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Proliposomes of lisinopril dihydrate for transdermal delivery: Formulation aspects and evaluation

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Abstract–We formulated and evaluated proliposomal gel of relatively low bioavailable drug lisinopril dihydrate (LDH) for transdermal delivery. Several proliposomal gel formulations of lisinopril dihydrate were prepared by modified coacervation phase separation method and examined for formation of liposomes by optical microscope and characterized by transmission electron microscopy. The formulations were evaluated for size, zeta potential, entrapment efficiency, rheological behavior, *ex vivo* drug permeation, skin irritation and stability. Differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) studies were performed to understand the phase transition behavior and mechanism for skin permeation, respectively. The microscopic examination revealed the formation of liposomes from proliposomal gel, and the size of the vesicles was found to be in the range of 385 nm to 635 nm. Entrapment efficiency was high for the formulation containing greater amounts of phosphatidylcholine. The DSC studies indicated the amorphous form of LDH in proliposomal gel formulation. *Ex vivo* permeation studies revealed sustained permeation of drug from proliposomal gels studied. The stability studies reveal that the proliposomal formulations are more stable when stored at refrigeration temperature (4 °C). In conclusion, proliposomal gels offer potential and prove to be efficient carriers for improved and sustained transdermal delivery of lisinopril dihydrate.

Key words: Lisinopril Dihydrate, Proliposomes, Microscopy, Transdermal, Permeability

INTRODUCTION

Lisinopril dihydrate (LDH), a synthetic peptide derivative, is an angiotensin converting enzyme inhibitor used for the treatment of hypertension and congestive heart failure. LDH is slowly and incompletely absorbed after oral administration with a bioavailability of 25-30% [1,2]. LDH was selected as a model drug because of its clinical need, low oral dose (2.5-20 mg), low molecular mass (441.5 g/mol) and low oral bioavailability (25%).

Colloidal systems of nano size range are constantly being developed as drug delivery carriers for diverse applications, such as the oral, transdermal, parenteral and ocular delivery [3-6]. Among those, transdermal drug delivery has been one of the potential applications for the local and systemic delivery of drugs [7,8]. However, the highly organized structure of stratum corneum forms an effective barrier to the permeation of drugs through skin, which is the rate-limiting step for drug permeation [7]. Thus, the major challenge in the transdermal route is permeation of drug across the stratum corneum impeding its barrier function.

Among the various colloidal systems developed, the liposomes have gained much interest and often been considered to be a potential option. In spite of many advantages, the use of liposomes is limited because of significant complications for drug delivery such as aggregation, sedimentation, phospholipids hydrolysis and oxidation [9]. However, these stability problems can be avoided by formulating liposomes as proliposomes [10,11]. Proliposomes, a semisolid liquid crystal (gel) product, are composed of drug and lipid portion [phosphatidyl choline (PC) and cholesterol (Chol)] with minimum quantities of ethanol and water. The formulation upon application onto the surface of skin gets hydrated with water from skin under occlusion and transform into liposomes, which favors drug delivery through skin [10]. Several mechanisms like fusion of vesicles on the surface of the skin [12] and vesicle intercalation into the intercellular lipid layers of the skin [13] could be explained for the ability of liposomes to modulate the diffusion across skin.

The characterization of proliposomes by microscopic techniques is essential to obtain reliable data about the actual transformation and morphology of the system. In the present study, proliposomes of LDH for transdermal delivery was investigated and evaluated. Microscopic analysis of these lipid-based drug delivery systems with particle sizes in the lower submicron range provides critical information about the size, shape and internal structure of the vesicles.

EXPERIMENTAL

1. Materials

Lisinopril dihydrate (LDH) was a gift sample from Aurobindo Pharmaceuticals, Hyderabad. Cholesterol was purchased from E. Merck. Mumbai, Maharashtra, India. Soy phosphatidyl choline [(PC), Phospholipon 90G] was a generous gift from Lipoid, Ludwigshafen, Germany. Dialysis membrane [DM-70; molecular weight cutoff (MWCO) 12,000 Da] was purchased from Himedia, Mumbai, Maharashtra, India. All other chemicals used were of analytical grade. Freshly collected double distilled water was used all throughout the study.

2. Methods

2-1. Preparation of Proliposomal Gels

The proliposomal gel formulations were prepared as per the reported method with slight modification [14] using PC, cholesterol

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Table 1. Composition of lisinopril dihydrate loaded proliposomal formulations

Formulation code	Molar ratio (PC : Chol)	PC (mg)	Cholesterol (mg)	Ethanol (μl)	Water (μl)
F0	1 : 0	750	-	400	160
F1	2 : 1	499	129	400	160
F2	1.5 : 1	450	154	400	160
F3	1 : 1	375	193	400	160
F4	1 : 1.5	300	232	400	160
F5	1 : 2	250	258	400	160

PC, phosphatidyl choline; Chol, cholesterol
Total 1 mM lipid mixture was used in all the preparations
All formulations contained 10 mg lisinopril dihydrate

as the lipid component. Accurately weighed amounts of PC and cholesterol were taken in a clean and dry, wide mouthed glass vials to make 1 mmol of total lipid. The composition of various proliposomal gel formulations is shown in Table 1. The drug was added to the lipid component mixture followed by the addition of 400 μL of absolute ethanol. After ensuring the homogeneous dispersion of the ingredients, the vials were tightly sealed to prevent the evaporation of the solvent and warmed in a thermostatic water bath at 55-60 °C for about 5 min with intermittent shaking until the ingredients were dissolved. To the resultant transparent solutions, 160 μL of double distilled water maintained at the same temperature was added while warming in the water bath till a clear or translucent solution was obtained, which upon cooling formed a yellowish translucent liquid or yellowish translucent gel or a white creamy proliposomal gel. The obtained gels were stored in the same closed glass vials in the dark until further characterization.

