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Development and evaluation of ultradeformable vesicles loaded transdermal film of boswellic acid

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Abstract

Background: Boswellic acid (BA), a phytoconstituent obtained from Boswellia serrata, suffers from several limitations after oral administration such as poor systemic absorption, high first-pass metabolism and high frequency of dose requirement, which creates a need to develop an alternative route for drug administration via novel drug delivery formulation. The present research work aims at developing ultradeformable vesicular carriers (transferosomes) for transdermal delivery of boswellic acid to effectively deliver the drug into deeper layers of the skin reaching the target site and thus improving its systemic bioavailability. Ultradeformable vesicles were prepared by thin-film hydration technique, and the formulation was optimized using 3^2 full factorial design where the amount of lecithin (mg) and concentration of surfactant (%) were considered as independent variables. The formulated boswellic acid-loaded vesicles were incorporated into transdermal film via solvent evaporation technique using the blend of polymers such as starch and HPMC K4M.

Results: The BA-loaded transferosomes were optimized based on vesicle size (nm) and drug entrapment efficiency (%EE), and the results were found to be 205.4 ± 1.215 nm and 86.39 ± 0.019%, respectively. Transmission electron microscopy (TEM) of optimized batch showed spherical shape of vesicles with identified lamellarity, surface charge of vesicles with high negative value − 15.2 mV that suggests electrostatic repulsion between vesicles, while the formulation showed good deformability index of 11.31 ± 0.032% due to use of Tween 80 as surfactant. In vitro permeation study demonstrated sustained release pattern of 96.53 ± 0.023% up to 24 h. Also, the in vitro drug diffusion study was carried out for transfersomal transdermal film which exhibited enhanced permeation and sustained retention of drug up to 94.71 ± 0.019% for 24 h.

Conclusion: Accordingly, the research work suggested that the transferosomes provided an efficient nanosized carriers for enhanced permeation of boswellic acid into deeper layers of skin and could successfully exhibit its therapeutic effect.

Keywords: Boswellic acid, Ultradeformable vesicles, Transdermal film, Deformability index, Edge activator, 3^2 Full factorial design, Characterization

Background

The skin is the largest protective barrier of human body, which acts as a major route for non-invasive delivery of therapeutic drugs [1]. Dermal and transdermal administration has significant influence for delivering a wide range of drugs through skin. However, human skin is the most complex organ, which limits the permeation of many macromolecules due to its barrier properties

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[2]. Transdermal drug delivery is the most non-invasive route for delivery of drug into the systemic circulation that offers many advantages as compared to conventional route of administration such as better alternative to achieve constant plasma levels for prolonged periods of time, which additionally could be advantageous because of less frequent dosing regimens, avoids hepatic first pass metabolism, increased patient compliance, predictable and extended duration of activity, minimum adverse effects and utility of short half-life drugs [1].

Many novel drug delivery systems have been developed to improve passage of drugs through skin such as active (microneedles, iontophoresis [3] and electroporation) and passive (penetration enhancers and drug delivery carriers) techniques [4] The most isolating method is the use of vesicular drug encapsulating system for dermal and transdermal delivery. Conventional approaches in vesicular drug delivery suggest liposomes and niosomes to be highly superior carriers for translation of drug into skin [5]. However, several studies also demonstrate the drawbacks of these conventional carriers, including their poor encapsulation capacity, drug leakage, inability to penetrate deeper layers of the skin due to their confinement, and reduced ability to increase systemic drug absorption [6]. Among these strategies, to overcome the major obstacle of penetration and drug loss, ultradeformable vesicles (transferosomes) appear promising as a novel vesicular drug carrier system. Transfersomes are a type of deformable or elastic vesicle that were initially discovered in the early 1990s [7]. According to several studies, when applied to an open biological barrier under nonocclusive conditions, they can permeate intact skin and transport drugs in therapeutic concentrations [8]. The transferosome is a highly adaptive and self-optimized elastic vesicle that can permeate through intact skin membranes by deforming along the stratum corneum barrier, delivering the medication at high concentrations in the deepest layers of the skin and even into the systemic circulation [9]. By adding the right surface-active substances, or more precisely "Edge activators," in the right proportions based on their nature, transferosomal membrane flexibility is achieved. When applied under nonocclusive conditions, the wonderful attribute of membrane elasticity reduces the chance of vesicle rupture in the skin, and it permits transfersomes to follow and maintain the natural water gradient throughout the epidermis [10].

Boswellic acid, a natural mixture of pentacyclic triterpene acids: beta-boswellic acid, 3-acetyl-beta-boswellic acid, 11-keto beta-boswellic acid and 3-acetyl-11-keto beta-boswellic acid, is isolated from oleo gum resin plant 'Boswellia Serrata' [11]. These constituents are widely effective for their use as anti-inflammatory agents; however, pharmacokinetic study has evidenced poor systemic absorption of boswellic acids due to their highly lipophilic nature and very little aqueous solubility [12]. Therefore, oral bioavailability is the major hurdle for translation of drug into strong therapeutic activity, resulting in high first-pass metabolism, high dosing frequency and adverse effects such as abdominal pain, nausea, epigastric pain and hyperacidity. To overcome above limitation of oral delivery, transdermal delivery of boswellic acids seems to be a preferred route rather than conventional dosage form, which could provide sustained and constant plasma level and reduce the frequency of drug administration.

Many studies have demonstrated that molecules with intermediate lipophilicity (log P value o/w of 2–3) can permeate via both lipid and polar environment of skin. In case of boswellic acid (log *P* value 8), they readily permeate through hydrophobic stratum corneum, but lower epidermal and dermal layers are hydrophilic in nature which limits transfer of drug into systemic circulation [13]. So poor permeability can also cause a major problem, which in turn proves the classification of Boswellic acid into BCS Class IV [14]. The current study's objectives were to create "Transferosomes" of Boswellic acid using a 32 full factorial statistical design, incorporate a drug-loaded vesicular dispersion into a transdermal film formulation using the appropriate methodology, and perform various evaluation and characterization studies on the resulting formulation. This approach would be an attractive pharmaceutical strategy to effectively deliver drugs at the target site, which may enhance permeability and bioavailability along with improving circulation time in blood and drug release.

Methods

Materials

Boswellic acid was obtained as a gift sample from Pharmanza Herbal Pvt. Limited, Anand, Gujarat. Lecithin was procured from The Lipid Company, India. Tween 80 (surfactant) was provided by Chemdyes Corporation, Rajkot, Gujarat. All other excipients used were of analytical grade.

Preformulation studies

Drug excipient compatibility study

By Fourier transform infrared spectroscopy, compatible interaction in between drug and additives was performed by using Jasco FTIR spectrophotometer, in the spectral range of 400–4000 cm−1. Samples were analysed and compared with the standard FTIR spectrum.

Solubility study of drug and lecithin in various solvents

Quantitative method was used to perform solubility study of boswellic acid and lecithin in different solvents.

