

Development and Characterization of Tacrolimus Liposomal Gel for Industrial Application

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ABSTRACT

Objective: The objective of the present study was to prepare tacrolimus liposomal gel formulations using ethanolic injection technique for efficacious and cost-effective treatment of atopic dermatitis. **Methods:** The liposomes were prepared using ethanolic injection technique at the drug concentration of 0.1% w/v and optimized by varying the lipid component/ cholesterol ratio and by monitoring particle size, polydispersity and entrapment efficiency. The optimized composition was incorporated into 0.5% Carbopol Ultrez 10 gel for topical application. The developed liposomal gel was evaluated with respect to physicochemical parameters such as pH, viscosity, rheometry and spreadability. Stability study was performed at different temperatures (4°C, 25°C and 40°C) to evaluate the long-term stability. *In vitro* permeation of tacrolimus gel was studied using freshly excised rat skin on Franz diffusion cell. The therapeutic efficacy study was performed on allergic contact dermatitis model in rats. **Results:** Stable tacrolimus liposomal gel was successfully formulated using ethanolic injection technique. *In-vitro* permeation study indicated higher release of the drug (69.7%) as compared to free drug in hydroalcoholic solution (32.8%) and marketed ointment (63.7%). The formulation also showed shear thinning performance, which

is a required property of topical formulation. The therapeutic efficacy study in rats indicated that liposomal gel containing 0.1% tacrolimus exhibited better activity as compared to 0.1% marketed tacrolimus ointment.

Conclusion: The study indicated that tacrolimus can be effectively incorporated in liposomes by commercially viable rapid ethanolic injection method and can be more efficient for the treatment of atopic dermatitis.

Key words: Cost-effective, Ethanol injection, Industrially relevant, Phospholipids, Topical delivery.

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INTRODUCTION

The phospholipids used in topical liposomal compositions exhibit similarity with membranes in the epidermis. This enables higher extent of penetration into the epidermal barrier in comparison to other topical dosage forms like creams and ointments. The liposomal encapsulation may also result in sustained drug release of drug due to a slow clearance of drug from the epidermal layer. The sustained release of drug in turn may also minimize systemic absorption of the drug into blood which is not a desirable attribute as far as topical mode of action is concerned.^{1,2} The liposomal drug delivery by topical route has resulted in reduction in side-effects, increased effectiveness and a higher patient compliance.^{3,4}

Literature suggest that liposomal encapsulation of tacrolimus in topical formulation enhances drug penetration of skin.^{5,6} It is also more user friendly to patients in the gel form than the conventional ointment as it is non greasy and sticky when applied on skin.^{7,8} Because of these characteristics need of occlusive dressings may be avoided for tacrolimus to be more effective. The reported studies suggest that liposomal encapsulation will be less toxic than free tacrolimus. Tacrolimus is commercially available as a topical ointment, but the ointment has been reported with low and highly variable absorption and doesn't ensure adequate topical delivery of the drug into deeper skin layers.^{9,10} It is interesting to note that despite reported positive studies no liposomal product of tacrolimus emerged in the market for topical application so far. The high manufacturing cost associated with liposomal technology is a significant deterrent. There is an evident need of providing of cost effective and industrially relevant solutions.

Ethanol injection is one of the methods for production of liposomes. This technique offers several advantages, e.g., its reproducibility, fast implementation and simplicity. Another advantage of the technique is that it does not cause oxidative alterations or lipid degradation.¹¹⁻¹³ The present study was aimed at developing tacrolimus liposomal formulation using ethanol injection technique which would ultimately lead to affordable and better treatment to vast majority of patient population.

