Journal of Liposome Research

http://informahealthcare.com/lpr
ISSN: 0898-2104 (print), 1532-2394 (electronic)

J Liposome Res, 2013; 23(3): 244–254
 DOI: 10.3109/08982104.2013.801489

informa
healthcare

RESEARCH ARTICLE

Transfersomal lyophilized gel of buspirone HCl: formulation, evaluation and statistical optimization

Rehab Nabil Shamma and Ibrahim Elsayed

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Abstract

Context: Buspirone HCl has very low oral bioavailability (4%) due to deactivation by extensive first pass effect. It also has very limited transdermal permeation due to its high hydrophilicity.
Objective: The aim of this study was to increase the transdermal permeation of buspirone HCl utilizing a stable dosage form.
Methods: Transfersomes were prepared using Tween-80 as a flexibility imparting agent to the vesicular walls. Oleic acid and/or ethanol, with different percentages, were utilized as a permeation enhancer. Formulations were characterized by analyzing particle size, polydispersity index, zeta potential, entrapment efficiency, *in vitro* release and *ex vivo* drug permeation. Factorial design (3^2) was planned for the optimization of formulations using Design-Expert® software. Lyophilized transfersomal gel of the optimized formulation was prepared using hydroxypropyl methylcellulose (HPMC) K100, carboxymethyl cellulose or sodium alginate with or without mannitol as a cryoprotectant. Physical characterization of the transfersomes and the lyophilized gel were carried out using transmission and scanning electron microscopy, respectively.
Results: The optimized formulation (T7), containing 35% oleic acid, had the highest desirability value (0.658) with high *ex vivo* drug flux (43.40 μg/h/cm^2) through rat skin when compared with the aqueous drug solution and formula T1 (without oleic acid). The T7 transfersomal gel containing HPMC K100 (G2) had the highest desirability value (0.640) among the lyophilized gel formulations with decreased *ex vivo* drug flux (38.98 μg/h/cm^2) in comparison with the original transfersomal formula (T7).
Conclusions: Lyophilized transfersomal gel containing oleic acid was considered as a promising transdermal delivery system for hydrophilic drugs.

Keywords

Ex vivo, factorial design, permeation, transdermal

History

Received 28 February 2013
Revised 16 April 2013
Accepted 29 April 2013
Published online 28 May 2013

Introduction

Buspirone hydrochloride (BH) is an orally ingested anxiolytic. The drug is used to treat generalized anxiety disorder, anxiety caused by alcohol craving or smoking cessation as well as attention-deficit hyperactivity disorder in children (Cadart et al., 2009). BH oral administration has a number of disadvantages. Although it is rapidly absorbed in the GI, this drug undergoes extensive first-pass metabolism and has a very short elimination half-life (Moffat et al., 2005). On the other hand, the transdermal route offers several biomedical advantages over oral route, including avoidance of first pass metabolism and providence of sustained plasma drug levels (Tiwary et al., 2007). However, BH is a hydrophilic molecule that does not readily permeate human skin and, so far, no transdermal system is commercially available (Meidan et al., 2003; Tsai et al., 2011).

Liposomes were first reported as carriers for transdermal drug delivery in 1980 (Mezei & Gulasekharam, 1980). However, liposomes were shown to be unsuitable vesicular carriers for the penetration of the skin barrier due to its rigidity which hinders their passage though the skin barrier (Cosco et al., 2008). Recently, novel vesicular carriers were designed, including ultradeformable liposomes, ethosomes, binary ethosomes, solid lipid nanoparticles and niosomes (Zhang et al., 2012).

Ultradeformable liposomes (transfersomes) consist of phospholipids, an edge activator that increases deformability of the phospholipid bilayers (Cevc & Blume, 1992). Applied on the skin surface, these elastic vesicles are able to squeeze through intercellular regions of the stratum corneum under the influence of the water-activity gradient. After passage, transfersomes were distributed between the cells via intercellular route, and accumulated in the subcutaneous tissue (Song & Kim, 2006). Moreover, transfersomes can effectively protect the drug against undesired rapid clearance from cutaneous blood vessels increasing the drug circulation time and bioavailability (Cevc et al., 2008). Transfersomes have been used as carriers for various compounds, among which are macromolecules such as various steroids, proteins (Paul et al., 1998), insulin (Cevc et al., 1998), corticosteroids

Address for correspondence: Ibrahim Elsayed, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt. Tel: +202-01149944306. E-mail: hema5th@yahoo.com

(Cevc & Blume, 2004), ketoprofen (Cevc et al., 2008) and anticancer drugs (Hiruta et al., 2006).

Penetration enhancers may be incorporated into formulations in order to improve drug flux through diverse membranes. Ethanol has been used to enhance the flux of levonorgestrel, estradiol, hydro-cortisone and 5-fluorouracil through rat skin (Williams & Barry, 2004). Ethanol can increase permeation through various mechanisms. Firstly, as a solvent, it can increase the drug solubility in the vehicle. Further, permeation of ethanol into the stratum corneum can alter the solubility properties of the tissue with a consequent improvement for drug partitioning into the membrane. Transethosomes (TELs) were prepared and characterized by Song et al. (2012) for enhancing voriconazole penetration through the skin. These nanovesicles contain high content of ethanol together with an edge activator or permeation enhancer.

Percutaneous drug absorption has also been increased by a wide variety of long-chain fatty acids, the most popular of which is oleic acid (Williams & Barry, 2004). Considerable efforts have been directed at investigating the mechanisms of action of oleic acid as a penetration enhancer in human skin. It is clear from numerous literature reports that the enhancer interacts with and modifies the lipid domains of the stratum corneum, as would be expected for a long-chain fatty acid with a *cis*-configuration (Ongpipattanakul et al., 1991).

The aim of this work is to study the effect of using different types, and concentrations of permeation enhancers on transfersomal vesicle characteristics employing a full factorial design. In addition, overcoming the instability issues related to the colloidal vesicles through formulation of easily reconstituted lyophilized transfersomal gel containing accurate divided doses.

Materials and methods

Materials

BH was kindly donated by GlaxoSmithKline (Cairo, Egypt). L-α-Phosphatidylcholine from egg yolk (PP), ethanol, acetonitrile (HPLC grade), chloroform, Tween-80, oleic acid, hydroxypropyl methylcellulose K100 (HPMC K100), sodium carboxymethyl cellulose (CMC) and sodium alginate were obtained from Sigma-Aldrich (St. Louis, MO). Ammonium acetate, potassium dihydrogen phosphate and disodium hydrogen phosphate were supplied by SISCO Research Laboratories Pvt. Ltd. (Mumbai, India).

