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PREPARATION AND CHARACTERISATION OF LORNOXICAM LOADED TRANS-FEROSOME: A NOVEL CARRIER FOR ENHANCED TRANSDERMAL DELIVERY

Anand K. Srivastava [1] and Nimisha [*2]

Buddha Institute of Pharmacy [1], Gorakhpur - 273209, Uttar Pradesh, India.
Amity Institute of Pharmacy [2], Lucknow, Amity University Uttar Pradesh, Sector 125, Noida - 201313, Uttar Pradesh, India.

Keywords:

Lornoxicam, Skin permeation, Surfactant, Transferosome, Phospholipid, Transdermal drug delivery

Correspondence to Author:
Nimisha

Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Sector 125, Noida - 201313, Uttar Pradesh, India.

E-mail: nsrivastava3@lko.amity.edu

ABSTRACT: To study and investigate transfersomes as a transdermal delivery system for the poorly soluble drug, Lornoxicam-loaded transfersomes were prepared with non-ionic surfactants like span 80 and tween 80 by the rotary evaporation-sonication method. Drug & polymer was dissolved in an organic solvent mixture of chloroform and ethanol (1:1, v/v). The outcome of the work is that prepared formulations were characterized for particle size analysis, drug entrapment, optical, phase contrast and transmission electron microscopy, *in-vitro* drug release through cellophane membrane, and drug release kinetic studies. FTIR studies showed compatibility of the drug with excipients. Based on the entrapment efficiency, drug content and *in-vitro* release studies, the formulation S2 containing span 80 (4% w/w) was selected for permeation studies through albino mice skin and compared with the pure lornoxicam solution. The cumulative percentage of lornoxicam permeated in all formulations was significantly ($p<0.05$) higher than the pure drug solution. Parameters like vesicular size, zeta potential, and drug entrapment were evaluated after one month. The study substantiated that the transfersome can be used as a feasible alternative formulation of Lornoxicam with advanced permeation characteristics for transdermal application.

INTRODUCTION: Transdermal drug delivery systems (TDDS) offer several potential advantages over conventional methods such as injectable and oral delivery [1]. The main barrier and rate-limiting step for the diffusion of drugs across the skin is provided by the outermost layer of the skin, the stratum corneum (SC) [2].

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Recent approaches in modulating vesicle compositions have been investigated to develop systems capable of carrying drugs and macromolecules to deeper tissues. These approaches have resulted in the design of two novel vesicular carriers, ethosomes and ultra-flexible lipid-based elastic vesicles, transfersomes [3, 4].

Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum [5-7]. Transfersomes are ultra-deformable vesicles possessing an aqueous core surrounded by a complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the

vesicle both self-regulating and self-optimizing [8]. Transfersomes are prepared from phospholipids and edge activators. An edge activator is often a single-chain surfactant with a high radius of curvature that destabilizes the lipid bilayers of the vesicles and increases the deformability of the bilayers. Sodium cholate, sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80 and dipotassium glycyrrhizinate have been employed as edge activators (EA) [9].

Lornoxicam (3E)-6-chloro-3-[hydroxyl (pyridin-2-yl amino) methylene]-2-methyl-2, 3-dihydro-4H-thieno [2, 3-e] [1, 2] thiazin-4-one 1, 1-dioxide (LXM) is used for the treatment of various types of pain, especially of the joints, osteoarthritis and sciatica 10-12. LXM is an NSAID of the oxicam class with analgesic, anti-inflammatory, and antipyretic properties. It is a potent inhibitor of both COX-1 and COX-2 enzymes.

LXM exhibits short plasma elimination with half life (3–5 hr), molecular weight of 371.8, partition coefficient of 1.7 [13]. LXM is characterized by lipophilic nature with a poor solubility in the acidic media of the stomach [14]. The aim of this present study is to formulate transfersomes for enhanced skin delivery of LXM. The system was developed and investigated by Fourier transform infrared (FTIR), optical microscopy, phase contrast microscopy (PCM), transmission electron microscopy (TEM), entrapment efficiency, surface charge and charge density, *in-vitro* drug release, and physical stability studies.

MATERIALS AND METHODS:

Materials: Lornoxicam (LXM) was received as a gift sample from Zydus Cadila Ltd., Ahmadabad, India; Phospholipids, Soya lecithin (PC) was purchased from HiMedia Labs. Pvt. Ltd., Mumbai, India; Span 80 and Tween 80 were obtained from SD Fine Chemical Ltd., Mumbai, India.

Ethanol and chloroform were obtained from Jiangsu Huaxi International Trade Co., China, and Qualigens. Fine Chemicals, Mumbai, India, respectively. All organic solvents used were of analytical grade.