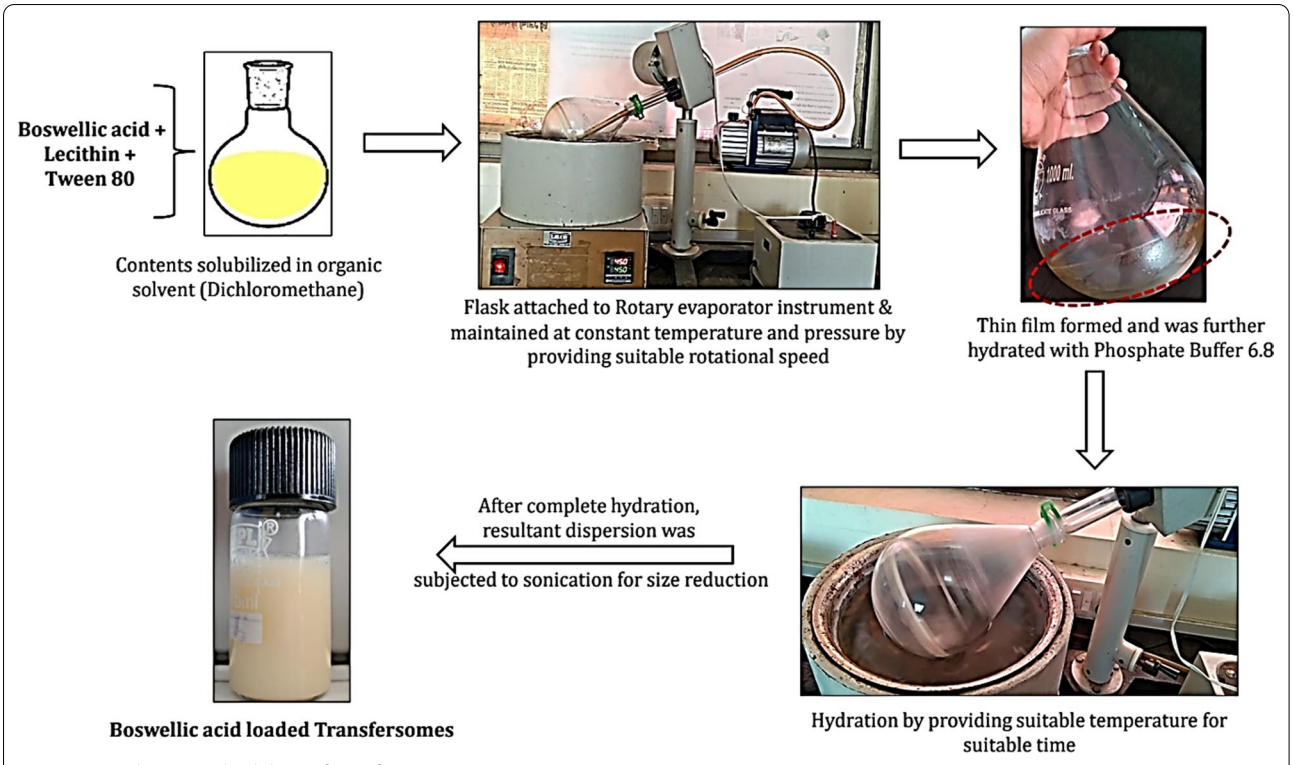

Fig. 1 Formulation methodology of transferosomes

Fixed 1 millilitre of solvents was added in different test tubes and labelled accordingly. Weigh 1 mg of drug and lecithin quantity separately and add into solvents. Shake the contents of the tube until the amount of drug and lecithin was soluble completely. Further, boswellic acid and lecithin were added in increments of 1 mg until it failed to solubilize in the given quantity of solvent [11].

Formulation and optimization of boswellic acid-loaded transferosomes

Formulation methodology

Thin-film hydration/rotary film evaporation technique was applied for formulation of nano-transferosomes by using Rota-Evaporator instrument. 300 mg boswellic acid and 200 mg lecithin were precisely weighed and dissolved in an adequate amount of 0.75% Tween 80 surfactant and dichloromethane solvent. The above mixture was transferred to the round bottom flask and attached to the Rota-evaporator instrument for solvent evaporation.

Table 2 Check-point batch formulation

Check-point batch	Coded value		Uncoded value	
	X_1	X_2	X_1 (mg)	X_2 (%)
BA 19	− 0.5	− 0.5	175	0.63
BA 20	0.5	0.5	225	0.88

Table 1 Optimization technique with levels and factors

Independent factors	Coded value			Uncoded value		
	Low	Medium	High	Low	Medium	High
X_1 = Amount of Lecithin (mg)	− 1	0	+ 1	150	200	250
X_2 = Conc. Of Tween 80 (%)	− 1	0	+ 1	0.5	0.75	1
Dependent factors						
Y_1 = Vesicle size (nm)			Y_2 = Entrapment efficiency (%)			

Table 3 Composition of transferosomal transdermal film

Formulation code	Starch (% w/w)	HPMC K4M (%w/w)	Water (ml)	Transferosomal dispersion (ml)
TF 1	3	3	5	15

The procedure was carried out at constant temperature (45°C) by providing vacuum pressure to ensure complete elimination of solvent traces and formation of thin film at the bottom of the flask. The deposited vesicular film was further hydrated by using 20 mL of phosphate buffer 6.8 for 90 min at 70°C considering absolute hydration of film from the bottom of flask. The resultant solution obtained was further subjected to bath sonication for 5 min in account to reduce the vesicle size of the formulation [15] [16] (Fig. 1).

Optimization of process parameters for transferosomes

The trial-and-error method was employed for the selection of best suitable process parameters such as film formation temperature (FFT) (°C), hydration temperature (HT) (°C), and hydration time (Ht) (minutes) where % entrapment efficiency (%EE) was evaluated and based on which comparison was done; thus, optimum process parameter values can be considered for further use. Three levels of 3 different processes in transferosomes formulation were considered, and in total 9 trial batches were formulated. Material composition selected was boswellic acid—300 mg, lecithin—200 mg, Tween 80—0.75% and hydration volume of PB 6.8-—20 mL.

Optimization of formulation parameters for transferosomes

Optimization of formulation-independent factors such as amount of lecithin (mg) and concentration of surfactant (Tween 80) (%) was performed by employing 3^2 full factorial statistical design basically to study the outcomes of dependent factors in formulation. Three levels of 2 different factors used in formulation of transferosomes were selected, and in total 9 factorial batches were prepared using Design-Expert software (DoE) as shown in Table 1. Dependent factors evaluated were vesicle size (nanometer) and %EE.

Formulation of check-point batch for evaluation of model

Check-point batch formulation was performed to evaluate the dependability of model and to verify the effectiveness of the established contour plot and reduced polynomial equation in the development of transferosomes. Two check-point batches were prepared, one from lower side and another from higher level. Each batch was fabricated 3 times and average value was calculated. Formulation is described in Table 2. Evaluation was done by comparing the predicted and observed experimental values of responses obtained (Table 3).

Formulation of optimized batch based on desirability function

Optimized batch was formulated to find out the effect of independent variables (X_1 and X_2) based on the provided results of dependent variables (Y_1 and Y_2). The main function of desirability was to unite the responses of required attributes and provide the probability of predicting the highest level for independent variables. When desired results were added to the software, a final formulation for the optimized batch was recommended, and the batch was made in accordance with that formulation. The evaluation was done by comparing the predicted value given by the software and the observed experimental value of responses obtained.

Evaluation study of transferosomes

Vesicle size & polydispersity index

Dynamic light scattering (DLS) technique was employed to determine vesicle size by using Malvern Zetasizer instrument. Sample for vesicle size analysis of formulated transferosomes was prepared by diluting 1 mL of supernatant obtained after centrifugation with distilled water and filtered using membrane filter. After suitable dilution and filtration, the sample was subjected to examination. Polydispersity index (PDI) is a parameter used to determine the size range of nanocarriers which describes the uniform vesicle size distribution for an efficient and stable formulation [6] [17].

Drug entrapment efficiency (%EE)

%EE was determined by Indirect method using Remi CM 12 Cooling centrifuge to determine the presence of unentrapped drug into the transferosomal vesicles. From the prepared Boswellic acid transferosomes, 2 ml suspension was centrifuged at 4°C temperature, 11,500 rpm for 30 min. After completion of process, the supernatant was separated and the remaining sediment containing lipids that are not used in transferosomes formulation were settled down. 1 mL of supernatant was pipetted out and was made up with 10 mL methanol where drug solubilizes in methanol and the lipid precipitates out [17] [18]. The solution was further filtered and analysed for presence of free drug by UV spectrophotometer at 248 nm. The concentration of free drug was obtained and thus, %EE was calculated by the equation as shown below.

$$\%EE = \frac{\text{Total amount of drug added} - \text{Amount of free drug present in supernatant}}{\text{Total amount of drug added}} \times 100$$

Zeta potential

The surface charge of the vesicles, which is the most useful characteristic for enhancing formulation stability and preventing vesicle aggregation, was measured using a Malvern Zetasizer in terms of zeta potential. Sample for zeta potential analysis of formulated transferosomes was prepared by diluting the 1 ml of transferosomal suspension with 10 ml of distilled water and filtered using membrane filter. After suitable dilution and filtration, the sample was subjected to examination [17].