MATERIALS AND METHODS

Materials

Tacrolimus was a gift sample from Concord Biotech (Ahmedabad, India). Lipoid S-75 and Lipoid S PC-3 were gifted by Lipoid, Germany. Absolute Ethanol USP and Cholesterol of purity ($\geq 99\%$) were procured from Sigma-Aldrich (India). Carbopol Ultrez 10 NF Polymers was kindly gifted by Lubrizol, USA. 2,4-Dinitrofluorobenzene (DNFB) was purchased from Sisco Research Laboratories, India. The marketed ointment Topgraf, Tacrolimus ointment (0.1%w/w) was from GlaxoSmithKline, India. Acetonitrile and Methanol were purchased from Merck Specialties Pvt. Ltd. (India). All the other reagents and ingredients were of analytical grade.

Preparation and optimization of tacrolimus loaded liposomes

Liposomes were prepared by rapid ethanol injection method.¹³ Various product-influencing variables viz. saturated vs unsaturated lipid, Drug

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Table 1: Formulation code and corresponding lipid composition of alcoholic lipid concentrate containing 1% w/v tacrolimus.

Code	Lipoid S-75 (% w/v)	Lipoid S PC-3 (%w/v)	Cholesterol (% w/v)	Absolute Alcohol
J1	0	7	0	q.s.
J2	0	5	2	q.s.
J3	10	0	0	q.s.
J4	5	0	0	q.s.
J5	5	0	2	q.s.
J6	5	0	1	q.s.

to lipid ratio, Drug to cholesterol ratio, were studied in order to produce liposomes of tacrolimus with desired attributes. The ratio of Lipoid S-75 (PC), Lipoid S PC- 3 (DSPC) and Cholesterol (CHOL) was varied as summarized Table 1. Three main criteria were targeted at optimization stage namely size (120 – 150 nm), polydispersity (<0.3) and entrapment efficiency (>98%). Briefly, Lipid component (Lipoid S-75 or Lipoid S PC-3 and/or Cholesterol) and tacrolimus were dissolved in absolute ethanol (40-60%). The liposomes were prepared by rapid injection of alcoholic drug lipid concentrate in purified water (Milli Q) in volume ratio of 1:9 through 29G needle.

The concentration of drug in lipid concentrate was kept at 1% w/v in all formulations. The data represents the varying concentration of Lipoid S-75, Lipoid S PC-3 and Cholesterol in corresponding alcoholic lipid concentrates. q.s.: quantity sufficient

The prepared batches of liposomes (0.1% w/v tacrolimus) were evaluated for targeted values of size, polydispersity and entrapment efficiency. The optimized composition was further characterized for charge (zeta potential) on the liposomal vesicles and size by transmission electron microscopy (TEM).

Characterization of the tacrolimus liposomes

Size distribution profile

Size distribution profile and polydispersity of various liposomal compositions were determined by dynamic light scattering (DLS) employing Malvern Zetasizer nano ZS, Malvern, UK with a scattering angle of 90°C at 25°C.

Drug entrapment efficiency

The entrapment efficiency (EE) of liposomes was determined by an ultracentrifugation method. First, Amicon ultra centrifugation filters (Amicon ultra, 0.5ML 10K, Merck, USA) were filled with 500uL samples of tacrolimus liposomes. After centrifugation at 10,000 rpm for 20 min in a micro centrifuge (TarsonMC-1, Spinwin), the ultra filtrate was injected into a high performance liquid chromatography (HPLC) system for free drug quantification. The EE of the vesicles was then calculated, based on the following formula (Equation 1).

$$\text{Entrapment efficiency (\%)} = [(TD - FD)/TD] \times 100 \quad (1)$$

Where TD is the theoretical amount of tacrolimus that is added and FD is the amount of drug determined in the filtrate. The results are expressed as the mean and standard deviation of three independent measurements.