2-2. Formation of Liposomes

Formation of liposomes from proliposomal gels upon hydration with phosphate buffer (pH 5.5) and their morphology was evaluated by observing at a magnification of 450X through an optical microscope (Coslabs micro, Ambala, Haryana, India) and transmission electron microscope (JEOL-200 CX; Jeol, Tokyo, Japan).

2-3. Characterization of Liposomes

2-3-1. Entrapment Efficiency

The proliposomal gels were hydrated with phosphate buffer (pH 5.5) and subjected to bath sonication (Sonica, Milano, Italy) for 3 min and the resultant dispersion was used for the determination of entrapment efficiency by dialysis method [15]. From the obtained liposomal dispersion unentrapped free drug was removed by placing about 2 ml of dispersion on a glass tube to which dialyzing membrane was attached to one side and dialyzing exhaustively for 15 min each time against 50 ml of pH 5.5 phosphate buffer; the dialysis was complete when no drug was detectable in the recipient solution. Amount of entrapped drug was obtained by subtracting amount of unentrapped drug from the total drug incorporated [16].

Entrapment efficiency (%)
=(Amount of drug entrapped/Total amount of drug)*100.

2-3-2. Surface Charge, Vesicle Size and Polydispersity Index

The mean size of liposomes obtained by hydrating the proliposomal gel, polydispersity index and surface charge (zeta potential) were determined by photon correlation spectroscopy using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, Worcestershire, UK). Each sample was suitably diluted and size analysis was performed at 25 °C with an angle of detection of 90°.

2-3-3. Rate of Spontaneity

The formation of abundant vesicles is one of the important characteristics for an optimized formulation. Spontaneity of liposomes formation is described as number of liposomes formed after hydration of proliposomal gels with phosphate buffer (pH 5.5) approximately for 15 min. The proliposomal gel (10 mg) was transferred into small stoppered glass vial and 2 ml of buffer was added carefully along the walls of the glass vial and kept aside without agitation. After 20 minutes, a drop of this solution was withdrawn and placed on a Neubauer chamber to count the number of vesicles [17].

2-3-4. Spreadability of Proliposomal Gels

The spreadability of the formulation was determined by placing 0.5 g of gel on a horizontal plate within a circle of 1 cm diameter which is previously marked, a second glass plate was placed over the first glass plate. A standardized weight of 500 g was placed on the upper plate. The increase in the diameter due to spreading of the test formulation was noted [18].

2-3-5. Rheological Behavior

The rheological characteristics of proliposomal gels were determined by using a Brookfield Programmable DVIII+ Digital Rheometer (Brookfield Engineering Laboratories Inc., Massachusetts, USA). The rheological measurements were performed using a controlled stress rheometer with the cone (24 mm) and plate geometry. The sample was equilibrated for 5 min before viscosity measurement and the torque sweep was in the range of 10-110%. All the measurements were performed in duplicate at ambient temperature. The consistency index and flow index were calculated from the Power-law equation:

$$\tau = Kr^n$$

where τ is the shear stress; r the shear rate; K the consistency index; n is the flow index. Taking the log on both sides

$$\log \tau = \log K + n \log r$$

Shear stress (dyne/cm^2)=viscosity (mPas)×rate of shear (s^{-1}).

Thus, from the plot of log of shear stress versus log of shear rate, the slope of the plot representing flow index and antilog of the y-intercept indicating consistency index was calculated.

2-3-6. Differential Scanning Calorimetry

The thermal characteristics of drug, phosphatidyl choline, cholesterol and proliposomal gel (F3) were studied by differential scanning calorimeter (Mettler DSC 821e, Mettler-Toledo, Switzerland). The sample weight of 5 mg was heated in a hermetically sealed aluminum pan over a temperature range of 20-350 °C under a constant nitrogen gas at a heating rate of 10 °C/min.

2-3-7. Fourier Transform Infrared Spectroscopy for the Skin

The FTIR spectroscopy of the rat skin treated with optimized gel for 24 h and the untreated skin (control) was carried out to determine the changes in the structure of the epidermis. After the treatment, the skin was wiped with a tissue and kept in a desiccator to remove traces of solvent. The FTIR spectrum of the control and proliposomal gel treated rat skin was recorded in the range of 4,000-400 cm^{-1} using FTIR spectrophotometer (Bruker USA).