Preparation of Lornoxicam Loaded Trasferosomes: Transfersomes were prepared by rotary evaporation-sonication method [15]. The lipid mixture (500mg) consisted of soya lecithin (PC), edge activator (span and tween 80), and Lornoxicam (50 mg) in different ratios was dissolved in an organic solvent mixture consisting of chloroform and ethanol (1:1, v/v) then placed in a clean, dry round bottom flask.

The organic solvent was carefully evaporated by rotary evaporation (Buchirotavapor R-3000, Switzerland) under reduced pressure above the lipid transition temperature (43^{o}C) to form a lipid film on the wall of the flask. Final traces of the solvents were removed by subjecting the flask to vacuum overnight.

The dried thin lipid film deposited on the wall of the flask was hydrated with a phosphate buffer solution (pH 7.4) by rotation for 1 hr at room temperature at 60 rpm. The resulting vesicles were sonicated for 15 min in a bath sonicator (Model Julabo Labortechnik GMBH- Germany) to reduce the size of the vesicles and then stored at 4^{o}C. The composition of formulations is shown in **Table I**.

TABLE 1: FORMULATION TABLE OF LORNOXICAM TRANSFEROSOME

Formulation code	Lornoxicam (mg)	Edge Activator(EA)	PC:EA ratio	Chloroform: ethanol
T-1	50	Tween 80	98:2	1:1
T-2	50	Tween 80	96:4	1:1
T-3	50	Tween 80	94:6	1:1
S-1	50	Span 80	98:2	1:1
S-2	50	Span 80	96:4	1:1
S-3	50	Span 80	94:6	1:1

Drug Excipient Interaction Study: Fourier Transform Infrared Spectroscopy (FTIR) conducted drug- polymer interaction studies. The spectra were recorded for pure LXM and LXM-PC in a Perkin Elmer FTIR spectrophotometer (RXIFT-IR system, USA) using KBr pellets at 450–4000 cm^{-1}. Also, the UV spectra of the pure drug and drug with excipient were also observed.

Particle Size, Vesicle Size and Zeta Potential of Vesicles: The particle size, vesicle size, and zeta potential of the transfersomes were measured by a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at room temperature. Analysis time was kept 60 sec, and the zeta potential of the vesicles was determined [16]. One hundred microliters of the transfersome were diluted with 900 µl deionized water. At least three independent samples (n=3) were prepared, each of which was measured thrice.

Entrapment Efficiency: Drug entrapment efficiencies (EE) of formed transferosomes were evaluated by taking transferosomal dispersion and centrifuged by using cooling ultracentrifuge (Remi, RM-12 C OX) at 4000 rpm in 3 subsequent rounds. The clear supernatant separated the unentrapped drug and the absorbance taken at λ max at 380 nm using UV spectrophotometer (Shimadzu UV 1800). Sediment was treated with 1 ml of 0.1 % isopropyl alcohol to lyses the vesicle and then diluted to 5 ml with buffer (pH 7.4), and absorbance was taken at 380 nm. The percent entrapment was calculated by using formula [17].

% entrapment = Amount in supernatant- amount in sediment / amount in supernatant ×100

Morphology Imaging of Transfersome Vesicles: Transfersomes vesicles can be visualized by Optical microscopy (OM) [18], Phase contrast microscopy (PCM), and Transmission electron microscopy (SEM) [19]. Non-sonicated transfersome vesicles were visualized under an optical microscope (Leica digital microscope, Germany). A thin film of transfersomes was spread on a slide and a cover slip was placed over it and then observed under the optical microscope. In Phase contrast microscopy (AE 2000, Motic Group USA) the light interacts with the matter itself in terms of refractive index variations. These refractive index variations together with the thickness through which the light interaction takes place, construct the image. The morphology of the vesicles was visualized by electron microscope (TEM) model TECNAI $20G^2$ with an accelerating voltage of 200 kV for surface appearance and shape.

***In-vitro* Drug Release Studies Through Cellophane Membrane:** Modified Franz diffusion cell with an effective diffusion area of 1.6 cm2 was used for this study. *An in-vitro* drug study was performed by using egg membrane in a phosphate buffer solution (pH 7.4). The egg membrane was separated by dipping the egg in concentrated HCL solution, with properly rotating the egg, the membrane was obtained by dissolving the Calcium carbonate coat of egg in a concentrated hydrochloric acid solution.