Tacrolimus formulation samples were quantified by an in-house HPLC method (EE and stability studies). The HPLC apparatus consisted of an Agilent 1260 Infinity II system equipped with diode array detector. Isocratic separation was performed by narrow bore chromatography using a Thermo Accucore C₁₈ column (4.6*50 mm, 2.6 mm particle size). The mobile phase comprised acetonitrile/phosphate buffer (KH₂PO₄,

20mM, pH3.5 (35:65, V/V). The flow rate and injection volume used were 0.5 mL min⁻¹ and 5 µL, respectively. The detection wavelength was set at 210 nm and the column temperature was maintained at 70°C. Under these conditions, tacrolimus was eluted at 3.42 min. The method was validated with respect to linearity, specificity, precision, intermediate precision, accuracy and solution stability as per International council for harmonization guidelines (ICH). The method was linear in the concentration range 3.12 to 100 µg/ml in diluent methanol and the lower limit of detection was 1 µg/ml.

Charge

The charge or zeta potential of the optimized liposomal composition was determined by measuring electrophoretic mobility of dispersion using electrophoretic light scattering. This was measured using Malvern Zetasizer nano ZS, Malvern, UK.

Transmission electron microscopy

Transmission electron microscope with an accelerating voltage of 300 kV, was used as a visualizing aid for liposomal vesicles (Make: FEI, Model: Tecnai G2, F30, Philips, Holland). Samples were negatively stained on a carbon-coated copper grid with a 1% aqueous solution of phosphotungstic acid. The grid was then mounted in the instrument and images were observed at different magnifications.

Preparation of tacrolimus liposomal gel

Tacrolimus liposomal gels (0.1% w/v) and corresponding placebo gel containing combination of Lipoid S-75 and Cholesterol were prepared utilizing Carbopol Ultrez 10 NF polymer and optimized liposomal dispersion (J5 and J6). Briefly, 1000 mg of Carbopol Ultrez 10 NF polymer was sprinkled gently to 200 ml of liposomal dispersion having tacrolimus concentration of 0.1% w/v with stirring at 500 rpm for 1 hrs. The hydrated polymer dispersion was neutralized with 18% NaOH solution to a pH of 6.0.

Characterization of the tacrolimus liposomal gel

Rheology study

Rheological behaviour was measured by a modular compact rheometer (MCR: P-PTD200, Anton Paar, Germany) at 25°C. Composition J6 was evaluated under shear rate sweep analysis ranged from 0.1 to 100 (1/s) with a data acquisition time varying in logarithmic scale from 10 sec to 1 sec at 25°C. In order to evaluate the shear rate (1/s) and viscosity as a function of shear stress (Pa), rheogram was processed for the best fit using Ostwald model (Equation 2).¹⁴

$$\tau = K\dot{\gamma}^n \quad (2)$$

where τ is the shear stress, $\dot{\gamma}$ is the shear rate, n is the index of flow and K is the index of consistency.

Spreadability study

It was evaluated for liposomal gel (J6) using the modified parallel plate method with known weights.^{14,15} Briefly, 600 mg sample was introduced into a central hole (1cm) of a mold glass plate. The mold plate was removed carefully and the sample was pressed with a circular disk of known weight (6g). The spreading area was plotted against added weights to obtain the spreading profiles for liposomal gel. The spreadability factor (S_f) was calculated using equation 3.

$$S_f = A/W \quad (3)$$

Where S_f (cm²/g) denotes the spreadability factor calculated by determining the ratio between (A) the maximum spread area (cm²) after the addition of the sequence of weights used in the experiment and (W) the total weight added (g).

Storage-stability studies

The stability of liposomal gel formulation was assessed by keeping the liposomal suspensions at three different temperature conditions, i.e. 2-8°C (Refrigerator; control), 25±2°/60% RH (Room temperature; RT) and 40±2°C/75%RH (Accelerated conditions) for a period of 3 Months. The liposomal suspensions were kept in sealed laminated aluminum tubes (10 ml capacity) after flushing with nitrogen. Samples were withdrawn periodically and analyzed for drug content, in the manner described under drug entrapment studies. The samples were also evaluated for change in description, pH and single shear point viscosity (Brookfield DV2T viscometer, 10 rpm, spindle F96) at specified time intervals of 0, 30, 60 and 90 days.

