RESEARCH ARTICLE

Liposomal hydrogel formulation for transdermal delivery of pirfenidone

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Abstract

Context: Pirfenidone (PFD) is an anti-fibrotic and anti-inflammatory agent indicated for the treatment of idiopathic pulmonary fibrosis (IPF). The current oral administration of PFD has several limitations including first pass metabolism and gastrointestinal irritation.

Objective: The aim of this study is to investigate the feasibility of transdermal delivery of PFD using liposomal carrier system.

Materials and methods: PFD-loaded liposomes were prepared using soy phosphatidylcholine (SPC) and sodium cholate (SC). Encapsulation efficiency (EE) of PFD in liposomes was optimized using different preparation techniques including thin film hydration (TFH) method, direct injection method (DIM) and drug encapsulation using freeze–thaw cycles. In vitro drug release study was performed using dialysis membrane method. The skin permeation studies were performed using excised porcine ear skin model in a Franz diffusion cell apparatus.

Results and discussion: The average particle size and zeta-potential of liposomes were 191 ± 4.1 nm and -40.4 ± 4.5 mV, respectively. The liposomes prepared by TFH followed by 10 freeze–thaw cycles showed the greatest EE of 22.7 ± 0.63%. The optimized liposome formulation was incorporated in hydroxypropyl methyl cellulose (HPMC) hydrogel containing different permeation enhancers including oleic acid (OA), isopropyl myristate (IPM) and propylene glycol (PG). PFD-loaded liposomes incorporated in hydrogel containing OA and IPM showed the greatest flux of 10.9 ± 1.04 µg/cm²/h across skin, which was 5-fold greater compared with free PFD. The cumulative amount of PFD permeated was 344 ± 28.8 µg/cm² with a lag time of 2.3 ± 1.3 h.

Conclusion: The hydrogel formulation containing PFD-loaded liposomes can be developed as a potential transdermal delivery system.

Keywords

Encapsulation efficiency, HPMC hydrogel, liposome, pirfenidone, skin permeation

History

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Introduction

Pirfenidone (5-methyl-1-phenylpyridin-2[H]-one, PFD) is a new pyridone derivative having anti-fibrotic and anti-inflammatory activity. PFD shows a wide range of therapeutic applications and has been evaluated for its safety and efficacy in multiple disorders including idiopathic pulmonary fibrosis (IPF), myelofibrosis, multiple sclerosis, neurofibromatosis, chronic hepatitis C, hypertrophic cardiomyopathy, fibrotic renal disorders and fibrosis caused by cancer radiation therapy (Barragán et al., 2010; Cho & Kopp, 2010).

The therapeutic functions of PFD are attributed to its efficacy in reducing fibroblast proliferation, decreased secretion of the fibrosis-associated cytokines, decreased biosynthesis of extracellular matrix and decreased accumulation of inflammatory cells (Cottin, 2013). PFD has been found to down regulate tumor necrosis factor-α (TNF-α) expression and transforming growth factor-β (TGF-β) (Jung et al., 2012). PFD has been approved for the treatment of IPF in European Union (2011), Canada (2012) and the United States (2014) (Duck et al., 2015).

The marketed formulations of PFD are available as solid oral dosage forms. The PFD is administered three times a day for a longer time to control chronic disease, such as IPF (Bruss et al., 2004). The clinical trial studies revealed several undesirable effects with oral administration of PFD including cough, head ache, diarrhea, fever, abnormality of hepatic function, dizziness and facial paralysis (King et al., 2014; Taylor & Leppert, 2012). Furthermore, PFD undergoes hepatic first-pass metabolism after oral administration (Arai et al., 2014). PFD has been reported to be metabolized by cytochrome P450 isoforms of CYP1A2, 2C9, 2C19, 2D6 and 2E1 (Arai et al., 2014).

Transdermal delivery of active molecules is a potential alternative to minimize the undesirable effects associated with oral administration. Transdermal drug delivery involves permeation of active molecules across the skin into the systemic circulation. This would avoid the irritation to gastrointestinal tract, hepatic first-pass metabolism and improvement in patient compliance (Alexander et al.,...
2012). However, drug permeation through skin is limited by its formidable barrier property. This barrier property of skin is mainly attributed to the outermost layer of skin, stratum corneum (SC) (Bouwstra & Ponec, 2006).