To perform an *in-vitro* release study, egg membrane was mounted horizontally on the receptor compartment with the membrane side facing upwards towards the Franz diffusion cell donor compartment. The receptor compartment was filled with 50 mL of phosphate buffer (pH 7.4) maintained at 37± 0.5^{o}C and stirred by a magnetic bar at 50 rpm. 1 mL formulation was placed in the donor compartment and occluded by parafilm. The study was carried out for 5 hr. An aliquot of 2 mL sample was withdrawn at predetermined time intervals via the sampling port and was quantified for LXM level [20].

Drug Release Kinetic Studies: To study the release kinetics, data obtained from *in-vitro* permeation studies were fitted in various kinetic models: zero order as the cumulative percent of drug permeated *vs.* time, first order as the log cumulative percentage of drug remaining *vs.* time, and Higuchi's model as the cumulative percent drug permeated *vs.* square root of time. To determine the mechanism of drug release, the data were fitted into the Korsmeyer- Peppas model as the log cumulative percentage of drug released *vs.* log time, and the exponent n was calculated from the slope of the straight line. According to this model if 'n' is below 0.45, Fickian mechanism governs drug release; if between 0.45 to 0.89, Non-Fickian mechanism governs drug release and if n is 0.89 or greater than 0.89, then release mechanism is governed by case-II transport or super case II transport mechanism respectively [21] .

***In- vitro* Skin Permeation Study:** The animals used for experiments were adult Swiss albino mice (6–8 weeks old) of either sex, weighing 25–30 g, from the Department of Pharmacology, Shambhunath Institute of Pharmacy, Allahabad. The animals were housed in polypropylene cages, four per cage, with free access to a standard laboratory diet (Lipton Feed, Mumbai, India) and water. They were kept under standard

environmental conditions (23±2°C; 55±5% relative humidity; 12-h light/dark cycle). The *in-vivo* experimental protocol was approved by the Institutional Animal Ethical Committee (CPCSEA registration no. 1632/PO/ERe/S/13/CPCSEA).

The permeation of LXM-loaded transfersomes through the mice skin was determined by using aside-by-side diffusion cells. The stratum corneum side of the skin faced the donor compartment, which was filled with the formulation containing LXM (1 mL). The receptor compartment was filled with 0.1 M phosphate buffer saline (pH7.4) and stirred with a star-head Teflon magnetic bar driven by a synchronous motor. The sink condition in the receptor medium was obtained in this study. At appropriate time intervals 2 mL aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh medium.

Stability Evaluation of Transfersomes: Transfersomes were stored in glass bottles with plastic plugs at 4±1°C, 25±1°C (room temperature, RT), and 45±1°C for 30 days to examine the stability of the formulations. The physicochemical stability of LXM-loaded transfersomes was evaluated by size, EE%, and zeta potential measurement. Samples from each vesicle were withdrawn after a month and the particle size, EE% and zeta potential of the vesicles were measured [22].

Statistical Analysis: Statistical analysis of the results was performed using Student's t test; $p<0.05$ was considered as significant. All values were reported as mean ± SEM and TEM results.

Lornoxicam Loaded Trasferosomes: Transferosome are vesicular system has ultra deformable properties and can easily penetrate the stratum corneum and regain size inside the skin. So, the aim was to deliver the drug at the site of action more efficiently and enhance pharmacological action. In the present study, span 80 and tween 80 were selected as the edge activator surfactants (2-6%w/w) for the transfersomal formulation as it is biocompatible and pharmaceutically acceptable [23]. 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 [24].

Drug Excipient Interaction Study: The FTIR spectral analysis of LXM alone showed that the principal peaks (Fig 1) should be at wave numbers of 3404 cm^{-1} (–NH Stretching), 1083 cm^{-1} (S=O group), 1646 cm^{-1} (C=0 group),1588 cm^{-1} (NH group in secondary amide) and 788 cm^{-1} (C-H bending with aromatic structure) as per established standards [25].