In-vitro skin permeation study

Comparative skin permeation studies with tacrolimus containing formulations (Liposomal gel J5, J6) and Marketed (0.1%) ointment were carried out using freshly excised hairless abdominal skin of Wistar rats on Franz-diffusion cells apparatus (Electrolab EDC-07 model). The results obtained were compared with that of non-liposomal formulation of tacrolimus in hydroalcoholic solution (50% v/v). Briefly, the excised skin was mounted on the receptor chamber with diameter of 1.5cm and cross-sectional area of 1.7cm² exposed to the receptor compartment. The receptor media was comprising of 12ml of distilled water: absolute ethanol (75:25v/v) and the temperature was maintained at 35±2°C.¹⁴ Liposomal or non-liposomal tacrolimus formulation were applied evenly in donor compartment. Aliquots of 400µL were withdrawn periodically at the specified time for the duration of 24 hrs. The amount of tacrolimus permeated was quantified by HPLC analysis.

In vivo evaluation of liposomal formulation in allergic contact dermatitis model

In this study the test formulation incorporated into 0.5% carbopol was compared with the reference product (marketed 0.1% tacrolimus ointment) in allergic contact dermatitis (ACD) model in Wistar rats.^{7,15} The experimental protocol for animal studies was approved by the Animal Ethical Committee of Y.B. Chavan College of Pharmacy.

Three groups of six animals were taken - one was used for control the second group was used for test and third one was used to study the activity of the reference product. To each group of control, test and reference was applied a blank carbopol gel (0.5% carbopol), tacrolimus liposomal gel (0.1%) and tacrolimus ointment (0.1%) respectively on both ear pinna for seven days. The rats from each group were sensitized onto the back with 2, 4-dinitro-1-fluorobenzene (DNFB) dissolved in olive oil and acetone (1:4) on the fifth day. The animals from each group were subsequently challenged on the seventh day with DNFB on the pinna. The resultant thickness of ear pinna before and after 24 hrs. of challenge with DNFB was measured using Digital External Micrometer (KT5-230-447, Kenta Technologies, Singapore).

RESULTS

Characterization of the tacrolimus liposomes

The results of optimization trials are summarized in Table 2. In trial J1 and J2 the liposomes were prepared with DSPC fell short of targeted criteria. In J3 and J4 trials unsaturated lipid with low phase transition temperature i.e. PC was used in place of DSPC. No significant improvement was observed in the targeted attributes. The compositions were further modified with inclusion of cholesterol in the lipid concentrate containing PC (J5, J6). The best results were observed with optimized composition (J6) which showed size of 140.9 nm, polydispersity of 0.14 and entrapment efficiency of 99.3%.

Table 2: Size, polydispersity and drug loading evaluation of tacrolimus liposomal gel.

Formulation code	TAC: PC: DSPC: CHOL (weight ratio in mg)	Liposomal size (nm)*	Polydispersity Index*	Entrapment efficiency (%)*
J1	10:0:100:0	195.0 + 2.75	0.60 ± 0.03	92.2 ± 0.39
J2	10:0:50:0	178.0 + 0.69	0.24 ± 0.02	93.9 ± 0.15
J3	10:100:0:0	199.7 + 1.07	0.40 ± 0.02	95.4 ± 0.75
J4	10:50:0:0	130.1 + 2.80	0.39 ± 0.06	97.9 ± 0.40
J5	10:50:0:10	143.0 + 0.79	0.27 ± 0.00	99.1 ± 0.90
J6	10:50:0:20	140.9 + 0.82	0.14 ± 0.01	99.3 ± 0.04

Formulations J1 to J6 with varying lipid compositions were evaluate for liposomal size, Polydispersity and entrapment efficiency. TAC: Tacrolimus, PC: Lipoid S-75, DSPC: Lipoid S PC- 3, CHOL: Cholesterol