To improve the skin permeation of active molecules which are otherwise impermeable through skin, various permeation enhancement techniques have been investigated. These approaches are generally classified into chemical permeation enhancers and physical permeation enhancers. Some of the widely used chemical permeation enhancers include fatty acids, terpenes and solvents, such as alcohols (Chen et al., 2014). Physical enhancement strategies include iontophoresis, electroporation, ultrasound, skin ablation using heat or laser and microneedles (Maghraby et al., 2006). Chemical permeation enhancers are incorporated into a formulation where they can improve the solubility and diffusivity of drugs through the skin by reducing the barrier property of skin (Alexander et al., 2012).

More recently, nanoparticle-based carrier systems have shown potential in transdermal delivery of drugs (Venuganti & Perumal, 2009). These carriers include liposomes, micelles, polymeric nanoparticles, lipid nanoparticles and metal nanoparticles. Of these, liposomal systems are the most widely characterized carriers with multiple clinically approved products (Allen & Cullis, 2013). Although, liposomes act as an efficient drug delivery system, the encapsulation of water soluble molecules, such as PFD, in liposomes presents a unique challenge during formulation design and processing. The high aqueous solubility of hydrophilic drugs allows them to be dissolved in the external aqueous phase during lipid film hydration, and become entrapped in the aqueous compartment of liposomes (Xu et al., 2012). The proportion of the aqueous medium getting entrapped in liposomal vesicles will be very less in comparison with the total bulk medium, leading to lower levels of encapsulation efficiency (EE).

The objective of this study was to investigate the feasibility of transdermal delivery of PFD using liposomes. Different preparation techniques were investigated to optimize liposomal formulations with maximum PFD encapsulation. The optimized liposome formulation was incorporated into hydroxypropyl methyl cellulose (HPMC) hydrogels for their efficient topical administration. To further improve the transdermal permeability of PFD, chemical permeation enhancers including isopropyl myristate (IPM), oleic acid (OA) and propylene glycol (PG) have been studied.

Materials and methods

Materials

PFD, soy phosphatidylcholine (SPC), sodium cholate, Triton-X100, methanol, chloroform, sodium chloride, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, potassium chloride, hydroxypropyl methyl cellulose (HPMC –average Mw: 90,000), OA, PG, IPM, HPLC grade acetonitrile and ammonium acetate were purchased from Sigma-Aldrich Chemical Company (Bangalore, India). All the chemicals were of analytical grade and used without further purification. Milli-Q (Millipore) water with 18.2 MΩ cm resistivity was used for all the experiments.

Preparation of liposomes

Liposomes were prepared using SPC and sodium cholate at 86:14% w/w ratio. Different preparation methods were studied to achieve maximum PFD EE. These include thin film hydration (TFH) method, direct injection method (DIM) and TFH followed by freeze–thaw cycles. Figure 1 shows the schematic for encapsulation of PFD using three methods.

In TFH method, SPC and sodium cholate (86:14% w/w) were dissolved in chloroform:methanol (2:1 v/v) in a round bottom flask. The organic solvent was evaporated using a rotary evaporator at 45 ± 1°C and 80 rpm under reduced pressure. The deposited lipid film was hydrated with PFD solution (5 mg/mL in deionized water) to obtain a final lipid composition of 10 mg/mL. The lipid vesicles were allowed to swell for 2 h at room temperature with intermittent vortexing followed by bath sonication for 3 min. This vesicle dispersion was extruded through a polycarbonate membrane (100 nm pore size) for 15 times.

The second method involved preparation of blank liposomes by TFH followed by drug encapsulation using freeze–thaw cycles. Blank liposomes were prepared using SPC and sodium cholate (86:14 w/w) by TFH method as described above. After evaporation of organic solvents, the lipid film was hydrated using deionized water to obtain liposomal dispersion with 20 mg/mL lipid concentration. These vesicles were allowed to swell for 2 h at room temperature with intermittent vortexing. The liposomes were then bath sonicated for 3 min followed by manual extrusion through polycarbonate membranes. This blank liposome (1 mL) sample was mixed with equal volume of PFD solution (5 mg/mL in deionized water) and vortexed for 3 min. The mixture was then frozen at −80°C for 5 min followed by thawing the sample in water bath maintained at 45°C for 10 min. These cycles were repeated for 5–10 times. Later the samples were extruded through a polycarbonate membrane (100 nm pore size) for 15 times.

In DIM, SPC and sodium cholate (86:14% w/w) were dissolved in chloroform:methanol (2:1% v/v). A weighed quantity of PFD was added to this lipid solution and dissolved in chloroform:methanol (2:1% v/v). The deposited lipid film was hydrated using deionized water under magnetic stirring to obtain liposomal dispersion. The organic solvent was evaporated using rotavapor and traces of organic solvents were removed by keeping the dispersion under vacuum overnight. Then the liposomal vesicles were bath sonicated for three minutes followed by extrusion through polycarbonate membranes (100 nm pore size) for 15 times.

Characterization of liposomes

The prepared liposomes were characterized for average particle size, size distribution and zeta-potential by dynamic light scattering (DLS) at 25°C using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). For zeta-potential measurement, samples were dispersed in deionized water and 0.75 mL of the dispersion was injected into clear folded capillary cell (DTS1060). The zeta-potential measurement was carried out using Smoluchowski model in automatic mode. The mean value of three different measurements was reported.
Encapsulation efficiency

The EE (%) of PFD in liposomes was determined after separation of the unentrapped PFD from the liposome dispersion. The free PFD from the dispersion was separated by ultracentrifugation of liposomes using Amicon 50 kDa filters at 6000 rpm for 12 min. The total PFD concentration (both free and entrapped) in liposomal dispersion was determined by lysing the liposomes using 6% v/v Triton-X100. The free PFD from filtrate and total PFD concentration were then determined using HPLC (Shimadzu, Japan). The HPLC was performed using a C8 column with mobile phase consisting of 10 mM ammonium acetate:acetonitrile (70:30% v/v). The sample (50 μL) was injected and PFD was detected at 310 nm wavelength. The EE was determined using Equation (1)

\[
\text{Encapsulation efficiency} = 1 - \frac{\text{Concentration of free PFD}}{\text{Concentration of total PFD}} \times 100
\]

Preparation of the PFD-loaded liposomal hydrogel

HPMC hydrogel was prepared for topical application of PFD-loaded liposomal dispersion. HPMC (2% w/w) was added to warm water and kept under magnetic stirring until it gets completely dissolved. Different permeation enhancers including OA, PG and IPM were added in different ratios into HPMC solution. This solution was allowed to cool resulting in the formation of hydrogel. The free PFD or PFD-loaded liposomes were spiked into these gels and mixed thoroughly for their uniform distribution. The liposomes were characterized for particle size and zeta-potential before and after incorporation in HPMC hydrogel. The hydrogel incorporated with liposomes was stored for 48 h. Later, the preparation was diluted using phosphate buffer at 1:10 ratio and centrifuged at 3000 rpm for 10 min. The supernatant was collected and analyzed for average particle size and zeta-potential as mentioned earlier.

Stability of the PFD-loaded liposomes

The stability of PFD-loaded liposomes was evaluated by determining the particle size, zeta-potential and PFD encapsulation for a period of four weeks. The prepared vesicles were stored in a transparent glass vial closed with screw caps at 2–8 °C and at room temperature (25 °C) for one month. Samples were withdrawn every seven days and were analyzed for particle size, zeta-potential and PFD EE as described above.

In vitro release studies

In vitro release of PFD from liposomes was determined using Franz diffusion cell apparatus having 0.637 cm² surface area and a receptor volume of 5 mL. A synthetic cellulose acetate membrane (12–14 kDa MWCO) was placed between the donor and receptor compartments of a diffusion cell. The receptor compartment was filled with phosphate buffered saline (PBS, pH 7.4) maintained at 37 ± 0.5 °C. The receptor medium was constantly stirred using a magnetic stir bar. The PFD release from liposomes and hydrogels was compared with diffusion of free PFD across the membrane. The free PFD solution (250 μg) as well as formulations containing similar amount of PFD was spiked in the donor compartment. Samples (300 μL) were collected from the receptor compartment at predetermined time intervals (0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h) and immediately replaced with fresh PBS. The collected samples were analyzed using the HPLC method as described above.

Skin permeation studies

The skin permeation studies were performed using excised porcine ear skin mounted on a Franz diffusion cell apparatus (PermeGear Inc., Hellertown, PA). Porcine ears were procured immediately after kill from a local abattoir and were cleaned under tap water. The hair on the dorsal side was removed using a hair clipper (HC70, Remington, China). Later, the full thickness skin from dorsal side of the ears was carefully excised using a scalpel and forceps. The underlying fat tissue was scrapped off using a blunt scalpel. These skin samples were stored at −80 °C for not more than eight weeks.

Before the experiment, the skin samples were thawed at room temperature. The thickness of the skin samples was measured using a digital micrometer (Baker Gauges India Pvt. Ltd, Mumbai, India). The integrity of skin was measured by determining the skin resistance (R). For that, direct current (I, 1 mA) was applied using DC power supply unit (V-care Meditech Pvt. Ltd, Bangalore, India) and voltage drop (V) was measured using a digital multimeter (17B, Fluke Corporation, China). The skin resistance (R) was calculated using Ohm’s law (V = IR).

For permeation studies, the skin sample was mounted between the receptor and donor compartments of a diffusion cell with SC facing the donor compartment. This Franz diffusion cell was having an effective diffusional area of 0.637 cm² and a receptor compartment volume of 5 mL. PBS (pH 7.4) maintained at 37 ± 0.5 °C was used as receptor medium. The free PFD solution as well as formulations containing similar amount of PFD was charged in the donor compartment. The PFD formulations investigated for skin permeation are shown in Table 3. Samples (300 μL) were withdrawn at predetermined time intervals (0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30, 36 and 48 h) from receptor compartment and immediately replaced with fresh PBS. The samples were then analyzed using HPLC method as described above.

Calculation of skin permeation parameters

The skin permeation parameters were calculated from the cumulative amount of PFD permeated per unit area versus time profile. The flux (J) was calculated from the slope of linear portion of the curve and lag time (tlag) was calculated by extrapolating the linear portion of the curve onto the time axis. The permeability coefficient (Kp) of PFD across porcine skin was calculated using the relationship derived from Fick’s law of diffusion.

\[
K_p = \frac{J}{C}
\]

where ‘J’ is the flux and ‘C’ is the initial drug concentration in the donor compartment.
The amount of PFD retained within skin samples was measured by tape stripping method. After 48 h of treatment, the skin sample was washed with PBS and tape stripping was performed using scotch book tape (845, 3M, St. Paul, MN). Tape stripping was repeated until the complete SC was removed. Skin resistance was measured to determine the complete removal of SC. The weight of SC removed was measured using a microbalance (XP6, Excellence plus, Mettler Toledo, Columbus, OH). The stripped tapes were placed in microcentrifuge tubes (2 mL) and the PFD was extracted using methanol (0.5 mL). The PFD retained in the viable skin after tape stripping was also extracted. The viable skin was cut into small pieces and 0.5 mL methanol was added to extract the PFD. The samples were sonicated for 30 min and centrifuged at 10,000 rpm for 10 min. Then the supernatant was collected and analyzed for PFD using HPLC method as described above.

Statistical analysis

All the results were presented as mean ± standard deviation (n = 3). Statistical analysis was performed using analysis of variance (ANOVA) (V6 GraphPAD Prism, La Jolla, CA) where p < 0.05 was considered to be the minimal level of significance.

Results

Preparation and characterisation of the PFD-loaded liposomes

Liposomes were prepared by three different techniques, TFH method, TFH followed by freeze–thaw cycles and DIM. Table 1 shows the average particle size, polydispersity index (PDI) and zeta-potential of liposomes prepared by the three strategies. The liposomes prepared by TFH method showed average particle size of 236 ± 6.4 nm (PDI: 0.42 ± 0.005). After extrusion through polycarbonate membrane for 15 times, the particle size reduced to 167 ± 6.0 (PDI: 0.24 ± 0.003). The average particle size was <200 nm for all the formulations prepared and change in preparation method did not show significant (p > 0.05) difference in particle size. Similarly, the PDI for all the formulations prepared was ≤0.3 (Table 1). The liposomes showed negative zeta-potential as both phosphatidylcholine and sodium cholate are negatively charged (Table 1). In general, increase in the number of freeze–thaw cycles decreased the zeta-potential.

Stability of the PFD-loaded liposomes

The stability of PFD-loaded liposomes was determined after storage at 2–8 and 25°C temperature conditions for up to 30 days. Table 2 shows the particle size, zeta-potential and percentage of PFD retained after every 7 days. There was no significant (p > 0.05) change in the particle size and zeta-potential for liposomes stored at both the temperature conditions. On the other hand, liposomes stored at 2–8°C retained 82% of PFD compared with only 55% of PFD for liposomes stored at 25°C after 30 days.

In vitro release of the PFD from liposomes

The diffusion of free PFD across the dialysis membrane and release of PFD from liposomes and liposomes incorporated in hydrogel were investigated. Figure 3 shows the in vitro release profile of PFD from these formulations. The free PFD diffused across the dialysis membrane within 2 h, while 100% release of PFD from liposomes was achieved in 4 h. On the other hand, the complete release of PFD from liposomal hydrogel formulation (LG2) was achieved only after 12 h (Figure 3).

Skin permeation of the PFD-loaded liposomes

The average thickness of the porcine ear skin used for the in vitro studies was found to be 0.92 ± 0.02 mm. The average skin resistance before the application of formulation was 5.88 ± 1.89 kΩ. Table 3 shows different formulations studied to enhance the skin penetration of PFD. In all the skin permeation studies, formulations were prepared with similar

| Formulation | Preparation technique | Lipid (mg/mL) | PFD (mg/mL) | Hydration medium | Freeze–thaw cycle | Particle size (nm) | PDIa | Zeta-potential (mV) |
|-------------|-----------------------|---------------|-------------|-----------------|-------------------|-------------------|------|-------------------|
| L1          | Thin film hydration method | 5             | 1           | Deionised water | NA                | 167 ± 6           | 0.24 ± 0.003 | −32 ± 6          |
| L2          | Thin film hydration method | 5             | 1           | Deionised water: ethanol (80:20) | NA | 198 ± 2 | 0.18 ± 0.003 | −35 ± 4          |
| L3          | Thin film hydration method | 5             | 1           | Deionised water | 5                 | 186 ± 8           | 0.28 ± 0.002 | −40 ± 8          |
| L4          | Thin film hydration method | 5             | 1           | Deionised water | 10               | 181 ± 6           | 0.27 ± 0.001 | −52 ± 5          |
| L5          | Thin film hydration method | 5             | 5           | Deionised water | 10               | 180 ± 13          | 0.28 ± 0.003 | −50 ± 6          |
| L6          | Thin film hydration method | 10            | 5           | Deionised water | 10               | 191 ± 4           | 0.28 ± 0.002 | −40 ± 4          |
| L7          | Direct injection method | 10            | 5           | NA              | NA               | 174 ± 6           | 0.23 ± 0.003 | −42 ± 4          |

Results are represented as mean ± standard deviation (n = 3).
aPDI – polydispersity index.
PFD content. Figure 4 shows the cumulative amount of PFD permeated across the skin after 48 h treatment for different formulations compared with free PFD.

In general, the cumulative amount of PFD permeated increased after a short lag time (Figure 4). Table 3 shows that the lag time for PFD permeation from solution was 8.63 ± 0.17 h. In contrast, the lag time significantly \( p < 0.05 \) decreased to 2.18 ± 0.56 h for PFD-loaded liposomal system (L6). The flux of PFD applied in the solution form was 2.55 ± 0.52 \( \mu \text{g/cm}^2/\text{h} \), whereas the PFD-loaded liposomes showed a significantly \( p < 0.05 \) greater flux of 7.66 ± 0.21 \( \mu \text{g/cm}^2/\text{h} \). Similarly, the cumulative amount of PFD permeated across the skin was found to be greater when delivered from PFD-loaded liposomes (251 ± 4.2 \( \mu \text{g/cm}^2 \)), compared with free PFD solution (85.3 ± 18.8 \( \mu \text{g/cm}^2 \)). Furthermore, incorporation of PFD in liposomes (L6) resulted in greater diffusion and permeability coefficient (Table 3).

For the ease of topical application, the liposomes were incorporated into HPMC hydrogel (2% w/w) containing different permeation enhancers. The liposomal formulation (L6) with higher EE was incorporated into HPMC hydrogel. This was compared with free PFD spiked in HPMC hydrogel with permeation enhancers. To study the stability of liposomes incorporated in hydrogel, we have performed particle size and zeta-potential measurement before and after incorporation in hydrogel. The average particle size after incorporation in hydrogel was found to be 214 ± 5.28 which was not significantly \( p > 0.05 \) different compared to particle size before incorporation in hydrogel. The zeta-potential of liposomes incorporated in hydrogel was \( \pm 46.4 ± 4.47 \) which was similar to free liposomes. Among the four hydrogel formulations of PFD investigated for skin permeation, the hydrogel containing PFD-loaded liposomes with 2.5% OA and 10% IPM (LG2) has shown the greatest flux of PFD.

### Table 2. Stability of liposomes (L6) stored over a period of 1 month at 2–8°C and 25°C.

| Time (days) | Stored at 2–8°C | Stored at 25°C |
|------------|----------------|----------------|
|            | Average size (nm) | Zeta-potential (mV) | PFD retained (%) | Average size (nm) | Zeta-potential (mV) | PFD retained (%) |
| 1          | 191.33 ± 4       | −40 ± 4         | 100.00          | 191.33 ± 4       | −40 ± 4         | 100.00          |
| 3          | 190.64 ± 12      | −44 ± 2         | 94.94           | 194.82 ± 8       | −43 ± 6         | 90.84           |
| 7          | 194.37 ± 8       | −45 ± 4         | 91.24           | 206.35 ± 11      | −48 ± 5         | 81.29           |
| 14         | 193.58 ± 6       | −44 ± 8         | 88.55           | 211.78 ± 6       | −44 ± 7         | 74.15           |
| 21         | 196.76 ± 11      | −44 ± 6         | 85.78           | 216.44 ± 14      | −46 ± 6         | 69.35           |
| 30         | 204.68 ± 7       | −46 ± 4         | 82.08           | 242.82 ± 12      | −42 ± 8         | 54.69           |

Figure 1. Schematic representation of PFD loading in liposomes prepared by thin film hydration (TFH) method (A), thin film hydration followed by freeze-thaw cycles (B), and direct injection method (C).

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10.9 ± 1.04 μg/cm²/h. This was significantly (p < 0.05) greater than PFD liposome (L6) and free PFD in hydrogel (PG2). Additionally, the cumulative amount of PFD permeated across the skin was greatest in case of LG2 (344 ± 28.8 μg/cm²) with a significantly (p < 0.05) lower lag time (2.30 ± 1.32 h). On the other hand, the hydrogel containing PG and OA (PG1 and LG1) showed lower flux and cumulative amount of PFD permeated with greater lag time. Similarly the diffusion coefficient and permeability coefficient were greater for PFD liposomal hydrogel with OA and IPM (LG2) compared with hydrogel containing OA and PG (LG1). Overall, the formulations where hydrogels were spiked with PFD solution showed lesser flux and greater lag time compared with hydrogels formulations spiked with liposomal PFD.

Furthermore, tape stripping was performed to evaluate the amount of PFD retained within SC and viable epidermis after treatment with different formulations. The complete removal of SC using tape stripping method was monitored by measuring skin resistance. The resistance values decreased to 3.14 ± 0.46 kΩ after complete removal of SC. The amount of PFD retained in SC and viable epidermis was determined by tape stripping method. Figure 5 shows the amount of PFD retained within SC and viable epidermis after treatment with different formulations. In general, PFD was retained more within SC compared with viable epidermis.

The PFD entrapped liposomes showed a 2-fold increase in SC retention compared with free PFD solution. The hydrogel formulations showed significantly (p < 0.05) greater PFD retention in SC compared with free PFD solution and PFD liposomes (Figure 5A). However, there was no significant (p > 0.05) difference among the hydrogel formulations in retention of PFD within SC. In the case of viable epidermis, PFD liposomal hydrogel containing OA and IPM (LG2) showed the greatest PFD retention, which was 4-fold greater than PFD liposomal formulation (L6) (Figure 5B). Similar to SC retention, all the hydrogel formulations showed greater PFD retention within viable epidermis.

### Discussion

The marketed product of PFD (Esbriet) is available as an oral solid dosage form against IPF (Duck et al., 2015). The frequency of administration is three times a day because of the lower half-life and high elimination rate (Lou et al., 2012). The PFD after oral administration has been reported to

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**Table 3. Skin permeation parameters for PFD formulations.**

| Formulation | Description                                      | Lag time (h) | Flux (μg/cm²/h) | Cumulative amount permeated (μg/cm²) | Diffusion coefficient (× 10⁻³) | Permeability coefficient (× 10⁻³, cm²/h) |
|-------------|--------------------------------------------------|--------------|-----------------|-------------------------------------|-------------------------------|-----------------------------------------|
| PFD         | PFD solution                                     | 8.63 ± 0.17  | 2.55 ± 0.52     | 85.3 ± 18.8                         | 1.91                          | 2.55                                    |
| L6          | PFD-loaded liposome                              | 2.18 ± 0.56  | 7.66 ± 0.21     | 251 ± 4.21                          | 0.26                          | 7.66                                    |
| PG1         | PFD solution in hydrogel containing 2% HPMC + 20% propylene glycol + 2.5% oleic acid | 6.88 ± 3.99  | 5.24 ± 0.67     | 167 ± 45.9                          | 0.37                          | 4.19                                    |
| PG2         | PFD solution in hydrogel containing 2% HPMC + 10% IPM + 2.5% oleic acid | 7.84 ± 1.66  | 6.14 ± 0.28     | 206 ± 13.0                          | 0.18                          | 6.14                                    |
| LG1         | L6 in hydrogel containing 2% HPMC + 20% propylene glycol + 2.5% oleic acid | 5.15 ± 1.29  | 7.60 ± 0.05     | 247 ± 8.20                          | 0.24                          | 6.07                                    |
| LG2         | L6 in hydrogel containing 2% HPMC + 10% IPM + 2.5% oleic acid | 2.30 ± 1.32  | 10.9 ± 1.04     | 343 ± 28.8                          | 0.85                          | 10.9                                    |

Results are represented as mean ± standard deviation (n = 3).
undergo extensive first-pass metabolism by cytochrome P450 class of enzymes (Arai et al., 2014). More importantly, it was reported that gastrointestinal adverse effects are the most important dose-limiting factors for PFD (Arai et al., 2014). Here, we have reported the development of a transdermal liposomal delivery system for PFD to improve the skin absorption, avoid first-pass metabolism and improve the overall patient compliance.

Because of the inherent permeability limitations of skin barrier, we have encapsulated PFD in a well-characterized liposomal delivery system. However, PFD with aqueous solubility of 15 mg/mL (Togami et al., 2015) poses a challenge in encapsulation into liposomal vesicles. The encapsulation of PFD in liposomes through conventional preparation method of TFH resulted in poor EE. Encapsulation of active molecule in liposomes formed through TFH method involves passive diffusion of hydrophilic molecule into the inner aqueous compartment (Bangham et al., 1965). However, high aqueous solubility of PFD results in less partitioning into lipid vesicles from bulk aqueous environment.

Further methods to improve encapsulation, including freeze–thaw cycles of prepared liposomes resulted in greater encapsulation of PFD in liposomes. The freeze–thaw cycle improves the encapsulation of active molecule in liposomes by two different strategies; physical disruption and fusion/destabilization of the lipid bilayer because of ice crystal formation, and diffusion of molecule in the frozen state accomplished by cryo-concentration (Costa et al., 2014; Hope et al., 1986).

In addition to passive drug encapsulation method, pH-induced entrapment of drug molecules has been reported (Lasic et al., 1995). We have studied the influence of pH on EE of PFD in liposomes. The EE was not significantly ($p>0.05$) different among the different pH conditions (4, 7 and 10) studied (Supplementary Figure S2). This is attributed to the neutral charge of PFD (Togami et al., 2015).

For application of formulations on skin, a suitable dosage form has to be developed. Recently, hydrogels have been widely used as topical formulations for their favorable properties, such as ease of preparation, skin compatibility, less irritation potential, entrapment of variety of active molecules and washability (Peppas et al., 2000). Of the many hydrophilic polymers, hydroxypropylmethyl cellulose has been commonly used in the preparation of stable hydrogels (Fyfe & Blazek, 1997). Furthermore, incorporation of liposomal vesicles in HPMC hydrogel would favor the partitioning of liposomes into skin membrane (El-nabarawi et al., 2013). The hydrogel formulation also allowed us to incorporate most widely studied skin chemical permeation enhancers including OA, PG and IPM.

The permeation enhancing effect of OA is mainly because of its ability to interact with SC lipids and disrupting the SC lamellar structure leading to increased diffusivity and partitioning of molecules (Moghadam et al., 2013). Previous reports found a synergistic effect of OA and PG on skin permeation enhancement of various drug molecules (Larrucea et al., 2001). This has been attributed to the facilitated incorporation of OA in the SC lipid alkyl domain and the interaction of PG at the polar head group region (Moreira et al., 2010). However, the combination of PG (20%) and OA (2.5%) did not significantly ($p>0.05$) enhance the skin permeation of PFD. This could be because of greater interaction of PG with HPMC hydrogel and reduced

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**Figure 4.** Cumulative amount of PFD permeated from different formulations across excised porcine skin. L6: PFD liposomal dispersion; PG1: PFD solution in hydrogel containing propylene glycol and oleic acid; PG2: PFD solution in hydrogel containing IPM and oleic acid; LG1: PFD liposomes in hydrogel containing propylene glycol and oleic acid; LG2: PFD liposomes in hydrogel containing IPM and oleic acid. Results are presented as mean ± standard deviation ($n=3$). Asterisk (*) indicates that the value is significantly ($p<0.05$) different compared with PG1, PG2, LG1, LG2 in other values.

**Figure 5.** The amount of PFD retained within SC (A) and viable epidermis (B) after treatment with different formulations. L6: PFD liposomal dispersion, PG1: PFD solution in hydrogel containing propylene glycol, PG2: PFD solution in hydrogel containing IPM and oleic acid; LG1: PFD liposomes in hydrogel containing IPM, LG2: PFD liposomes in hydrogel containing propylene glycol. Results are presented as mean ± standard deviation ($n=3$). Asterisk (*) indicates that the value is significantly ($p<0.05$) different compared with PG1, PG2, LG1, LG2 in case of “A” and PFD and L6 in case of “B”.

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*Transdermal delivery of liposomal PFD*
partitioning into the skin membrane. Furthermore, the partitioning of PFD in skin membrane from hydrogel containing PG could be less because of its greater solubility in hydrogel than in skin tissue. When free PFD was incorporated in hydrogel containing PG or IPM, there was no significant difference in skin permeation characteristics of PFD. However, when PFD liposomes were incorporated in hydrogel containing PG or IPM, hydrogel with IPM showed significantly better skin permeation properties compared with hydrogel containing PG. This is attributed to the interaction of IPM with liposomal lamellar structure and its fluidization, apart from the skin penetration enhancement of IPM through interactions with skin lipids.

On the other hand, the combination of IPM and OA showed the greatest permeation enhancement for PFD after incorporation in hydrogel formulation. This could be because of greater partitioning of IPM in skin than in hydrogel. It is interesting to note that combination of IPM and OA significantly ($p < 0.05$) enhanced the skin permeation of liposomal PFD in hydrogel compared with free PFD in hydrogel.

The particle size of a carrier is an important attribute for efficient skin transport of active molecules. In general, liposomes with <200 nm particle size have been shown to penetrate the viable epidermis through intercellular lipid matrix pathways (Liu et al., 1992). Here, we have prepared liposomes containing 14% w/w of sodium cholate. Originally reported by Cevc, these liposomes act as highly deformable vesicles which can squeeze through the minute pores of SC (Cevc & Blume, 1992, 2004). Therefore, there was a 3-fold increase in PFD flux through skin after encapsulation in liposomes compared to free PFD solution. Furthermore, with the incorporation of chemical permeation enhancers in the hydrogel with liposomal PFD, the flux increased to 10.9 ± 1.04 μg/cm²/h. This increased partitioning of liposomal PFD is attributed to a significant ($p < 0.05$) decrease in lag time compared to free PFD. Overall, we have shown that PFD can be delivered through skin for transdermal application using liposomal carrier system with suitable hydrogel formulation.

Conclusion

The EE of PFD in liposomes can be improved by preparing liposomes with TFH method followed by freeze–thaw cycles. The PFD-loaded liposomes showed greater skin permeation compared with free PFD solution. The PFD-loaded liposomes incorporated in hydrogel containing OA and IPM as permeation enhancers showed greater skin permeation in comparison with PFD-loaded liposomes. The derived results of this work support the possibility of considering liposomal hydrogel containing a mixture of chemical permeation enhancers as a potential transdermal delivery system for PFD.

Declaration of interest

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The authors report no declarations of interest.

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Supplementary material available online
Supplementary material.