Methods

Preparation of BH transfersomal vesicles

Transfersomal formulations of BH were prepared according to the thin-layer evaporation technique (Bangham et al., 1965). L-α-PP and Tween-80 were used in the molar ratio 5:1 to which a constant weight of drug (20 mg) was added. Oleic acid was incorporated in some formulations as a transdermal permeation enhancer, as shown in Table 1. The ingredients were dissolved in 15 mL chloroform/methanol mixture in the ratio of 2:1 v/v, respectively, using bath sonicator (Ultrasonic bath sonicator, Model SH 150-41, PCI Analytics Pvt. Ltd, Mumbai, India) for 2 min The resulting solution was then transferred into a 250 mL round-bottomed flask and the organic solvent was slowly evaporated under reduced pressure using a rotary evaporator (Rotavapor, Heidolph VV 2000, Burladingen, Germany) for 5 min at 60 °C. A thin dry film was formed on the inner wall of the rotating flask. The dried film was hydrated with 15 mL distilled water or hydro-alcoholic solution by rotation for 1 h at 60 °C. The formed vesicles were sonicated for 1 min in a bath sonicator for size reduction (Mishra et al., 2007).

Table 1. Full factorial design used to optimize the transferosomal formulations.

	Level		
	−1	0	1
Factors (independent variables)			
X_1: Concentration of oleic acid (%)	0	17.5	35
X_2: Concentration of ethanol (%)	0	15	30
Response (dependent variables)		Constraints	
Y_1: Particle size		Minimize	
Y_2: Polydispersity index		Minimize	
Y_2: Zeta potential		Maximize	
Y_3: Entrapment efficiency		Maximize	

Characterization of BH transfersomal vesicles

Determination of transfersomal particle size (PS), polydispersity index (PDI) and zeta potential (ZP). The mean PS, PDI and ZP were determined by photon correlation spectroscopy with a Malvern Zetasizer Nano ZS at an angle of 90° in 10 mm diameter cells at 25 °C (Malvern Instrument Ltd., Worcestershire, UK). Before measurement, the formulation was diluted appropriately with double-distilled water to have a suitable scattering intensity. The PDI was used as an indication for the vesicle size distribution. The ZP of the vesicles was determined by observing their electrophoretic mobility in an electrical field.

Determination of BH entrapment efficiency. Transfersomal vesicles were separated from the un-entrapped BH by centrifugation at 15 000 rpm for 2 h at 4 °C. Concentrations of BH in the supernatant solutions were determined spectrophotometrically at the predetermined λ_{max} (240 nm).

Drug entrapment efficiency was determined according to the following equation (Priyanka & Sathali, 2012):

$$\%EE = \frac{TD - FD}{TD} \times 100,$$

where %EE is the percentage of drug entrapped, FD is the amount of free drug and TD is the amount of total drug.

Study on the influence of different permeation enhancers using 3^2 full factorial experimental design

A 3^2 full factorial experimental design was employed in order to investigate the joint influence of formulation variables on the vesicle characteristics using Design-Expert® 8 software (Stat-Ease, Inc., Minneapolis, MN). In this design, two factors were evaluated, each at three levels and experimental trials are performed at all nine possible combinations. The independent variables were concentration of oleic acid (X_1) and ethanol (X_2). The PS (Y_1), PDI (Y_2), ZP (Y_3) and %EE (Y_4)

Table 2. Experimental runs, independent variables, and measured responses of the 3^2 full factorial experimental design of the transferosomal formulations.

Runs	X_1: Concentration of oleic acid (%)	X_2: Concentration of ethanol (%)	Y_1: PS (nm)	Y_2: PDI	Y_3: ZP (mV)	Y_4: %EE
T1	0	0	283.20 ± 10.94	0.48 ± 0.03	−67.10 ± 1.17	29.01 ± 5.54
T2	0	15	415.40 ± 6.46	0.44 ± 0.05	−57.70 ± 2.28	14.21 ± 1.89
T3	0	30	506.30 ± 10.78	0.52 ± 0.06	−55.70 ± 3.39	16.05 ± 1.51
T4	17.5	0	542.30 ±3.23	0.48 ± 0.06	−59.80 ± 2.62	44.73 ± 2.21
T5	17.5	15	612.00 ± 9.60	0.47 ± 0.06	−56.00 ± 4.91	38.85 ± 2.19
T6	17.5	30	519.40 ± 7.63	0.37 ± 0.05	−59.30 ± 5.04	21.67 ± 1.75
T7	35	0	327.50 ± 2.90	0.46 ± 0.02	−59.10 ± 5.31	46.82 ± 3.31
T8	35	15	497.30 ± 7.17	0.27 ± 0.03	−57.40 ± 1.78	45.56 ± 4.65
T9	35	30	227.10 ± 11.32	0.19 ± 0.06	−55.70 ± 4.53	4.58 ± 0.37

were selected as the dependent variables. Table 2 depicts the composition of the prepared formulations.

In vitro *drug release*

The release of BH from transfersomal nanovesicles was performed using the *in vitro* dialyzing method (Franz diffusion cells with hydrophilic membrane of regenerated cellulose; Dialysis tubing D-0530; Sigma) at 37 °C, 100 rpm, for 8 h (Song et al., 2012). A weighed amount of prepared transfersomal suspension, equivalent to 2 mg drug, was poured into the donor cell and dialyzed against 100 mL phosphate buffer pH 7.4 as a dialyzing medium. Drug aqueous solution was considered as a control (Paolino et al., 2012). Aliquots were taken at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h time intervals and analyzed for drug content at the predetermined λ_{max}. Experiments were repeated three times and the results were expressed as the mean values ± SD. The *in vitro* release data were analyzed according to the equations of zero order, first order and Higuchi diffusion.

The release half-life was calculated and statistically analyzed using one-way analysis of variance (ANOVA) to test the significance of difference at $p \leq 0.05$. A subsequent Tukey honestly significant difference test was also performed. SPSS 15.0 software (SPSS, Inc., Chicago, IL) was used to carry out these statistical analyses.

