Original Research Article

Cavamax W7 composite psoralen ethosomal gel versus cavamax W7 psoralen solid complex gel for topical delivery: A comparative evaluation

Smriti Kumari, Kamla Pathak

Department of Pharmaceutics, Rajiv Academy for Pharmacy, Mathura, Uttar Pradesh, India

Abstract

Aim: The present research work was aimed to formulate and characterize psoralen-encapsulated cavamax W7 composite ethosomal gel and compare its *in vitro* and *ex vivo* behavior against psoralen-cavamax W7-complex reference gel. **Materials and Methods:** A total of nine formulations of composite ethosomes were prepared by injection method using 3^2 factorial design and entrapment efficiency was designated as dependent variable. Concomitantly, psoralen was complexed with cavamax W7 (1:1 molar ratio) by kneading method and formation of complex was confirmed by Diffuse reflectance spectroscopy (DRS), scanning electron microscopy (SEM), X-ray diffraction (XRD), and differential scanning calorimetry (DSC). **Results:** F9 with vesicle size of 183 ± 2.8 nm, and highest % entrapment efficiency of 98.12 ± 1.15 was selected as optimized formulation. Transmission electron microscopy (TEM) revealed uniform and spherical shaped vesicles. The optimized formulation F9 was formulated as carbapol gel and compared against ethosomal gel, psoralen gel, and psoralen cavamax W7 complex gel. The gels were evaluated for permeation characteristics and the rank order was composite ethosomal gel > ethosomal gel > psoralen-cavamax W7 complex gel > psoralen gel. The ethosomal gel (G5) with highest *in vitro* permeation of 82.48 ± 2.23% was subjected to *in vivo* Confocal laser scanning microscopy (CLSM) studies using rhodamine B as tracer. The penetration of rhodamine B was uniform, deeper, and two times faster into epidermis than control gel. **Conclusion:** Conclusively, cavamax W7 composite ethosomes present themselves as efficient carrier for superior topical delivery of psoralen and have potential for clinical applications in minimizing side effects associated with photosensitivity of psoralen.

Key words: Composite ethosomes, confocal laser scanning microscopy, permeation, psoralen, psoralen-cavamax W7-complex

INTRODUCTION

Psoralen, a tricyclic furanocoumarin (7H-furo [3, 2-g] [I] benzopyran-7-one) with photosensitizing activity, is an anti-vitiligo and anti-psoriatic drug widely used for psorasias. Psoriasis is a non-infectious chronic immunological disorder manifested with dermatological condition where localized or widespread erythematous scaling lesions or plaques are very common symptoms along with hyper-proliferation attended by dermal inflammation.[1] The skin condition in psoriasis is extremely dry, lipid and moisture deficient, with increased thickness due to hyper proliferation of skin cells almost by seven times, challenging topical delivery.[2]

Clinically, psoralen is used to diminish the lesions by its regular application via PUVA therapy (psoralen + ultraviolet (UV) therapy).[3] Recent trends in photo chemotherapy of psoralen involve oral or topical administration of a photosensitizing psoralen followed by exposure to long-wavelength (320-400 nm) UVA irradiation. However, topical route remains the cornerstone of the treatment during psoriasis. Cream and gels of psoralen have been formulated utilizing permeation enhancers such as menthol[4] but commercially, it is available as an oily solution of Babchi's oil (per 10 ml contains 30% *Psoralea corylifolia* and 70% *Sesamum indicum*). Reports can be traced for Babchi oil microemulsion[3] and nanostructured solid lipid carriers of Babchi oil,[5] but no report can be found on vesicular topical drug delivery system of psoralen.

Ethosomes appear to be very promising vesicular system in dermal drug delivery and are reported to have five to 10-fold

Address for correspondence:
Dr. Kamla Pathak, Department of Pharmaceutics,
Rajiv Academy for Pharmacy, NH-2, P. O. Chattikara,
Mathura-281 001, India.
E-mail: kamlapathak5@gmail.com

Access this article online

Quick Response Code:

Website: www.jpionline.org

DOI: 10.4103/2230-973X.121284

higher skin permeation and deposition in comparison to conventional liposomal formulation.[6] The enhanced delivery of actives using ethosomes over liposomes can be ascribed to the interaction between ethosomes and skin lipids. The structural composition of ethosomes imparts excellent penetration property that can be specifically useful in psoriatic skin conditions of dry, scaled and thickened with lipid imbalance.

Investigations have revealed that encapsulation of drug in the form of cyclodextrin-drug complex or inclusion of drug inside cyclodextrin in vesicular formulation could be an innovative new strategy for integrating the dual advantages of the two types of carrier into a distinct solitary system.[7] Therefore, the focus of present investigation was to analyze the composite vesicular approach which would help in increasing the skin permeation and deposition of psoralen deep into the skin delivered via topical gel and, to compare permeation and penetration behavior against topical gel of solid cavamax W7 complex of psoralen.

MATERIALS AND METHODS

Materials

Psoralen was procured from Kumaoun chemicals, Nainital, India. Soya Lecithin of Himedia Laboratories Pvt. Ltd., Mumbai, India), ethanol (95% v/v) from (SD Fine Chem Ltd, Mumbai, India), and propylene glycol of Sigma Aldrich Chemie GmbH, Netherlands) were used. Cavamax W6, W7, and W8 were kind gift from (ISP Specialty Product Limited, USA). The gels in study were prepared using Carbopol 934 LR (Central Drug House (P), Ltd., New Delhi, India).

Preliminary studies

Preliminary studies were carried out to optimize the blank composite ethosomes prepared by injection method (described later). Blank composite ethosomes of cavamax W6, W7, and W8 were prepared and the ethosomal suspensions were visualized by optical photomicrograph at 100× (HICON Enterprises, India). Based on the physical attributes, cavamax W7 was selected to prepare composite ethosomes by varying the concentration of cavamax W7 in the range of 0.1-3.0% w/v. Photomicrographic analysis revealed the optimum concentration of cavamax W7 as 0.5% w/v. Further, *in vitro* adsorption,[8] phase solubility,[9] and solubility studies detailed in Table 1 were done to trace out various excipients and their levels that can influence ethosomal formulations. The quantification of psoralen in all the studies was done spectrophotometrically at 243 nm (Pharma Spec 1700 Shimadzu, Kyoto, Japan).

Preparation of cavamax W7 composite ethosomes

Cavamax W7 composite ethosomes of psoralen were formulated by injection method[8] using 3^2 factorial design [Table 2]. Encapsulation efficiency was considered as dependent variable and levels of cavamax W7 and soya lecithin as independent variables. To the solution of soya lecithin in ethanol (30% w/v), drug was added. Propylene glycol was added to the mixture and heated to 30°C ± 1°C in a water bath. Solution of cavamax W7 in distilled water (preheated at 30°C ± 1°C) was injected slowly to the lipid mixture with constant stirring on magnetic stirrer for 1.5 hr while maintaining the system at 30°C ± 1°C. The suspension of ethosomes was left to cool at room temperature for 30 min and subjected to probe sonication for three cycles

Table 1: Solubility data of psoralen in distilled water in the presence of excipients utilized for preparation of cavamax W7 composite ethosomes of psoralen

Sample composition	Drug+Excipient (% w/v)	Solubility (mg/ml)	% Solubility enhancement
Drug	0.05	0.55	—
Drug+soya lecithin	0.05+0.5	1.009	83.49
Drug+cavamax W7	0.05+0.5	1.113	102.50
Drug+soya lecithin+ethanol+propylene glycol	0.05+0.5+30+10	2.436	343.63
Drug+cavamax W7+ethanol+propylene glycol	0.05+0.5+30+10	3.492	535.71
Drug+soya lecithin+cavamax W7+ethanol+propylene glycol	0.05+0.5+0.5+30+10	3.85	600.32

Table 2: 3^2 full factorial design of ethosomal and cavamax W7 composite ethosomal formulations of psoralen