2-3-8. *Ex Vivo* Permeation Study using Excised Rat Abdominal Skin

All the animal studies were conducted with the prior approval of the Institutional Animal Ethical Committee, St. Peter's Institute of Pharmaceutical Sciences, Hanamkonda. The epidermis from rat abdominal skin was prepared by a heat separation technique as reported earlier [19,20]. The permeation of LDH from proliposomal formulations and lisinopril dihydrate powder (control) were carried by fabricated Franz diffusion cells with an effective diffusion area of 4.153 cm^2. The skin was brought to room temperature and sandwiched between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The skin was allowed to equilibrate and about 0.5 g of the proliposomal gel was placed on the skin surface in the donor compartment which was covered with parafilm. The receptor compartment of the cell was filled with 14 ml of phosphate buffer (pH 5.5). During the experiments, the solution in receptor compartment was maintained at 37±0.5 ºC and stirred at 400 rpm throughout the study. The amount of drug permeated was determined by removing 1 ml aliquots of sample at appropriate time intervals up to 24 h; the volume was replenished with an equal volume of phosphate buffer to maintain sink conditions. At the end of the study, the samples were suitably diluted and the amount of drug was determined spectrophotometrically at 212 nm.

2-3-9. Permeation Data Analysis

The cumulative amount of drug permeated through a unit area of skin was plotted as a function of time.

i) Flux can be calculated by using the following equation:

$$J_{ss} = \frac{dQ}{dt} \times \frac{1}{A}$$

where, J_{ss} - steady state flux (μg/cm^2/h) calculated by using the slope of the graph containing cumulative amount permeated through unit area vs time, A - surface area, dQ/dt - cumulative amount permeated per unit area per unit time.

ii) Permeability coefficient (K_p) represents the correlation between the flux and initial drug load, which was calculated using the following equation:

$$K_p = J_{ss}/C$$

where, K_p - Permeability coefficient (cm/hr), J_{ss} - steady state flux, C - initial concentration of LDH in the donor compartment.

2-3-10. Microscopic Examination of Rat Skin

Proliposomal gels were applied for 24 h on the excised rat skin mounted on the diffusion cell. The histological examination was carried out for the respective skin after removing the preparation and the skin was fixed in 10% formalin by the conventional procedure followed by staining with hematoxylin-eosin. Then the stained sections were examined under an optical microscope (Coslabmicro, India). Untreated skin served as a control [21].

2-3-11. Skin Irritation Test

The optimized proliposomal formulation was assessed for skin irritancy potential in rabbits [22] since skin protection is prime consideration for transdermal drug delivery. The rabbits (n=3) weighing 2-2.5 kg were acclimatized before the beginning of the study and were maintained under controlled condition of temperature. The hair of rabbit ear was exposed and hair was removed with a hair clipper 24 h prior to application of the proliposomal gel. Then 0.5 g of gel was applied on the hair free skin of rabbit by uniform spreading within the area of 4 cm^2. The skin was examined for any signs of erythema (redness) or oedema (swelling) over a period of seven days.

2-3-12. Stability Studies

Optimized formulation F3 was studied for stability aspects by monitoring at room temperature and refrigeration temperature (4± 2 ºC) for six weeks. The stability of gels was evaluated for encapsulation efficiency, any change in consistency of gels, surface charge, vesicle size and polydispersity index.

2-3-13. Statistical Analysis

Statistical analysis of the data obtained was performed using Student's *t* test with GraphPad Prism software (version 4.00; GraphPad Software, San Diego, California). The level of statistical significance was chosen as less than $p<0.05$.

RESULTS AND DISCUSSION

1. Preparation of Proliposomes

Proliposomes of LDH were prepared by modified coacervation phase separation method by varying the concentrations of PC to cholesterol. At 0% concentration of cholesterol (F0) with PC alone the formulation was flowable yellowish liquid with poor stability and low entrapment efficiency. To improve the stability and entrapment efficiency of formulations, cholesterol, a structural lipid, was employed at varying concentrations. The appropriate ratio of PC and cholesterol is the prime factor in maintaining the integrity of proliposomes. As reported earlier in our previous report the alteration in concentrations of PC and cholesterol leads to disruption and leakage of drug from vesicles before union of vesicles with the skin [23].

2. Formation of Liposomes

Hydration of the proliposomal formulation resulted in formation of liposomes as seen from Figs. 1(a)-1(d). Fig. 1(a) represents the surface of dry proliposomal gel, (b) upon contact with water, (c) tubular structures formed upon swelling of phospholipids in the formulation, and (d) spherical vesicles formed upon gentle shaking. However, the TEM micrograph confirmed the spherical nature of liposomes formed. The physical appearance of the formulations was converted from translucent yellowish liquid gel state to creamy white gel upon increasing the concentration of cholesterol in the formulation. However, at further increased concentration of cholesterol (F5), the formulation obtained was white compact solid, which was not further evaluated.

3. Characterization of Proliposomes

Spreadability is a vital parameter for better patient compliance and can result in greater penetration and absorptivity of the gel by providing more contact area. The spreadabilty of all formulations was found to be in the range of 4.3-10 cm. With increase in cholesterol content the spreadability of the proliposomal gel was found to be reduced. All the formulations are spreadable with good skin feel except the higher concentration of cholesterol (F5).

