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Lornoxicam Loaded Transfersomes: Formulation And Evaluation

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ABSTRACT

Transfersomes loaded with lornoxicam, a potent non-steroidal anti-inflammatory drug, were developed by thin film hydration method for transdermal delivery of lornoxicam. The composition of phospholipid (soya lecithin), edge activator (span 80) and the drug (lornoxicam) was optimized based on vesicle size and entrapment efficiency. The optimized formulation was compared with lornoxicam loaded vesicles without the edge activator. The developed transfersomes of lornoxicam and lornoxicam loaded vesicles without edge activator were compared for *in vitro* permeation of lornoxicam through dialysis membrane and for *ex vivo* permeation through porcine ear skin. The average vesicle diameter of the optimized formulation was 678 nm with average drug entrapment efficiency of 65.3%. The *in vitro* flux obtained for the optimized formulation was 79.1µg/cm^2/h while that for formulation without edge activator was found to be 70.2µg/cm^2/hr. The *ex vivo* flux of lornoxicam through porcine ear skin obtained for optimized formulation of lornoxicam loaded transfersomes was 13.2µg/cm^2/h while that for formulation without edge activator was 7.5µg/cm^2/h. The developed transfersomal formulation was stable on storage for 30 days at 4 ± 1^o C with respect to drug content and vesicle size.

Keywords: Deformable vesicles, non-steroidal anti-inflammatory, edge activator, vesicle size, entrapment efficiency, *in vitro* and *ex vivo* permeation

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INTRODUCTION

Lornoxicam (chlortenoxicam) (LRN) is a nonsteroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. Lornoxicam is also reported to be effective in relieving symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute sciatica and low back pain and has potential as an alternative to other NSAIDs for the management of painful inflammatory conditions including arthritis. Most common adverse events associated with LRN are gastrointestinal disturbances like abdominal pain, nausea, diarrhea, indigestion, inflammation of pancreas and mouth ulcer. Presently LRN is administered orally in the form of tablets, as well as by parenteral route [1-2]. Research is ongoing for exploring various non-invasive, painless and effective methods to deliver LRN. Transdermal drug delivery has a niche in the area of non-invasive drug delivery. Transdermally delivered LRN is likely to be better accepted than that given by oral route especially in chronic arthritis conditions due to direct application to the affected area. Transdermal LRN is also likely to have lesser intensity of gastrointestinal adverse events than the oral route. Various approaches to deliver LRN through dermal route e.g. transdermal patches, solid lipid nano particles, niosomes, nanoemulsion, have been studied by researchers [3-12].

Transfersomes (Idea AG) or deformable vesicles (also called as elastic liposomes or ultraflexible liposomes) were first introduced in the early 1990s [13]. Elasticity is imparted to these vesicles by addition of an edge activator in the lipid bilayer structure. These deformable vesicles when applied to the skin non-occluded, due to their flexibility, permeate through the lipid lamellar regions of stratum corneum due to hydration in the skin [14].

Appropriately formulated deformable vesicles can penetrate pores of even upto five times lesser diameter [15]. These elastic liposomes have been studied as drug carriers for a range of small molecules, peptides, proteins and vaccines[16-20]. The present work reports development and evaluation of LRN loaded transfersomes. Phospholipid lecithin was used to prepare these deformable vesicles along with surfactant span 80 as edge activator to impart flexibility to the vesicle membrane[21]. Transfersomes were prepared by thin film hydration technique. The formulation was optimized based on vesicle size, drug content and entrapment efficiency. The optimized formulation was evaluated for appearance, vesicle size, morphology, *in vitro* and *ex vivo* permeation and stability in comparison to LRN loaded vesicles without edge activator.

MATERIALS AND METHOD

Materials

Lornoxicam (LRN) was a generous sample provided by Abbott Healthcare Pvt. Ltd, India. Soya lecithin (Leciva 90, Phosphatidyl choline) (lecithin) was gifted by VAV Life Sciences, Mumbai, India. Span 80 and triethanolamine (TEA) were purchased from SD Fine Chemicals, India. All other chemicals were of analytical grade.

Development of LRN loaded transfersomes

Optimization of hydration medium:

Table1 gives the amount and strength of triethanolamine (TEA) solution used in trials taken to optimize the hydration medium.