Formulation code	Drug (mg)	Soya Lecithin (% w/v) (X_1)	Cavamax W7 (% w/v) (X_2)	Ethanol (% w/v)	Propylene glycol (% w/v)	Dependent variable
F1	10	0.2 (−)	0.3 (−)	30	10	% Entrapment efficiency
F2	10	0.2 (−)	0.5 (0)	30	10	
F3	10	0.2 (−)	0.8 (+)	30	10	
F4	10	0.5 (0)	0.3 (−)	30	10	
F5	10	0.5 (0)	0.5 (0)	30	10	
F6	10	0.5 (0)	0.8 (+)	30	10	
F7	10	1 (+)	0.3 (−)	30	10	
F8	10	1 (+)	0.5 (0)	30	10	
F9	10	1 (+)	0.8 (+)	30	10	
Fx*	10	0.75	0.4	30	10	

Fx*: Extra design check point formulation; (−): low level; (0): zero level; (1): high level

of 5 min with relaxation of 5 min between the cycles at 4,000 rpm (894.0 × g) to get ethosomes. The ethosomal suspension was purified by dialysis across Himedia Dialysis membrane 150 (Qualigens Fine Chemicals, Mumbai, India) in 200 ml of phosphate buffer pH 6.8 stirred magnetically at 500 rpm (13.98 × g) for 20 min. The purification time was optimized statistically using paired t- test (Table 3)

Evaluation of cavamax W7 composite ethosomes

Vesicle size and zeta potential

Vesicle size, zeta potential and polydispersity index were measured by Zetasizer (Malvern Zetasizer ver. 6.0, U.K) by diluting one drop of ethosomal and cavamax W7 composite ethosomal suspension with hydroethanolic solution at 25°C in clear disposable zeta cells. All the measurements were done in triplicate for each sample.

Entrapment efficiency

Purified vesicular suspension was transferred into centrifuge tube and centrifuged for 1 h at 4000 rpm (894.0 × g). The sediment was lysed by using ethanol (95% v/v) and filtered through nylon filter disc (0.22 μm). The drug was assayed both in the sediment and supernatant to determine the entrapment efficiency by Eq 1

$$\text{Entrapment efficiency} = Wa - (Ws + Wp)/Wa \quad ...(1)$$

Where W_a = amount of drug added in the system; W_s = amount of drug in the supernatant after centrifugation; W_P = amount of drug in the purification medium.

Selection of optimized formulation and validation of design

The optimized formulation was selected on the basis of maximum entrapment efficiency. Using Design Expert Software version 8.0.5 (Stat-Ease, Minneapolis, USA Inc.), polynomial equation for entrapment efficiency was generated that was transformed by removing insignificant coefficients. An extra design check point formulation (Fx) was developed by selecting the levels as 0.75% w/v of soya lecithin and 0.4% w/v of cavamax W7 and evaluated. The experimental value was compared with predicted value obtained from transformed polynomial equation and evaluated statistically by one way analysis of variance (ANOVA) at 5% level of significance.

Table 3: Optimization of purification time using paired *t*-test

Purification time interval (min)	t_{cal}	t_{tab} (d.f.=2)	Result	Conclusion
10-20	4.82	2.92	$t_{cal} > t_{tab}$	Significant difference (P<0.05)
20-30	1.69	2.92	$t_{cal} < t_{tab}$	No significant difference (P>0.05)

d.f.:Degree of freedom; t_{cal}:Calculated *t* value; t_{tab}:Tabled *t* value

Vesicular visualization by transmission electron microscopy

One drop of formulation was placed on a copper grid for 2-3 min and negatively stained by phosphotungstic acid. The air dried sample was visualized under transmission electron microscope (TEM) at 20,000 × magnification at an accelerating voltage of 200 KV.

Stability study

Stability of the optimized (F9) and formulation was determined by storing the vesicles at 4°C for 6 months in accordance with International Conference on Harmonization (ICH) Q1A (R2) guidelines. After appropriate time intervals, samples were withdrawn and evaluated for their mean particle size, zeta potential, and percent entrapment efficiency.

Preparation and evaluation of solid complex of psoralen and cavamax W7

Kneading

The complex was prepared by kneading method, by adding small amount of water to Cavamax W7, in (1:1) molar ratio, placed in a mortar, and mixing to obtain a homogeneous paste. Psoralen was gradually incorporated and kneaded for 60 min with introduction of few drops of water to maintain suitable consistency. The resulting paste was forced through 100 mesh sieve and the granules obtained were dried in oven at 50°C for 24 h. The dried complex was pulverized into fine powder and characterized.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed using auto-sampler equipped with a differential scanning calorimeter (SIIO 6300, Tokyo, Japan). Alumina was used as a reference material and the scanning rate was 10°C/min, with a scanning temperature range of 25-300°C.

Scanning electron microscopy

Morphology of the psoralen, cavamaxW7, physical mixture and binary complex was studied by scanning electron microscope (SEM) (JEOL 5400, Tokyo, Japan) operated at an accelerating voltage of 4 kV. Samples were coated with gold ion for 5-6 min under argon atmosphere to make them conductive and were photographed at 4.0 nm resolution, at different magnifications ranging from 100×-3,500×.

Diffuse reflectance infra red spectroscopy

Psoralen, cavamaxW7, physical mixture, and binary complex were grounded, mixed with potassium bromide (IR grade) and analyzed by Fourier transform infrared spectroscopy (FTIR) 8400S Shimadzu, Kyoto, Japan. The scanning range of 500-4,000 cm^{-1} was used with 1 cm^{-1} resolution.

X-ray diffraction

The diffractograms were recorded at room temperature using D8 Advance diffractometer (D8 Advance, Bruker, Germany). The samples were irradiated with Ni filtered 2.2 KW Cu Anode,

Dermic X-ray tube equipped with a sample holder with Zero Background and Polymethyl methacrylate (PMMA) and Lynux Eye detector. A scanning rate of 2°/min over a diffraction angle (2θ) was used in the range of 3-70°.

Preparation and evaluation of gels

Carbopol 934 was soaked in distilled water (1% w/v) for 2 hr to form its aqueous dispersion to which triethanolamine was added under continuous stirring. The gels were formulated with or without permeation enhancers (menthol, isopropyl myristate) as per the composition given in Table 4 and evaluated. The pH of gels was measured by Digital pH meter; model 111 E (HICON, New Delhi, India). Drug content was determined by diluting 100 mg of the gel to 5 ml with ethanol (95%) and vortexing for 5 min. The volume was made up to 10 ml with phosphate buffer, pH 6.8 and assayed.

Ex vivo skin permeability

All the animal experiments have been conducted in full compliance with the institutional ethical and regulatory principles and as per the spirit of Association for Assessment and Accreditation of Laboratory Animal Care and International's expectations for animal care and use/ethics committees. The investigations were performed after obtaining approval by the Institutional Animal Ethical Committee of Rajiv Academy for Pharmacy, Mathura, India (IAEC no: IAEC/02/12). For the *ex vivo* permeability study, three albino rats (Wistar strain) 6-8 weeks old, weighing 120-150 gm were sacrificed by spinal cord delocalization. The pre-shaved abdominal skin was separated from the underlying connective tissue with the scalpel. The excised skin was placed on aluminum foil, and the dermal side of the skin was gently teased off for any adhering fat or subcutaneous tissue. Prepared skin was mounted on Franz diffusion cell (area 3.14 cm^2) and the test formulation (2 mg/cm^2) was applied on the epidermal side of the skin. Two milliliters of sample was withdrawn from the receptor compartment containing 11 ml of the phosphate buffer pH 6.8 maintained at 32°C ± 1°C, at appropriate time intervals and analyzed at 246 nm. An equal volume of fresh phosphate buffer, pH 6.8 was replaced after each sampling. The cumulative amount of drug permeated across the skin per square surface area was plotted against time to calculate the steady state flux (J_{SS}).

Confocal laser scanning microscopy

Depth of permeation of rhodamine B (tracer) loaded cavamax W7 composite ethosomal vesicles via Carbapol gel were investigated by confocal laser scanning microscopy. The gel was applied to the dorsal skin of albino rats (Wistar strain) for 6 hr. The rats were sacrificed and the skin was excised, washed, blotted dry, and placed on the aluminum foil. Sections of the skin samples were prepared and examined under microscope (Olympus, Japan) at 20× at an optical excitation of 488 nm argon laser beam and fluorescence emission was detected above 560 nm.