The results reveal that the entrapment efficiency is dependent on the composition of proliposomes. The entrapment efficiency of cholesterol free proliposomal formulation (F0) was found to be 71.6± 1.9%, whereas the entrapment efficiency was significantly increased (83.5-92.5%) in proliposome formulations (F1 to F4) containing varying ratios of PC to cholesterol ($p<0.01$). However, with higher

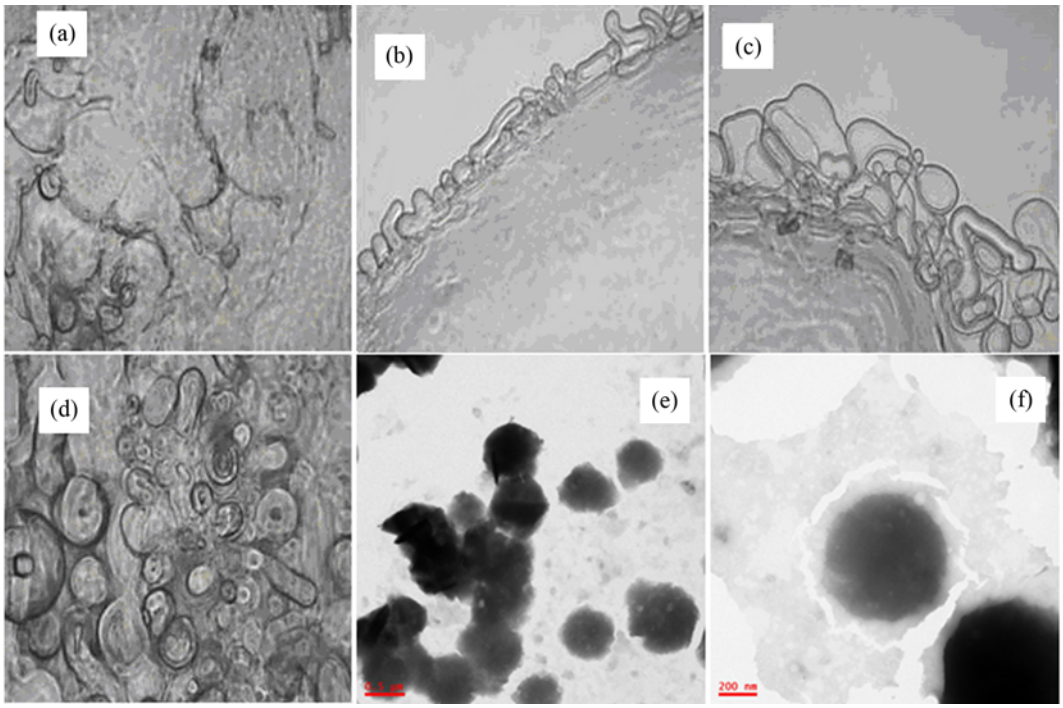

Fig. 1. Optical micrographs (450× magnification) representing formation of liposomes from proliposomal gel (F3 formulation) (a) dry proliposomal gel (b) upon hydration (c) swelling results in tubular structures (d) spherical multilamellar vesicles ((e) and (f)) Transmission electron micrograph of liposomes from proliposome at different scales.

cholesterol content in F4 the entrapment efficiency was decreased. This may be because the higher amounts of cholesterol beyond a certain quantity may start disrupting the regular bi-layered structure followed by leakage of entrapped drug from the vesicle.

The surface charge of liposomes formed from proliposomal formulations was in the range of −5.08 to −13.6 (mv). All the liposomes produced from proliposomal formulations upon hydration showed negative zeta potential; this might be due to the ionization of surface fatty acid functional groups of PC. The surface charge of proliposomal formulations F1 (2 : 1 molar ratios of PC : Chol) and F3 (1 : 1 molar ratios of PC : Chol) was found to be −13.6± 3.6 and −12.4±2.4 mv, respectively; as they contain higher amounts of PC in their formulations, more will be the ionization of surface fatty acid functional groups of PC and lead to higher negative surface charge compared to other proliposomal formulations. Due to negative surface charge, repulsive forces generate between liposomes formed, which prevents the coalescence of the liposomes formed and also helps to incorporate hydrophilic drug in the aqueous compartment of the vesicle [24,25].

Vesicle size and size distribution of the liposomes formed from the proliposomal systems are vital parameters for understanding the entrapment efficiency. The size of liposomes formed from all the formulations was within the range of 385.5±18 to 635.1±26 nm (Table 3). However, we could observe a linear relationship between the size of the vesicles and concentration of cholesterol. As the concentration of cholesterol increased, the size was also increased. As shown in Table 3, the F3 and F4 formulations with larger vesicular size showed higher entrapment efficiencies. Generally, liposomes of size 1,000 nm and above are reported to be multilamellar vesicles [26] with small aqueous compartment. In our study liposomes formed are of <1,000 nm. They are advantageous for incorporation of hydrophilic drug in the larger aqueous compartment, which leads to the improved entrapment of drug into the vesicles. Polydispersity index, which is a dimensionless measurement of broadness of particle size distribution, was also calculated for all the formulations. Small value of PI (<0.1) indicates a homogeneity, whereas a PI (>0.3) indicates a higher heterogeneity. The PI employed as a measure of uniformity in size distribution was in the acceptable limits for all