% Deformability index/ elasticity

Deformability is an important parameter that reflects the ability of transferosomes to transverse the narrow channels of the skin and yet retain its shape and size. Extrusion measurement technique was used to measure deformability of formulation. A conical flask containing one side hole was fixed with a vacuum pump whereas, on the mouth of the flask, a rubber bung attached to a stainless-steel filter holder was fixed. A membrane filter of pore size 0.20 µm was kept upon mesh plate, and the formulation was allowed to pass through it (Fig. 2). Fixed volume of vesicular formulation was allowed to pass through the filter membrane under constant vacuum pressure. The amount of transferosome formulation, which was extruded during 15 min of time duration, was measured, and vesicle size of formulation before and after extrusion was monitored by DLS technique using Malvern Zetasizer [17] [19] [20]. The experiment was done in triplicate, and %DI is calculated by the following equation:

$$D = J(rv/rp)^2$$

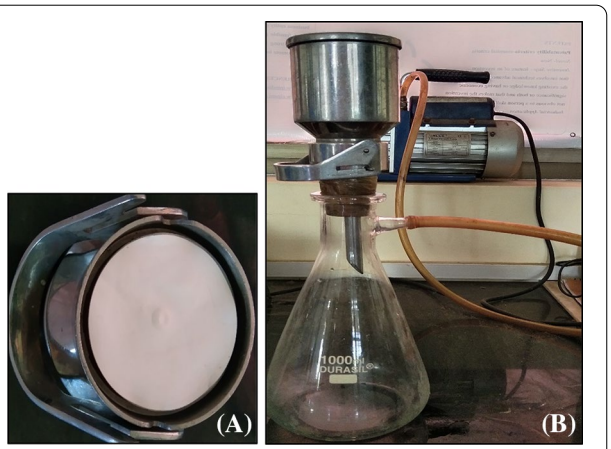

Fig. 2 **A** Filter membrane placed on membrane holder and **B** apparatus design for deformability Index

where D = Deformability index, J = Amount of transferosome suspension extruded, rv = Vesicle size of formulation after extrusion, rp = Pore size of the membrane filter = 0.20 µm.

Vesicle morphology by transmission electron microscope (TEM)

Transmission electron microscopy (TEM) was used to visualize the morphology of optimized transferosomes. Sample of 1 ml transferosomes was diluted with 10 ml distilled water and a drop of it was placed to dry on a copper-coated grid microscopic slide. After then, dried sample was stained by using a drop of 1% aqueous solution of phosphotungstic acid. Excess solution was wiped off using filter paper, and the prepared specimen was viewed under the microscope to capture image [17] [7]

FTIR study of transferosomal dispersion

The characterization of transferosomal dispersion by FTIR was done to check the compatible interaction between drug and excipients in the formulation. FTIR spectrum of boswellic acid transferosomal dispersion was scanned and compared with the standard spectrum of boswellic acid drug.

In vitro drug permeation study

Dialysis sac diffusion method was used for the permeation study. Dialysis bag used was of molecular weight 12,000–14,000. First step of activation is for removal of glycerol and was done by firstly washing the tube under tap running water for about 3–4 h. Second step is for removal of sulphur compound and was done by treating the bag with 0.3% w/v of sodium sulphide solution at 70° C for 1 min followed by washing with hot water (60°C) for 2 min. Third step is acidification of tube by 0.2% sulphuric acid and finally rinsed with hot water to remove acid; thus, dialysis bag was further used for the study [21]. One millilitre transferosomal formulation was filled in dialysis tube by sealing the tube thoroughly from both the sides. The dispersion filled tube was immersed in 200 ml of phosphate buffer 6.8 and maintained at 37 ± 0.5 °C. The whole assembly was fixed on magnetic stirrer and continuous agitation was provided with the help of magnetic bead. At different time intervals, 5 ml of aliquots was withdrawn from the dissolution medium and analysed by UV spectrophotometer at 248 nm. Equivalent amount of PB 6.8 was added to the medium after each sampling to maintain constant volume of dissolution medium. The cumulative amount of drug released during predefined time intervals was calculated [19] [22] [23].

Formulation of transferosomal transdermal film of boswellic acid

Transdermal films containing optimized batch of transferosomal dispersion were prepared by using blend of polymers such as starch and hydroxypropyl methyl cellulose (HPMC K4M), which were dissolved in preheated distilled water of appropriate quantity and transferosomal dispersion equivalent to 250 mg dose of drug was added. Propylene glycol was added as plasticizer to the resultant dispersion and stirred at 100 rpm on magnetic stirrer for appropriate time to form homogeneous dispersion. The resultant dispersion was poured into the Petri dish; inverted funnel was kept above the dish for uniform solvent evaporation and allowed to air-dry for 24 h. The prepared films were stored in a tightly closed container and further used for evaluation [24].

Evaluation parameters of transferosomal transdermal film of boswellic acid

Thickness

To determine the uniformity of the film from all the sides, which reflects the accuracy of dose in the whole film, film thickness was determined by taking measurements by Micrometre Screw gauze from three different places of the prepared transferosomal film and plain drug film, and the mean value was calculated [24] [25].

Moisture content

The prepared film was weighed and kept in a desiccator containing activated silica at room temperature for 24 h. The film was weighed repeatedly until it showed the constant weight [24] [25]. The difference between initial and final weights with respect to final weight was taken as a percent moisture content.

$$\text{Moisture Content} = \frac{\text{Initial weight of Film} - \text{Final weight of Film}}{\text{Initial weight of film}} \times 100$$

Moisture uptake study

The prepared film was weighed and kept in desiccators at room temperature for 24 h containing a saturated solution of potassium chloride in order to maintain 84% relative humidity [24] [25]. After 24 h, the film was reweighed and determined the percentage moisture uptake.

$$\text{Moisture Content} = \frac{\text{Final weight of Film} - \text{Initial weight of Film}}{\text{Final weight of film}} \times 100$$

Folding endurance

Main purpose was to check the ability of the films to withstand rupture. The film was repeatedly folded from the specific area, at the same place until it was broken. The number of times the film could be folded at the same place without breaking was folding endurance value [25] [26]. Study was performed in triplicate.

Drug content

The specified area of film of length 2×2 cm was cut and dissolved in 100 mL methanol in which drug was soluble , and then, the solution was stirred continuously on magnetic stirrer for 8 h. Then, the whole solution was sonicated. After sonication and subsequent filtration, drug in solution was estimated spectrophotometrically by appropriate dilution [27]. The absorbance of the solution was measured at 248 nm by UV spectrophotometer and drug content was determined.

In vitro drug permeation study

The study was performed in phosphate buffer pH 6.8 using Franz Diffusion cell and semi-permeable synthetic membrane (Strat-M®) as artificial skin membrane. Permeation studies were performed by placing the fabricated transdermal patch with synthetic skin membrane in between receptor and donor compartment in a vertical diffusion cell such as Franz diffusion cell. The transdermal system was mounted in the diffusion cell in donor compartment, which was in contact with the receptor fluid. The receiver compartment was maintained at specific temperature and was continuously stirred at a constant rate. The samples were withdrawn at different time intervals, and equal amount of buffer was replaced each time. The samples were diluted appropriately, and absorbance was determined at 248 nm spectrophotometrically. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic bead at 50 rpm; the temperature was maintained at 37 ± 0.5 °C [28].

Stability study

Stability study of final formulation was done to evaluate the effect of storage in context to various parameters such as temperature, humidity, light and time on optimized boswellic acid-loaded transferosomal film. To examine the characteristic change in the drug or excipients, it will also helpful to know shelf life of the formulation and prescribed storage condition. The optimized transferosomal film of BA was packed in aluminium foil and analysed at conditions in accordance with ICH Q1A (R2) guidelines for stability study [28]. Stability storage criteria set were as follows: temperature 40 °C ± 2 °C, relative Humidity 75% RH ± 5% RH and time period of 1 month [29]. The parameters to be analysed were moisture content, moisture uptake study, folding endurance and drug content.