RESULTS AND DISCUSSION

Preliminary trials

The photomicrographs (data not shown) revealed poor structural features of cavamax W6 ethosomal vesicles and vesicle formation could not be facilitated by cavamax W8. Ethosomes prepared with cavamax W7 were spherical in shape with uniformity in their structure, inferring cavamax W7 to be appropriate for preparing composite ethosomes. The optimum concentration of cavamax W7 was identified as 0.5% w/v below which vesicles could not be formed and beyond it the size of vesicle increased with loss in uniformity in size and shape. The results are concordant with those observed by Akhtar and Pathak[8] and guided the selection of levels of cavamax W7 for 3^2 factorial experimental design.

Stirring of the reaction mixture is an important consideration that facilitates contact between the drug and phospholipids, and plays major role in determining the entrapment efficiency of the system, as the drug in a vesicular system is loaded either in the ethanolic core or is adsorbed on the phospholipid bilayer depending on its characteristics. Psoralen, being a lipophilic drug,[9] will be adsorbed on the phospholipid bilayer and that in turn will be dependent on the processing conditions. Hence, *in vitro* adsorption studies were carried out that revealed an increase in percent drug adsorbed with time and attained plateau level beyond 1.5 hr. As the percent adsorbed at 1.5 hr (61.11 ± 0.086) was non-significantly ($P < 0.05$) different from the percent drug adsorbed at 2 hr (61.30 ± 0.098%), 1.5 hr was considered optimum time for stirring to affect maximum drug entrapment.

The effect of cavamax W7 on the solubility of psoralen was analysed through phase solubility studies that guided the levels of cavamax W7 for the experimental design as well to

Table 4: Composition of psoralen gels

Ingredient	Ethosomal formulations			Cavamax W7 composite ethosomal gel		Cavamax W7-psoralen complex gels			Psoralen gels			
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
Psoralen (mg)	10	10	10	10	10	10	10	10	10	10	10	10
Carbopol 934P (% w/w)	1	1	1	1	1	1	1	1	1	1	1	1
Menthol (% w/w)	—	5	—	—	5		—	5	—	—	5	—
Isopropyl myristate (% w/w)	—	—	5	—	—	5			5			5
Triethanolamine (% w/w)	2	2	2	2	2	2	2	2	2	2	2	2
Double distilled water q.s.	100	100	100	100	100	100	100	100	100	100	100	100

find stoichiometric ratio of cavamax W7 and psoralen, for the preparation of binary complex in order to explore its utility as an effective carrier for delivery of psoralen across skin in comparison to ethosomal gel system. Phase solubility of psoralen with cavamax W7 indicated increase in its solubility on increasing the concentration of cavamax W7. A_L type curve[10] with slope of 0.998 was recorded in the 0.2-0.6% w/v of cavamax W7. Thus the optimum stoichiometric ratio for cavamax W7-psoralen complex was deduced to be 1:1 [Figure 1]. The stability constant ($K_{1:1}$) was 982 M^{-1} which is well within the literature values ranging from 50-2000 M^{-1} for beta-cyclodextrin (β-CD) complexes.[11] This value suggests that the complex formed is of sufficient strength but at it would not hinder the process of dissociation of drug during its release. It is worth mentioning that the phase solubility profile does not verify the formation of inclusion complexes, it solely describes how the increasing concentration of solubilizer influences drug solubility.[12] Based on the phase solubility results, the levels of cavamax W7 selected were 0.3% w/v, 0.5% w/v, and 0.8% w/v for designing cavamax W7 composite ethosomes of psoralen.

Another aspect of preliminary studies was to assess the effect of excipients on solubility of psoralen. The solubility of drug in distilled water was found to be 0.55 mg/ml. In presence of cavamax W7 and soya lecithin, its solubility was enhanced by 2.02 and 1.8 folds, respectively [Table 1]. Cavamax W7 improved the solubility of psoralen by enclosing the drug in its hydrophobic cavity thus forming soluble complexes with the drug. On the other hand, the hydrophobic tails and hydrophilic head region in soya lecithin's structure decrease the surface tension of drug particles in aqueous media facilitating solubilization.[13] The liquids, ethanol, and propylene glycol to be used in formulation of composite displayed considerable cumulative solubility enhancement effect due to the co-solvent action. Finally, all the proposed excipients combined together displayed 7-folds enhancement in solubility of psoralen. Thus, high entrapment of drug can be expected in the presence of proposed excipients. Based on these considerations, cavamax W7 composite ethosomes were designed by solvent injection method and purified by dialysis.

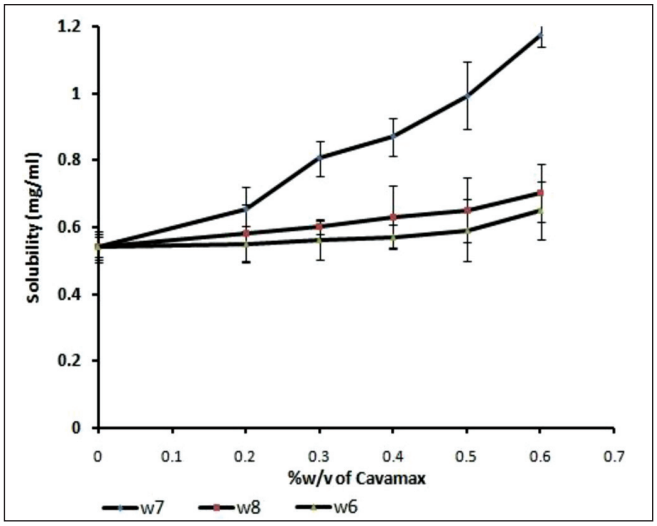

Figure 1: Phase solubility profiles of psoralen in distilled water at increasing concentration of cavamax W7, cavamax W6, cavamax W8

Evaluation of cavamax composite ethosomes

Particle size and zeta potential

The vesicles of composite ethosomal formulations (F1-F9) varied in the size range of 183.73 ± 5.94 nm and 254.3 ± 0.11 nm [Table 5]. For a given strength of soya lecithin, the vesicle size decreased with increasing concentration of cavamax W7. The small sized vesicles were the result of displacement of phospholipids from the outer half of the vesicle membrane by cavamax W7 that resulted in an inward bending of the phospholipid layer contributing to the reduction in vesicle size.[12,14] Therefore, increasing the amount of cavamax W7 in formulation resulted in reduced vesicle size though not significantly ($P > 0.05$). Vesicle size is an important consideration in a topical drug delivery system. For drug delivery to the skin, optimum vesicle size of ethosomes is considered to be less than 200 nm,[13] although vesicle size less than 300 nm is considered to be efficient enough in localizing the drug deep into the skin up to some extent. Considering the latter fact, all the vesicular formulations were appropriate for topical delivery.

The polydispersity index (PDI) of less than 0.3 indicates the homogeneous distribution of particles within the formulation whereas a value greater than 0.3 suggests its heterogeneous nature.[15] Correspondingly, F1, F5, F8, and F9 can be deduced as homogeneous formulations. The PDI was found to be minimum

Table 5: Pharmacotechnical properties of cavamax W7 composite ethosomal vesicles of psoralen

Formulation code	Vesicle size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%)
F1	254.00±0.11	0.258±0.003	−54.17±0.23	72.13±1.05
F2	238.61±5.63	0.323±0.008	−59.67±0.45	85.43±0.98
F3	220.00±2.92	0.322±0.001	−60.12±0.03	89.25±0.56
F4	245.85±4.41	0.363±0.004	−61.23±0.54	76.11±1.04
F5	208.92±2.32	0.268±0.009	−63.76±0.49	90.15±1.25
F6	187.61±6.13	0.377±0.010	−67.89±0.34	94.04±0.56
F7	212.70±4.84	0.314±0.011	−69.02±0.39	79.63±1.92
F8	194.34±2.56	0.268±0.004	−70.11±0.53	90.24±1.07
F9	183.73±5.94	0.241±0.024	−79.87±0.67	98.82±1.18
F_x	189.67±3.46	0.250±0.005	−72.67±0.34	86.96±7.85

F_x: Extra design check point

for F9 (0.241 ± 0.024) probably due to surfactant action of highest level of soya lecithin[8] and the inward bending effect contributed by highest level of cavamax W7 in the formulation. Zeta potential is another parameter that infers thermodynamic stability of the nanosized vesicular system. The vesicular systems were negatively charged and F9 displayed highest zeta potential of 79.87 ± 0.67mV as compared to other formulations. The negative charge on the vesicles might be either due to the ethanol or soya lecithin. Careful analysis of the charge status of each ingredient inferred that psoralen carries no charge, is stable and electrically neutral; and Cavamax W7 is electrically neutral too.[14] So, the net charge on the vesicles can be attributed to soya lecithin and ethanol and highest levels of these imparted highest zeta potential value to F9.