Ex vivo *drug permeation*

The same procedure as in the *in vitro* release studies was carried out except replacing the cellulose membrane with mice skin (Song et al., 2012). Fresh hairless skin specimens of newly born mice were used with the epidermis side facing the donor compartment. For each formulation, drug permeation through hairless mouse skin was studied in triplicate. The quantitative determination of drug in *ex vivo* skin permeation study was performed by HPLC (Shimadzu, Tokyo, Japan) using acetonitrile:water:ammonium acetate (61:37:2) adjusted to pH 7 using acetic acid as mobile phase delivered at 1 mL/min. The column was a reversed-phase column (Nucleosil C18, PS, 10 mL, 250 mm × 4.6 mm, Germany). The detector was an ultraviolet variable wavelength detector (Model SPD-10 A; Shimadzu). The detection wavelength was 240 nm. Peak areas were determined with C-R6A chromatopac integrator (Shimadzu). This method had been validated. All assays were performed at ambient conditions.

Table 3. Full factorial design used to optimize the transfersomal gel formulations.

	Level		
	−1	0	1
Factors (independent variables)			
X_1: Type of gel forming polymer	HPMC	CMC	Na alginate
X_2: Concentration of mannitol (%)	0		5
Response (dependent variables)		Constraints	
Y_1: Particle size		Minimize	
Y_2: Polydispersity index		Minimize	
Y_2: Zeta potential		Maximize	
Y_3: Entrapment efficiency		Maximize	

The cumulative amount of drug permeated through the skin per unit area (μg/cm^2) was plotted as a function of time (h) for each formulation. The flux (J) at 8 h was calculated from the following equation (El Zaafarany et al., 2010):

$$J = \frac{\text{Amount of permeated drug}}{\text{Time} \times \text{Area of the membrane}}$$

The difference in flux values were statistically analyzed using one-way ANOVA with subsequent Tukey honestly significant difference test.

Transmission electron microscopy

The morphologic examination of the optimum formulation was performed by transmission electron microscopy (TEM) operating at 100 kV (model JEM-1230; Jeol, Tokyo, Japan). One drop of the diluted vesicular dispersion was deposited on the surface of a carbon-coated copper grid, negatively stained with 1% phosphotungstic acid then allowed to dry at room temperature for 10 min for investigation by TEM (Mishra et al., 2007).

Preparation of transfersomal lyophilized gel

The optimized transfersomal formulation was incorporated into a 1% aqueous solution of three gelling agents; namely HPMC K100, CMC or sodium alginate with or without the incorporation of 1% mannitol as a cryoprotectant, as shown in Table 3 (Chen et al., 2010). The formed gel (2.5 mL) was poured into the holes of a PVC blister of diameter 2 cm, frozen at −20 °C, and subsequently freeze-dried for 24 h at −45 °C and a pressure of 7×10^{-2} mbar for 24 h using a

Table 4. Experimental runs, independent variables and measured responses of the 3×2 full factorial experimental design of the transfersomal gel formulations.

Formula	Gelling agent	Mannitol	PS (nm)	PDI	Zeta potential (mV)	%EE
G1	HPMC	0	475.80 ± 4.05	0.45 ± 0.03	-50.80 ± 3.64	70.60 ± 1.52
G2	HPMC	5	514.70 ± 8.52	0.36 ± 0.02	-56.50 ± 2.40	80.38 ± 2.45
G3	CMC	0	446.40 ± 10.81	0.45 ± 0.06	-77.30 ± 1.99	73.75 ± 1.51
G4	CMC	5	675.80 ± 3.34	0.45 ± 0.02	-77.80 ± 4.15	84.88 ± 3.71
G5	Na alginate	0	476.70 ± 2.25	0.83 ± 0.02	-82.90 ± 2.67	82.15 ± 2.39
G6	Na alginate	5	795.90 ± 5.91	0.83 ± 0.04	-90.60 ± 4.37	91.06 ± 2.97

Table 5. Output data of the 3^2 factorial analysis of the transfersomal formulations.

Responses	R^2	Adjusted R^2	Predicted R^2	Adequate precision	Significant factors
PS (nm)	0.7707	0.6943	0.5763	7.95	B
PDI	0.9009	0.8414	0.8307	11.15	A, B and AB
ZP (mV)	0.4222	0.0755	−1.8800	3.36	–
%EE	0.5175	0.4485	0.2693	4.75	B

freeze-dryer (Novalyphe-NL 500; Savant Instruments Corp., Hicksville, NY) (Li et al., 2009).

Evaluation of transfersomal lyophilized gel

Each lyophilized gel disc was reconstituted in 2.5 mL distilled water and tested for PS, PDI, ZP, %EE and *ex vivo* permeation studies as mentioned before. One-way ANOVA with subsequent Tukey test was imparted to compare the reconstituted gels with the original formula (T7) using SPSS® 17 software (SPSS, Inc.). On the other hand, a $3^1.2^1$ full factorial experimental design was employed in order to investigate the joint influence of formulation variables on the lyophilized gel characteristics using Design-Expert® 8 software. The independent variables were concentration of type of the gel forming polymer (X_1) and concentration of mannitol (X_2). The PS (Y_1), PDI (Y_2), ZP (Y_3) and %EE (Y_4) were selected as the dependent variables. Table 4 depicts the composition of the prepared formulations.

Scanning electron microscopy (SEM)

The surface characteristics of a lyophilized transfersomal gel sample were observed using a scanning electron microscope (JXA-840; JEOL). The sample was gold-coated under vacuum and then examined (Yehia et al., 2012).

Results and discussion

Analysis of 3^2 factorial design

The factorial design, a commonly used statistical approach for planning and optimization of experimental series, was used. The used design comprises a full 3^2 factorial design with statistical analysis through Design-Expert® Software. The predicted R^2 values were in a reasonable agreement with the adjusted R^2 in all responses except the ZP (Table 5). The latter had a negative predicted R^2 value implying that the overall mean was a better predictor of the response (Basalious et al., 2011; Quinten et al., 2009). Adequate precision measured the signal-to-noise ratio to ensure that the model can be used to navigate the design space (de Lima et al., 2011). A ratio greater than 4 (the desirable value) was observed in all responses, except ZP.