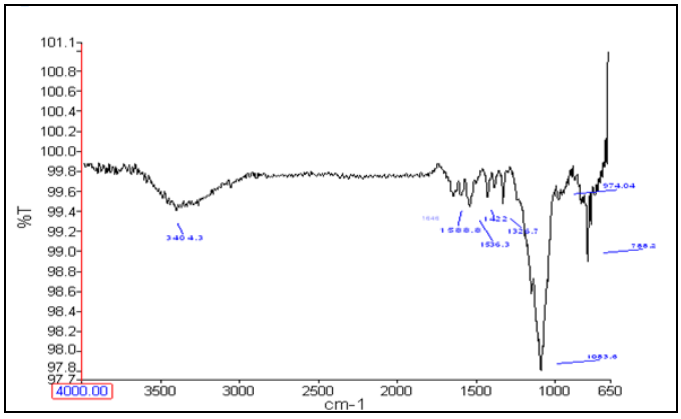

FIG. 1/A: IR SPECTRUM OF PURE DRUG LORNOXICAM

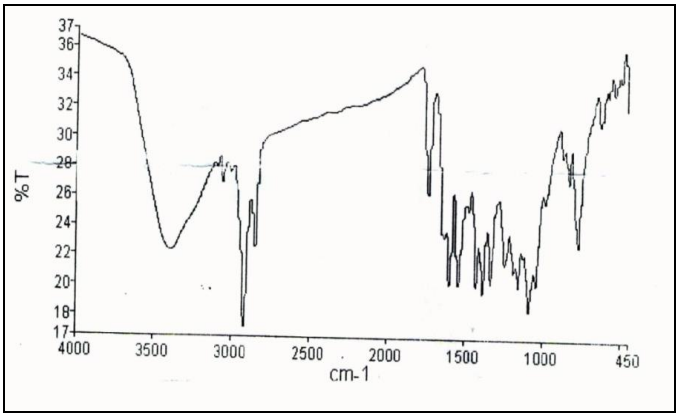

FIG.1/B: IR SPECTRUM OF PHYSICAL MIXTURE (LORNOXICAM + PC (LECITHIN)

FIG. 1: FTIR SPECTRUM OF PURE DRUG LORNOXICAM (A) AND PHYSICAL MIXTURE OF LORNOXICAM AND SOYA LECITHIN (B) 278X158MM (300 X 300 DPI)

Particle Size, Vesicle Size, and Zeta Potential of Vesicles: The mean particle size of tranferosome vesicles were in the range of 330-550 nm with the polydispersity index (PDI) of 0.4-0.8 indicating that the transfersomes were homogeneously dispersed. The zeta potential of the transfersomes was between -19 to -29 mV **Table 2.**

TABLE 2: THE PHYSICOCHEMICAL CHARACTERISTICS OF TRANSFEROSOMES

Formulation code	Mean Particle Size (nm)	Vesicle size (nm)	PDI	Zeta Potential	% EE
T-1	423±1.59	947±5.63	0.26±0.01	-30.1±1.45	22.72±0.37
T-2	546±2.95	1152±7.82	0.53±0.04	-22.4±1.04	27.5±0.84
T-3	384±1.77	883±4.96	0.66±0.03	-19.5±1.01	35.95±1.22
S-1	334±3.27	692±4.61	0.77±0.03	-28.9±1.09	38.46±0.95
S-2	396±1.92	810±3.26	0.40±0.02	-18.9±1.24	42.65±1.54
S-3	487±2.38	1086±6.15	0.32±0.01	-14.8±1.38	53.33±1.62

Entrapment Efficiency: The entrapment efficiency of transfersomes was between 22% and 54% **Table 2**. Entrapment efficiency was found to increase with the increase of the surfactant concentration (span 80) while no specified increase was found in entrapment efficiency with the increase in tween 80 concentration. Maximum % entrapment was found in S3 formulation as 53.33 % and minimum entrapment were found in T1 formulation as 22.72 %.

Morphology Imaging of Transfersome Vesicles: Optical inspection indicated the vesicles appeared as multilamellar vesicles, with the lamellae of vesicles evenly spaced to the core **Fig. 2** and no aggregation irregularities were observed in the system. Lornoxicam S2, S3, and T2, T3 batches showed oval and semi-spherical structures. Transferosome dispersion was prepared, and S2 and S3 batches were evaluated **Fig. 3** under a phase contrast microscope (PCM) and got the semi-spherical shape. Results of Transmission Electron Microscopy (TEM) of S2 formulation revealed a positive image in which transfersomes appeared as spherical structures, confirming the vesicular characteristics.