Entrapment efficiency

The tabulated entrapment data [Table 5] revealed an increase in entrapment efficiency on increasing soya lecithin levels; and for a given strength of soya lecithin, the entrapment efficiency increased with increasing levels of cavamax W7. The maximum entrapment efficiency was recorded for the formulation F9 (98.8 ± 1.12%). This data pattern is similar to the vesicle size data and hence same factors and their interplay is attributable to drug entrapment. Apart for this psoralen being water insoluble, lipophilic drug gets easily enclosed in the hydrophobic cavity of cavamax W7 and will be readily entrapped within the ethanolic milieu of ethosomal vesicle. Thus entrapment of psoralen can be visualized both-within the vesicular system as ethanolic solution and adsorbed on the lipidic layer of ethosomes. Thus presence of cavamax W7 in the phospholipid layer has a positive impact on percent drug entrapment of the vesicular carrier.[8] On the basis of particle size, zeta potential and entrapment efficiency (dependent variable), formulation F9 was selected as optimized formulation for preparation of different topical gels with or without using permeation enhancers. In all, 12 gels with different compositions were prepared with or without menthol and isopropyl myristate (both included in Food and Drug Association (FDA) Inactive Ingredients Guide) as permeation enhancers.

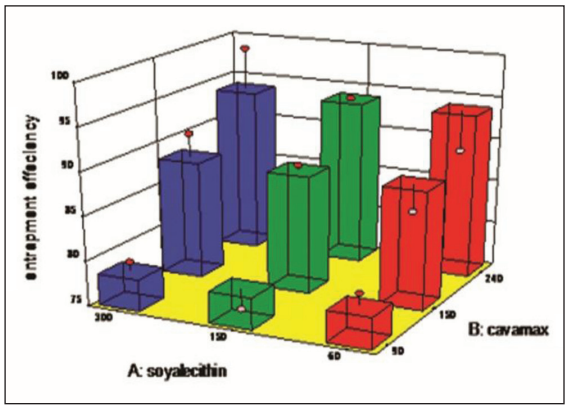

Figure 2: Response surface plot showing the effects of levels of soya lecithin and cavamax W7 on entrapment efficiency

Validation of design

The 3^2 factorial design was validated by Design Expert Software version 8.0.5 (Stat-Ease, Minneapolis, USA Inc.). A general statistical model can be depicted with respect to the data obtained from the formulations subjected to optimization. The model developed can be characterized by polynomial equation representing the response data. The final transformed obtained by removing all the insignificant values was

$$\% \text{ Entrapment efficiency} = 87.11 - 8.45\ (X_1) + 1.52\ (X_2) \quad ...(2)$$

From the equation, response surface plot [Figure 2] was generated and it was observed that at highest levels of soya lecithin and cavamax W7 (1% and 0.8%, respectively) the entrapment efficiency increased. The percent entrapment efficiency of the extra design check point formulation (F_x) was found to be 86.96 ± 7.85%. Comparison with predicted value of 81.16% obtained from the transformed equation indicated no significant difference ($P < 0.05$) between the two values when checked using one way ANOVA, thus validating the design.

Selection of optimized formulation

The optimized formulation of composite ethosomal formulation was identified as F9 on the basis of highest entrapment efficiency of 98.8 ± 1.12%. Its least vesicle size of 183.73 ± 5.94 nm, lowest PDI of 0.241 and highest zeta potential of –79.87 strengthened its selection.

Vesicular visualization

Electron microscope images of F9 depicted the presence of uniformly distributed spherical-shaped [Figure 3a], nano-sized vesicles and absence of aggregates. Careful observation of the vesicle image revealed dense thick periphery with light transparent core, confirming vesicle formation. The vesicles were close to 200 nm [Figure 3b] which is desirable for ethosomal formulations for topical delivery.[13]

Stability

The stability data of optimized cavamax W7 composite ethosomal (F9) formulation has been summarized in Table 6. As indicated that there was no significant difference ($P > 0.05$) in vesicular size, zeta potential and percent entrapment efficiency of cavamax

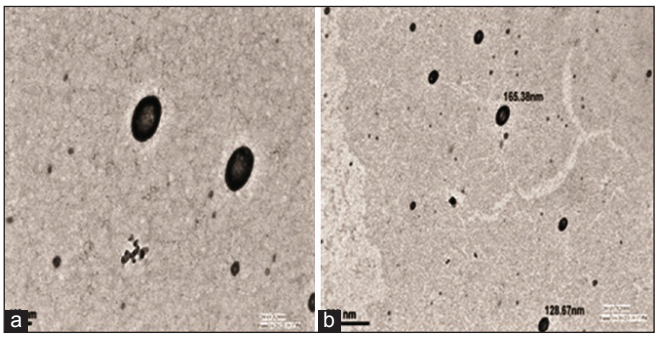

Figure 3: Transmission electron micrographs of composite ethosomes of psoralen (F9)

W7 composite ethosomal formulation before and after storage for 6 months indicating good stability of cavamax W7 composite ethosomal formulation. It was due to the stabilizing effect exerted by both cavamax W7 and ethanol. Incorporation of β-CD is reported to improve the stability and imparts rigidity to the vesicles[14] and presence of ethanol avoids aggregation of vesicles arising out of electrostatic repulsion due to net negative charge exerted on the surface of the particles.[16]

Characterization of cavamax W7 psoralen complex

Differential scanning calorimetry

The thermogram of psoralen exhibited sharp endothermic peak at 165.4°C, corresponding to its melting point[17] [Figure 4a]. The thermogram of Cavamax W7 [Figure 4b] showed a broad endothermic peak at 110.8°C, possibly due to the release of water molecules and a decomposition peak at 326.9°C.[18] Their physical mixture [Figure 4c] was an additive spectrum of the two with slight shift and decreased peak height of psoralen and widened peak of cavamax W7. The thermal profile of the psoralen-cavamax W7 complex [Figure 4d] showed complete disappearance of the psoralen endothermic peak, indicating formation of an amorphous complex and deposition of the drug in the cavamax W7 cavity.[19] The melting point of the guest molecule when embedded in the cavamax W7 cavity generally shifts to a different temperature or disappears within the temperature range where cavamax W7 decomposes.[20] Thermal analysis provided significant indication of amorphous product.

Scanning electron microscopy

The scanning electron micrographs are a mode to study the changes at morphological levels. Scanning electron microphotographs of psoralen, cavamax W7, and psoralen-cavamax W7 binary complexes and physical mixture are shown in Figure 5. Psoralen appeared as rod-shaped crystal [Figure 5a] and Cavamax W7 [Figure 5b] presented parallelogram shaped crystals. The physical mixture of psoralen and cavamax W7 [Figure 5c] indicated physical deposition of drug particles on surface of cavamax W7. In the micrograph of the complex original morphology of the raw materials disappeared and it was not possible to differentiate the two components as the crystalline structure of psoralen disappeared [Figure 5d] and amorphous agglomerates were formed. This change in particle morphology indicates formation of a new solid phase, in accordance to the report by Lee *et al.*[21]

Table 6: Stability study data of the optimized psoralen loaded cavamax W7 composite ethosomes (F9)

Formulation code	Time period (months)	Parameters		
		Vesicle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
F9	0	183.73±5.94	−79.87±0.67	98.80±2.12
	6	196.78±6.97	−74.98±0.89	91.28±1.98