The effect of formulation variables on the PS and PDI

Transfersomal vesicles prepared using three concentrations of permeation enhancers showed a PS ranging from 280 to 620 nm. Figure 1(a) shows the effect of oleic acid (X_1) and ethanol (X_2) concentrations on the PS of BH transfersomal formulations. Increasing the concentration of ethanol resulted in a significant increase in PS ($p = 0.0052$). This might be due to the liposomal aggregation and fusion induced by ethanol incorporation (Komatsu & Okada, 1995). These results are in contrast with the findings of other authors, who concluded a generalized reduction in the mean vesicle diameters due to the presence of ethanol in the vesicles (Ainbinder & Touitou, 2005; Lopez-Pinto et al., 2005; Paolino et al., 2005). Conversely, increasing oleic acid concentration decreased the PS but with no significant difference ($p = 0.2313$). Similar results were also observed upon oleic acid incorporation as a penetration enhancer in the preparation of voriconazole TELs (Song et al., 2012).

The PDI of all measured formulations was acceptable at values <0.5, thus indicating a narrow size distribution of the measured formulations and consequently a homogenous distribution, except formula T3 ($PDI = 0.52 \pm 0.06$), as shown in Table 2 (Essa et al., 2002). Table 5 shows that oleic acid, ethanol concentrations and their interaction had a significant effect on the PDI ($p = 0.005$, 0.024 and 0.0168, respectively). The lowest PDI value was observed at the combination of oleic acid and ethanol high concentrations (0.198) as shown in Figure 1(b).

The effect of formulation variables on ZP

The surface-charge properties of the prepared formulations were investigated and results showed negative charges on their surfaces with ZP values ranging from −55.7 to −67.1 mV (Table 2 and Figure 1c). The ZP findings

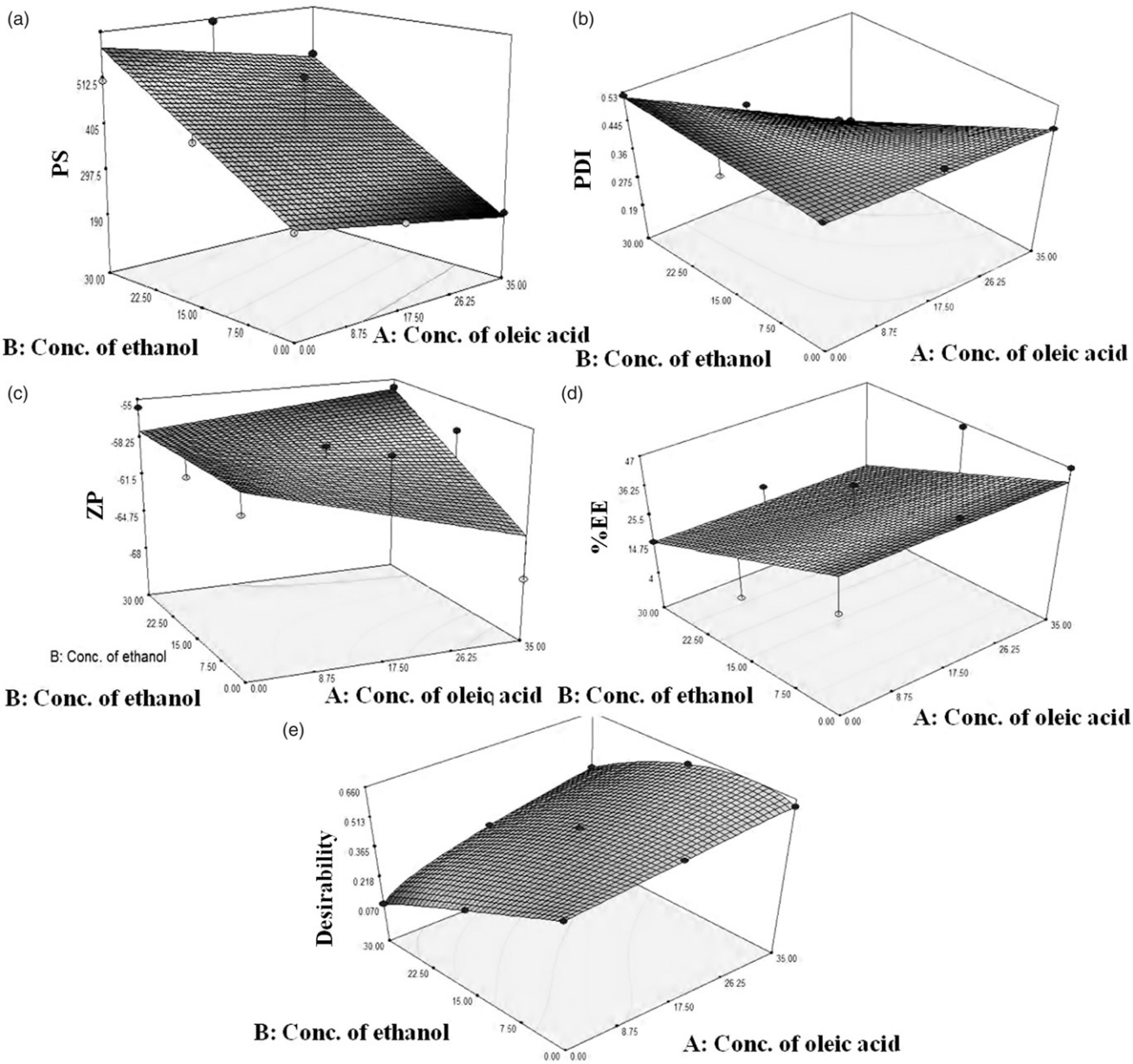

Figure 1. Response surface plot for the effect of oleic acid (X_1) and ethanol (X_2) concentrations on the particle size (a), polydispersity index (b), zeta potential (c), entrapment efficiency percentage (d) and desirability (e) of BS transfersomal formulae.

showed a good degree of stability for all the prepared formulations (Yu & Xie, 2012).

The effect of formulation variables on the %EE

The %EE of all transfersomal vesicles investigated ranged from 4.57% to 46.82% as shown in Table 2. A significant impact of the ethanol concentration on the %EE of BH-transfersomal formulation was demonstrated. It was observed that increasing ethanol concentration resulted in a significant decrease in the %EE of BH-transfersomal formulations ($p = 0.0289$), as shown in Figure 1(d). This may be attributed to the increase in the permeability of the phospholipids bilayer by ethanol, which promoted leakage of the encapsulated drug (Komatsu & Okada, 1997). However, changing the concentration of oleic acid has a non-significant effect on the %EE ($p = 0.2083$). This was in contrast with the previously reported significant decrease in the PS upon incorporation of oleic acid by Song et al. (2012).