Results

Preformulation studies

Drug excipient compatibility study

FTIR spectrum of pure boswellic acid (BA), BA + Lecithin, BA + Dichloromethane and BA + Tween 80 is shown in Fig. 3. The spectrum of boswellic acid showed characteristic peaks at 2941.89 cm^{-1} (C–H alkyl stretching), 1490.20 (C=C aromatic ring) stretching, 1696.97 (C=O symmetric stretching of carboxylates) stretching and 787.17 (C–H aromatic).

Solubility study of drug and lecithin in various solvents

Boswellic acid was almost insoluble in water, while it was highly soluble in dichloromethane, chloroform and methanol with the range of 601.35 ± 1.753, 534.26 ± 1.167 and 114.72 ± 0.503 mg/ml, respectively. Lecithin was partly soluble in water, partially soluble in methanol and practically insoluble in acetone. It was highly soluble in chloroform and dichloromethane.

Optimization outcomes of process parameters for transferosomes

Trial-and-error method was employed for selection of process parameters. Results of process-controlled parameters are shown in Table 4, and graphical representation for the effect of film formation temperature (℃), hydration temperature (℃) and hydration time (minutes) is shown in Fig. 4, Fig. 5, Fig. 6.

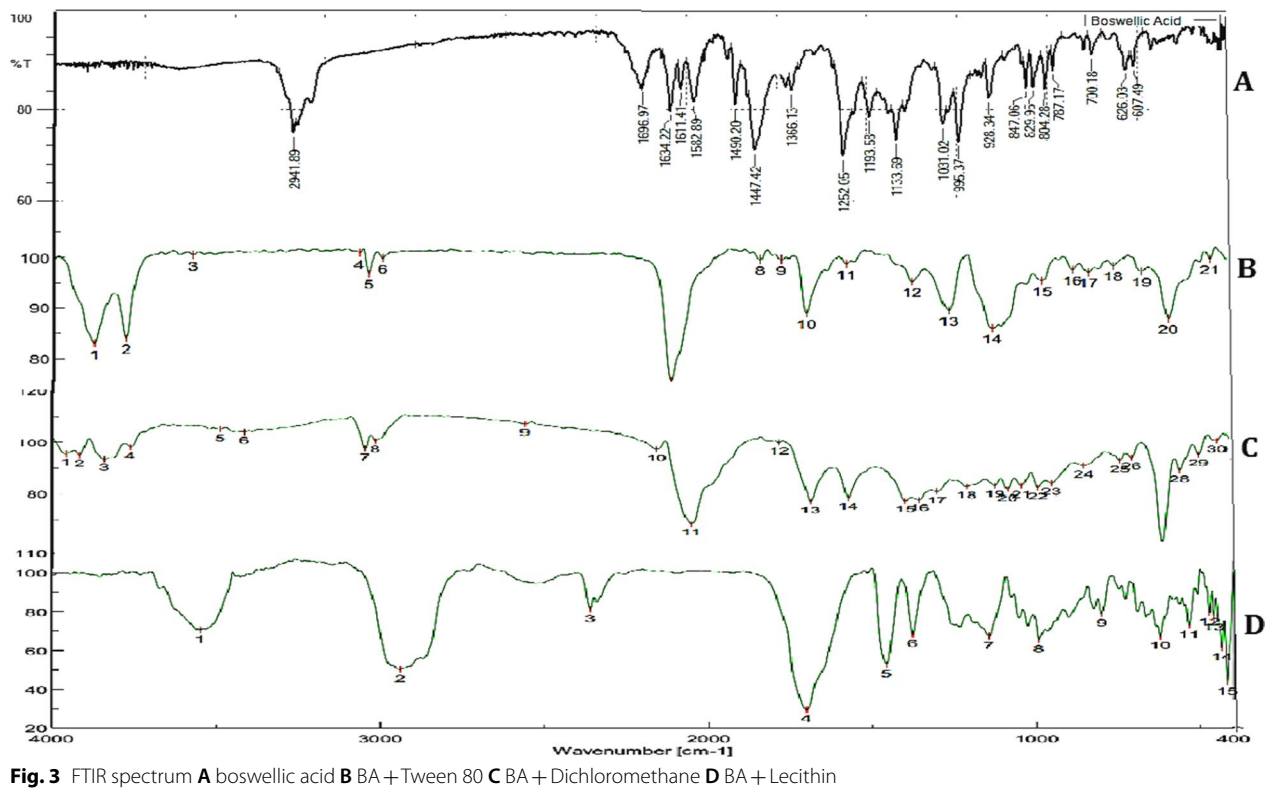

Fig. 3 FTIR spectrum **A** boswellic acid **B** BA + Tween 80 **C** BA + Dichloromethane **D** BA + Lecithin

Table 4 Process-controlled parameters optimization by trial-and-error method

Batch no	Boswellic acid (mg)	Conc. Of tween 80 (%)	FFT (℃)	HT (°c)	H time (minutes)	%EE
BA 1	300	0.75	**40**	70	90	88.81 ± 0.533
BA 2	300	0.75	**45**	70	90	**92.10 ± 0.511**
BA 3	300	0.75	**50**	70	90	85.30 ± 0.236
BA 4	300	0.75	45	**60**	90	84.60 ± 0.482
BA 5	300	0.75	45	**70**	90	**90.89 ± 0.623**
BA 6	300	0.75	45	**80**	90	86.57 ± 0.319
BA 7	300	0.75	45	70	**60**	81.93 ± 0.587
BA 8	300	0.75	45	70	**90**	**86.70 ± 0.498**
BA 9	300	0.75	45	70	**120**	86.07 ± 0.617

The bold digit in column 4, 5 and 6 differentiate the condition while bold digit in last column indicate based on that result process parameters were selected

(Mean ± SD, where $n = 3$)

Optimization outcomes of formulation parameters for transferosomes

3^2 full factorial statistical design was employed for optimization of formulation parameters. Results of vesicle size and drug entrapment efficiency, which were considered as dependent factors, are shown in Table 5. By the assistance of ANOVA and constructing polynomial equation, variation in responses such as vesicle size and %EE was evaluated. ℃

$$Y_1(\text{Vesicle size}) = +258.80 + 49.23 * A - 19.66 * B + 10.875 * AB - 5.40 * A^2 + 8.20 * B^2$$

$$Y_2(\%EE) = +89.59 + 5.071 * A - 0.93 * B + 1.31 * AB + 0.081 * A^2 + 0.58 * B^2$$

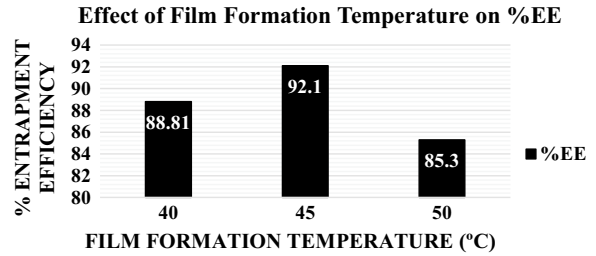

Fig. 4 Graphical comparison of effect of film formation temperature on %EE

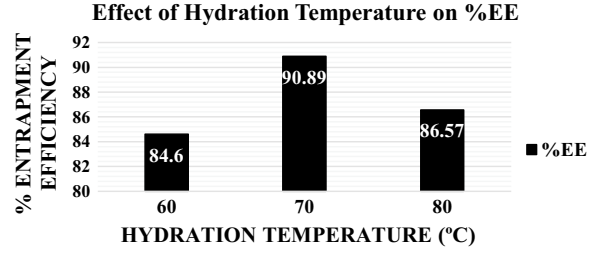

Fig. 5 Graphical comparison of effect of hydration temperature on %EE

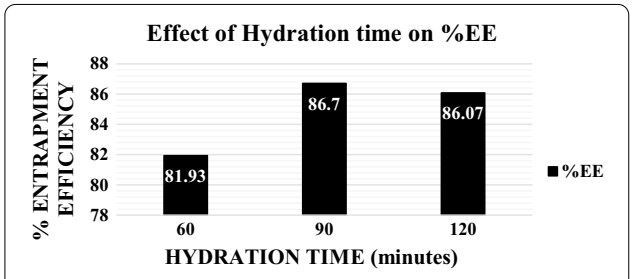

Fig. 6 Graphical comparison of effect of hydration time on %EE

Evaluation of check-point batch

Check-point batch analysis data are shown in Table 6.