*Data represent mean ± SD, n = 3

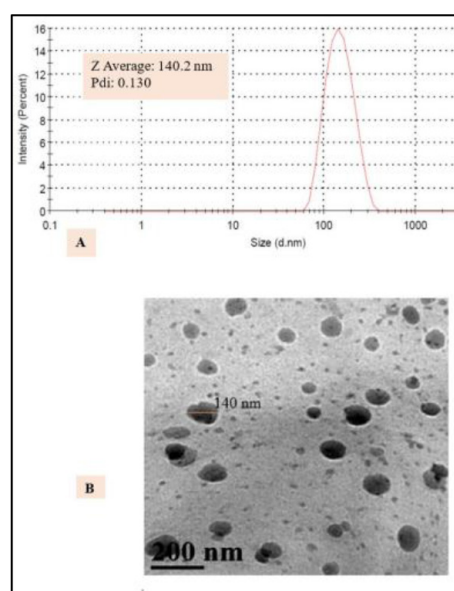


Figure 1: Size characterization of optimized liposomal composition J6. (A) The typical size distribution profile depicted one narrow peak and the polydispersity index (PDI) below 0.2 (B) TEM indicated the particle size of liposomes in the range of approximately 120-160 nm and spherical morphology.

The liposomal formulation J6 was additionally subjected to charge and TEM evaluation. The charge observed on the vesicles was found to be negative i.e. -39.1 mv. The overall size evaluation data by Malvern zetasizer and transmission electron microscopy is shown in Figure 1.

Characterization of the Tacrolimus Liposomal Gel

Rheological evaluation

Tacrolimus liposomal gel (J6) was subjected to rheological evaluation. The rheogram obtained by plotting shear rate $\dot{\gamma}$ [1/s] versus viscosity η [Pas] revealed no significant hysteresis effects. Under the considered experimental conditions, the up curve and down curve practically coincided. The gel began to flow after a shearing stress, corresponding to the yield value, after which the viscosity decreased with increasing rate of shear (Figure 2A). The value of n was found to be 0.258 which confirmed the shear thinning property of gel.

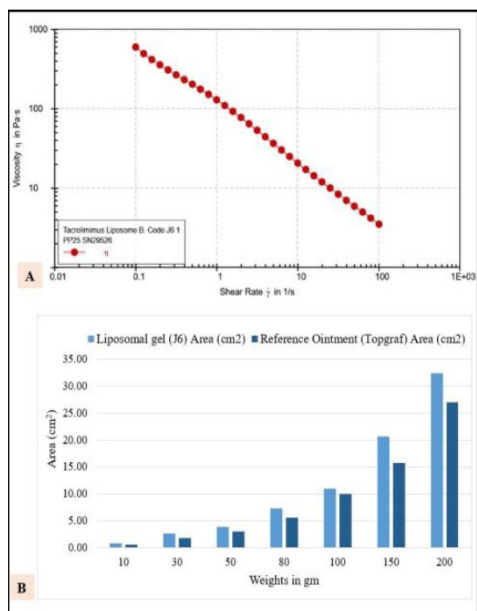


Figure 2: Flow characteristic evaluation of optimized Liposomal gel J6. (A) Rheological study depicting graph of shear rate D [1/s] versus viscosity η [Pa.s], (B) Spreadability evaluation of liposomal gel against marketed ointment formulation depicting spreading area with added weights.

Spreadability evaluation

Comparison of the spreading area of liposomal gel (J6) with marketed tacrolimus ointment with the increments of weight is presented in Figure 2B. The spreading area of liposomal gel is higher than ointment due to shear thinning behaviour and favorable spreading characteristics of Carbopol based system. The calculated spreadability factors for liposomal gel and marketed ointment were 0.14 and 0.11 cm²/g respectively, indicating better spreading behaviour of gel.

Stability Study

The liposomal gel subjected to specified stability conditions were evaluated with respect to description, assay, pH and viscosity (Table 3). The assay values for the samples stored at 25°C/60% RH were found to be in the range between 99.7% and 99.1% and compared to the initial value of 101.0% the observed differences were not statistically significant. Similar was the case with pH and viscosity values wherein no significant change in data trend was observed with time.