Optimization BH – transfersomal formulations

BH – transfersomal formulations were optimized for the responses Y_1 (PS), Y_2 (ZP), Y_3 (PDI) and Y_4 (%EE). The aim was to minimize PS and PDI and maximize ZP and entrapment efficiency. The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert® 8 software and based on the criterion of desirability (Basalious et al., 2010). The optimized BH – transfersomal formula (T7), composed of 35% oleic acid as a permeation enhancer, had the highest desirability value (0.658) as shown in Figure 1(e).

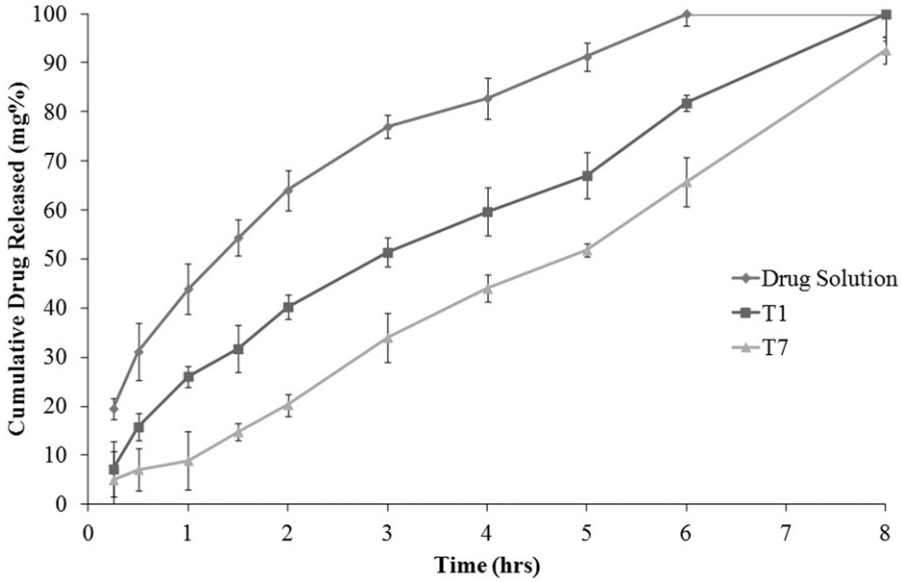

Figure 2. Release profile of BH through cellophane membrane from the drug solution, transfersomal formula containing no permeation enhancer (T1) and the corresponding formula containing oleic acid (T7).

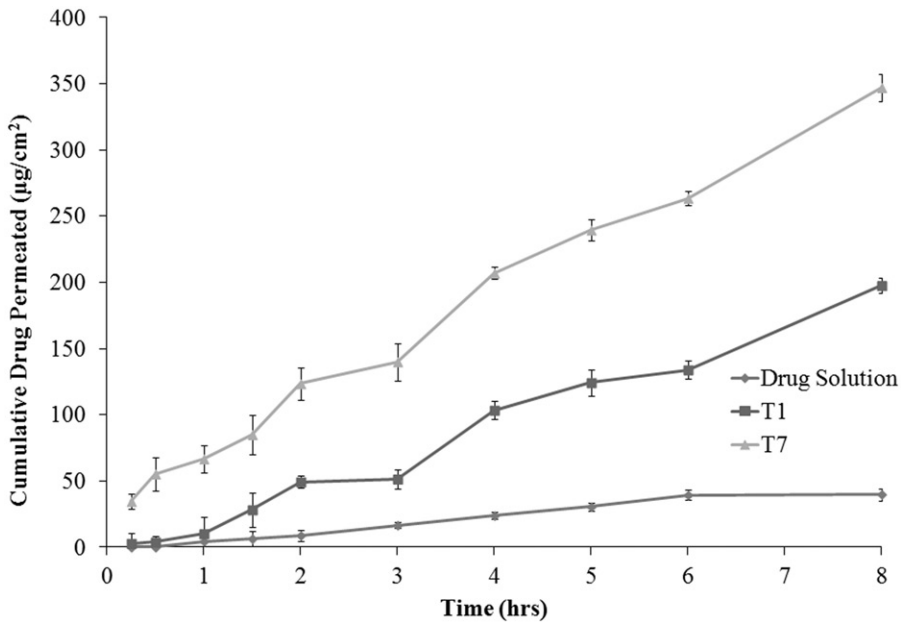

Figure 3. Permeation profile of BH through rat skin from the drug solution, transfersomal formula containing no permeation enhancer (T1) and the corresponding formula containing oleic acid (T7).

In vitro drug release

In vitro release rates are important indicators for the quality of the transdermal drug delivery system. Artificial cellophane membrane was used for studying the composition effect of the prepared transfersomal formulations. The optimized formula (T7) underwent *in vitro* release study and compared with T1 formula, containing no permeation enhancer, and the drug solution (as a control).

The drug took about 6 h to be completely diffused from its solution, as shown in Figure 2. This might be due to the stagnant donor cell model utilized in the study (Tanojo et al., 1997). Moreover, transfersomes decreased the rate of the drug release. The release half-life ($t_{50\%}$) increased significantly from 1.11 h, in the case of drug solution, to be 2.06 h, in the case of T1 ($p<0.001$). It might be due to the lipid bilayer which controlled the release of the entrapped drug (Jukanti et al., 2011; Mishra et al., 2007). The mathematical modeling of the data revealed that the drug release from transfersomal formulation T1 and the drug solution followed diffusion controlled kinetics (higher R^2 values). Similar results were previously reported by Jukanti et al. (2011) who studied the drug release models from proliposomes through artificial cellulose membrane fitted to Franz diffusion cell.

