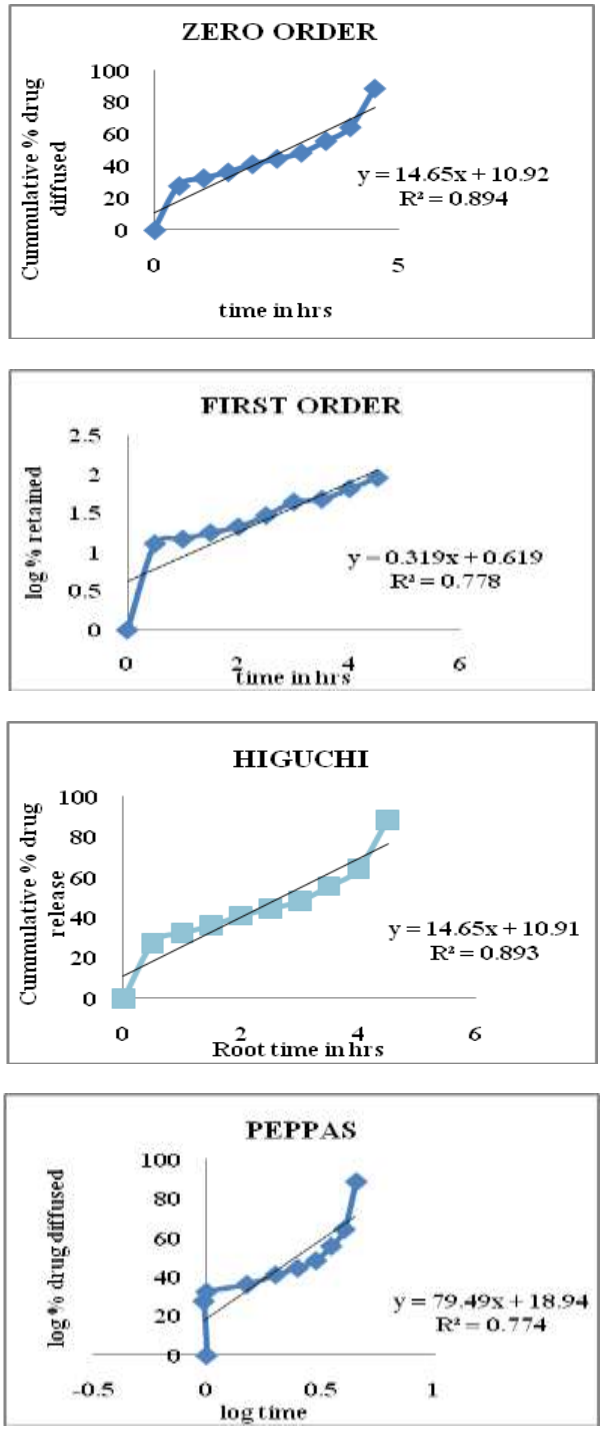

Figure 7: Kinetic Release profiles of formulation f6

Table 3: Regression values of formulations

Formulations	Zero order (r^2)	First order (r^2)	Korsemeyer-Peppas (r^2)	Higuchi (r^2)	n value
f2	0.843	0.759	0.862	0.884	1.02
f6	0.894	0.778	0.774	0.893	1.06
f8	0.858	0.765	0.848	0.884	1.02
f11	0.881	0.772	0.849	0.877	1.04

Stability studies

The stability studies were carried out according for % entrapment efficiency and drug content at 25±2°C for period of 30 days. It is clear from the results obtained that the transfersomes have shown the minimum drug loss at 25±2°C for period of 30 days. The percentage entrapment efficiency and percentage drug content were comparatively good.

The results are shown in the Table 4.

Table 4: % Entrapment efficiency and % Drug content after stability studies

Number of Days	% Entrapment Efficiency		% Drug Content	
	Before (25±2°C)	After (25±2°C)	Before (25±2°C)	After (25±2°C)
30	91.87	89.66	92.04	91.26

Based on the above data, it was confirmed that prepared Lornoxicam transfersome gel (f6) can be considered as a good approach to reduce the dosing frequency and to maintain drug concentration at the desired site of application.