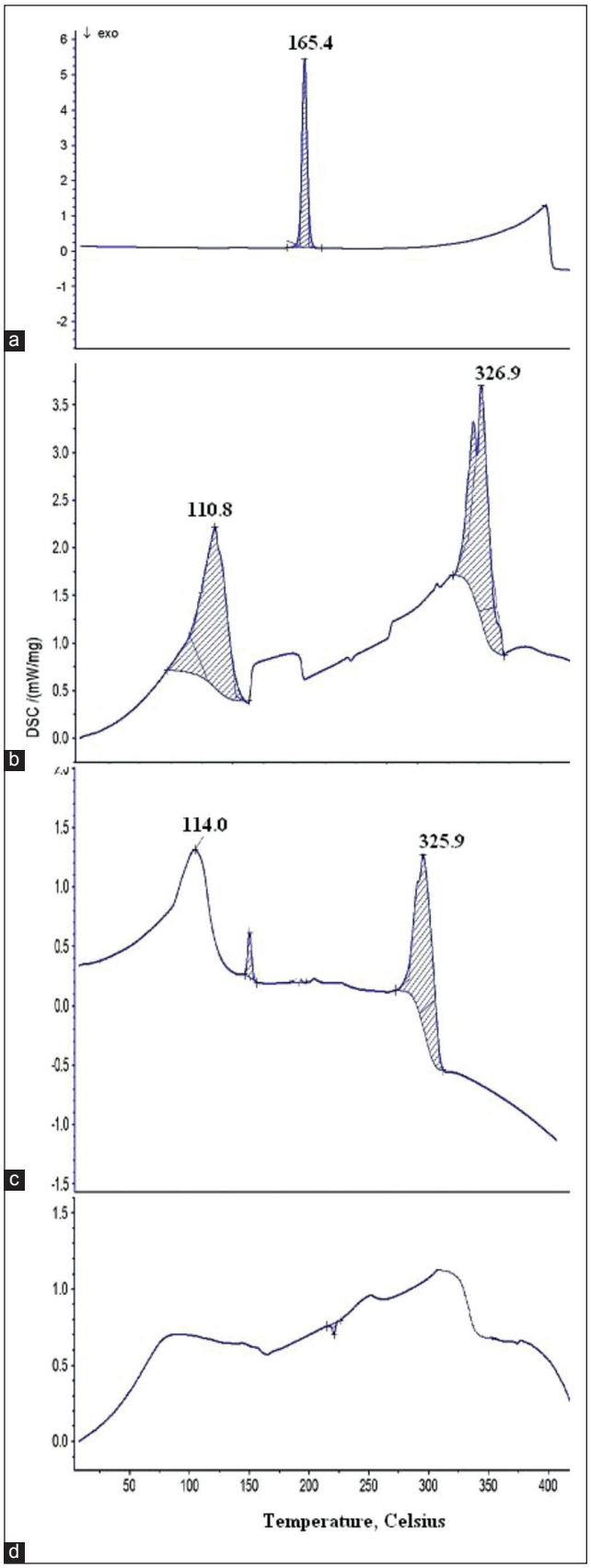

Figure 4: Differential Scanning thermograms of (a) psoralen, (b) cavamax W7, (c) physical mixture and (d) psoralen: cavamax W7 binary complex

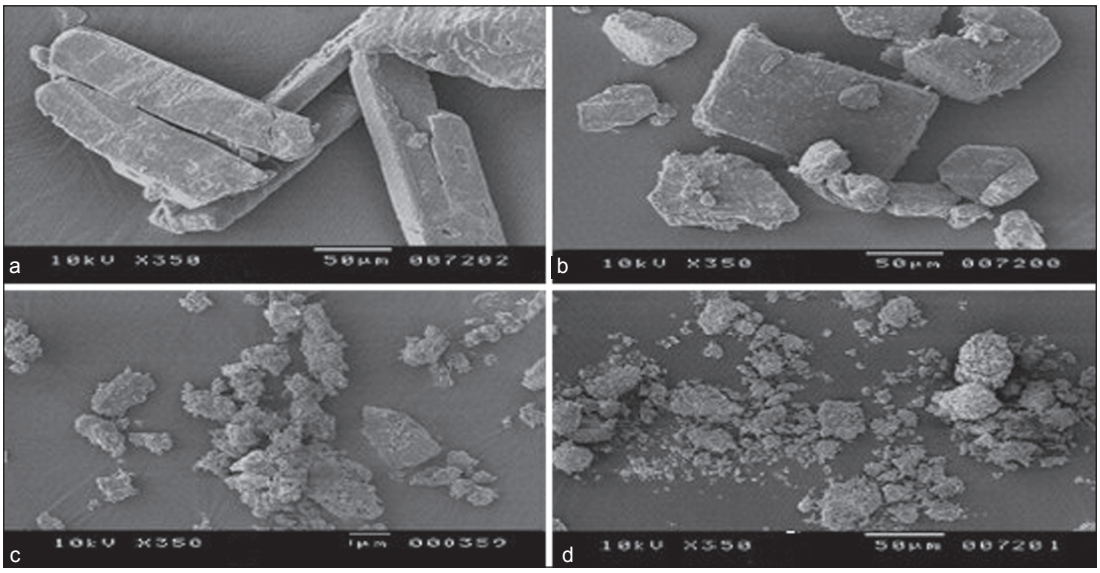

Figure 5: Scanning electron micrographs of (a) psoralen crystals, (b) cavamax W7, (c) physical mixture, (d) psoralen cavamax complex

Diffuse reflectance spectroscopy

Another additional indicator of the formation of a drug: Cavamax W7 complex was the changes observed in the diffuse reflectance spectrographs. Figure 6 displays the diffuse reflectance spectra for psoralen, cavamax W7, complex, and the physical mixture. The spectral analysis of psoralen showed characteristic (aromatic C-H stretch), 2402, 2284, 2002 cm^{-1} (overtone band), 1787 (C = O stretching vibration of unsaturated lactones), 1609 (C = C of ring), (C-C(= O)-C stretching vibration), 1189 (O-C-C band), 1052 (symmetric C-O-C stretch), and 753 (out of plane C-H bend), for psoralen.[4] The spectrum of physical mixture was an additive spectrum of psoralen and cavamax W7, with peak broadening and lowering of peak intensity. It can be clearly observed that the diffuse reflectance spectrum for the complex is almost identical to that of cavamax W7 alone. On the other hand, in the complex psoralen aromatic region peaks i.e., principal peaks at wave numbers 3057 cm^{-1} C-H stretch, overtone band 2402, 2284, 2002, 1609 C = C of ring, and 1310 cm^{-1} C-C = O-C stretching vibration disappeared which suggests that these groups of the drug are included in the cavity, while C = O stretching vibration of unsaturated lactones at 1787 cm^{-1} was retained suggesting it to be outside the cavamax W7 cavity.

X-Ray diffraction

X-ray diffractogram confirmed the crystalline nature of psoralen [Figure 7a], and cavamax W7 [Figure 7b] by the presence of multiple sharp peaks. The diffractogram of the physical mixture [Figure 7c] could be considered as the superimposition of the patterns of the pure components with some variations in the shapes and intensities of the characteristic diffraction peaks, while the inclusion complex [Figure 7d] showed reduction in crystallinity marked by lower intensity of the diffraction peaks due to particle size reduction during complex formation by kneading. These results are in strong agreement with the work of Bayomi *et al.*[22] X-Ray diffraction pattern of psoralen-cavamax W7 complex indicated characteristic peaks of psoralen but with reduced intensity. This indicates the presence of psoralen in the complex and reduction of intensity with slight significant difference in peak width and with uplifted base line indicates reduced crystalline nature of the formulation. The shift in base line is due to the fact that amorphous powder do not have sharp edges and when X-rays are passed through them, it is not diffracted completely, thereby, the spectra is blurred and peaks seems to be fused together.[23] All the spectral studies strongly indicated formation of amorphous new solid phase of the psoralen cavamax W7 complex. The aim for the formation of complex was to analyze and evidence the superiority of ethosomal formulation over the complex of psoralen. Literature scan revealed numerous research reports on topical delivery systems wherein β-CD complexes of drugs have been prepared for improved topical delivery. But improvement in topical delivery by enhancement in solubility of drug is limited considering the barrier properties of stratum corneum. Consequently, topical gels were formulated using vesicular formulations (composite ethosomes and ethosomes) of psoralen and compared with complexed drug and pure drug formulations as reference. The gels were evaluated for various parameters to identify the best formulation in terms of physical and *in vitro* permeation characteristics.