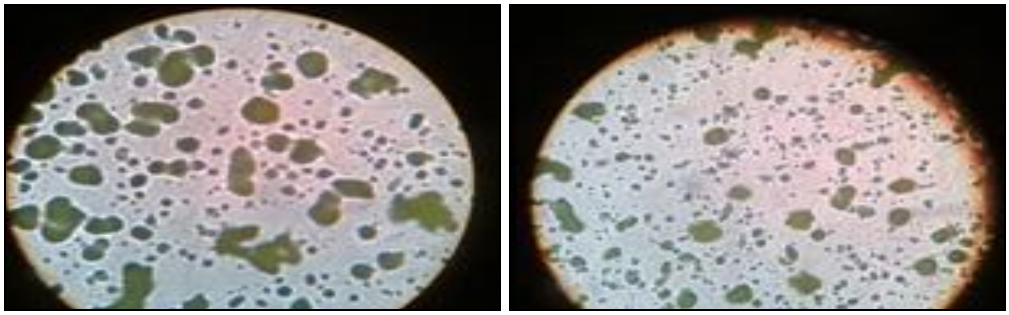

FIG. 2A: OPTICAL MICROSCOPY IMAGE OF LXM S3 & LMX2 FORMULATION

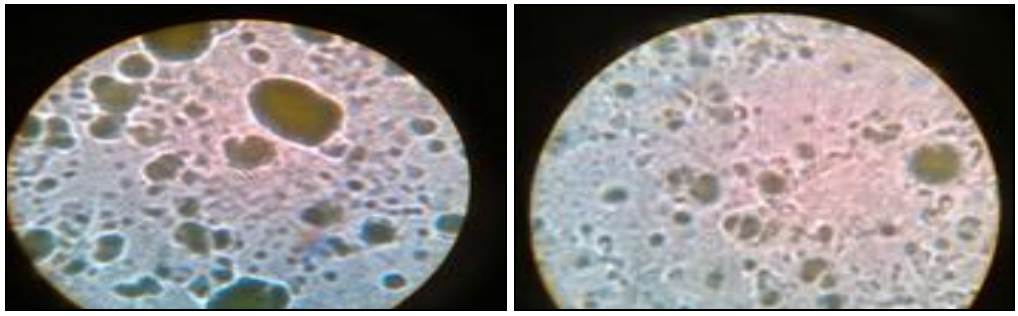

FIG. 2B: OPTICAL MICROSCOPY IMAGE OF LXM T2 & LXM T3 FORMULATION

FIG. 2: OPTICAL MICROSCOPY IMAGES OF LORNOXICAM S2 (A), S3 (B), T2 (C) AND T3 (D) FORMULATIONS 278X158MM (300 X 300 DPI)

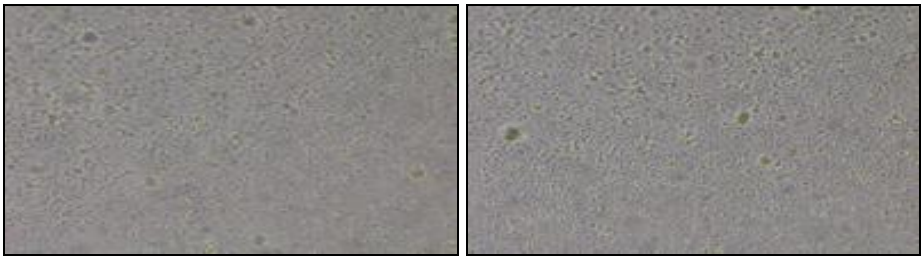

FIG. 3A: PCM PHOTOGRAPH OF LXM S3 & LXM S2 FORMULATION

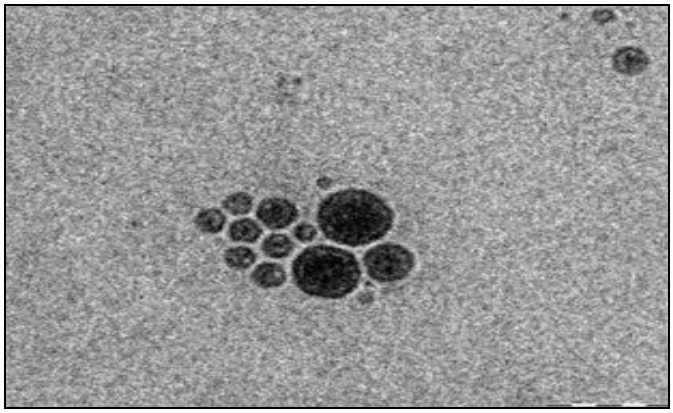

FIG. 3B: TEM IMAGE OF LXM S2 FORMULATION

FIG. 3: PHASE CONTRAST MICROSCOPY OF LORNOXICAM S2 (A), S3 (B), AND TRANSMISSION ELECTRON MICROSCOPY OF LORNOXICAM S2 (C) FORMULATIONS 278X158MM (300 X 300 DP

***In-vitro* Drug Release Studies Through Cellophane Membrane:** *In-vitro* drug release carried out of different formulations through egg membrane and graph was plotted **Fig. 4**.

The drug release was performed for 5 hr. The % CDR (Cumulative Drug Release) of the different formulations was found to be 6.43% to 49.18% **Table 3**.

The result indicates that S2 is the best formulation because it denoted a high percentage of drug release as 49.18 % in 5 hr. Formulation S2 was optimized and found to be suitable for further studies among various types of formulations. The release rate of Lornoxicam from S2 was significantly higher (P <0.05, t-test) than the other formulations.