CONCLUSION

Lornoxicam is an oxicam derivative used to reduce pain, inflammation and stiffness caused by rheumatoid arthritis and osteo arthritis. It is well absorbed following oral administration however; its use has been associated with a number of undesirable side effects on the stomach and kidneys in addition to gastric mucosal damage. These side effects can be avoided by topical administration of the drug. Despite note worthy advances have been made over recent years for the management of rheumatoid arthritis, the currently available methods, have a dose limiting therapeutic index with compromised safety implications. These studies have shown promising results, hence there is feasibility of delivering Lornoxicam through transfersomal transdermal gel. Thus, the developed transdermal transfersomal formulation may prove to be a promising carrier for Lornoxicam and other drugs, especially due to their simple production and simplistic scale-up.

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REFERENCES

1. Allen LV, Popovich NG and Ansel HC. "Transdermal Drug Delivery System. In: Ansel's Pharmaceutical dosage form and drug delivery systems", 8thed. Lippincott Williams and Wilkins. 2005; 298-315.
2. Waugh A and Grant A. The Skin. In: Ross and Wilson. Anatomy and Physiology in health and illness. 10th ed. Churchill Livingstone. 2006; 358-367.
3. William AC and Barry BW. "Penetration enhancers". Adv Drug Deliv Rev., 56. 2004; 603-618
4. Cevc G. "Overcoming semipermeable barriers, such as the skin, with ultradeformable mixed lipid vesicles, transfersomes, liposomes, or mixed lipid micelles". Langmuir. 2003; 19(26):10753-10763.
5. Jain S, Jain P, Umamaheshwari RB, Jain NK. Transfersomes–A novel vesicular carrier for enhanced transdermal delivery: Development, characterization and performance evaluation. Drug Dev Ind Pharm. 2003; 29: 1013–1026.
6. Cristina Dinu Pirvu, Cristina Hlevca, Alina ortan, Razvan Prisada. "Elastic vesicles as drugs carriers through the skin". Farmacia., 2010; 58(2): 128.
7. Mishra D., Dubey V., Asthana A., Saraf D.K. and Jain N.K. Elastic liposomes mediated transcutaneous immunization against hepatitis B. Vaccine. 2006; 24: 4847-4855
8. Shailesh T.Prajapati, Charmi G.Patel &Chhagan N.Patel, Transfersomes: Vesicular Carrier System For Transdermal Drug Delivery. Asian Journal of Biochemical&Pharmaceutical Research. 2011; 2 (1): 507-524.
9. Gupta A, Prajapati SK, Balamurugan M, Singh M, Bhatia D. Design and development of a proniosomal transdermal drug delivery system for Captopril. Trop J Pharma Res. 2007; 6: 687–693.
10. Irfan M, Verma S, Ram A. Preparation and characterization of ibuprofen loaded transfersome as a novel carrier for transdermal drug delivery system. Asian j pharm clin resear. 2012; 5(3): 162-5.
11. Walve JR, Bakliwal SR, Rane BR, Pawar SP. Transfersomes: A syrrogated carrier for transdermal drug delivery system. Int J App Bio Pharm Tech, 2011; 2(1): 204-13
12. W. A. Herrmann and M. S. Geertsen. Efficacy and safety of lornoxicam compared with placebo and diclofenac in acute sciatica/lumbo-sciatica: an analysis from a randomised, double-blind, multicentre, parallel-group study. International Journal of Clinical Practice, 2009; 63(11): 1613–1621.
13. G. Yener, M. Üner, U. Gönüllü et al. Design of meloxicam and lornoxicam transdermal patches: preparation, physical characterization, ex vivo and in vivo studies. Chemical and Pharmaceutical Bulletin, 2010; 58(11): 1466–1473.
14. K. Kavitha and M. M. Rajendra. Design and evaluation of transdermal films of lornoxicam.

International Journal of Pharma and Biosciences, 2011; 2(2): 54–62.

15. C. Valenta and B. G. Auner. The use of polymers for dermal and transdermal delivery. European Journal of Pharmaceutics and Biopharmaceutics, 2004; 58 (2): 279-289.