Table 1: Trials for optimization of hydration medium for development of LRN loaded transfersomes

Formulation code	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8
Strength of TEA used for hydration(% w/v)	1	2	3	4	5	5	5	5
Amount of TEA solution used for hydration (ml)	5	5	5	5	5	5.5	6.6	7.5

(LRN: lornoxicam; TEA: triethanolamine)

Preparation of LRN loaded deformable vesicles:

LRN loaded deformable vesicles were prepared by thin-film hydration technique [22]. Lecithin and span 80 were dissolved in methanol in a round bottom flask. The solvent was evaporated in a rotary vacuum evaporator under reduced pressure at 45^{o}C, 90 rpm, to get thin film. The flask was rotated under reduced pressure at room temperature till there were no traces of solvent. LRN solution in triethanolamine (TEA) was used to hydrate the thin film obtained. The resultant suspension was stored in refrigerator (4±1^{o}C) for 16-18 hours and then vesicle size reduction was carried out by sonication for 30 minutes.

Feasibility trials:

LRN loaded transfersomes were prepared for each composition as indicated in Table 2 as per the procedure given earlier for preparation of LRN loaded deformable vesicles.

Table 2: Compositions of feasibility trials for development of LRN loaded transfersomes

Formulation code	Lecithin %	Span 80 %	LRN %
T-9	61.11	11.11	27.78
T-10	74.07	7.41	18.52
T-11	80.00	8.00	12.00
T-12	83.33	8.33	8.33
T-13	83.33	6.67	10.00
T-14	78.13	12.50	9.37
T-15	85.71	5.71	8.57

Optimization of composition of LRN loaded transfersomes:

Trials were taken as per the compositions given in Table 3 to optimize the concentrations of LRN, lecithin and span 80. Position of (LRN: lornoxicam) should be directly below the table

Table 3: Optimization trials for development of LRN loaded transfersomes

Formulation code	Lecithin: Span 80	LRN (mg)
T-16	12.5:1	30
T-17	25:1	30
T-18	10:1	30
T-19	12.5:1	20
T-20	20:1	30
T-21	25:1	20
T-22	10:1	20
T-23	20:1	20

Preparation of LRN loaded vesicles without edge activator: (LPO)

LRN loaded vesicles without span 80 were prepared by thin-film hydration technique as described earlier for preparation of LRN loaded vesicles except that span 80 was not added to the formulation.

Preparation of blank (placebo) formulations: T- 18(PL) and LPO(PL)

Transfersomes without LRN, referred to as T- 18(PL), were prepared by thin film hydration technique described earlier except that the hydration medium constituted only of TEA solution. Blank vesicles without span 80 and LRN, referred to as LPO(PL), were also prepared corresponding to LPO.

Drug Content

The drug content of the formulations was determined by diluting 1ml of the vesicular suspension to 25ml with methanol in a volumetric flask and sonicating for 15 minutes. 1ml of this solution was further diluted to 10 ml with methanol. The absorbance of this solution was taken on ultraviolet spectrophotometer (Perkin Elmer, Lambda 25) at wavelength of 377nm. Three independent measurements were carried out for each sample and an average of these was considered.

Entrapment Efficiency [23]

The entrapment efficiency was determined by measurement of free drug in the hydration medium. The vesicular suspension was subjected to centrifugation and the supernatant was withdrawn. The supernatant was filtered through 0.45µ syringe filter. It was suitably diluted with methanol and phosphate buffer pH 7.5 and the absorbance determined on ultraviolet spectrophotometer. The entrapment efficiency was calculated using the following equation

$$\% EE = (C_i - C_u)/C_i * 100$$

Where, EE= entrapment efficiency, C_i is the initial amount of LRN added to the formulation, C_u is the amount of unentrapped drug in the supernatant.

Evaluation

Appearance:

The LRN loaded deformable vesicular suspension was visually observed for appearance with respect to colour and clarity.

Measurement of vesicle size:

The vesicle size of optimized formulation of LRN deformable vesicles, blank deformable vesicles, T-18(PL), vesicular formulation without edge activator LPO, and corresponding blank vesicles LPO(PL) were measured on a Malvern Zetasizer, Nano ZS90. The measurement was performed on vesicular suspension diluted with deionised water at room temperature. Average of three independent measurements was considered.