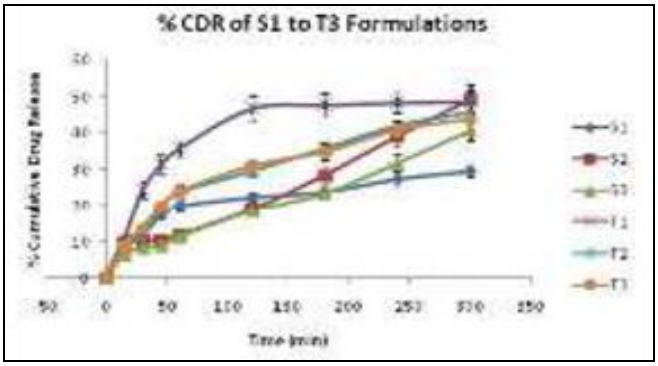

FIG. 4: *IN-VITRO* % CUMULATIVE DRUG RELEASE IN S1 TO T3 FORMULATIONS

TABLE 3: *IN-VITRO* DRUG RELEASE CHART FOR S-1 TO T-3 FORMULATIONS

	%cumulative Drug Release(% CDR)					
Time (min)	**S-1**	**S-2**	**S-3**	**T-1**	**T-2**	**T-3**
0	0.00	0.00	0.00	0.00	0.00	0.00
15	10.3±0.8	9.65±0.75	6.43±0.24	10.82±0.33	8.14±0.57	8.75±0.62
30	11.70±0.65	10.20±0.46	8.24±0.18	24.28±2.31	11.54±0.51	13.63±0.58
45	17.25±1.14	10.45±0.92	8.84±0.2	31.28±2.52	19.34±1.14	19/85±1.22
60	19.85±1.21	11.78±0.55	11.29±0.48	35.68±1.96	23.60±1.64	23.89±1.71
120	22.29±1.4	18.97±1.19	18.48±1.05	46.67±3.55	29.51±1.48	30.93±2.1
180	23.86±1.36	28.48±2.37	23.42±1.29	47.64±3.19	35.68±1.95	34.82±2.28
240	27.41±2.08	39.10±2.82	31.91±2.16	48.26±2.84	41.86±1.37	40.8±2.37
300	29.57±1.81	49.18±3.68	40.74±2.67	48.47±2.99	45.56±2.26	43.78±2.94

Drug Release Kinetic Studies: Plots of Zero order, First order, Higuchi matrix, Korsmayer Pappas, and Hixson Crowell for the formulations were plotted and the regression coefficient (r^2) values and the 'n' values for Korsmayer pappas are tabulated in **Table 4** which exhibits that the best fit model was Zero order, while 'n' exponent value, for Pappas model, for formulations S3 and T3 is greater than 0.45 indicating that formulation is released by Non-Fickian diffusion mechanism.

While for formulations S1, S2, T1 and T2 the 'n' exponent value for Pappas model is less than 0.45, indicating that formulation is released by Fickian diffusion mechanism.

TABLE 4: REGRESSION CO-EFFICIENT FOR VARIOUS LORNOXICAM TRANSFERSOMAL FORMULATIONS

	r^2				Korsmayer			
Formulation code	**Zero**	**First**	**Higuchi**	**Hixson Crowell**	**r^2**	**n**	**Best Fit Model**	**Release Mechanism**
S-1	0.998	0.974	0.958	0.989	0.994	0.281269	Zero	Fickian
S-2	0.996	0.984	0.955	0.991	0.946	0.111014	Zero	Fickian
S-3	0.997	0.976	0.957	0.989	0.970	0.540977	Zero	Non Fickian
T-1	0.995	0.987	0.958	0.992	0.976	0.304906	Zero	Fickian
T-2	0.998	0.984	0.959	0.993	0.992	0.19627	Zero	Fickian
T-3	0.998	0.991	0.953	0.995	0.981	0.45134	Zero	Non Fickian

***In-vitro* Skin permeation Study:** Based on the entrapment efficiency, drug content and *in-vitro* release studies, the formulation S2 containing span 80 (4% w/w) was selected for permeation studies through albino mice skin and compared with the pure Lornoxicam solution **Fig. 5**.