Table 2. Appearance and rheological characteristics of proliposomal formulations

Formulation code	Appearance	Spreadability	Flow index	Consistency index
F0	Yellowish liquid	10	0.913	214
F1	Yellowish translucent gel	8	0.838	202
F2	White creamy gel	6.6	0.461	133
F3	White creamy gel	5.5	0.568	165
F4	White creamy gel	4.3	0.899	243
F5	White compact solid	-	-	-

Table 3. Physicochemical characteristics of lisinopril dihydrate liposomes

Formulation code	Encapsulation efficiency	Vesicle size (nm)	Zeta potential (mv)	Rate of spontaneity	Polydispersity index
F0	71.6±1.9	385.5±18	−5.08±2.2	5.9×10^4	0.223
F1	83.5±2.4	426.2±22	−13.6±3.6	6.5×10^4	0.271
F2	86.4±2.9	467.6±34	−10.4±2.5	6.9×10^4	0.232
F3	92.5±3.1	543.0±41	−12.4±2.4	6.6×10^4	0.253
F4	90.2±3.4	635.1±26	−11.6±3.1	7.0×10^3	0.286

the proliposomal formulations.

Rheological behavior or viscosity is a critical parameter to consider for percutaneous application of proliposomal gels. The viscosity of proliposomal formulations increased with increased amounts of cholesterol in the formulations. The flow index and consistency index values of the formulations are shown in Table 2. The developed proliposomal gels were observed to be flowable liquid, translucent gel, creamy gel and compact solid with increasing amounts of cholesterol.

4. Differential Scanning Calorimeter

The LDH and cholesterol has shown sharp endothermic peaks at 165.0 °C and 148.42 °C, respectively (Fig. 2). The disappearance of the peak corresponding to the melting point of LDH in proliposomal formulation is obviously due to the transformation of crystalline form of the drug to amorphous form in PC and cholesterol mixture. Furthermore, the absence of the characteristic peak of cholesterol in proliposomal formulation indicates a change in the phase transition behavior of pure lipids and could be due to the formation of liquid crystalline state.

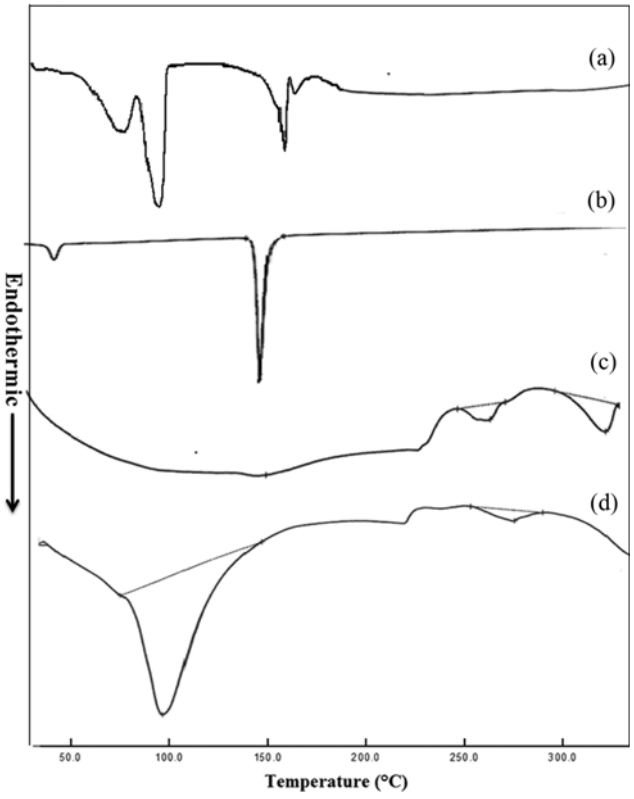

Fig. 2. DSC thermograms of (a) lisinopril dihydrate (b) cholesterol, (c) phosphatidylcholine and (d) proliposomal formulation (F3).

5. Fourier Transform Infrared Analysis

FTIR spectra (Fig. 3) of skin demonstrate relative peaks around 2,800-3,000 cm^{-1} in relation to C-H stretching vibrations from hydrophobic alkyl chains of cholesterol and fatty acids. The prominent peaks around 2,852 and 2,922 cm^{-1} highlighted CH_2 symmetric and asymmetric vibrations, respectively, originating primarily from extracellular lipids of stratum corneum. Both the peak height and area are proportional to the amount of lipids present, and any extraction of lipids from the stratum corneum results in decrease of peak height and area. The reduction in area of these peaks is symptomatic of fluidization of the stratum corneum.