Evaluation of transferosomal optimized batch

Vesicle size and PDI

Vesicle size of prepared transferosomal optimized batch was found to be 205.4 nm, which was nearer enough to the predicted data 208.93 nm given by the software, while the PDI was found to be 0.343.

Table 5 Formulation parameters optimization by 3^2 full factorial design

Batch no.	Amount of lecithin (X_1) (mg)	Concentration of surfactant (X_2) (%)	Vesicle size (Y1) (nm)	Drug EE(Y2) (%)
BA 10	150	0.5	247.9 ± 0.219	86.68 ± 0.553
BA 11	200	0.5	283.6 ± 0.355	91.17 ± 0.267
BA 12	250	0.5	317.7 ± 0.314	95.63 ± 0.392
BA 13	150	0.75	193.5 ± 0.268	85.57 ± 0.561
BA 14	200	0.75	266.3 ± 0.347	90.52 ± 1.133
BA 15	250	0.75	305.8 ± 0.532	92.85 ± 0.619
BA 16	150	1	187.5 ± 1.122	82.72 ± 0.546
BA 17	200	1	242.9 ± 0.598	88.25 ± 0.367
BA 18	250	1	300.8 ± 0.361	96.92 ± 0.466

(Mean ± SD, where $n = 3$)

Table 6 Check-point batch analysis

Check-point batch	Predicted value		Experimental value	
	Vesicle size (nm)	% EE	Vesicle size (nm)	% EE
BA 19	247.43	88.01	242.80 ± 0.326	86.72 ± 0.548
BA 20	276.88	92.16	257.60 ± 0.269	89.96 ± 0.391

(Mean ± SD, where $n = 3$)

% Entrapment efficiency

% Entrapment Efficiency of prepared transferosomal optimized batch was found to be 86.39 ± 0.019%, which was closer to the predicted value 84.95% given by the software.

Zeta potential

Zeta potential value of optimized batch was found to be − 15.2 mV which indicated good homogeneity and storage stability of the vesicles present in dispersion medium.

% Deformability index

$$D = J\left(\frac{rv}{rp}\right)^2$$

where D = Deformability index, J = Amount of transferosome suspension extruded = 11.2 ml, rv = Vesicle size of formulation after extrusion = 201.06 nm = 0.201 µm, rp = Pore size of the membrane filter = 0.20 µm.

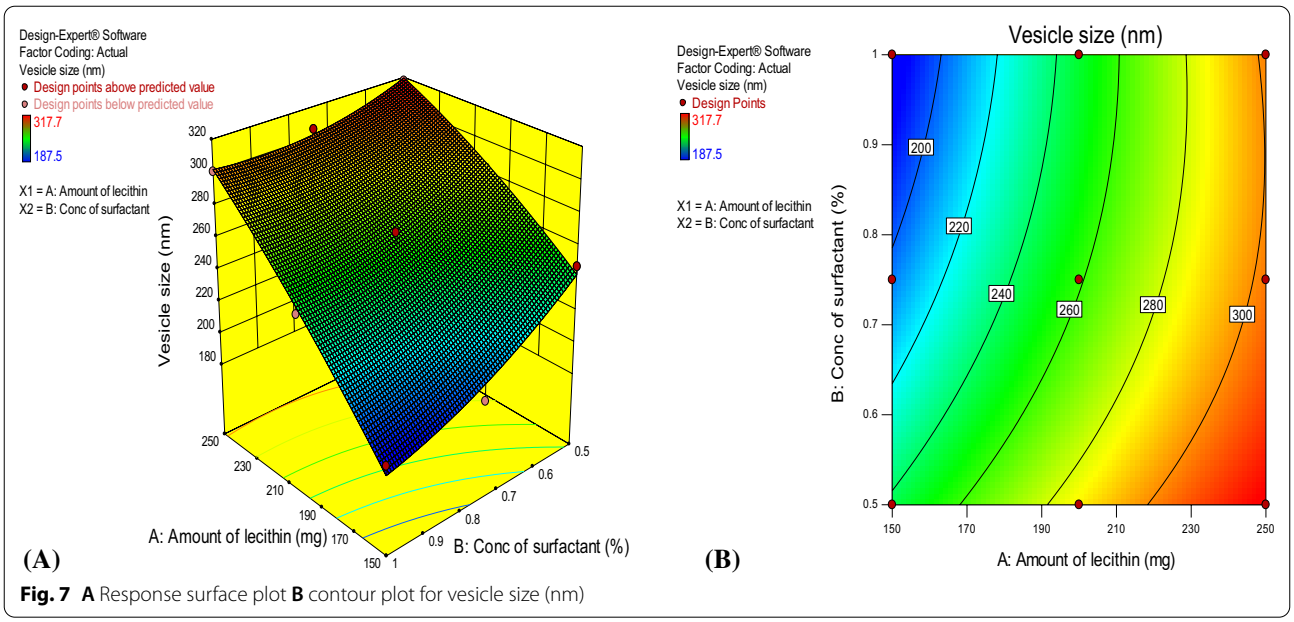

Fig. 7 **A** Response surface plot **B** contour plot for vesicle size (nm)

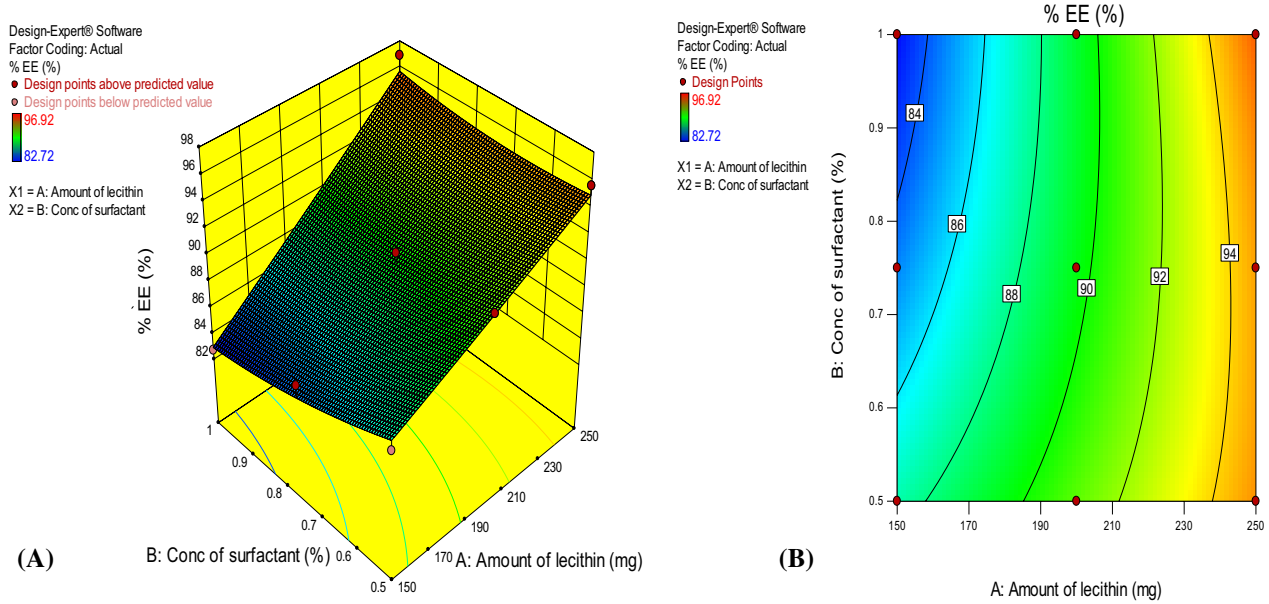

Fig. 8 **A** Response surface plot **B** contour plot for entrapment efficiency

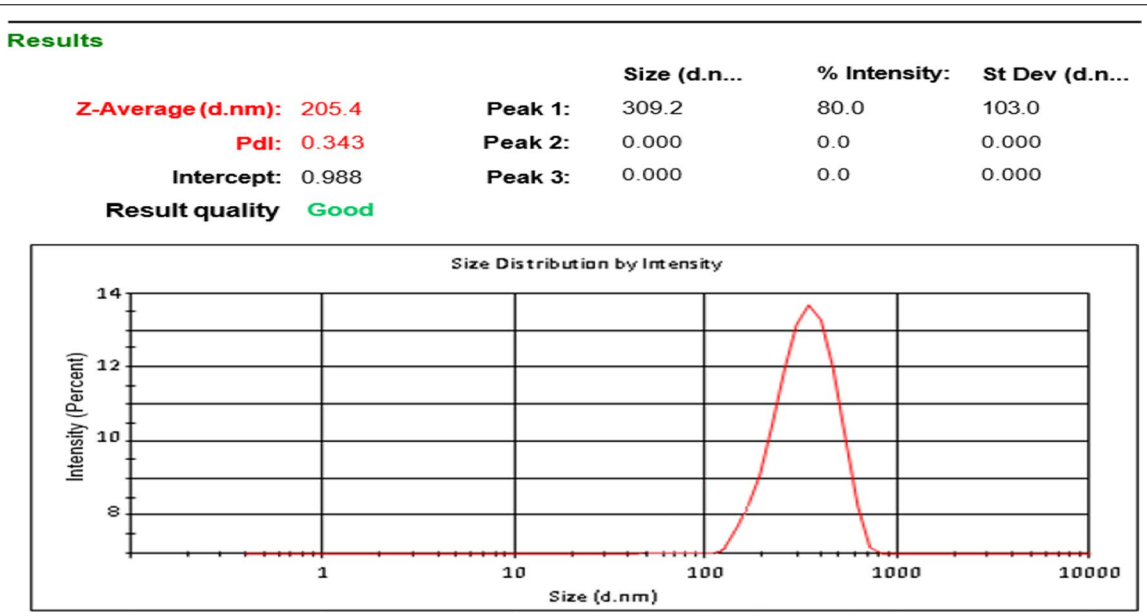

Fig. 9 Vesicle size distribution graph of optimized batch

The optimized batch of boswellic acid transferosomes showed % deformability index of 11.31 ± 0.032%, which suggests that the addition of edge activator Tween 80 in dispersion had major effect in elasticity of transferosome vesicle and their ability to retain their vesicle size after extrusion (Figs. 7, 8, 9 and 10).

Vesicle morphology by TEM

Results of TEM analysis of BA transferosomal formulation are shown in Fig. 11.

FTIR study of transferosomal dispersion

FTIR spectra of transferosomal dispersion are shown in Fig. 12. The characteristic IR absorption bands'

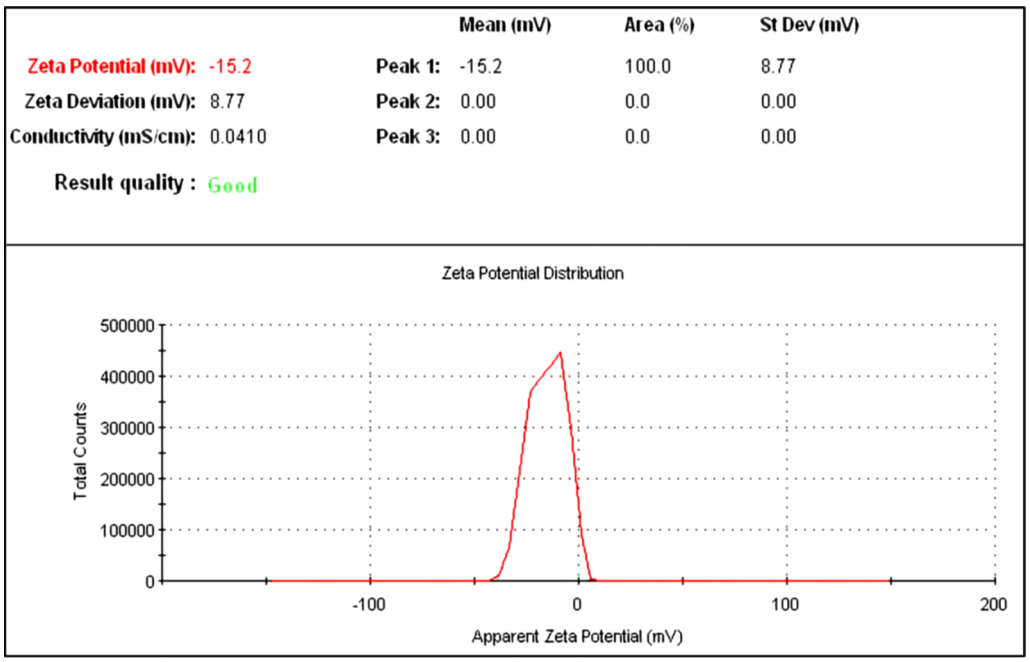

Fig. 10 Zeta potential graph of optimized batch

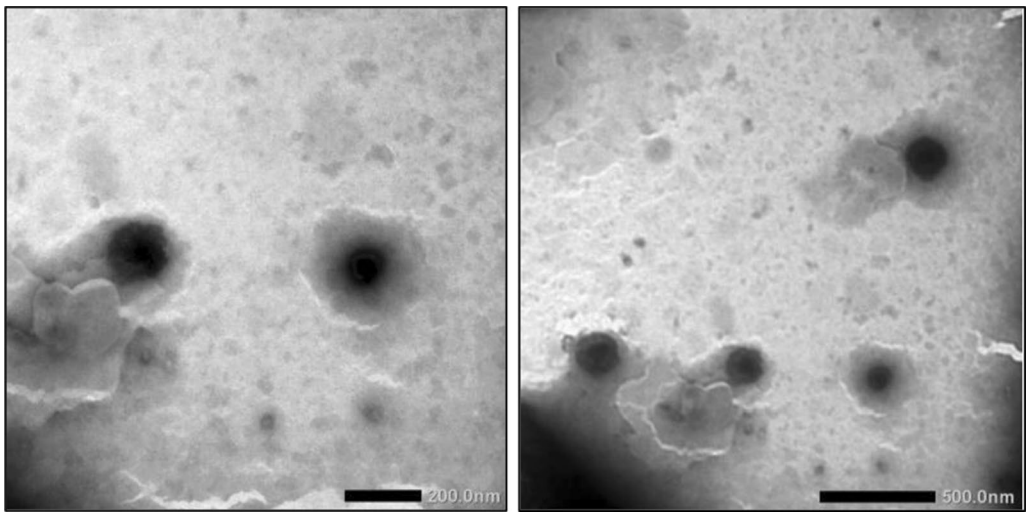

Fig. 11 TEM analysis of optimized boswellic acid transferosomes at 20,000 × and 10,000 × magnification

comparison peaks at 2947.61 cm^{-1} (C-H alkyl stretching), 1507.88 (C=C aromatic ring) stretching, 1705.23 (C=O symmetric stretching of carboxylates) stretching and 802.23 (C–H aromatic), which concludes no major changes were observable in the frequencies of the functional group of transferosomal dispersion when compared with FTIR spectra of pure boswellic acid drug.