In-vitro skin permeation study

The *in vitro* permeation studies were performed for a period of 24 hrs duration as the formulations are expected to retain on skin for 24 hrs. A comparative permeation profile is shown in Figure 3. The liposomal gel formulation showed better extent of drug permeation (69.69%) in 24 hrs. as compared to marketed ointment and Batch J5 which showed slightly lesser permeation of 63.53% and 60.07% respectively. The hydroalcoholic solution devoid of any phospholipidic carrier served the purpose of control. It exhibited the least extent of drug permeation (32.80%). The mean flux value across rat skin was calculated for each formulation. As shown in Table 4, the obtained flux values were higher and comparable in case of tacrolimus liposomes J6 and commercial ointment i.e. 25.15 µg/hr/cm² and 26.56 µg/hr/cm² respectively.

In-vivo pharmacodynamic evaluation

Figure 4 presents the ear thickness profile in left and right ear of wistar rats after application of blank gel, 0.1% liposomal gel and marketed 0.1% ointment in control, test and reference group respectively. No significant

Table 3: Stability data of Optimized Liposomal Gel (Batch code: J6) as per ICH guidelines showing results of description, Assay, pH and viscosity at specified storage conditions.

Storage Condition	Test Parameters			
	Description	Assay (%)*	pH	Viscosity (Pa.S)*
Initial	Translucent gel	101.0 ± 0.87	6.2	68.2 ± 2.13
1M/ 2-8 °C	Translucent gel	99.3 ± 1.12	6.1	68.8 ± 3.23
1M/ 25 °C/60% RH	Translucent gel	99.7 ± 0.86	6.5	72.3 ± 2.12
1M/ 40 °C/75% RH	Translucent gel	99.0 ± 0.98	6.3	70.9 ± 1.13
2M/ 2-8 °C	Translucent gel	99.0 ± 0.76	6.2	67.4 ± 2.01
2M/ 25 °C/60% RH	Translucent gel	99.4 ± 1.13	6.5	68.3 ± 0.89
2M/ 40 °C/75% RH	Translucent gel	98.8 ± 0.56	6.3	74.5 ± 2.02
3M/ 2-8 °C	Translucent gel	98.5 ± 0.45	6.4	70.2 ± 1.11
3M/ 25 °C/60% RH	Translucent gel	99.1 ± 1.12	6.2	73.4 ± 1.76
3M/ 40 °C/75% RH	Translucent gel	97.9 ± 0.78	6.4	69.5 ± 1.54

M: month, RH: relative humidity,

*Data represent mean ± standard deviation, n = 3

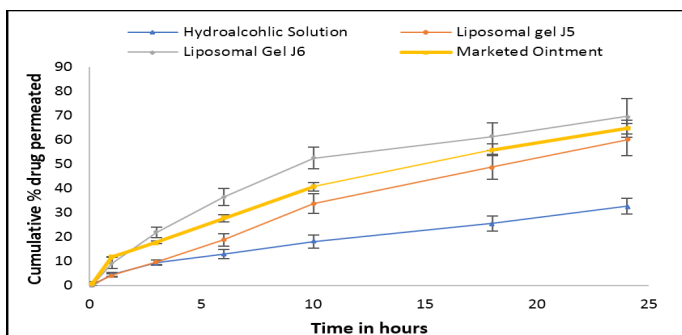


Figure 3: Comparative permeation profile of Tacrolimus containing compositions (Hydroalcoholic solution, liposomal gel J5, liposomal gel J6 and marketed ointment) across rat skin. The y-axis represents % drug permeated and the x-axis denotes times in hours. Data represent mean ± standard deviation, n = 3.

Table 4: Comparative summary of mean cumulative percentage drug permeated and permeation flux for different formulations across rat skin.

Tacrolimus Formulation	Mean cumulative drug permeated in 24 hr (%)*	Permeation flux (µg/cm ² /hr)*
Hydroalcoholic solution	32.80 ± 3.24	10.36 ± 0.23
Liposomal gel (batch code J5)	60.07 ± 6.59	16.21 ± 1.23
Liposomal gel (batch code J6)	69.69 ± 7.24	25.15 ± 2.01
Marketed ointment (Topgraf)	64.63 ± 4.71	26.56 ± 1.57

* Data represents mean ± standard deviation (n = 3) values for hydroalcoholic solution, liposomal gel J5, liposomal gel J6 and marketed ointment

difference in thickness of ear pinna was found before the application of DNFB on ear pinna to 3 groups. After the application of DNFB in 3 said groups, significant decrease in ear thickness in test group was found as compared with control group. The test formulation showed slightly better performance in reducing the ear thickness than reference formulation. The result obtained shows that 0.1% tacrolimus liposome

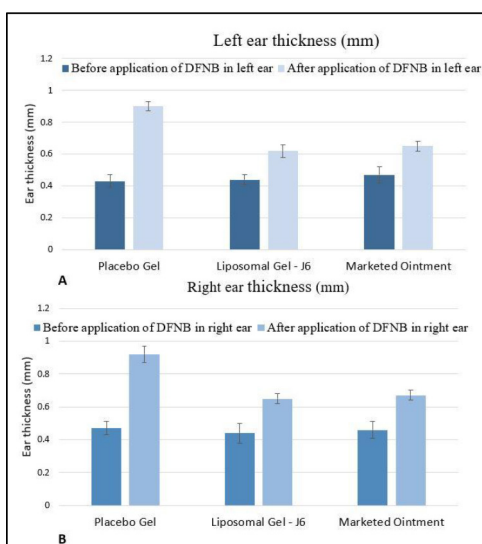


Figure 4: The evaluation of ear thickness in left ear (A) and right ear (B) after application of placebo gel, liposomal gel (Batch code J6) and marketed ointment. Data represent mean \pm standard deviation, $n = 6$. The statistical significance of differences were analyzed utilizing analysis of variance with Bonferroni test. Differences were considered statistically significant at $P < 0.05$.

was effective in ACD model in rats with slightly better efficacy compared to 0.1% marketed ointment.

DISCUSSION

Tacrolimus has lipophilic properties and it is highly challenging to develop a suitable carrier for its dermal administration. The delivery systems such as ointments show only limited liberation of the drug into the epidermal and dermal skin layers. Utilization of a liposomal gel-based carrier for tacrolimus with desired resemblance with skin lipids and enhanced penetrative properties of alcohol would be predicted to increase the bioavailability of the drug in the skin.^{16,17}

In the present study liposomal gel-based delivery system was developed for tacrolimus using industrially relevant ethanol injection method. One of the key features of the technology is that it doesn't require mechanical force to generate homogeneous and narrow distributed liposomes.^{18,19} So, it provides an edge over the other technologies like the thin film hydration method which is normally followed by size reduction through extrusion, high pressure homogenization or ultrasonication.^{7,8} The technique enables liposome preparation regardless of production scale. Liposome size can be controlled by the process parameters like injection pressure, injection rate, temperature and lipid concentration. The technique conveniently enables an aseptic production process in typical topical plant setup as all raw materials can be transferred into the sterilized/sanitized vessel system via 0.2 μm membrane filters.

Lipoid S-75 was tactically used as the core lipid in the formulation because of two reasons. One, it is approved for topical application which is partially purified and comprises of about 70% phosphatidyl choline. This makes it cost effective alternative against costlier purified phosphatidyl choline like Lipoid S-100 and HSPC. Second, it contains about 8% of phosphatidyl ethanolamine which facilitated attaining negative charge on the optimized lipidic vesicles (J6). The charge contributed in achieving unilamellar vesicles of smaller size and narrow polydispersity.

Vesicle size is important for topical route of drug delivery. The liposomal vesicles smaller than 300 nm are known to deliver their contents into deeper layers of the skin.^{20,21} In our study, the average diameters of lipo-

somes were below 150nm. This suggested that the optimized liposomes had potential for delivery of tacrolimus through the skin. The size and polydispersity of the liposome was also found to be dependent on lipid-drug ratio. This is evident by the fact that batch J1 and J3 both having comparatively higher lipid content yielded bigger liposomal size of 195 nm and 199.7 nm respectively. These were also found to be more polydisperse as compared to other batches with low lipid content i.e. drug to lipid ratio of 10:50. It was also observed that decreasing the lipid content (J4, J5, J6) didn't show any adverse effect on EE. Additionally, the lipids displayed higher particle size with higher phase transition temperature which is in line with studies reported in literature.¹² As expected among different core lipids, Lipoid S-75 resulted in smallest particle size of liposomes so it was selected for further optimization. CHOL is a well-known component of liposomal systems that helps to stabilize the bilayer lipidic vesicles by virtue of its cementing effect. Considerable enhancement in the polydispersity and encapsulation efficiency was observed with presence of CHOL in batch J5 and J6 which is consistent with previous report.⁷ Increase in the amount of CHOL from 10mg to 20mg resulted in best results in composition J6.

The optimized gel formulation comprising of Carbopol® Ultrez™ 10 indicated non-newtonian system with pseudoplastic flow behavior, which is preferred for dermatological dosage form because the formulation flow resistance should be lower when applied under medium to high shear conditions.²² The stability data showed that the observed differences in assay, pH and viscosity were not significant against initial value. This suggest good stability of the developed gel at the recommended real time storage condition of 25°C/60% RH for at least one year.

In Atopic dermatitis a thickened skin barrier is formed due to plaque formation and keratinocyte hyper-proliferation resulting in a bigger blockade to penetration of drugs.¹⁰ An improved delivery of tacrolimus to the target site is necessary in such cases to allow for successful therapy. The drug permeation data obtained in the present study (Table 4) reveal that the penetrative behavior of tacrolimus was enhanced using liposomal systems and that the rat skin barrier was permeated to a greater degree compared with the commercial ointment preparation. The higher amount of tacrolimus permeation is consistent with the greater inhibition of DNFB effects, compared with commercial ointments.

CONCLUSION

Tacrolimus could be successfully entrapped in the liposomes by cost effective and commercially viable ethanol injection methodology. The optimized liposomal formulation showed good drug loading and desired vesicle specific characters. The developed liposomal gel system displayed more effective permeation in the epidermis compared with aqueous drug system and showed a superior *in vivo* inhibitory action on mouse ear swelling induced by DNFB to a commercial ointment. Higher extent of drug permeation across the rat skin with liposomal tacrolimus indicates that the drug in its phospholipid-based carrier has gained facilitated entry into the stratum corneum which is otherwise a tough barrier. The non-greasy feel and better spreadability of the formulation would render it more user friendly. From these *in vitro* and *in vivo* studies, it can be concluded that the developed lipogel system may be useful as a more efficacious and cost-effective therapeutic agent for atopic dermatitis. The findings from this study can be further explored to substantiate the clinical / commercial potential of the delivery system.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AD: Atopic dermatitis; **USP:** United states pharmacopeia; **NF:** National formulary; **USA:** United states of America; **UK:** United kingdom; **DNFB:** 2,4-Dinitrofluorobenzene; **HPLC:** High performance liquid chromatography; **PC:** Lipoid S-75; **DSPC:** Lipoid S PC- 3; **CHO:** Cholesterol; **TEM:** Transmission electron microscopy; **DLS:** dynamic light scattering; **EE:** Entrapment efficiency; **ICH:** International council for harmonisation; **RT:** Room temperature; **RH:** Relative humidity; **ACD:** Allergic contact dermatitis; **DNFB:** 2, 4-dinitro-1-fluorobenzene; **DSPC:** Hydrogenated phospholipid; **PDI:** Polydispersity index; **HSPC:** Hydrogenated soybean phosphatidylcholine.

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