On the other hand, oleic acid in T7 led to further sustainment of the drug release ($t_{50\%}=4.79$ h, $p<0.001$) and

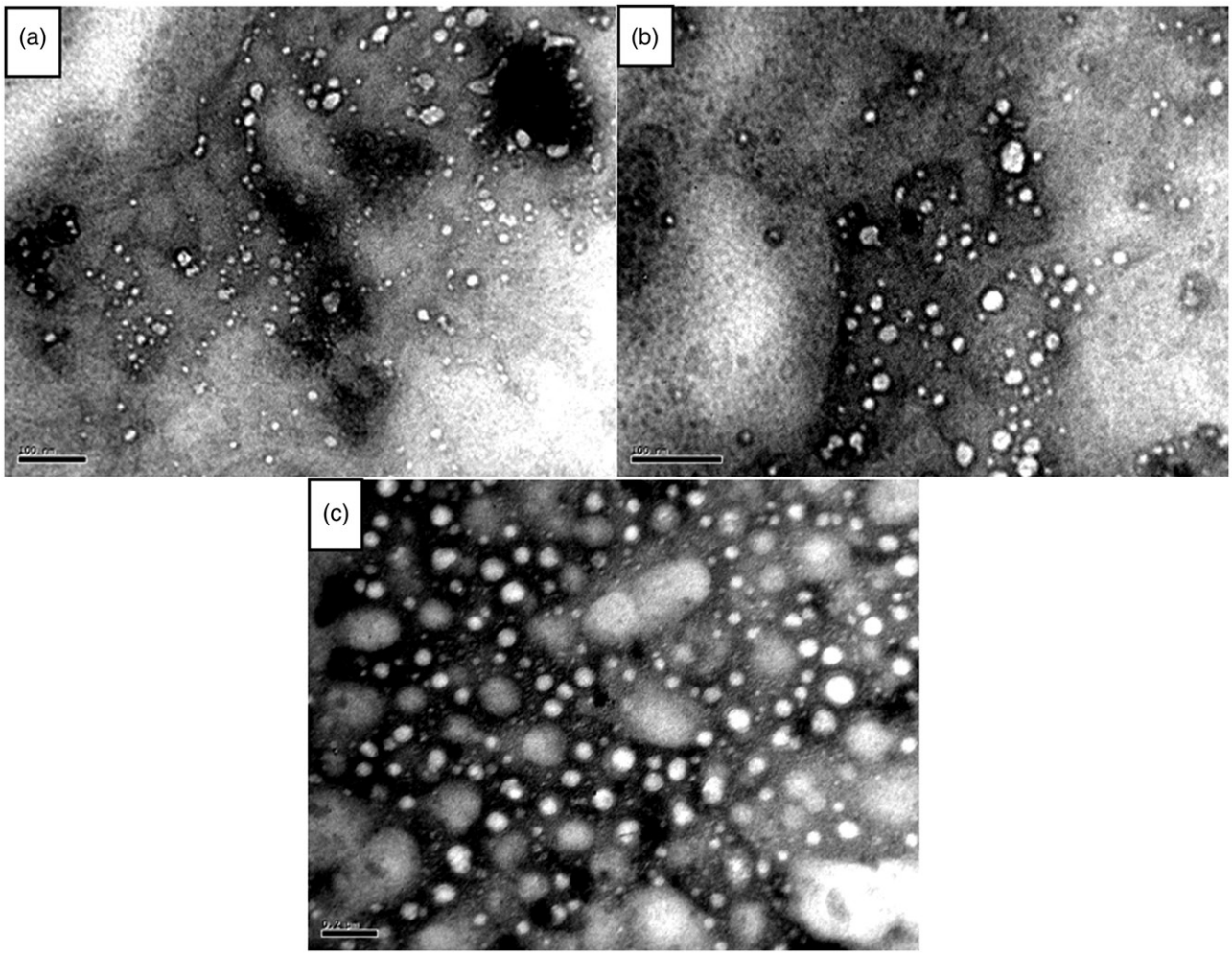

Figure 4. Transmission electron micrographs of the optimized transfersomal formula (T7).

Table 6. Output data of the $3^1.2^1$ factorial analysis of the transfersomal gel formulations.

Responses	R^2	Adjusted R^2	Predicted R^2	Adequate precision	Significant factors
PS (nm)	0.7908	0.4770	−0.8827	4.08	–
PDI	0.9880	0.9701	0.8924	15.32	A
ZP (mV)	0.9886	0.9715	0.8976	17.58	A
%EE	0.9955	0.9887	0.9593	32.60	A and B

zero-order mechanism ($R^2 = 0.9908$) but with a decrease in the release extent (92.56%). It might be due to the high hydrophobicity of oleic acid (Rowe et al., 2009).

Ex vivo drug permeation

The drug solution showed very low skin permeation ability (Figure 3), with a drug flux of 4.99 µg/h/cm^2. The drug flux increased significantly to 24.69 µg/h/cm^2 upon encapsulation in the transfersomal formulation T1 ($p < 0.001$). It might be due to Tween-80 which rendered the transfersomal wall flexible and prevented its rupture in the skin (Malakar et al., 2012). Nonionic surfactant vesicles have previously been reported as a successful carrier for transdermal delivery of furosemide with enhanced permeation (Azeem et al., 2008).

The incorporation of oleic acid as a permeation enhancer further increased the drug flux up to 43.40 µg/h/cm^2 ($p < 0.001$). It might be due to the disruption of the stratum corneum lipid domain (Williams & Barry, 2004). Moreover, the presence of unsaturated fatty acids, such as oleic acid (Cho & Gwak, 2004), in the vesicle structure could increase the deformability features of the carriers (Celia et al., 2012; Rowat et al., 2006). Oleic acid is known to intercalate between the liposomal bilayers leading to a decreased phase transition temperature of skin lipids as well as an increase in their fluidity (Song et al., 2012). This could explain the improved skin delivery of drugs from liposomes containing oleic acid (El Maghraby et al., 2004). It was previously reported that oleic acid increased the flux of salicylic acid 28-fold and 5-flourouracil flux 56-fold through human skin membrane *in vitro* (Goodman & Barry, 1989).