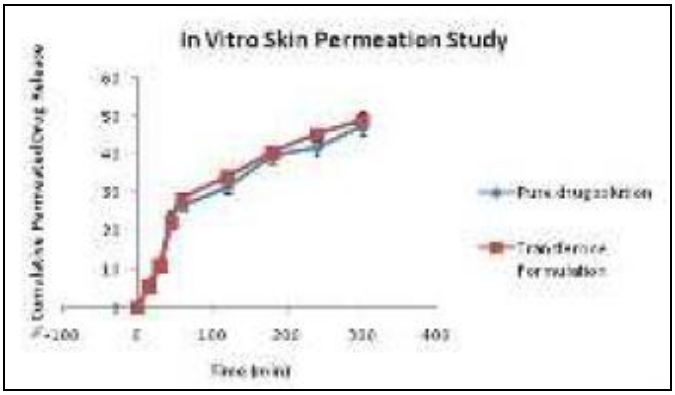

FIG. 5: *IN-VITRO* SKIN PERMEATION STUDY OF PURE DRUG SOLUTION AND TRANSFEROSOME FORMULATION

The *in-vitro* permeation studies provide valuable information about the formulation behaviour *in-vivo* since they indicate the amount of drug available for absorption. The cumulative amount of LXM permeated through the abdominal mice skin in all vesicle formulations was significantly ($p<0.05$) higher than the pure drug solution. The amount of LMX permeated from different transfersomal formulations with different types of EA is found in the following order: Span 80 > Tween 80. The higher skin permeation of LXM from Span 80 (S2) may be related to the lipophilic nature of the drug.

Stability Evaluation of Transfersomes: Physical stability study was performed at room temperature for one month **Table 5.** Results suggest that keeping the vesicular product at room temperature minimizes the stability of vesicles, but vesicular product may be stable in refrigerated condition (4^oC). Parameters like vesicular size, zeta potential, and drug entrapment were evaluated after one month.

The vesicular size of transferosome formulations (S1- S3 and T1-T3) had a slight change from (692±4.61 to 1152±7.82 nm earlier) to (716±5.22 to 1169±10.42 nm) after one month. Same way zeta potential values (-30.1±1.45 to -14.8±1.38mV earlier) changed to (-33.4±0.97 to -20.8±1.65mV). % EE values also decreased slightly, indicating that formulations were stable.

TABLE 5: STABILITY OF PREPARED VESICLES DURING STORAGE AT ROOM TEMPERATURE FOR ONE MONTH

Formulation code	vesicle size (nm)		Zeta potential (mv)		% EE	
	Initial	**After one month**	**Initial**	**After one month**	**Initial**	**After one month**
S-1	692±4.61	716±5.22	-28.9±1.09	-31.5±1.51	38.46±0.95	33.65±0.84
S-2	810±3.26	828±5.73	-18.9±1.24	-22.1±1.34	42.65±1.54	37.84±1.16
S-3	1086±6.15	1107±9.56	-14.8±1.38	-20.8±1.65	53.33±1.62	50.16±2.14
T-1	947±5.63	956±7.31	-30.1±1.45	-33.4±0.97	22.72±0.37	18.23±0.53
T-2	1152±7.82	1169±10.42	-22.4±1.04	-26.7±1.83	27.5±0.84	23.42±1.28
T-3	883±4.96	899±6.95	-19.5±1.01	-24.5±1.47	35.95±1.22	30.36±1.3

DISCUSSION:

Lornoxicam Loaded Trasferosomes: These results are related to the HLB values of these edge activators (EA). They are 4.3 and 15 for Span 80 and Tween 80, respectively. Based on these HLB values, the affinity for lipids was expected to be in the order of Span 80 > Tween 80. This consideration explains the higher EE% encountered with Span 80 as compared to Tween 80. The entrapment efficiency of the Span 80 formulation

was high because of the increase in lipid volume ratio in the vesicles compared to the encapsulated aqueous volume [26].

Drug Excipient Interaction Study: The FTIR spectra of LXM - PC were compared with the FTIR of the pure drug (Lornoxicam), which indicates no interaction between drug and polymer, so drug was compatible with the polymer.

Particle Size, Vesicle Size and Zeta Potential of Vesicles: An addition of a lower concentration of surfactants leads to higher zeta potential values. The drug-loaded transfersomes had lower surface charge when a high level of cholesterol was incorporated. The results agreed with a previous study, which reported that increasing the level of cholesterol in a phospholipid membrane decreases surface charge in the physiological environment [27].

Entrapment Efficiency: Entrapment efficiency of the formulation was observed to increase with increasing surfactant and decreasing polymer concentration. Incorporating ethanol and chloroform increases the fluidity and the interlamellar distance of vesicular membranes, which is probably responsible for better entrapment efficiency [28].

Morphology Imaging of Transfersome Vesicles: Transferosome particles were uniformly scattered, and lump masses were seen at rare places. This was due to a rise in the concentration of the surfactant; molecules may start forming micelles in abilayer resulting in pore formation in vesicle membranes and complete conversion of vesicle membranes into mixed micelles; these mixed micelles were reported to have a lower drug-carrying capacity and poor skin permeation [29].