Evaluation of gels

pH and drug content

The gels were translucent with faint odor of raw materials used. The pH of psoralen loaded cavamax W7 composite ethosomal, ethosomal, complexed psoralen and psoralen gels was found to be in range of 6.72 ± 0.10 and 7.25 ± 0.06 [Table 7]. All the formulations seem to be non-irritating to the skin as pH of all the formulations lied between the normal physiological pH range of the skin i.e., 3.0-9.0.[24] Higher pH or overly acidic pH of the topical products alters the pH of the skin, thereby causing redness, dryness and irritation that is not clinically

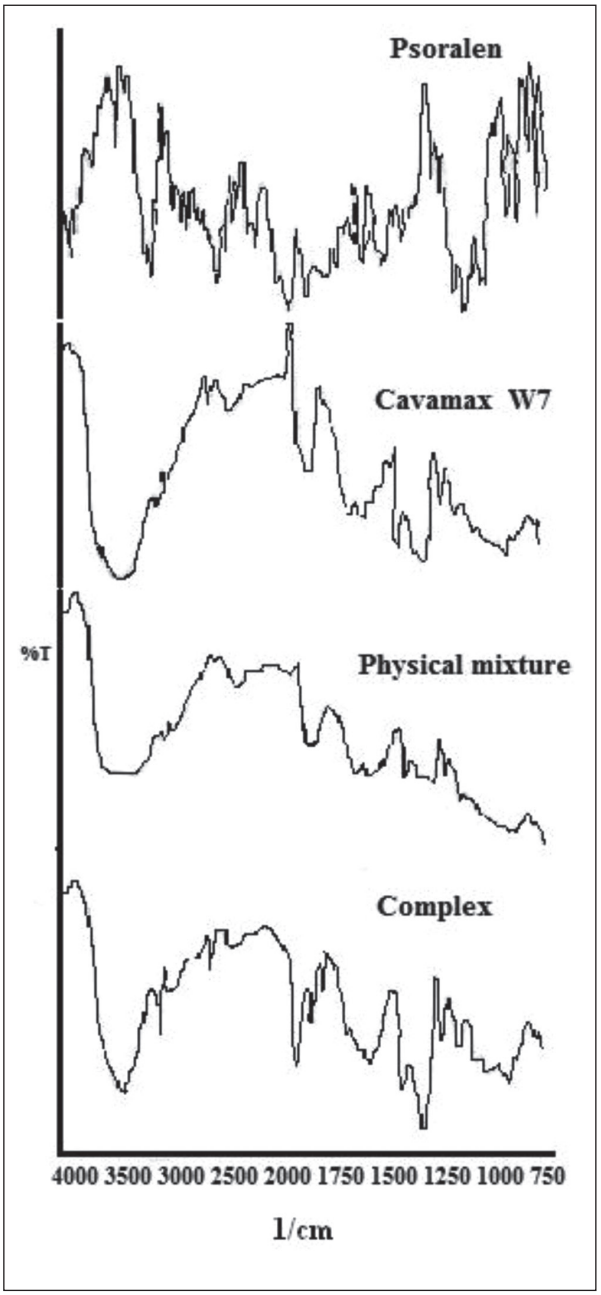

Figure 6: Diffuse reflectance spectrographs of psoralen, cavamax W7, and physical mixture, complex

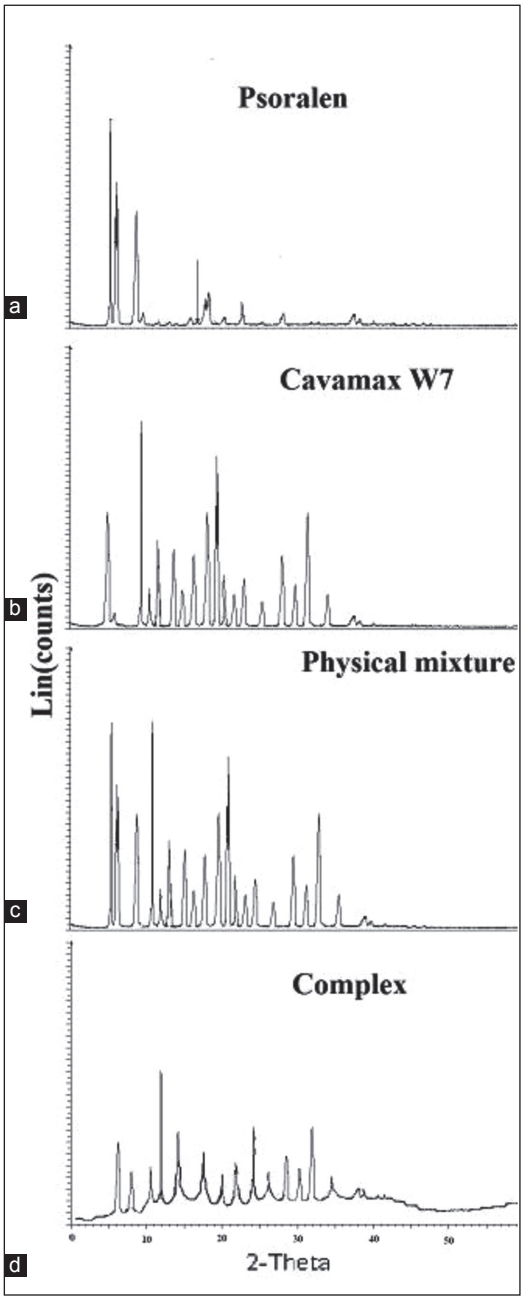

Figure 7: X-Ray diffractograms of psoralen, cavamax W7, physical mixture, and psoralen cavamax complex

desirable in case of pre-existing psoriatic condition. Drug content of cavamax W7 composite ethosomal gel ranged between 85.88 ± 1.65 and 94.36 ± 3.15 clarifying that the drug was dispersed homogeneously throughout the gels. Drug content of cavamax W7 composite ethosomes was found to be higher as compared to reference ethosomes due to presence of cavamax W7 in composite ethosomes that led to higher solubilization and hence higher entrapment efficiency.[8] On comparing psoralen gels (G10-G12) and cavamax W7-Psoralen complex gels (G7-G9), the latter showed higher entrapment efficiency than psoralen gels (G10-G12). Drug content of G7-G9 was in the range of 82.00 ± 1.19 and 92.70 ± 1.39%, which is less than the ethosomal content inferring that ethosomal preparation possesses higher drug content than the gels formed using conventional formulae.

Ex-vivo skin permeability

Ex vivo skin permeability studies were done to evaluate the role of cavamax W7 and ethosomes as a promising and effective carrier in enhancing the permeation across the rat skin. *In vitro* permeability profiles of the formulations in phosphate buffer pH 6.8 [Figure 8] were used to calculate *in vitro* permeability parameters [Table 7]. Higher permeation rates from cavamax W7 composite ethosomal gels was due to higher percent of drug content present in it, which increased the cumulative amount

Table 7: Pharmaceutical parameters of psoralen loaded ethosomal gels (G1-G3) and cavamax W7 composite ethosomal gels (G4-G6), complex gel (G7-G9), psoralen gel (G10-G12)

Formulation code	pH	Drug content %	Model	r^2	Flux (μg/cm²/hr)	Enhancement ratio	Similarity factor (f_2)
G1	7.21±0.13	85.88±1.65	Zero-order	0.960	3.367	4.15	35.50
G2	7.20±0.06	92.79±1.89	Hixcon-crowell	0.981	3.630	4.47	35.00
G3	7.21±0.02	88.64±0.98	Zero-Order	0.987	3.473	4.28	36.20
G4	6.93±0.10	88.34±4.36	Zero-order	0.976	4.988	5.90	55.57
G5*	6.71± 0.54	94.36±3.15	Zero-order	0.967	5.527	6.81	100
G6	6.82±0.10	89.78±1.64	Peppas	0.940	3.792	6.14	42.30
G7	6.86±0.21	89.08±2.45	Peppas	0.981	3.140	3.87	24.50
G8	7.25±0.06	92.70±1.39	Zero-order	0.982	4.420	5.45	44.00
G9	6.72±0.15	91.43±2.39	Peppas	0.980	3.871	4.77	25.25
G10**	7.14±0.34	82.00±1.19	Zero-order	0.976	0.811	-	25.00
G11	7.25±0.34	88.69±2.15	Peppas	0.800	2.960	3.64	25.50
G12	6.94±0.17	86.10±0.50	Peppas	0.931	1.870	2.30	26.50

*Reference formulation for calculation of f_2; **Reference formulation for calculation of enhancement ratio

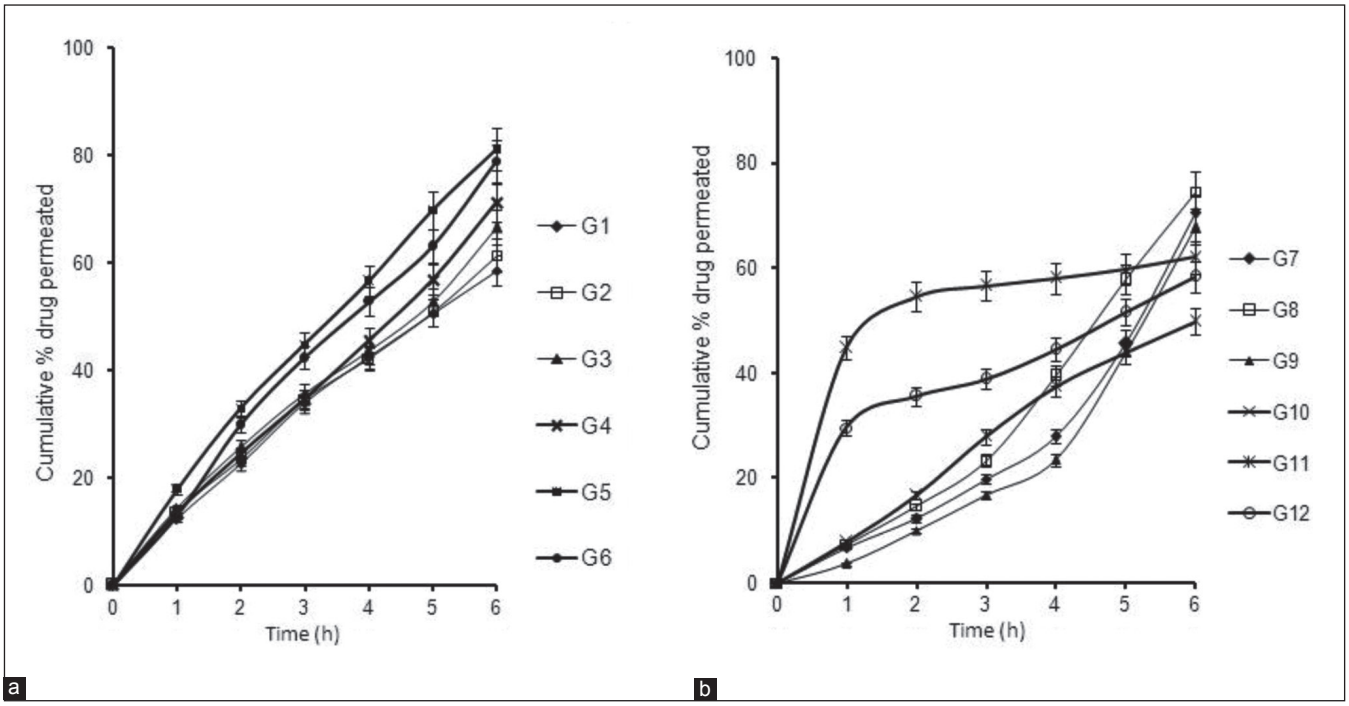

Figure 8: Comparative *ex-vivo* permeability profiles of psoralen from ethosomal (G1-G3) gels (G1-G3), cavamax W7 ethosomal gels (G4-G6), psoralen complex gels (G7-G9) and psoralen gels (G10-G12) in phosphate buffer pH 6.8 in 6 hr across rat skin

of drug permeated. As well as due to the synergistic effect exerted by ethanol and cavamax W7 in cavamax W7 composite ethosomes, permeation rate further enhanced thus, exhibited higher permeation rate as compared to ethosomal gels. In the presence of permeation enhancers, isopropyl myristate and menthol, enhancement in permeation was observed in ethosomal gel, psoralen complex gels and psoralen gels as expected in all, except cavamax W7 composite ethosomal gel. Isopropyl myristate is an aliphatic ester and is extensively employed as permeation enhancer in dermatological products. Though, its mode of action is not clear, but, it is supposed to acts by penetrating between and disrupting the organization of lipid bilayer of stratum corneum as described by Mortazavi and Aboofazeli[25] whereas, menthol enhances permeation by its distribution preferentially into the intercellular spaces of stratum corneum and the possible reversible disruption of the intercellular lipid domain.[26] This enhancement in permeation may be due to permeation of drugs by both the lipid and pore pathways, thus enhancing the permeation of drugs.[27] On comparing the permeation profiles with that of control gel profile (reference) using one way ANOVA, significant difference ($P > 0.05$) was observed (d.f = 11).

Similarity factor (f_2) that is used to compare the similarity in dissolution profiles was also calculated. For this purpose, G5 was selected as reference formulation based on zero order maximum % cumulative drug permeation of 82.48%. A f_2 value of greater than 50 indicates similarity in profiles and in our case, the f_2 value of all the formulations was less than 50, except for

G4 (composite ethosomal gel) that showed an f_2 value of 55.57, slightly higher than 50 indicating similarity in its permeation profile to G5 [Table 7]. The permeation profiles of rest of the formulations namely ethosomal gels (G1-G3), psoralen complex gels (G7-G9), and psoralen gels (G10-G12) were dissimilar to G5 as the values of f_2 ranged from 25-44.

Among ethosomal gels (G1-G3), G2 was selected as best formulation as it showed a steady state flux of 3.630 μg/cm²/min that followed zero order release kinetics. On the other hand, G4 was selected as optimized gel among cavamax W7 composite ethosomal gels (G4-G6) as it followed zero order permeation kinetics and reached the target flux with a steady state flux 4.89 μg/cm²/min. However, G5 also showed a permeation profile comparable to G4 with no significant difference ($P < 0.05$) in percent cumulative drug permeated thus suggesting negligible influence of menthol as permeation enhancer incorporated in G5. The flux achieved by G4 was found to be equivalent to the flux showed by ethosomal gels made with permeation enhancers and was significantly different ($P > 0.05$) from ethosomal gel (G1). This suggests that permeation time and flux of psoralen has been significantly enhanced utilizing cavamax W7 composite ethosomes, thereby, inferring its superiority over convention gels as a carrier system for psoralen. This system may be a superior alternate for delivery of psoralen across skin within a short period of time.

Enhancement ratio was calculated taking psoralen gel without permeation enhancer (G10) as reference formulation [Table 7]. Effect of permeation enhancer is clearly observed with both menthol and isopropyl myristate but marked increase in enhancement ratio was exhibited in the formulations incorporated with menthol. However, the permeation properties of cavamax W7 composite ethosomes were least affected by both the permeation enhancers as the enhancement ratio values are insignificant when compared to other formulations.

Patil *et al.*,[28] had reported that cavamax W7 enhanced the permeability by acting as permeation enhancers, thus, assisting in carrying the drug to the biological membrane i.e., skin. In presence of cavamax W7, increase in flux is expected, due to the fact that as the flux is related to the concentration of free drug present and cavamax can only complex with the drug present in excess of its solubility, thus enhancing its flux. Also, the interaction of cavamax W7 with the skin causes extraction of stratum corneum lipids, thereby, enhancing the skin permeation.[29]

Confocal laser scanning microscopy

The confocal laser scanning microscopy revealed that the penetration of rhodamine from control gel [Figure 9a] was confined to the stratum corneum layer (9 μm) of the skin in 12 h. While in case of cavamax composite ethosomal gel [Figure 9b], enhanced permeation of rhodamine B was observed in and beyond the stratum corneum, till stratum spinosum (16 μm-55 μm) in 6 h. Increased permeation of rhodamine from composite ethosomes was due to the presence of ethanol that possesses the ability to interact with the lipid molecules, resulting in increased stratum corneum fluidity. This in turn enhances inter- and intracellular permeability of the ethosomes. Ethanol also provides flexibility to the ethosomal membrane that enhances the skin penetration.[30] The results indicate that cavamax ethosomes were two times faster in traversing the stratum corneum than control gel that was predominantly accumulated in the upper layer (stratum corneum) of epidermis. Melanoma, a common side effect of psoralen which occurs on prolonged contact with sunlight can be a potential side effect of non ethosomal gel[10] and possibly be overcome by encapsulating psoralen in ethosomes.

CONCLUSION

A topical delivery system of psoralen was successfully designed as composite ethosomal vesicular gel that was efficacious in comparison to ethosomal formulation in its permeation characteristics. The permeation capacity of the developed formulation was much higher than reference ethosomal formulation even in the absence of permeation enhancers.

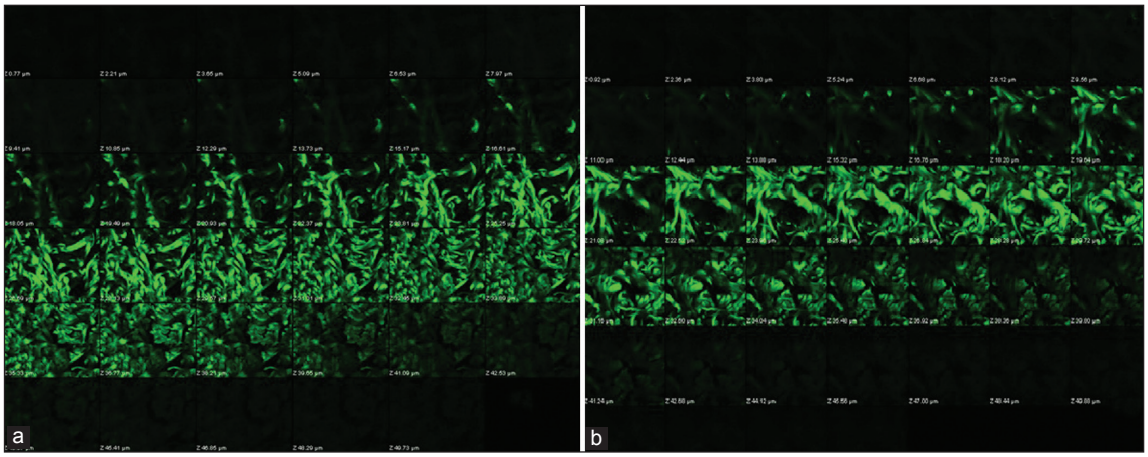

Figure 9: Confocal laser scanning micrographs of (a) control gel after 12 hr, and (b) cavamax W7 composite ethosomal gel after 6 hr using rhodamine as the fluorescent tracer

This is of clinical importance particularly in psoriatic condition where the skin conditions are scaly and non facile for delivery of drugs. Conclusively, cavamax W7 psoralen composite ethosomes present themselves as efficient carrier for superior topical delivery of psoralen and have potential for clinical applications in minimizing severe side effects associated with photosensitivity of psoralen.

REFERENCES

1. Ghazizadeh R, Tosa M, Ghazizadeh M. Clinical improvement in psoriasis with treatment of associated hyperlipidemia. Am J Med Sci 2011;341:394-8.
2. Katare OP, Raza K, Singh B, Dogra S. Novel drug delivery systems in topical treatment of psoriasis: Rigors and vigors. Indian J Dermatol Venereol Leprol 2010;76:612-21.
3. Ali J, Akhtar N, Sultana Y, Baboota S, Ahuja A. Antipsoriatic microemulsion gel formulations for topical drug delivery of babchi oil (Psoralea corylifolia). Methods Find Exp Clin Pharmacol 2008;30:277-85.
4. Patel NA, Patel NJ, Patel RP. Comparative development and evaluation of topical gel and cream formulation of psoralen. Drug Discov Ther 2009;3:234-42.
5. Faiyazuddin M, Akhtar N, Akhter J, Suri S, Shakeel F, Shafiq S, *et al*. Production, characterization, *in vitro* and *ex vivo* studies of babchi oil-encapsulated nanostructured solid lipid carriers produced by a hot aqueous titration method. Pharmazie 2010;65:348-55.
6. Singh HP, Utreja P, Tiwary AK, Jain S. Elastic liposomal formulation for sustained delivery of colchicine: *In vitro* characterization and *in vivo* evaluation of anti-gout activity. AAPS J 2009;11:54-64.
7. Maestrelli F, González-Rodriguez ML, Rabasco AM, Mura P. Effect of preparation technique on the properties of liposomes encapsulating ketoprofen-cyclodextrin complexes aimed for transdermal delivery. Int J Pharm 2006;312:53-60.
8. Akhtar N, Pathak K. Cavamax composite ethosomal gel of clotrimazole for improved topical delivery: Development and comparison with ethosomal gel. AAPS PharmSciTech 2012;13:344-55.
9. Masood Q, Majid I. Photo chemotherapy and its variants: Safe and useful modes of therapy. JK Pract 2004;1:166-70.
10. Higuchi T, Connors K. Phase-solubility techniques. Adv Anal Chem Instrum 1965;4:117-212.
11. Loftsson T, Jarto P, Masson M, Jarvinen T. Cyclodextrins in drug delivery. Expert Opin Drug Deliv 2005;2:335-51.
12. Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: An updated review. AAPS PharmSciTech 2005;6:329-57.
13. Verma P, Pathak K. Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep skin infections through topical gel formulation. Nanomedicine 2012;8:89-96.
14. Pandey S, Kumar B, Swamy SM, Gupta A. A review on pharmaceutical application of cyclodextrins. Int J Pharm Tech 2010;2:281-319.
15. Shah M, Pathak K. Development and statistical optimization of solid lipid nanoparticles of simvastatin by using 2^3 full factorial design. AAPS PharmSciTech 2010;11:489-96.
16. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS PharmSciTech 2007;111:1-15.
17. Mahendra AA, Baudara GH, Rohinie GA, Disala JC. Studies on the composition and standardization of "Bakuchi Oil": An ayurvedic medicinal oil prepared from *Psoralea corilyfolia* used in the treatment of vitiligo. Int J Res Ayurveda Pharm 2012;3:411-5.
18. Hassan M, Suleiman M, Najib N. Improvement of the *in vitro* dissolution characteristics of famotidine by inclusion in cyclodextrin. Int J Pharm 1990;58:19-24.
19. Sami F, Philip B, Pathak K. Effect of auxiliary substances on complexation efficiency and intrinsic dissolution rate of gemfibrozil-β-CD complexes. AAPS PharmSciTech 2010;11:27-35.
20. Cabral-Marques H, Hadgraft J, Kllaway I. Studies of cyclodextrin inclusion complexes: The salbutamol-cyclodextrin complex as studies by phase solubility and DSC. Int J Pharm 1990;63:259-66.
21. Lee PS, Han JY, Song TW, Sung JH, Kwon OS, Song S, *et al*. Physicochemical characteristics and bioavailability of a novel intestinal metabolite of ginseng saponin (IH901) complexed with-cyclodextrin. Int J Pharm 2006;3:29-36.
22. Bayomi MA. Abanumay KA, Al-Angray AA. Effect of inclusion complexation with cyclodextrins on photostability of nifedipine in solid state. Int J Pharm 2002;243:107-17.
23. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. Mumbai: Himalaya Publishing House (India); 2004. p. 2.303-2.331.
24. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. J Adv Pharm Technol Res 2010;1:274-82.
25. Mortazavi SA, Aboofazeli R. An investigation into the effect of various penetration enhancers on percutaneous absorption of piroxicam. Iran J Pharm Res 2003;2:135-40.
26. Kunta JR, Goskonda VR, Brotherton HO, Khan MA, Reddy IK. Effect of menthol and related terpenes on the percutaneous absorption of propanolol across excised hairless mouse skin. J Pharm Sci 1997;86:1369-73.
27. Mohanty B, Nayak AK, Sen KK. Comparative evaluation of *in vitro* diclofenac sodium permeability across excised mouse skin from different common pharmaceutical vehicles. Int J PharmTech Res 2010;2:920-30.
28. Patil JS, Kadam DV, Marapur SC, Kamalapur MV. Inclusion complex system; a novel technique to improve the solubility and bioavailability of poorly soluble drugs: A review. Int J Pharm Sci Rev Res 2010;2:29-33.
29. Vemula UR, Lagishetty V, Lingala S. Solubility enhancement techniques. Int J Pharm Sci Rev Res 2011;5:41-51.
30. Aggarwal G, Goel A, Dhawan S, