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Proposome for transdermal delivery of tofacitinib

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ABSTRACT

Tofacitinib citrate (TC) has recently gained interest in treating skin disorders such as psoriasis, atopic dermatitis and baldness. Unfortunately, the oral administration shows side effects, such as decreased neutrophil counts. To this end, the topical delivery of TC can be used to reduce the risk associated with systemic exposure. However, TC shows minimal absorption via skin. Hence, the objective of this study is to enhance the skin delivery of TC using a non-invasive approach. The liposomes based on propylene glycol, named as proposomes, carrying TC, were studied. The vesicle characteristics and *in vitro* skin permeation were assessed. The proposomes enhanced the skin permeability of TC by 4–11 folds. The composition of proposomes was found to affect the skin permeation and deposition of TC. The proposomes were stable for at least 6 months. Overall, proposomes were effective for targeted topical drug delivery.

1. Introduction

Tofacitinib citrate (TC) is a Janus kinase inhibitor. It is used for the treatment of rheumatoid arthritis in patients who do not respond well with methotrexate. Recently, it is found effective against multiple skin disorders, including chronic plaque psoriasis, atopic dermatitis, vitiligo and alopecia areata (Anzengruber et al., 2016; Bissonnette et al., 2016; Craiglow and King, 2014, 2015; Levy et al., 2015). However, the oral administration of TC is associated with several side effects, including opportunistic infections, decreased neutrophil counts, and increased cholesterol levels (Kremer et al., 2013; Winthrop et al., 2014). To reduce the side effects caused by systemic administration of TC, topical delivery may be used. However, topical TC has minimal absorption through skin (Murphy et al., 2012).

Liposomes are an effective means for topical drug delivery (Gupta et al., 2012; Rahman et al., 2015). They are extensively used in both cosmetic and pharmaceutical applications (Akbarzadeh et al., 2013). In the past few decades, various forms of liposomal formulations and nanovesicles have been developed, for instance, ethosomes (ethanol-based liposomes), niosomes (non-ionic surfactant-based vesicles), glycerosomes (glycerol-based liposomes) (Chourasia et al., 2011; Harisa et al., 2018; Manca et al., 2017).

Alcohols, such as ethanol and propylene glycol (PG), have been used as an ingredient to offer flexibility to the liposomal structure to enhance skin permeation (Elmoslemany et al., 2012; Elsayed et al., 2007; Harvey et al., 2013; Ingebrigtsen et al., 2017; Li et al., 2016; Vanic et al., 2014; Zhao et al., 2013). Ethosomes have been reported to enhance skin penetration of several drugs (Touitou et al., 2000). However, ethanol is not suitable for certain skin diseases because it could potentially aggravate the conditions. In addition, it can also cause patient incompliance because of its smell (Farkas et al., 2003; Sakazaki et al., 2014).

In contrast, PG is widely used in various topical formulations (Carrer et al., 2019; Chen et al., 2014; El Maghraby et al., 2000; Joo et al., 2019; Lee et al., 2014; Limpongsa et al., 2015; Manconi et al., 2019; Pople and Singh, 2011; Thombre et al., 2019; Williams and Barry, 2004). It has been shown that PG solubilized the stratum corneum lipids which helps in enhanced skin permeation of drugs (Kang et al., 2006). It has also been reported that significant cutaneous and percutaneous penetration of drugs could be achieved via the synergic effect of PG and phospholipids (Cooper et al., 1985; Yokomizo and Sagitani, 1996). In addition, being more viscous and less volatile, PG could provide the improved liposomal stability whereas no alcoholic smell could improve patient compliance. We hypothesize that PG-based liposome, named as proposome, is a suitable carrier for the transdermal delivery of TC. The word proposome is coined by the combination on

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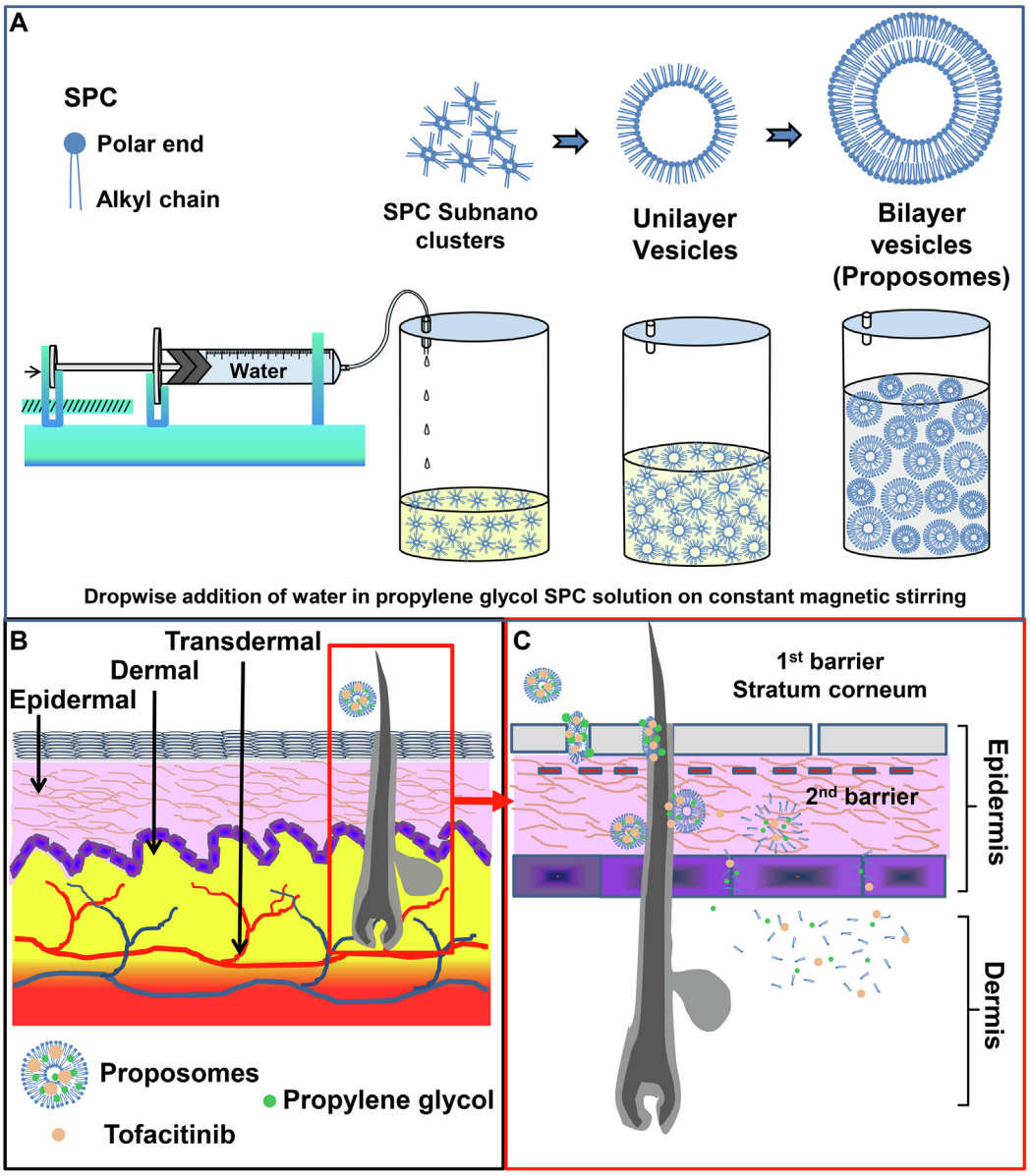

Fig. 1. The schematic representation of A) proposome preparation process, B) the targets of skin delivery and C) the proposed mechanism of proposome on skin permeation enhancement. SPC: Soya phosphatidylcholine.

'prop' (first 4 letters from propylene glycol) and 'osome' (last 5 letters from liposome).

In this study, we prepared proposomes using a cold mixing method (Fig. 1A). The proposome was characterized for its stability, vesicle morphology, polydispersity index (PDI), zeta potential and entrapment efficiency. In addition, the skin absorption of TC through proposomes was also assessed. The proposome composition was found to affect the absorption of TC into the different skin layers. (Fig. 1B-C).

2. Experimental section

2.1. Materials

The materials include: soya phosphatidylcholine (SPC) (Phospholipon 90 G, Lipoid), PG (Chempure Pte Ltd, Singapore), dialysis tubing (Thermo Scientific, USA), ethanol (Thermo Fischer, Singapore), Tofacitinib citrate (Sigma, Singapore), uranyless for transmission electron microscopy (TEM) (Sigma, Singapore), copper mesh grids for TEM (Sigma, Singapore), rhodamine (Sigma, Singapore), calcein (Sigma, Singapore), phosphate buffered saline (PBS) (Vivantis, Singapore) and water purified by Milli-Q system. All materials are used as supplied without further purification.

2.2. Preparation of proposomes

The proposome was prepared by cold mixing process as previously reported (Chourasia et al., 2011), with slight modification of setup (Fig. 1A, SI1). The proposomes with 1%, 2% and 3% w/v SPC and PG concentration of 20%, 30% and 40% v/v were prepared to form 9 formulations. The TC was dissolved in PG before adding SPC. The final concentration of TC was at 0.2% w/v in all formulations. The critical micelle concentration (CMC) of SPC in PG was measured, using the surface tension method. The various mixtures were prepared at different concentrations of SPC up to 4 mg/mL in 3 solutions containing 20%, 30%, and 40% w/v of PG and equilibrated for 24 h at room temperature with continuous agitation.

As a control, ethosome was also prepared in a similar way. The composition of ethosome formulation was 30% v/v ethanol, 0.2% w/v

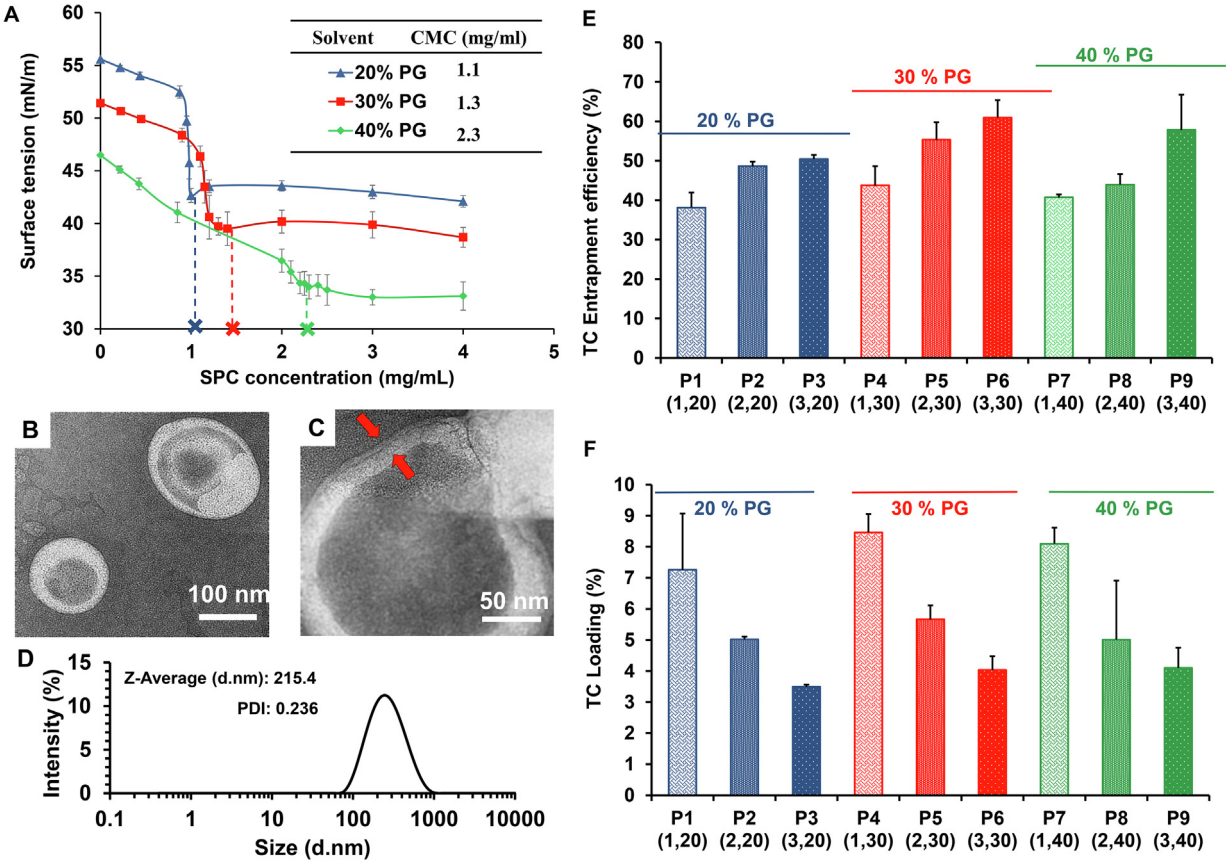

Fig. 2. Proposome characterization. A) The CMC of SPC in aqueous solution containing 20%, 30%, and 40% PG. B) The TEM images of P4 (1, 30). C) Magnified TEM image shows the vesicular structure. The arrow indicates the lipidic layering structure. D) Hydrodynamic diameter of P4 (1, 30) based on the % intensity. The proposomes are uniform with average diameter of 215 nm. E) Entrapment efficiency and F) Loading efficiency of TC in 9 formulations.

Table 1
Characteristics of various formulations, where P0 (1,30) is blank proposome and P1 to P9 had 0.2% w/v TC. The values in bracket with labels show the SPC concentration (1%, 2% and 3% w/v) and PG concentration (20%, 30% and 40% v/v).

Code	Size (nm) (d-Avg.)	PDI	Zeta potential (mV)	% TC loading	EE (%)
P0 (1, 30)	372 ± 12.00	0.554 ± 0.056	−28.7 ± 1.06	–	–
P1 (1, 20)	315 ± 2.82	0.299 ± 0.028	−5.97 ± 0.33	7.26 ± 1.80	38.14 ± 3.82
P2 (2, 20)	278 ± 9.67	0.237 ± 0.016	−6.3 ± 0.33	5.02 ± 0.09	48.63 ± 0.96
P3 (3, 20)	290 ± 4.06	0.222 ± 0.042	−5.97 ± 0.23	3.49 ± 0.07	50.41 ± 1.04
P4 (1, 30)	250 ± 2.55	0.231 ± 0.032	−4.59 ± 0.06	8.46 ± 0.59	43.76 ± 4.85
P5 (2, 30)	257 ± 5.92	0.222 ± 0.009	−4.51 ± 0.20	5.66 ± 0.45	55.32 ± 4.41
P6 (3, 30)	282 ± 1.60	0.152 ± 0.030	−6.23 ± 0.20	4.03 ± 0.44	58.24 ± 6.40
P7 (1, 40)	220 ± 0.30	0.265 ± 0.027	−6.97 ± 0.30	8.10 ± 0.51	40.70 ± 0.79
P8 (2, 40)	221 ± 4.80	0.198 ± 0.042	−4.68 ± 0.55	5.01 ± 1.9	43.96 ± 2.65
P9 (3, 40)	258 ± 5.83	0.198 ± 0.009	−5.07 ± 0.11	4.10 ± 0.65	57.84 ± 8.94

TC and 1% w/v SPC. In addition, the process of proposome preparation was studied by analysing the vesicle characteristics after addition of water into PG at various intervals (SI2).

2.3. Proposome characterization

Surface tension of the equilibrated mixtures was measured using a Sigma 700/701 Force Tensiometer (Dyne Technologies, the UK). Dynamic light scattering technique was used to determine the average size, PDI, and zeta potential of various formulations, with the Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK). The samples were diluted with appropriate medium, i.e., 20% or 30% or 40% of PG in water, respectively. The samples were equilibrated at 25 °C before measurement. All measurements were carried out in triplicates at automatic measurement mode.

The release of TC from proposomes was measured using dialysis membranes with the molecular cut-off weight of 3500 Da (SnakeSkin dialysis tubing, Thermo Scientific, USA). It was placed in the vertical Franz diffusion cell with the effective exposure area of 1 cm^2. The formulation of 200 µL was placed in the donor compartment, and the receptor compartment was filled with 5 mL of PBS with constant magnetic stirring of 250 rpm, maintained at 32 °C. A volume of 100 µL of receptor solution was withdrawn and an equal quantity of fresh medium was replaced hourly for up to 7 h. The samples were analysed using the validated HPLC method.

For the TEM imaging, the proposome was loaded on TEM grids (Carbon coated 300 mesh copper grids) by placing a drop of proposome solution on the grids. The excess solution was removed by blotting using tissue paper. The samples were negatively stained with uranyless solution for 2 min; then excess solution was drained off. The grid was

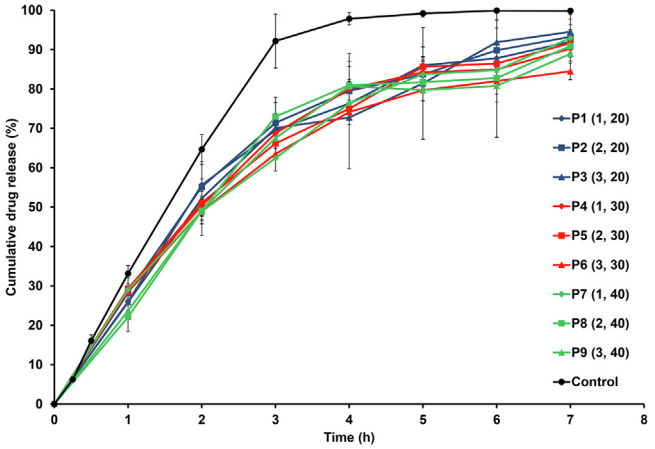

Fig. 3. Cumulative release of TC from proposomes and control solution through a membrane. The values in bracket show the SPC concentration (1%, 2% and 3% w/v) and PG concentration, 20% (blue), 30% (red) and 40% (green) v/v. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dried in air, and the samples were examined using the FEI Tecnai T12 system, with magnification of 30,000x to 97,000x.

2.4. Drug encapsulation

The drug entrapment efficiency and loading efficiency were obtained by ultrafiltration using the dialysis bag made of regenerated cellulose membrane, molecular cut-off 3,500 Da (Thermo Scientific SnakeSkin Dialysis Tubing, USA) (Ravi et al., 2014). 0.4 mL of formulation was added into dialysis bag (~3 cm length including the clipping on both sides) and dialyzed against Milli-Q water (80 mL) for 1 h with constant stirring using magnetic bead at 75 rpm. The time (1 h) of dialysis to get free TC was based on time of filtration (> 90%) of free TC solution (0.4 mL 0.2% w/v) from the dialysis membrane. The filtrate was analysed by HPLC to determine the amount of free drug (SI3). It was calculated using the following equation:

$$\text{Entrapment efficiency}(\%) = \frac{W_{total} - W_{free}}{W_{total}} \times 100$$

$$\text{Drugloading}(\%) = \frac{W_{total} - W_{free}}{W_{totalSPC}} \times 100$$

where W_{total} = total amount of drug in proposome, W_{free} = amount of free drug obtained from filtrate after dialysis and $W_{totalSPC}$ = total weight of SPC used in proposome formulation.

2.5. In vitro skin permeation

Human dermatome skin samples, donated by a 55-year-old, white male, was obtained from Science Care (Phoenix, AZ, USA). The use of cadaver human skin for this study was reviewed by the National University of Singapore Institutional Review Board and subsequently exempted because the cadaveric tissues were without identifier. The study was carried out using the method reported previously, using vertical Franz diffusion cells at 32 °C with an effective exposed area of 0.25 cm^2 (Kathuria et al., 2016). The skin pieces with different thickness from a cadaver were studied for TC deposition (SI4). 40 µL of each formulation and a control (TC solution in 30% PG) were applied onto the skin pieces. The samples were collected after 48 h, processed and

Fig. 4. (A-D) *In vitro* skin permeation of 9 formulations and control (TC in 30% PG). The amount of TC in A) epidermis, B) dermis and C) receptor compartment. D) Total amount in epidermis, dermis and receptor compartment. E) Overall effect of proposome composition on TC delivery to different skin layers. The amount of TC in proposomes and control was 0.2 %w/v. (N = 3, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$, compared with control).

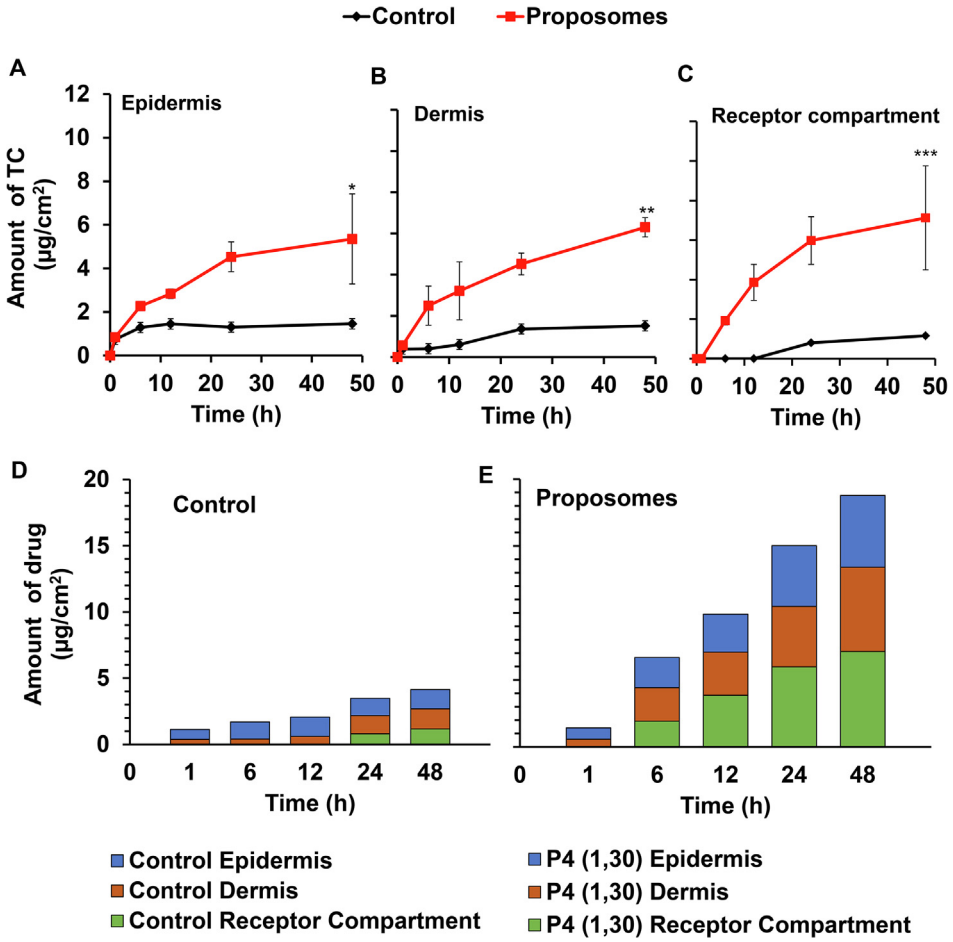

Fig. 5. Skin permeation of TC. (A-C) Distribution and permeation of TC from P4 (1,30) and control (TC in 30% PG). Amount of TC measured in A) epidermis, B) dermis and C) receptor compartment. Total amount of TC (epidermis, dermis and receptor compartment) for D) control and E) proposomes (P4 (1,30)). The amount of TC in proposomes and control was 0.2 %w/v. (N = 3, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$, compared with control).

analysed using the validated HPLC method. The 5 mL of PBS in the receptor compartment was enough to maintain the sink conditions over the period of testing. The enhancement ratio (ER), as defined in SI4, was calculated for all formulations for epidermis, dermis and receptor compartment.

A kinetic permeation experiment was also carried out for proposomes P4 (1, 30). The samples were collected at 1 h, 6 h, 12 h, 24 h and 48 h. At specified time, the receptor solution was withdrawn and analysed by HPLC to determine the amount of TC permeated. For both experiments, skin surface was washed with PBS to remove residual TC on skin surface. The washed skin samples were separated into epidermis and dermis using the forceps and stored at −20 °C till further processing. Later, the skin samples were digested by proteinase K solution (200 µg/mL) for 8 h at 60 °C (Kathuria et al., 2016). The digested samples were processed and analysed (SI5).

2.6. Confocal laser scanning microscopy

The penetration of proposomes into the skin layers was visualized with calcein (log P = 1.61) and rhodamine (log P = 2.43) loaded into proposomes. The calcein is a suitbale flurosecnt probe for TC, which has a log P of 1.24 (Jain et al., 2019). The fluroscence from the human cadaver skin was observed after applying either calcein-loaded proposomes or respective control for 24 h on the skin placed in Franz diffusion cell. After 24 h of application, the formulation was taken out using pipette. The skin was removed and washed off carefully to remove any remains of proposomes on the stratum corneum while also avoiding the contact of washed solution with dermis area. The unexposed area of the skin was trimmed, and only exposed area was examined. The composition of proposomes was 1% SPC, 30% PG with 0.05% calcein/rhodamine. Calcein/rhodamine (0.05%) in 30% PG was used as control.

2.7. Proposome stability

The stability of proposomes and ethosomes were assessed by their physical appearance, average vesicle size, PDI and zeta potential, at different time points over 180 days under storage conditions of 4 °C. The physical appearance was assessed by signs of sedimentation or separation which was captured using a digital camera.

2.8. TC stability

TC solutions in water (0.2% w/v) was stored in closed glass bottles at 4 °C in refrigerator and in chamber maintained at 32 °C using hot air circulation. The TC concentration was examined after 30 and 60 days at both conditions. The formulation P4 (1, 30) was also stored at 4 °C in refrigerator and drug content was measured for 6 months. TC concentration in the proposomes was assayed by mixing one part with nine parts of ethanol. The ethanolic mixture was vortexed for 2 min and then centrifuged at 10,000 rpm for 5 min, before the HPLC analysis.

2.9. Statistical analysis

All the results were presented as the mean ± standard deviation.

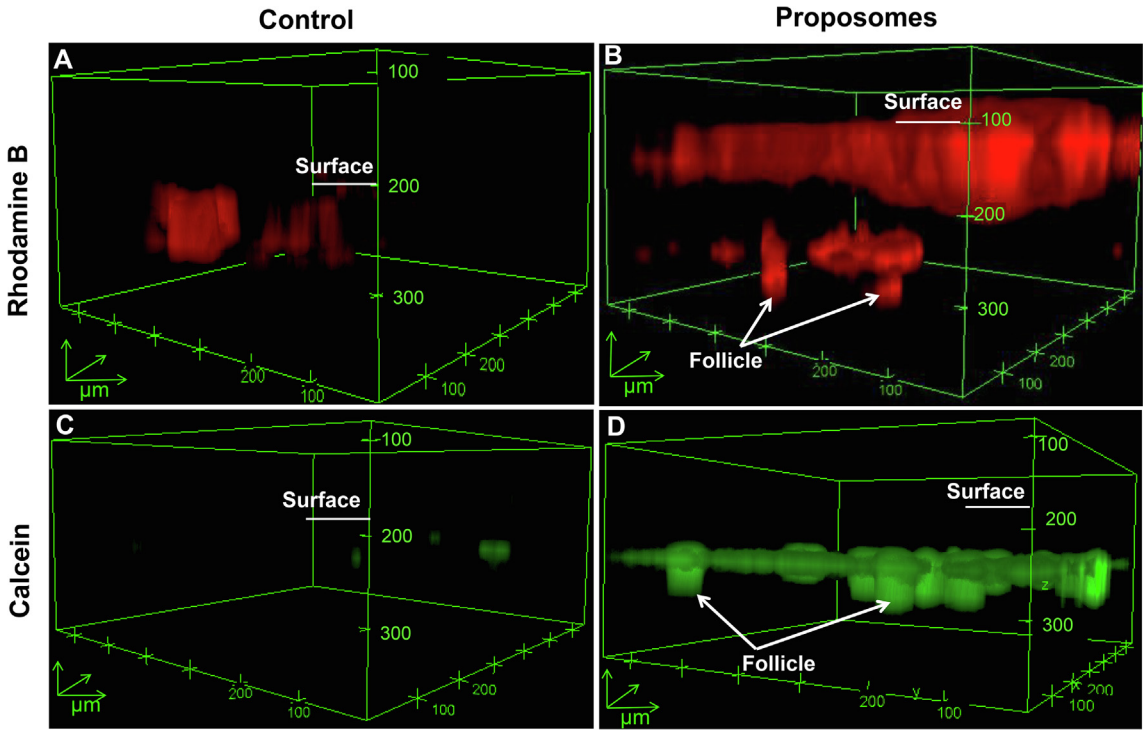

Fig. 6. Confocal microscopy images showing fluorescent calcein (green) and rhodamine B (red) penetration into human skin after 24 h treatment. A) Rhodamine B control B) Rhodamine B proposome. C) Calcein control. D) Calcein proposome. The control was 0.2% w/v calcein or rhodamine B solution in 30% PG. The concentration of calcein and rhodamine B was same for proposomes as in control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

One-way analysis of variance (ANOVA) was performed to compare groups of data and *p*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Formulation characterization

The SPC is dissolved in pure PG. It was found that higher PG percentage was associated with higher CMC values of SPC in PG aqueous solution (Fig. 2A). When water was slowly and continuously incorporated, the CMC decreased accordingly, leading to vesicle formation (Fig. 1A).

The TEM images confirmed the vesicular structure of the proposome (Fig. 2B-C). The uniform size distribution was demonstrated by the PDI measurements (Table 1 and Fig. 2D).

Entrapment efficiency is defined as the amount of drug entrapped by the proposome over total amount of drug dissolved in the solution. The higher SPC concentration was also correlated with the higher entrapment efficiency (Fig. 2E), which could be attributed to the increased number of proposomes for more entrapment. Because of hydrophobic nature of TC (log P = 1.81), most of the TC could have been localized within the lipid bilayers, which was consistent with the previously reports (Ascenso et al., 2015; Touitou et al., 2000).