In-vitro **Drug Release Studies Through Cellophane Membrane:** In the case of S2, a biphasic release was observed, *i.e.*, initial burst effect and subsequent controlled effect. The initial burst effect after 1 hr was due to the rapid dissolution of the surface drug. The rapid leaching out of LXM results in the formation of pores. It thus leads to the decrease of the mean diffusional path length of the drug molecules to release into dissolution medium and, hence, higher release rates [30]. The release of LXM from transfersomal formulations first increased with increasing Span 80 concentration and then decreased by increasing the concentration of EA. A possible explanation for lower drug release at low edge activator concentrations may be that the lipid membranes were more ordered and less leaky, which impeded drug release due to the loss of vesicular structure and formation of rigid mixed micelles.

In-vitro **Skin Permeation Study:** Transfersomes have shown to be successful in the delivery of drugs into the skin because they are composed of phospholipid and surfactants. Also, they can squeeze through the pores in the skin's stratum corneum (SC) [31]. They can also adsorb onto or fuse with the stratum corneum, and the intact vesicle can penetrate into and through the intact skin. Moreover, surfactants are enhancers that solubilize the lipophilic compound; they also have the potential to solubilize the lipid within the SC. Surfactants swell the SC, interact with the intercellular keratin, and fluidize the SC lipids to create channels that allow increased drug delivery. The higher drug skin retention in the case of transfersomes may be due to the creation of reservoir effect for the drug in the skin due to the deposition of other components of transfersomes with the drug into the skin, thereby increasing the drug retention capacity into the skin. This gave an understanding that transfersomes could prolong the penetration of drug molecules but also help to localize the drug in the skin.

The probable reason for the high permeation of the S2 formulation may be the partitioning of vesicles into the stratum corneum, which is an important process as it drives the partitioning of vesicle-bound drug into the skin. It can, therefore, strongly influence the flux and lag times obtained. One of the reasons for the better skin permeation of the transfersomes was their better partitioning with the stratum corneum and in the deeper layer of skin under the influence of the trans-epidermal osmotic gradient. The transfersomal formulation consists of polar lipids (Phospholipid + Surfactant) that attract water due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water. So, when the transfersomal formulation was applied on the skin surface that was partly dehydrated by water loss due to evaporation, the lipid vesicles felt this osmotic gradient and tried to escape complete drying by

moving along this gradient, resulting in the faster partitioning of vesicles into the stratum corneum and other deeper layers of the skin [32].

Stability Evaluation of Transfersomes: The stability of the prepared vesicles showed drug leakage after one month. This may be due to the higher fluidity of lipid bilayers at higher temperatures resulting in higher drug leakage. Drug leakage study data analysis revealed that transferosome dispersion was not more stable at room temperature. Vesicles were also reasonably stable in terms of aggregation and fusion. In accordance with the results, it can be concluded that at room temperature, there was a slight but insignificantly increase in the particle size.

CONCLUSION: Clinical efficacy is the most important criterion for any drug delivery system. A novel transferosomal system has been developed for transdermal delivery. The principal object of present research is to make Lornoxicam loaded transferosomes for transdermal delivery, which remove gastrointestinal disorders by overcoming the first-pass metabolism and also help reduce dose-related side effects, increasing bioavailability, increasing the residence time of the drug and better patient compliance.

Lornoxicam is used for pain and inflammation management. It is generally given by oral route. However, it has poor bioavailability by an oral route which makes oral treatment unsatisfactory. Transdermal route may be a viable alternative for self-application where the limitations of the oral route could be overcome. Ethanol and chloroform could be employed to increase the permeability of drug to enhance its bioavailability.

The Lornoxicam loaded transferosomes were prepared by rotary evaporation-sonication methodand characterized for their compatibility study by FTIR spectroscopy, particle and vesicular size, morphological studies by OM (optical microscopy), PCM (phase contrast microscopy) and TEM (transmission electron microscopy), entrapment efficiency percentage, in-vitro drug release (cellophane), drug release kinetic studies, in-vitro skin permeation studies (albino mice skin) and stability studies. The present research work concluded that ultra-deformable vesicles can provide a novel solution for transport-related problems. They are free from the rigid nature of conventional vehicles and can transport even large molecules. They work on several mechanisms to provide an excellent carrier system for drug transport. Transferosomescan deform and pass through narrow constrictions (5 to 10 times less than their own diameter) without measurable loss. Ultra-deformable vesicles hold great perspective in delivering a huge range of drug substances, including large molecules like peptides, hormones and antibiotics, drugs with poor penetration due to unfavorable physicochemical characteristics, and drugs for quicker and targeted action. This technology's above-discussed properties strongly advocate its good future in transdermal drug delivery.

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