Morphology:

Morphology of the vesicles was studied using Motic phase contrast microscope (B1 advanced series). The morphology of optimized LRN loaded deformable vesicles was also studied with scanning electron microscope.

Measurement of Zeta Potential:

Zeta potential of the optimized formulation T-18 was measured on a Malvern Zeta Sizer, Nano ZS90. The measurement was performed on vesicular suspension diluted with deionised water at room temperature.

In Vitro study [24-25:]

Optimized formulation of LRN loaded transfersomes and LPO were studied for *in vitro* drug release through dialysis membrane (dialysis membrane-150, Hi-media, molecular weight cutoff-12000 to 14000). A vertical diffusion cell was used for the study. Phosphate buffer pH 7.5 was used as the receptor medium. The receptor medium was continuously stirred and the temperature was maintained at $32^oC \pm 1^oC$ with the help of a water jacket. 0.25ml of vesicular suspension was placed in the donor compartment over an effective diffusion area of 3.8 cm^2. Aliquot of 1ml was withdrawn from the receptor compartment at 30 min, 2 h, 4 h, 6 h and 8 h time intervals. Sink conditions were maintained by replacing with equal volume of phosphate buffer pH 7.5. Phosphate buffer pH 7.5 was used to suitably dilute the aliquots and absorbance was read on ultraviolet spectrophotometer at detection wavelength of 377 nm. Cumulative amount of LRN released was calculated and plotted against time. The flux of LRN through dialysis membrane per unit area per

hour was calculated by dividing the slope of the straight line portion of the graph by the effective area of diffusion.

*Ex vivo study***:**

Porcine ear skin was used to study the *ex vivo* permeation of LRN from T-18 and LPO. It was stored in normal saline at -20^{o}C after clearing the hair and the fatty layer and used for study within a week [26].

During the study, the prepared skin was brought to room temperature and was mounted in between the donor and the receptor compartment of the vertical diffusion cell. The receptor compartment contained phosphate buffer pH 7.5. The receptor medium was continuously stirred and the temperature was maintained at 32^{o}C ± 1^{o}C with the help of a water jacket. The skin was equilibrated with the receptor medium for 30 minutes. At the end of 30 minutes, 1ml aliquot was withdrawn from the receptor compartment so as to get a blank reading. It was replaced with an equal amount of phosphate buffer pH 7.5. 0.25 ml of vesicular suspension was added to the donor compartment which had an effective diffusion area of 3.8cm^{2}. 1ml aliquot was withdrawn at 30minutes, 2h, 4h, 6h and 8h time points. The aliquots were replaced by adding equal amount of phosphate buffer pH 7.5 to the receptor compartment so as to maintain sink conditions. Absorbance was read on ultraviolet spectrophotometer, after appropriate dilutions with phosphate buffer pH 7.5, at a detection wavelength of 377 nm. Cumulative amount of LRN released was calculated and was plotted against time. Slope of the straight line portion of the graph was divided by effective area of permeation to calculate flux of LRN through porcine ear skin per unit area per hour.

Data Analysis:

The statistical analysis of the data was carried out by applying Student's t-test using GraphPad Prism 5. A P-value of less than 0.05 was considered to be significant.

Stability

T-18 and LPO were stored in glass bottles with plastic caps at 4 ± 1°C and 30°C/65 %RH for 30 days to evaluate the stability of the formulations. The formulations were evaluated for sedimentation, vesicle size and measurement of the LRN content remaining in the formulation after 30 days.

RESULTS AND DISCUSSION

Results

Development of deformable vesicles of LRN

Optimization of hydration medium:

Clarity of solution was not achieved when LRN was added to the amount of triethanolamine solution of the corresponding strengths as per T-1 to T-7 as indicated in Table 1**.** Addition of LRN to T-8 gave clear LRN solution.

Feasibility trials:

Table 4 gives the values of LRN content and entrapment efficiency of initial feasibility trials mentioned in Table 2. The average entrapment efficiency of the formulations ranged from 6.5 % to 76.2%. The LRN content for all the formulations was more than 95% of the added amount.