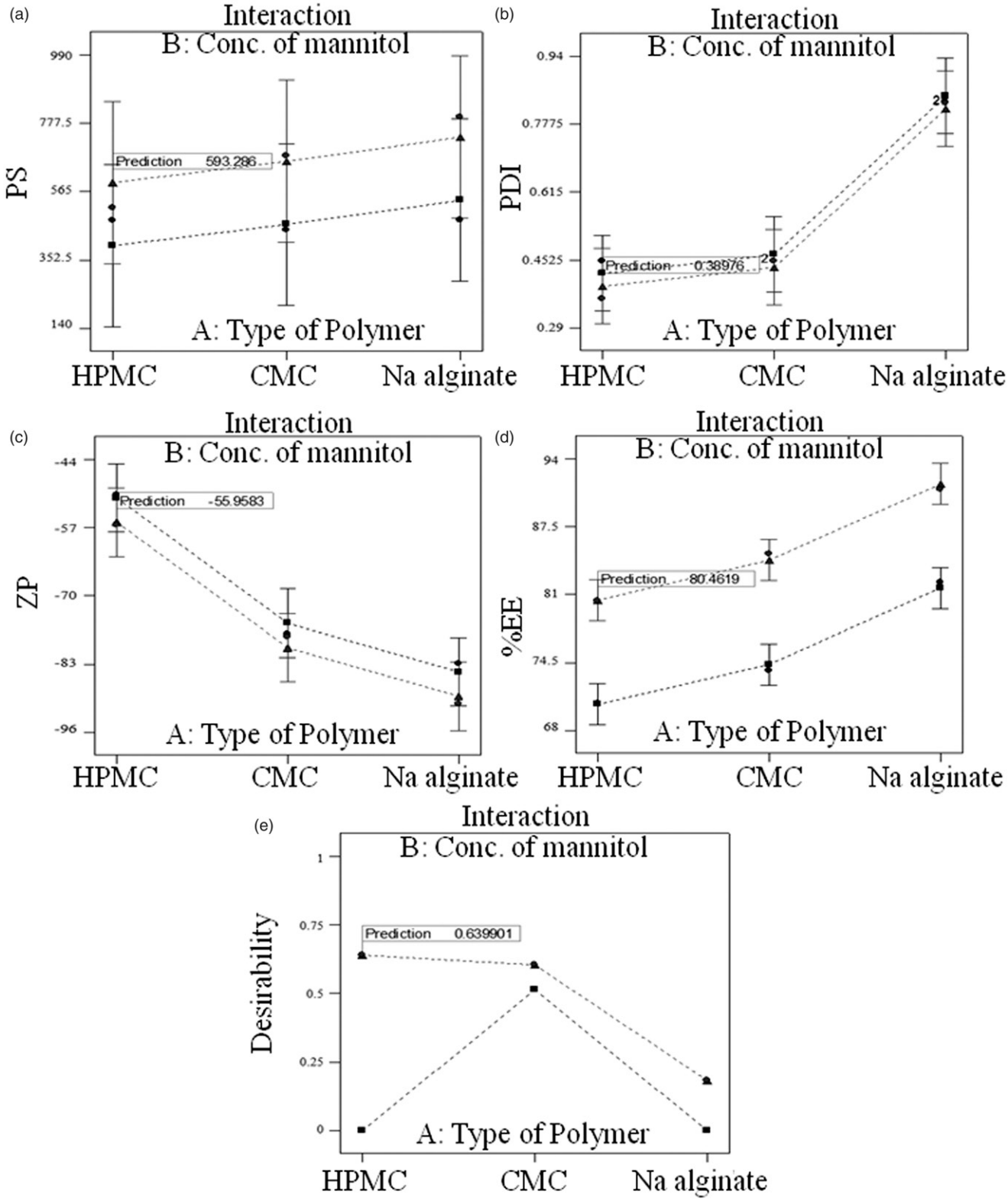

Figure 5. Interaction plot for the effect of the gel forming polymer (X_1) concentration of mannitol (X_2) on the particle size (a), polydispersity index (b), zeta potential (c), encapsulation efficiency percentage (d) and desirability (e) of the lyophilized transfersomal gel.

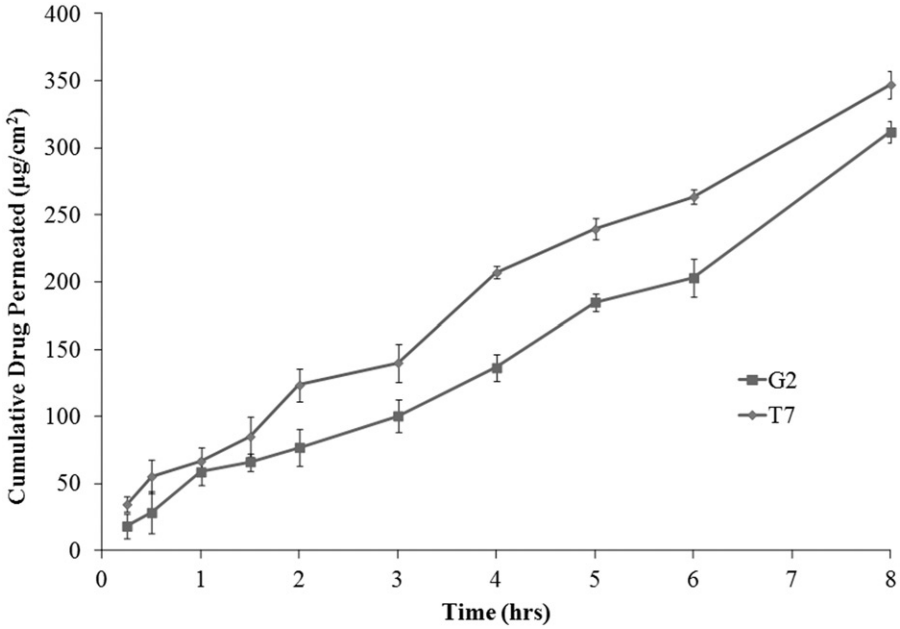

Figure 6. Permeation profile of BH through rat skin from transfersomal formula (T7) and the corresponding lyophilized gel after reconstitution (G2).

Transmission electron microscopy

The morphology of the optimized formulation was observed using TEM. The TEM micrographs showed vesicles with uniform spherical shape. The vesicles appeared non-aggregated characterized by smooth surface with narrow size distribution (Figure 4).

Characterization of the lyophilized transfersomal gel

Freeze-drying may generate many stresses that could destabilize colloidal nanosuspension, especially, the stress of freezing and dehydration. The most popular cryoprotectants encountered in the literature for freeze-drying nanoparticles are sugars, such as trehalose, sucrose, glucose and mannitol. Mannitol was used as cryoprotectant in this study. The immobilization of nanovesicles within a glassy matrix of cryoprotectant can prevent their aggregation and protect them against the mechanical stress of ice crystals (Abdelwahed et al., 2006).

After reconstitution of the lyophilized gel, PS of the transfersomes significantly increased in comparison with the original formula (T7), nevertheless the polymer used ($p < 0.001$), as shown in Table 4. This could be attributed to the coating of transfersomes with the hydrophilic polymers during freeze-drying. This result is in accordance with the previously reported results by Takeuchi et al. (1999, 2000, 2001) upon characterization of the polyvinyl alcohol-coated liposomes.

PDI was not significantly changed in the case of HPMC and CMC ($p > 0.05$), whereas a significant increase was observed upon the use of Na alginate as gelling agent ($p < 0.001$).