In the *in vitro* drug release testing, the formulations showed similar release profiles, with delayed TC release as compared to the control (TC in 30% PG solution), from which most of the drug was released within 3 h (Fig. 3).

3.2. In vitro skin permeation

The proposomes containing 40% PG resulted in the highest relative proportion of drug permeated into receptor compartment (Fig. 4). In contrast, higher proportion of the TC was recovered from epidermis for proposomes containing 20% PG while higher proportion was recovered in the dermis for proposomes containing 30% PG. All formulations showed higher permeability, i.e., 4.79–12.93 µg/cm^2, compared with 1.58–4.6 µg/cm^2 of PEG-based ointment as reported (Murphy et al., 2012). The formulation P4 (1,30) was chosen for further study as it was suitable for both epidermal and dermal targeting.

3.3. In vitro skin kinetics

The TC permeation through skin was much faster in proposomes than control (Fig. 5). In the first 12 h, 6 to 8-fold higher amount of drug appeared in dermis than control. After 6 to 12 h, around 0.5–1 µg TC was detected in the receptor compartment for proposome, whereas negligible drug was detected for control.

3.4. Confocal laser scanning microscopy

Proposomes loaded with calcein and rhodamine were applied on human cadaver skin samples for 24 h. In general, the proposomes showed higher fluorescence intensity than control (Fig. 6). Furthermore, the calcein and rhodamine, when delivered through proposomes, penetrated into deeper skin layers. The circular structures were also observed in the deeper layers in case of proposome (SI6). This was also evident in the three-dimensional view of stacked images showing the follicular structure. The observation indicates the transfollicular route was one of the skin penetration mechanisms. In addition, the improved absorption of chemicals with different log P values suggested proposomes as a potential vehicle for other drugs as well.

3.5. Enhanced stability of TC in proposomes

TC loaded ethosomes showed visible sedimentation within 7 days of preparation. In contrast, TC-proposome solution remained stable in 180 days (Fig. 7A). The vesicles size, PDI, and zeta potentials did not

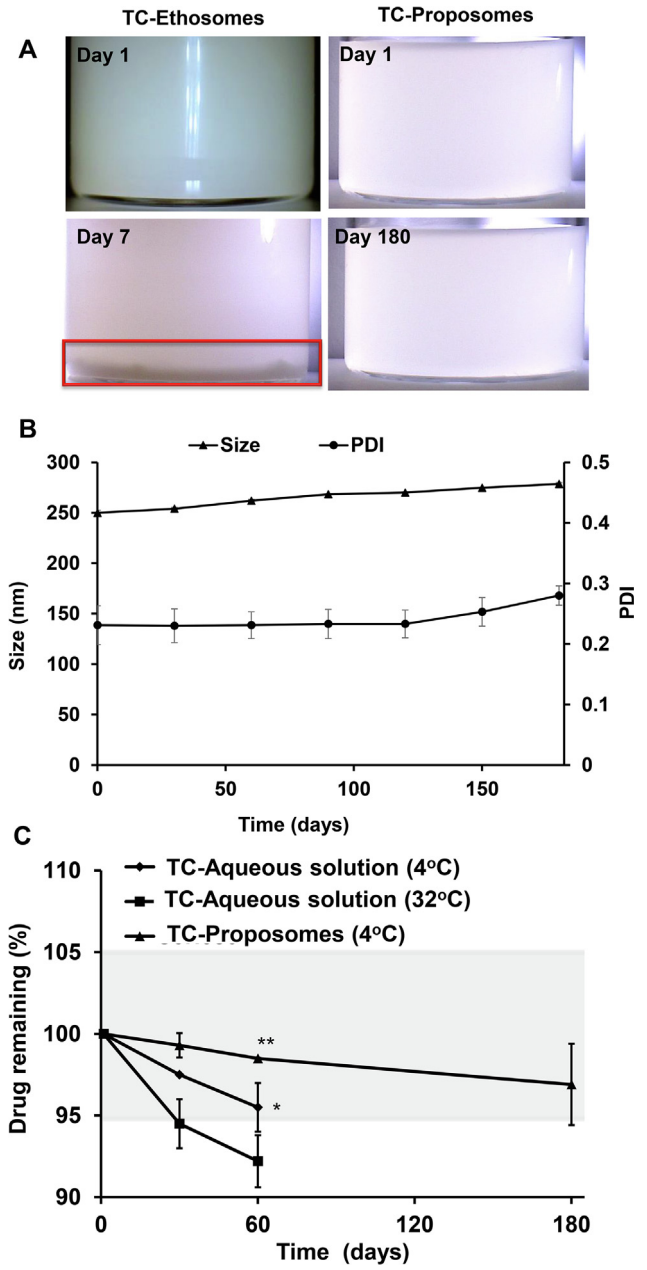

Fig. 7. Stability of proposomes and TC. A) Appearance of TC-loaded ethosome and proposome. The sedimentation in ethosomes within 7 days shows physical instability while proposomes were stable for 180 days at least. B) Average vesicle size and PDI of P4 (1, 30) formulation. C) Stability of TC in proposome and aqueous solution. The gray region shows the limits of ± 5% drug remaining. (N = 3, * $p \leq 0.05$ and ** $p \leq 0.01$).

change significantly for 180 days (Fig. 7B). The amount of TC was reduced to 95% at 4 °C and 92% at 32 °C after 120 days of storage. In contrast, proposomes retarded TC degradation, as 97% of drug remained intact after 180 days (Fig. 7C). This could be attributed to entrapment of drug inside proposome. The improved TC stability may be ascribed to the presence of PG as an aldehyde scavenger (Murphy et al., 2012). Murphy et al reported improved stability of TC with glycerine in formulation, which was further improved by addition of antioxidants (Murphy et al., 2012).