Table 4: Drug content and entrapment efficiency (EE) values for feasibility trials of LRN loaded transfersomes

Formulation code	Drug Content (LRN)(%)	EE (%)
T-9	97.3 ± 0.8	6.5 ± 2.45
T-10	98.2 ± 0.5	28.9 ± 2.43
T-11	96.9 ± 0.4	64.8 ± 0.82
T-12	97.6 ± 0.8	76.2 ± 0.86
T-13	98.4 ± 0.3	67.9 ± 0.73
T-14	98.9 ± 0.5	68.7 ± 0.83
T-15	97.8 ± 0.6	64.2 ± 0.71

(LRN: lornoxicam)

Optimization of composition of LRN loaded transfersomes:

Table 5 gives the values obtained for drug content, entrapment efficiency, vesicle size and polydispersibility index of optimization trials of LRN loaded transfersomes given in Table 3. The entrapment efficiency values ranged from 15.9% ± 1.35% to 77.3%± 0.88. The average vesicle size ranged from 678 nm to 930 nm with the polydispersibility index ranging within 0.2 to 0.4.

Table 5: Drug content, entrapment efficiency (EE)**, vesicle size and polydispersibility index (PDI) values of optimization trials for development of LRN loaded transfersomes**

Formulation code	Drug Content (LRN) (%)	EE (%)	Average vesicle size (nm)	PDI
T-16	96.9 ± 0.7	67.9 ± 0.82	744	0.297
T-17	97.4 ± 1.3	33.7 ± 1.38	771	0.304
T-18	97.1 ± 0.8	65.3 ± 0.76	678	0.348
T-19	97.9 ± 0.7	77.3 ± 0.88	910	0.224
T-20	96.9 ± 1.2	17.4 ± 0.83	758	0.306
T-21	97.2 ± 0.8	15.9 ± 1.35	907	0.332

T-22	97.7 ± 0.6	76.2 ± 0.74	774	0.222
T-23	98.1 ± 0.8	10.6 ± 1.43	930	0.370

Evaluation of LRN deformable vesicles

*Appearance**:***

The LRN loaded transfersomal suspension was bright yellow coloured, non-transparent suspension with no visible solid particles.

*Measurement of vesicle size**:***

Table 6 gives the comparative values of vesicle size and polydispersibility index for T-18, LPO,T-18(PL) and LPO(PL). The vesicle size for these formulations ranged from 678 to 955 nm with polydispersibility index ranging within 0.1 to 0.4.

Table 6: Comparative results of vesicle size and polydispersibility index (PDI) for T-18(PL), T-18, LPO(PL) and LPO

Formulation code	Average vesicle size (nm)	PDI
T-18(PL)	764	0.10
T-18	678	0.35
LPO(PL)	955	0.15
LPO	816	0.40

Morphology:

The vesicles exhibited spherical morphology. Figure 1A and 1B depict the morphology of vesicles of optimized T-18 formulation and LPO respectively as observed under Motic phase contrast microscope. Figure 1C is the scanning electron microscope image of the optimized T-18 formulation.

Measurement of zeta potential:

The optimized formulation T-18 exhibited a negative zeta potential of -52.3mV, Figure 2.