In vitro drug permeation study

The in vitro drug permeation study, from optimized batch BA21 transferosomal dispersion, was performed using dialysis tube, and the % cumulative permeated was found to be 96.53 ± 0.023% after 24 h. Figure 13 Graphical representation of in vitro drug permeation from BA transferosomal dispersion (Table 7).

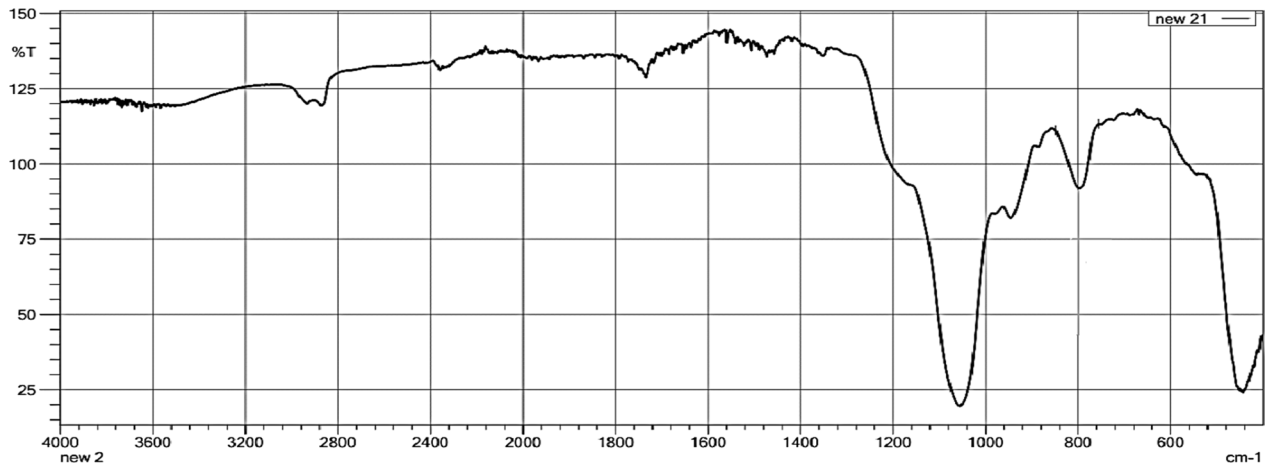

Fig. 12 FTIR spectrum of BA transferosomal dispersion

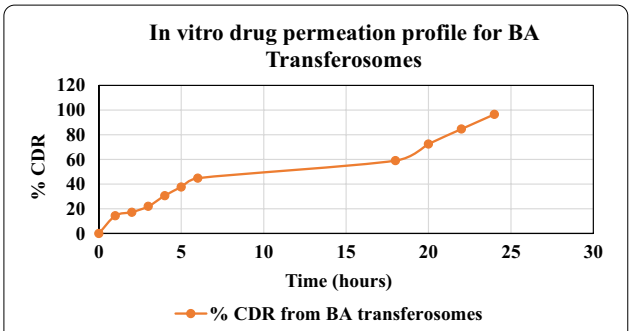

Fig. 13 Graphical representation of in vitro drug permeation from BA transferosomal dispersion

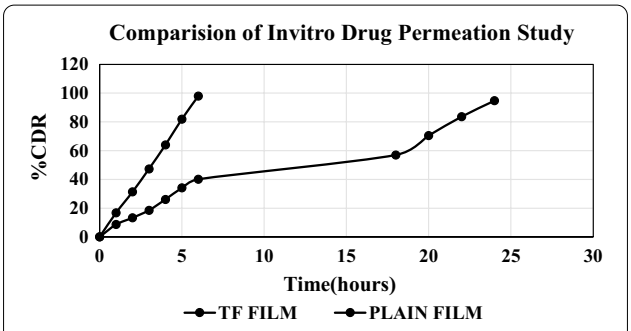

Fig. 14 Graphical representation of comparison profile of in vitro drug permeation study

Table 7 Optimized batch formulation

Optimized batch no.	Amount of lecithin (mg)	Conc. Of surfactant (%)	Drug (mg)	PB 6.8 (ml)	Desirability
BA21	150	0.72	300	20	0.914

Table 8 Results of BA-loaded transferosomal film

Parameters	Results
Thickness (μm)	96.62 ± 0.008
Moisture content (%)	3.5 ± 0.008
Moisture uptake (%)	2.6 ± 0.113
Folding endurance	49 ± 0.017 folds
Drug content (%)	95.41 ± 0.512

(Mean ± SD, where $n = 3$)

Evaluation of transferosomal transdermal film of boswellic acid

Transferosomal transdermal film was prepared by solvent evaporation technique using HPMC K4M and starch as rate-controlling polymers. The results of boswellic acid-loaded transferosomal film are shown in Table 8.

In vitro drug permeation study

The in vitro drug diffusion study, from transferosomal transdermal film and plain drug-loaded transdermal film, was performed using semipermeable membrane by Franz diffusion cell. The % cumulative drug diffused from BA-loaded transferosomal film was found to be 94.71 ± 0.019% after 24 h as compared to plain drug transdermal film which diffused drug 97.95 ± 0.034% after 6 h' time duration. Figure 14 Graphical Representation of Comparison profile of in vitro drug permeation study.

Stability study

Samples were analysed for stability study at different time intervals such as after 15 days and after 1 month. Initial results for moisture content, moisture uptake, folding endurance and drug content were found to be $3.5 \pm 0.008\%$, $2.6 \pm 0.113\%$, 49 ± 0.017 folds and $95.41 \pm 0.512\%$, respectively. After 15 days, results for the evaluated parameters were found to be $3.7 \pm 0.012\%$, $2.4 \pm 0.033\%$, 47 ± 0.018 folds and $93.98 \pm 0.004\%$, respectively (about same as initial). After one month, there was no much change observed in the results and was found to be $3.8 \pm 0.032\%$, $2.5 \pm 0.005\%$, 47 ± 0.118 folds, $92.37 \pm 0.054\%$.

Discussion

In the current study, attempt was made to formulate and characterize boswellic acid-loaded transferosomes via thin-film hydration technique using Rota-evaporator instrument. Preformulation studies were carried out such as drug-excipient compatibility study and solubility study of drug and lecithin in various solvents. Observing the FTIR spectra of pure boswellic acid and mixture of boswellic acid with various excipients, no specific change in the characteristic IR absorption bands was found when compared with the standard peaks. Thus, ultimately it can be concluded that there was no specific interaction between boswellic acid and excipients that are to be used in the formulation of transferosomes. For solubility study, boswellic acid was almost insoluble in water, maybe due to its highly hydrophobic nature. It was sparingly soluble in acetonitrile and acetone. Further it was highly soluble in dichloromethane, chloroform and methanol. Following lecithin solubility, it was partly soluble in water, partially soluble in methanol and practically insoluble in acetone. It was highly soluble in chloroform and dichloromethane.

Transferosomes preparation includes optimization of two types of parameters such as process and formulation, selection of which would be useful in further preparation of the optimized batch. Process parameters were optimized by trial-and-error method, three parameters with three different variables were selected and in total 9 batches were prepared of transferosomes.

Effect of film formation temperature

Varying range of temperature to form film at bottom of flask was selected as 40–50° C. Looking over the results for drug entrapment efficiency as the temperature increases more quickly the solvent would evaporate forming thin film of vesicles at the bottom of the flask that ultimately results in increase in drug entrapment into vesicles. But further increase in temperature would result in decreased drug entrapment into vesicles. This may be due to over boiling of the solvent from its optimum range, which results in the formation of cracked film, and thus, low amount of drug was entrapped. Optimum selected: 45 °C.

Effect of hydration temperature

Hydration temperature varies from 60 to 80 °C. After hydration with suitable hydration medium, as temperature increase for hydration, drug entrapment efficiency would increase. But further increasing the temperature for hydration results in decreased entrapment of drug, which may be due to rupture of vesicles at high temperature. Optimum selected: 70° C.

Effect of hydration time

Drug entrapment efficiency increases with increase in hydration time period from 60 to 90 min. But further increase to 120 min, the capability of drug entrapment into transferosome vesicles decreases. Optimum selected: 90 min.