Zeta potential increased significantly ($p < 0.001$) in the case of CMC and Na alginate polymers to be -77.3 and -82.9 mV, respectively, whereas there was no significant change in the ZP upon use of HPMC polymer ($p = 0.410$). This might be due to that the negative charge carried on Na Alginate and CMC polymers (Rowe et al., 2009).

Upon analysis of the factorial design, it was observed that the predicted R^2 values were in a reasonable agreement with the adjusted R^2 in all responses except the PS (Table 6). The latter had a negative predicted R^2 value implying that the overall mean was a better predictor of the response (Basalious et al., 2011; Quinten et al., 2009). Adequate precision measured the signal-to-noise ratio to ensure that the model can be used to navigate the design space (de Lima et al., 2011). A ratio greater than 4 (the desirable value) was observed in all responses.

Type of the gel forming polymer and concentration of mannitol had no significant effect on the PS of the reconstituted vesicles ($p = 0.5070$ and 0.1413, respectively). PDI and ZP were significantly affected by type of polymer only ($p = 0.0120$ and 0.0117, respectively). On the other hand, the two studied factors significantly affected the entrapment efficiency ($p = 0.0097$ and 0.0042, respectively). The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert® 8 software and based on the criterion of desirability (Basalious et al., 2010). The optimized transfersomal gel (G2) had the highest desirability value (0.640) as shown in Figure 5.

Ex vivo permeation was performed for the optimized reconstituted gel (G2), as shown in Figure 6, and it was observed that the drug flux through the rat skin decreased from 43.40 to 38.98 µg/h/cm^2. This might be due to the presence of viscosity imparting polymer which hindered the drug release and diffusion.

Scanning electron microscopy

Scanning electron micrographs of the lyophilized HPMC transfersomal gel formula are shown in Figure 7. The micrographs show the highly porous nature of the prepared lyophilized transfersomal gel formulation. The highly porous nature of the transfersomal gel formulation explains the rapid penetration of water, which results in rapid reconstitution of the transfersomal gel.

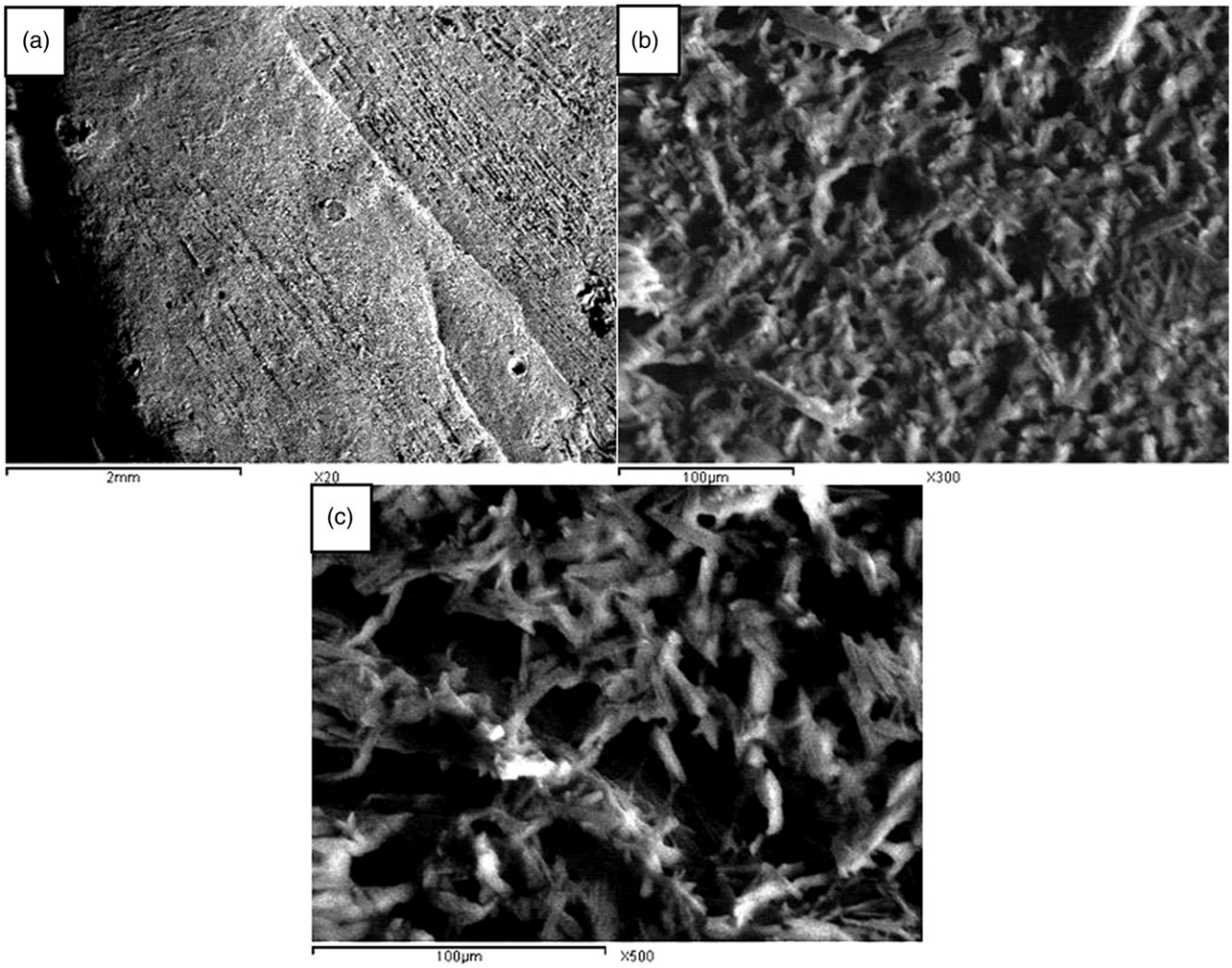

Figure 7. Scanning electron micrographs of the optimized lyophilized gel formula (G2).

Conclusions

It is a challenge to permeate a highly hydrophilic drug, such as buspirone HCl through the lipophilic skin barriers. Transfersomes, as highly deformable vesicles, succeeded to increase the drug permeation with further improvement in the incorporation of oleic acid as a permeation enhancer. Easily reconstituted lyophilized transfersomal gel achieved a physically stable multiple unit transdermal dosage form allowing an accurate dosing for the potent hydrophilic drugs.

Declaration of interest

The authors declare conflicts of interest.

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