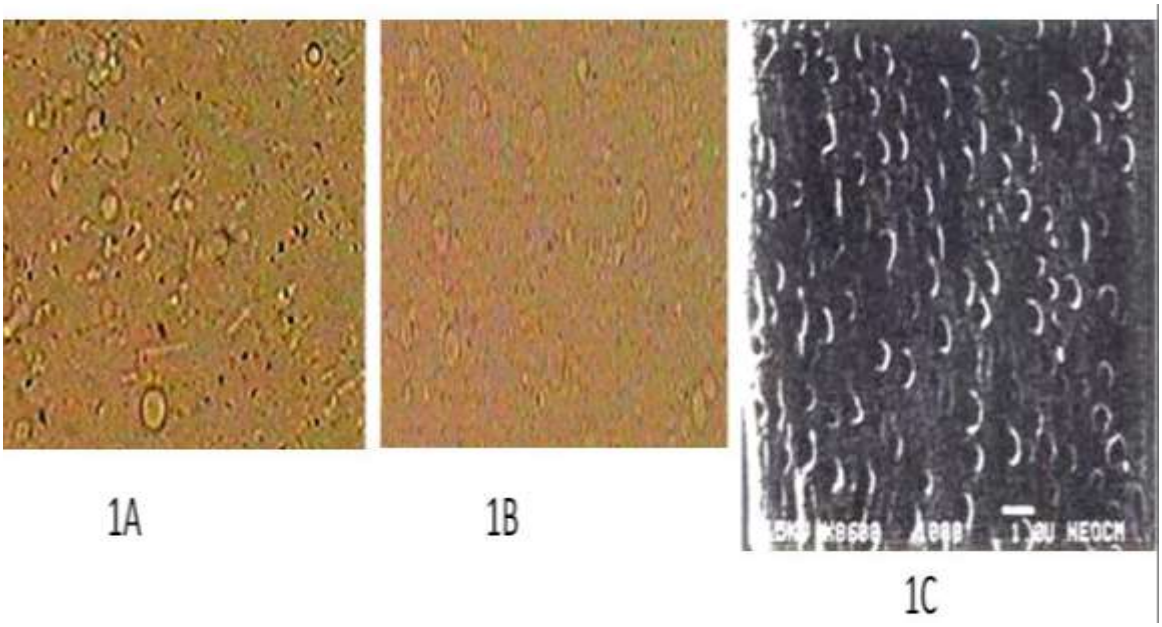

Figure 1: **Morphology of vesicles: 1A: Phase contrast photomicrograph(Motic,40x):Vesicle morphology of optimized LRN deformable vesicle formulation, T-18, 1B: phase contrast photomicrograph (Motic ,40x) :Morphology of vesicles without edge activator, LPO, 1C: Scanning electron microscope image of optimized LRN loaded transfersomes, T-18.**

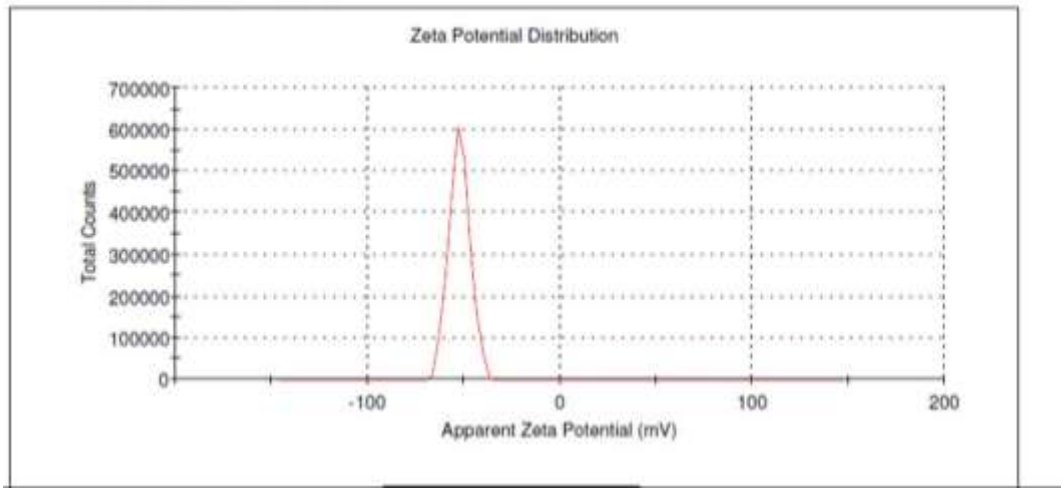

Figure 2: **Zeta potential graph of optimized formulation (T-18) of lornoxicam loaded transfersomes**

In Vitro study: Figure 3 depicts the comparative percent of LRN permeated *in vitro* with time for T-18 and LPO. Figure 4 depicts cumulative amount of LRN permeated *in vitro* with time for the two formulations. The *in vitro* flux of LRN obtained for T-18 was 79.1µg/cm^2/hr while that obtained for LPO was 70.2µg/cm^2/hr.

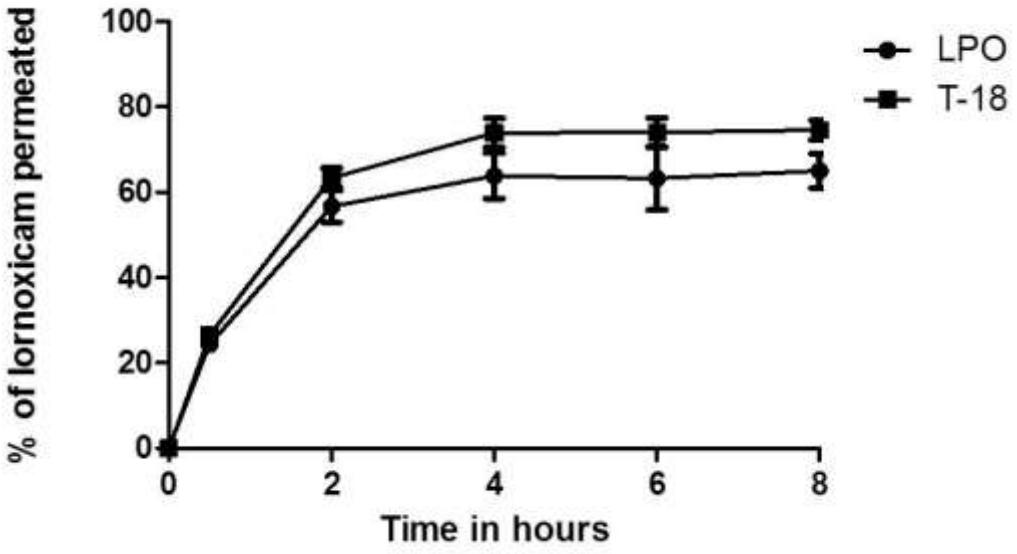

Figure 3: **Comparative percent lornoxicam permeated *in vitro* from T-18 and LPO.**
(T-18: optimized LRN loaded transfersomes; LPO: LRN vesicular formulation without edge activator).

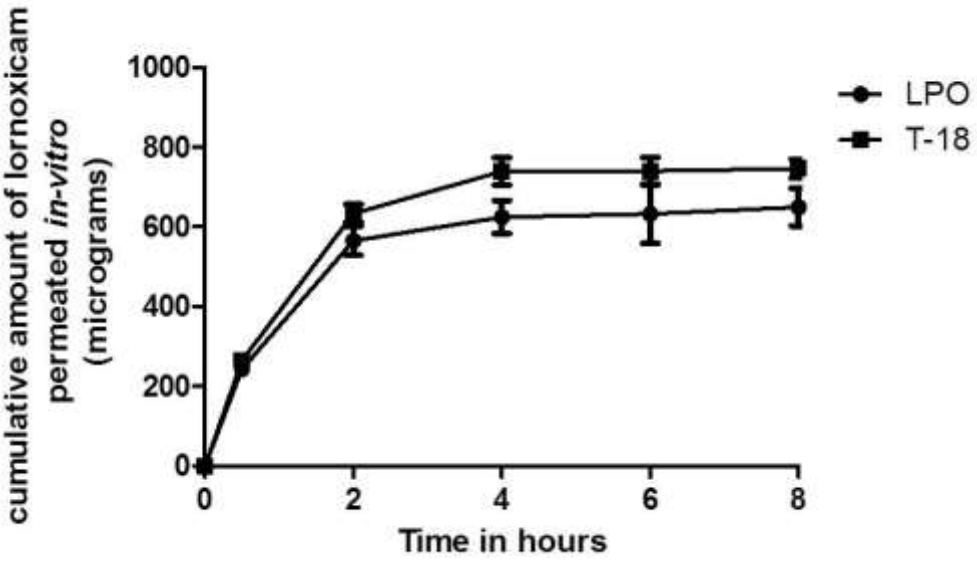

Figure 4: Cumulative amount of LRN permeated *in vitro* from T-18 and LPO.
(T-18: optimized LRN deformable vesicular formulation; LPO: LRN vesicular formulation without edge activator).

Ex Vivo study:

The permeation profile of LRN through porcine ear skin from T-18 in comparison with LPO is depicted in figure 5. Flux value of LRN through porcine ear skin obtained for T-18 was 13.2µg /cm^2/h. while that for LPO was 7.5µg/cm^2/h.

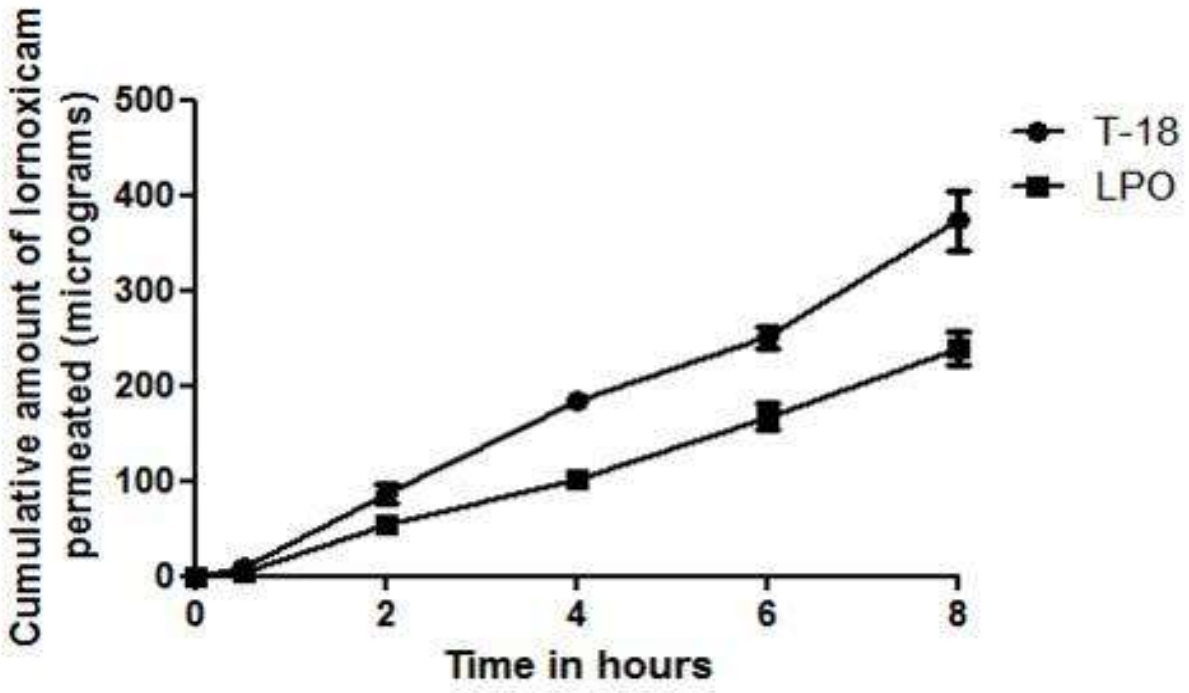

Figure 5: Cumulative amount of LRN permeated *ex vivo* through porcine ear skin from T-18 and LPO.

(T-18: optimized LRN deformable vesicular formulation; LPO: LRN vesicular formulation without edge activator).

Stability

The stability results of drug content and vesicle size for T-18 and LPO are given in Table 7. There was no appreciable change in the drug content of the two formulations at the end of 30 days. Vesicle size remained unchanged at 4^{o}C after 30 days while it was found to slightly increase at 30^{o}C/ 65% RH after 30 days. Some sediment was observed in LPO at 30 ^{o}C / 65%, however it was easily redispersible. No sediment was observed in case of T-18 at 30 ^{o}C / 65% RH.

Table 7: Average vesicle size and drug content of T-18 and LPO after 30 days storage period

Formulation code	Average Vesicle size (nm)			Drug (LRN) content		
	Initial	4^{o}C±1 ^{o} C 30 days	30 ^{o} C/60%RH 30 days	Initial	4^{o}C±1 ^{o} C 30 days	30 ^{o}C/60%RH 30 days
T-18	678	657.0	782.3	97.1%	95.5%	95.3%
LPO	816	816.5	833.9	96.1%	94.8%	94.5%

DISCUSSION

Transfersomes, or deformable vesicles, have unique structural properties which result in increased permeability of these vesicles through the skin. The phospholipids constituting the bilayer of the

vesicles have a high affinity for biological membranes. The vesicle-phospholipid bilayer mixes with the intercellular lipid layer of the skin thereby enhancing permeability of the vesicles. Thus, phospholipid bilayer forms the essential component of the vesicular systems having the similar lipid composition as that of the skin. Lecithin (phosphatidylcholine) is commonly used phospholipid for vesicular formulations. Edge activators are mainly incorporated in the lipid bilayer to increase the deformability (flexibility/elasticity) of the bilayer [27,28,29.]