Formulation parameters were optimized by applying 3^2 full factorial statistical design, where influence of independent factors such as amount of lecithin (mg) and concentration of surfactant (Tween 80) (%) was studied on dependent factors such as vesicle size (nm) and drug entrapment efficiency (%).

Influence of amount of lecithin (X_1) and concentration of surfactant (X_2) on vesicle size (Y_1) (nm)

Vesicle size is an important parameter in transferosomes evaluation. Co-relation results between amount of lecithin and vesicle size revealed positive effect of lecithin on vesicle size of transferosomes. Increase in the amount of lecithin from 150 to 250 mg significantly resulted in increment of vesicle size from 187.5 to 317.7 nm. This might be due to the formation of multilamellar vesicles of transferosomes with increasing lecithin content, thus increasing the vesicle size. On the other side, the concentration of surfactant showed negative correlation effect on vesicle size, i.e. increase in the concentration of surfactant (0.5–1%) resulted in decreased vesicle size of formulation. This might be due to the capability of Tween 80 surfactant to solubilize within lipid bilayer due to its long hydrocarbon chain forming hydrogen bond with the hydrophilic polar head of lecithin and resulting in reduction of vesicle size.

Influence of amount of lecithin (X_1) and concentration of surfactant (X_2) on drug entrapment efficiency (Y_2) (%)

Drug entrapment efficiency indicated the amount of drug encapsulated in final formulation. There is a positive correlation effect of amount of lecithin on drug entrapment into vesicles. It suggests that with increasing lecithin strength from 150 to 250 mg more drug was entrapped

into the transferosome vesicles, which might due to the fact that increasing lecithin amount would result in formation of more rigid and multilamellar vesicles, thus encapsulating more drug concentration. On the other side, in case of concentration of surfactant T-80, moving towards higher concentration resulted in decreased entrapment efficiency which shows negative correlation influence of surfactant conc. on drug encapsulation. The reason behind this finding is increasing concentration of surfactant results in lecithin solubilization forming mixed micelles and pore generation, which ultimately causes the leakage of entrapped drug into vesicles and thus low drug entrapment.

Various evaluation parameters were performed for the final optimized batch of transferosomes. The vesicle size of 205.4 nm was desirable for penetration of vesicles into the skin, thus reaching the site of action, and the highest entrapment efficiency was obtained because Tween 80 was used as a surfactant which contributed in high drug entrapment. Boswellic acid, free drug (not entrapped into transferosomal vesicles), was soluble in methanol solvent, while transferosomes vesicular outer layer made up of phospholipids were insoluble in methanol. Hence, the lipids will precipitate out and the free drug will dissolve after suitable dilution with methanol, which ultimately gives the presence of free drug concentration and thus drug entrapped into vesicles was calculated.

The charge present on the surface of the transferosomal vesicle in a suspension is called zeta potential, which depends on the types of ion present in formulation. By analysing the surface charge on vesicles, zeta potential indicates the degree of repulsion between two adjacent vesicles that is, in turn, helpful in determining the velocity of vesicles due to electrophoretic mobility. Zeta potential value between + 30 and − 30 mV results in high tendency for flocculation of vesicles. Values of potential more positive than + 30 mV or more negative than − 30 mV results in a stable formulation. Zeta potential value − 15.2 mV indicated good homogeneity and storage stability of the vesicles present in dispersion medium. The optimized batch of boswellic acid transferosomes showed % deformability index of 11.31 ± 0.032%, which suggests that the addition of edge activator such as Tween 80 destabilizes the phospholipid bilayer while improving the elasticity and deformability, thus enhancing the ability of transferosomal vesicles to squeeze through narrow channels of skin and also maintain its integrity. TEM was utilized for evaluating vesicle morphology that pointed out well-identified outline and core of the prepared vesicles confirming its vesicular characteristics such as lamellarity and lipid bilayer encapsulating the drug with smooth surface. In vitro drug permeation study from transferosomal dispersion of boswellic acid was done, and an initial rapid release from boswellic acid transferosome dispersion was observed in the first one hour followed by sustained release pattern for up to 24 h. The fast release of drug in the initial phase may be due to the presence of free drug on the surface of prepared vesicles, that unentrapped drug passes through the initial release phase earlier while the entrapped drug into the vesicles permeates slowly.

Optimized batch of BA transferosomal dispersion was loaded into transdermal film by solvent evaporation technique. Various evaluation parameters were performed for the final transferosomal film formulation where, results of moisture content indicated good resistance of brittleness through long-term storage of the film, moisture uptake by the prepared films suggests capability of films for protection against microbial contamination and reduce bulkiness. The results of folding endurance were satisfactory, which indicated high capability of films to maintain their integrity without breaking with respect to general skin folding after application to skin. Good uniformity of drug content was found, which suggests that the formulation methodology employed for film preparation has the ability to produce transdermal films with minimum variability and uniform drug content.

The in vitro drug permeation study of boswellic acid from transferosomal film was compared with plain BA transdermal film and % cumulative drug diffusion from both formulations was studied for the period of 24 h, and it is clear from the given results (Fig. 14) that BA-loaded transferosomal film exhibited enhanced permeation and sustained retention up to 94.71 ± 0.019% of drug diffusion which suggests higher penetration capability of BA-loaded transferosomal film rather than plain BA transdermal film. The stability study results revealed good stability of final transferosomal film under accelerated conditions. Overall, the current study concluded that by utilizing transferosomal vesicles for drug encapsulation it minimized drug loss, improved penetration ability of drug into deeper skin layers and exhibited required therapeutic effect.

Conclusion

Boswellic acid-loaded transferosomes were successfully formulated with the help of Rota evaporator by using thin-film hydration technique. Selection of formulation variables such as amount of lecithin and concentration of surfactant are critical parameters to obtain a desired formulation. 3^2 Full Factorial design was employed as optimization technique where vesicle size (nm) and drug entrapment efficiency (%) were considered as dependent factors. Based on significant desirability optimized batch of BA transferosomes, dispersion was obtained and further evaluated. Vesicle size and %EE of optimized

batch were 205.4 ± 1.215 nm and 86.39 ± 0.019%, respectively, whereas % deformability index was found to be 11.31 ± 0.032%. Zeta potential value of − 15.2 concluded good homogeneity and storage stability of the vesicles present in dispersion medium, while vesicle morphology by TEM showed fine outline and core of the prepared vesicles confirming its vesicular characteristics. FTIR study concludes no major changes were observable in the frequencies of the functional group of transferosomal dispersion when compared with FTIR spectra of pure boswellic acid drug. The results of In vitro drug permeation for transferosome dispersion showed up to 96.53 ± 0.023% drug diffusion for 24 h across the membrane. Optimized transferosomal dispersion of BA was further incorporated into transdermal film and evaluated for various parameters where the results of invitro drug permeation from BA-loaded transferosomal film depicted enhanced permeation and sustained retention of drug up to 94.71 ± 0.019% for 24 h across the membrane when compared to plain BA transdermal film which showed rapid drug permeation up to 97.95 ± 0.034% for 6 h. Accordingly, the research work concludes that the transferosomes could be efficient nanosized carriers for enhanced permeation of boswellic acid into deeper layers of skin and could successfully exhibit its therapeutic effect.

Abbreviations

BA: Boswellic acid; FTIR: Fourier transform infrared; BCS: Biopharmaceutical classification system; DCM: Dichloromethane; PB: Phosphate buffer; %EE: % Entrapment efficiency; UV: Ultraviolet; PDI: Polydispersity index; HPMC: Hydroxypropyl methylcellulose; ANOVA: Analysis of variance; RH: Relative humidity; T-80: Tween 80; mV: Millivolt.

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Author contributions

Author DJ performed all the above research work and prepared the complete manuscript. Authors UV and MJ guided and monitored the research activities. Authors HK, KD and VS contributed in final drafting of the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors declare no conflict of interest.

Competing interests

The authors declare that they have no competing interests.

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