4. Discussion

The study described the formulation of PG based nanovesicles named as 'proposomes'. The TC loaded proposomes were characterised and investigated for stability over time, skin delivery and deposition in different dermal layers. The proposome composition was found to affect the vesicle's characteristics and permeation of drug through skin. The varying proposome composition caused variations in the entrapment efficiency of TC that can be explained by the CMC of SPC (Fig. 2A), vesicle size, and solubility of TC in PG (SI7). Below CMC, the SPC molecules would be distributed as non-aggregates in medium, while the SPC molecules would be aggregated above CMC. The CMC in 40% PG was 2.3 mg/mL (Fig. 2A) which was almost twice higher than that in 20% or 30% PG. This shows that with the same amount of SPC, the number of proposomes would be lowest in the 40% PG.

The higher concentration of PG resulted in higher CMC of SPC and reduction in vesicle size (Table 1). This finding is consistent with another report that concentration of 40% PG imparted increasing disorders on lipid bilayers structure compared with 20% PG (Harvey et al., 2013). In contrast, a positive correlation was seen between SPC concentration and vesicle size. The lower absolute zeta potential was seen for TC loaded proposomes (-4.6 mV to −6.97 mV) compared to blank proposomes (-27 mV), which may be attributed to ionic interactions between SPC (negatively charged) and TC (positively charged). The size for TC loaded proposomes as compared to blank proposomes was also lower, which may be attributed to tighter packing of SPC in proposomes with TC due to ionic interactions.

In this study, the targeted topical application was defined as epidermal, dermal and transdermal delivery. For epidermal delivery and dermal delivery, the epidermis and dermis are the targeted delivery sites, respectively. For transdermal, the targeted site is hypodermis, i.e., for the drug to be absorbed into systematic circulation. The pharmacological targets of TC, i.e., T cells and B-cells are located at dermal layer. Hence, the ideal delivery system for TC should target dermal layer. However, it has been reported that liposomes could not cross the granular layer of epidermis (Kirjavainen et al., 1996). Bäsler *et al.* had also emphasized the role of tight junctions in epidermis (SI8) as absorption barrier that hinders the intradermal drug delivery (Basler et al., 2016). Deli M.A. *et al* reported the phospholipid's ability to reversibly modulate tight junction, by mechanism of redistribution of tight junctional proteins (Deli, 2009). Although there were no studies showing the effect of PG on tight junctions, it is known that PG can enhance drug permeation by interacting with intercellular lipids (Kang et al., 2006).

The proposome composition modulated the deposition of the TC in different skin layers. The higher amount of TC was retained inside skin for proposomes containing 20% PG while lower amount for those containing 40% PG. Limpongsa et al also reported that PG (10% and 20%) can be used as deposition enhancer for finasteride (Limpongsa et al., 2015). The proposome P9 (3, 40) showed the highest amount of TC permeation, while least being retained. In contrast, the transdermal permeation was lower with from formulations with lower SPC, i.e., P7(1,40) and P8 (2,40). In short, the composition of proposome would affect the target of delivery, i.e., 20% PG proposomes for epidermal targeting, 30% PG proposomes for balanced targeting and 40% PG for transdermal targeting. Hence, the proposome containing 30% PG with high ER for dermis, would be most suitable for the intradermal delivery of TC to act on T cells and B-cells.

The greater skin permeation of TC from proposomes than control may be ascribed to the synergism of SPC and PG. The SPC and PG acted as permeation enhancers, possibly by interacting with stratum corneum intercellular lipids and/or tight junctional proteins. Moreover, the bright follicle-like fluorescent structures in skin may suggest that proposomes increased the drug permeation via the hair follicular route (Fig. 6).

PG is a common ingredient, widely used in topical formulations as solvents or skin penetration enhancers. It is a weak contact sensitiser that exhibits very low sensitization risk for normal skin condition (Lessmann et al., 2005). However, the inclusion of PG in topical

formulations together with other techniques, such as ultrasound, could cause inflammation potentially (Horiguchi et al., 2005). In terms of efficacy, PG in topical formulations is not necessarily increasing skin permeation in all scenarios (Lee et al., 2014). Therefore, the skin condition as well as the mode of drug delivery should be considered before PG incorporation.

5. Conclusion

The proposomes are uniform in size with suitable entrapment efficiency to deliver TC into the skin. The SPC and PG synergistically enhance the skin permeation of TC. The enhanced absorption of two fluorescent probes with different log P values suggests proposomes are potentially useful for transdermal delivery of different drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2020.119558.

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