Discussion

Surfactants are usually used as edge activators due to their ability to reduce the interfacial tension of the bilayer hence increasing its flexibility. Span 80 has a hydrophilic-lipophilic balance value of 4.3 and hence is suitable for entrapment of hydrophobic drugs. Lecithin and span 80 were therefore selected as the lipid and the edge activator respectively to prepare deformable vesicles of LRN. Selection of hydration medium was an important aspect in the development of LRN loaded deformable vesicles due to solubility characteristics of LRN. Poor water solubility and pH dependent solubility of LRN led to the selection of triethanolamine as the hydration medium [30.] Accordingly, T-8 from Table 1was selected as hydration medium. LRN was dissolved in this medium to prepare the LRN loaded vesicles.

Formulations T-9 to T-15 (Table 2) were the trials taken to arrive at a suitable composition of lecithin, span 80 and LRN. It was observed that T-9 and T-10 showed undissolved particles settling at the bottom of the container. Amount of lecithin was therefore increased and trials were taken to arrive at a suitable concentration of ingredients to achieve better entrapment. It was observed from the results given in Table 4 that entrapment efficiency was influenced by drug concentration. For formulations T-11 to T-15, increase in lipid or the surfactant concentration did not exhibit substantial change in entrapment efficiency. From these trials, considering the maximum drug loading, T-11 was the composition considered for further optimization. Optimization of this composition was carried out as given in Table 3 in which effect of concentration of lipid, surfactant and drug was studied on vesicle size as well as entrapment efficiency. The drug content of all the formulations was above 95% of the input drug amount. The results of optimization trials are given in Table 5. The values obtained indicate that for a given drug concentration an optimum lipid to surfactant ratio is required for better entrapment efficiency. For an optimum lipid to surfactant ratio, decrease in drug concentration increased the entrapment efficiency. From these results, 10:1 ratio of lecithin:span 80 was considered optimum. Formulation T-18 was, therefore, selected for further studies as it exhibited better results for vesicle size as well as entrapment efficiency.

In order to study the effect of span 80 and LRN on vesicle size, the vesicle size of T-18 was compared with vesicle size of LPO which is the vesicular formulation without span 80. T-18 and LPO were also compared for vesicle size with corresponding blank (placebo) vesicles, T-18(PL) and LPO (PL). The data given in Table 6 indicates that addition of span 80 to the lipid vesicles decreased the size of resultant vesicles. This effect was exhibited by the blank vesicles as well. This effect may be due to the decrease in the surface energy as a result of addition of the surfactant span 80 leading to formation of smaller vesicles [31]. The drug loaded vesicles exhibited lower vesicle size than the corresponding placebo vesicles.

Surface charge on the vesicles has shown to influence the physicochemical properties of the vesicles [32]. In this study a negative zeta potential was observed for the optimized formulation. Lecithin (phosphatidylcholine) is a zwitterionic compound with isoelectric point (pI) between 6 and 7 [33]. Under the conditions of the present study (pH >7), where the pH was higher than its pI, phosphatidylcholine would carry a net negative charge. Also, the anion form of LRN would be the predominant form under these conditions [34]. As a result the formulation exhibited a negative charge. Negatively charged liposome formulations have been reported to exhibit strongly improved skin permeation of drugs[35].

Figure 3 and figure 4 are graphical representations of results obtained for *in vitro* diffusion study. The cumulative amount of LRN permeated *in vitro* from T-18 was significantly higher ($P<0.05$) than that permeated for LPO at time points beyond 0.5h. T-18 exhibited significantly higher and faster permeation of LRN through the biological membrane than LPO ($P<0.05$) as depicted in Figure 5. The effect of edge activator on permeation was more prominently observed in the *ex vivo* study than in the *in vitro* study.

The stability results given in Table 7 indicate that the vesicle size of the formulations and drug content did not change appreciably on storage at 4^{o}C for 30 days. At 30^{o}C/65% RH there was an increase in the vesicle size of both, T-18 and LPO, as compared to initial values. However, at 30^{o}C/65%RH, there was slight settling at the bottom of the container for LPO, which was redispersible. The settling may be attributed to the absence of surfactant span 80 in the formulation. Such settling was not observed for T-18.

CONCLUSION

The study indicates the feasibility of formulation of LRN loaded transfersomes using lecithin and span 80 for transdermal delivery of LRN. The developed deformable vesicular formulation exhibited better performance than corresponding vesicular formulation without edge activator with

respect to vesicle size and *in vitro* and *ex vivo* permeation characteristics. Detailed characterization study of the vesicles and *in vivo* studies form the further scope of this work to substantiate the results obtained in *in vitro* and *ex vivo* studies in the present work.

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