6. *Ex Vivo* Permeation of Lisinopril Dihydrate from Proliposomes

The cumulative amount of LDH permeated from various proliposomal formulations and LDH powder (control) were revealed in Table 4 and in Fig. 4. The amount permeated was detected at the initial samplings of 0.5 h, which indicates the conversion of proliposomes to liposomal vesicles occurred immediately when the water present in the receptor compartment got in contact with the skin. Thus, the drug from the vesicles formed permeated across the skin. Better skin permeating ability of proliposomal gels was perhaps due to structural modification of stratum corneum due to the presence of lipids and ethanol in the proliposomal formulations. Intercellular lipid barrier in stratum corneum would be dramatically looser and more permeable following treatment with liposomes. The fusion of vesicles to skin can result in greater concentration gradients and thus improved drug permeability. The cumulative amount of drug permeated was in the order F0<F1<F4<F2<F3<Control as shown in Fig. 4. Among the formulations, F3 showed greater permeation and F4 showed decreased permeation. This may be due to optimum cholesterol and lecithin ratio (1 : 1 ratio) in F3 and due to the high cholesterol content in F4. The formulation with high cholesterol content showed lower permeation, which may be due to formation of a film of cholesterol around the vesicles, which increased the micro viscosity of the bilayers by getting intercalated into the bilayers and thus reducing the permeability of the drug through the film. As shown in Fig. 4, the drug permeation from the powder was higher than proliposomal formulations, suggesting sustained release of the LDH from the proliposomal formulation at constant rate. Hwang et al., 1997 [27] also prepared proliposomal formulation of nicotine and observed constant flux of the nicotine from the proliposomal formulation compared to the nicotine powder, indicating sustained delivery of the nicotine across the rat skin at constant rate from proliposomes.

7. Histological Examination of the Rat Skin

The photomicrographs of untreated or control rat skin show nor-

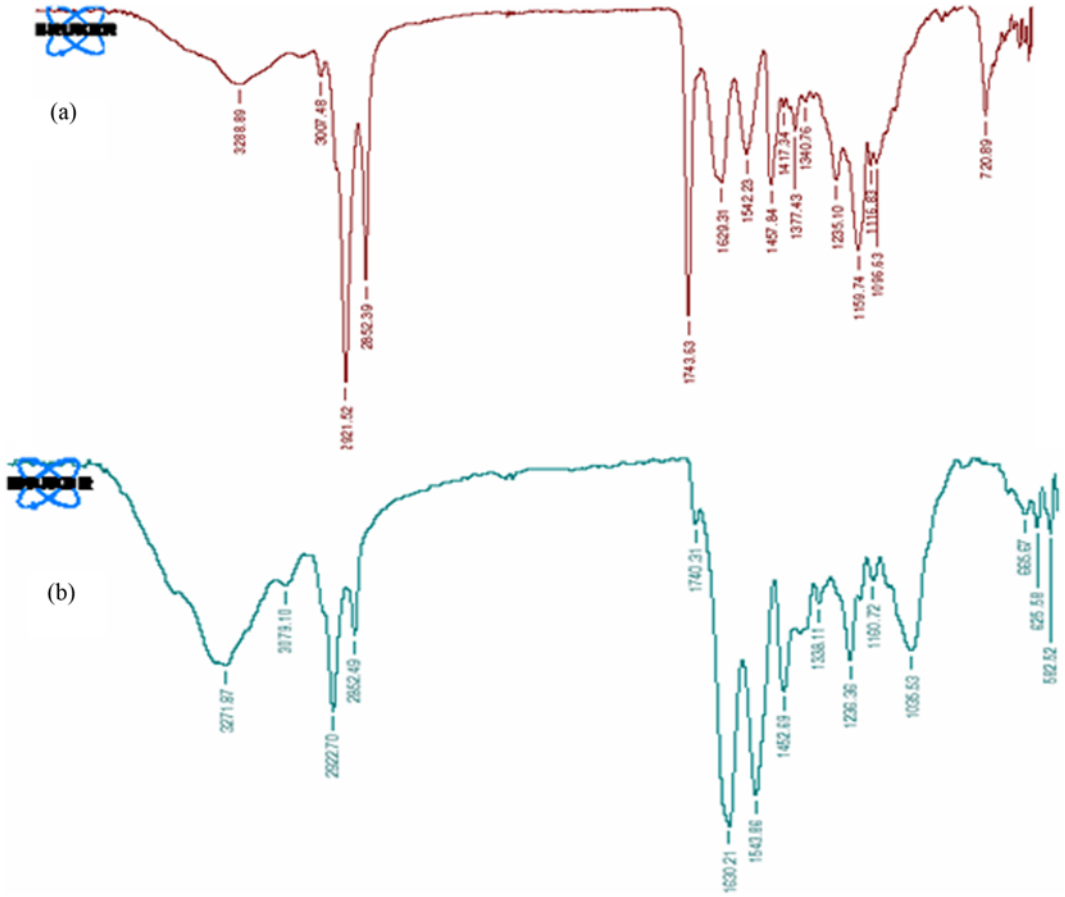

Fig. 3. FTIR spectra of (a) untreated skin (control) (b) skin treated with optimized proliposome formulation (F3).

Table 4. Permeation parameters of lisinopril dihydrate proliposomal formulations through rat skin (mean±SD; n=3)

Formulation code	J_{ss} (μg/cm^2/h)	K_p (cm/h)×10^{-3}
Control	72.31±2.83	14.46±0.59
F0	22.70±0.70	4.54±1.06
F1	25.20±1.48	5.04±0.98
F2	31.85±1.28	6.37±1.45
F3	44.20±1.64	8.84±1.14
F4	27.89±1.03	5.58±0.76

mal skin layers (Fig. 5(a)). After 24 h treatment of skin with proliposomal gel, definite changes were observed in the skin morphology (Fig. 5(b)) that could be attributed due to the effect of ethanol and phospholipids on stratum corneum. The dense structure of the stratum corneum was faintly altered by the application of proliposomal gel.

8. Skin Irritation Studies

The primary observations of skin irritation studies showed no obvious signs of erythema and oedema during 7 days, demonstrating that LDH proliposomal gel is nonirritant.

9. Stability Studies

The physical appearance and entrapment efficiency of the proliposomal formulations were evaluated over a period of six weeks. Formation of drug crystals was not observed at refrigeration and

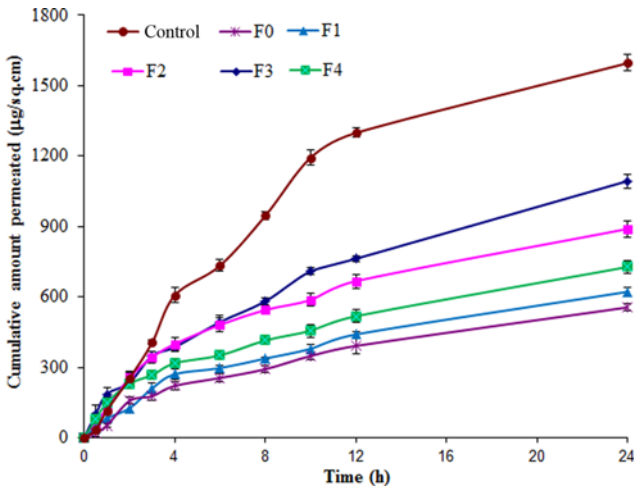

Fig. 4. *Ex vivo* skin permeation of lisinopril dihydrate across rat abdominal skin from proliposomal gel formulation and LDH powder (mean±SD; n=3).

room temperatures. The consistency of the gels was increased because of the loss of alcohol over storage. The microscopic studies revealed the formation of liposomes from proliposomal gel, but we could not notice any appreciable change in the morphology of liposomes formed. The encapsulation efficiency of proliposomes stored at room temperature decreased when compared to formulation stored

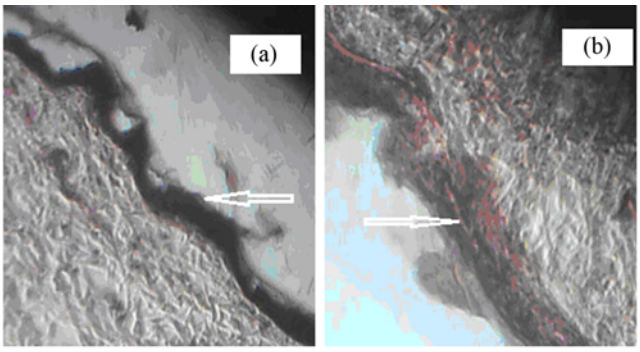

Fig. 5. Histological microphotographs (100× magnifications) of rat skin (a) control skin with intact skin layers without any swelling (b) treated skin showing swelling of the skin layers.

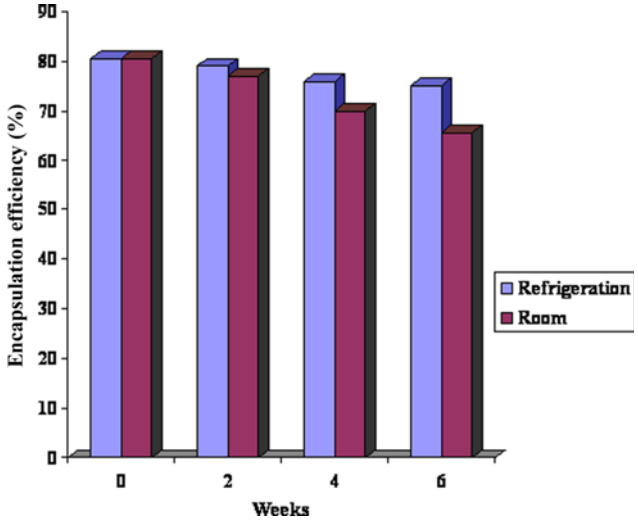

Fig. 6. Encapsulation efficiency of optimized proliposomal gel (F3) upon storage at refrigeration and room temperature.

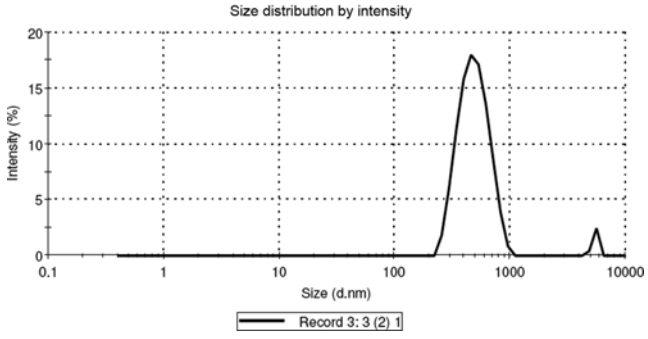

Fig. 7. Size distribution of liposomes formed from optimized proliposomal formulation (F3) upon hydration.

in refrigerator (Fig. 6). The amount of drug present within the vesicles under definite conditions ultimately governs the shelf life of the drug. We could not notice any appreciable change in vesicle size (565.1± 39 nm), polydispersity index (0.192) and zeta potential (−13.5±3.1 mv) of the liposomes obtained by hydrating the proliposomal gel formulation when stored at refrigerated conditions. However, the liposomes formed from the formulation stored at room temperature showed significant ($p<0.05$) increase in vesicle size (685.2± 41 nm) without any change in zeta potential. The data clearly indicates the influence of temperature on the stability of proliposomes. The formulations stored in a refrigerator at 4 °C were comparatively more stable than the formulations stored at room temperature, as not much leakage of the drug was found at these temperatures. Decreased stability of proliposomal gels stored at room temperature compared to refrigeration temperature (4 °C) might be due to more loss of ethanol at room temperature than at low temperature (4 °C).

CONCLUSION

Lisinopril dihydrate proliposomal formulations for transdermal delivery were successfully developed. Optical microscopic evaluation of proliposomes revealed the formation of spherical vesicles of liposomes from proliposomes upon hydration, which was further confirmed from transmission electron microscopy. The physicochemical evaluations of proliposomes clearly expose the significance of cholesterol and phosphatidyl choline composition in formulation. Optimized proliposomal formulation F3 (1 : 1 ratio of phasphatidyl choline and cholesterol) showed good physicochemical characteristics: entrapment efficiency (92.5±3.1) and higher permeation compared to other formulations. The stability studies accomplish that the proliposomal formulations are more stable at refrigeration conditions compared to room temperature. Further studies are suggested to prove the therapeutic efficacy of the formulations in humans.

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REFERENCES

1. S. G. Lancaster and P. A. Todd, *Drugs*, **35**, 646 (1988).
2. H. Marc, *Angiotensin converting enzyme inhibitors, angiotensin and calcium channel blockers*, In: A. D. Williams, L. L. Thamas Eds., *Foye's principles of medicinal chemistry*, 5th Ed., M. D. Baltimore: Lippincott Williams and Wilkins (2002).
3. A. Gurrapu, R. Jukanti, S. R. Bobbala, S. Kanuganti and J. B. Jeevana, *Adv. Powder Technol.*, **23**, 583 (2012).
4. D. Vaibhav, M. Dinesh and N. K. Jain, *Eur. J. Pharm. Biopharm.*, **67**, 398 (2007).
5. P. C. Calder, G. L. Jensen, B. V. Koletzko, P. Singer and G. J. Wanten, *Intensive Care Med.*, **36**, 735 (2010).
6. S. Tamilvanan and S. Benita, *Eur. J. Pharm. Biopharm.*, **58**, 357 (2004).
7. M. R. Prausnitz, S. Mitragotri and R. Langer, *Nat. Rev. Drug Discov.*, **3**, 115 (2004).
8. P. C. Wu, Y. B. Huang, J. J. F. Chang, J. S. Chang and Y. H. Tsai, *Int. J. Pharm.*, **209**, 87 (2000).
9. K. Rajesh, R. B. Gupta and G. V. Betageri, *Drug Deliv.*, **8**, 25 (2001).
10. N. I. Payne, P. Timmis, C. V. Ambrose and M. D. Warel, *J. Pharm. Sci.*, **75**, 325 (1986).

11. G. V. Betageri, US Patent, **6**, 249 (2005).
12. E. Touitou, H. E. Junginger, N. D. Weiner, T. Nagai and M. Mezei, *J. Pharm. Sci*., **83**, 1189 (1994).
13 H. Schreier and J. Bouwstra, *J. Control Release*., **30**, 1 (1994).
14. M. Mokhtar, O. A. Sammour, M. A. Hammada and N. A. Megrab, *Int. J. Pharm.*, **361**, 104 (2008).
15. N. Udupa, K. S. Chandraprakash, P. Umadevi and G. K. Pillai, *Drug Dev. Ind. Pharm.*, **19**, 1331 (1993).
16. A. Deepika and P. K. Indu, *Int. J. Pharm*., **290**, 155 (2005).
17. B. Vora, A. J. Khopade and N. K. Jain, *J. Control Release*, **54**, 149 (1998).
18. D. M. Sagar and B. P. Vandana, *Int. J. Pharm.*, **363**, 132 (2008).
19. K. Zhao and J. Singh, *J. Control Release*, **62**, 359 (1999).
20. T. N. Sunil and P. Ramesh, *J. Control Release*, **95**, 367 (2004).
21. A. F. Azarbayjani, E. H. En Hui Tan, Y. W. Chan and S. Y. Chan, *Biol. Pharm. Bull.*, **32**, 1453 (2009).
22. M. D. Joshi and V. B. Patravale, *Drug Dev. Ind. Pharm*., **32**, 911 (2006).
23. R. Jukanti, S. Sheela, S. Bandari and P. R. Veerareddy, *J. Pharm. Sci.*, **100**, 3208 (2011).
24. J. Varshosaz, A. Pardakbty and S. M. H. Baharanchi, *Drug Deliv.*, **12**, 75 (2005).
25. M. S. EI-Ridy, R. M. Khalil, D. M. Moustafa, M. Mohamed and A. S. Monem, *Drug Dev. Ind. Pharm.*, **23**, 771 (1997).
26. P. S. Hiremath, K. S. Soppimath and G. V. Betageri, *Int. J. Pharm.*, **380**, 96 (2009).
27. B. Y. Hwang, B. H. Jung, S. J. Chung, M. H. Lee and C. K. Shim, *J. Control Release*, **49**, 177 (1997).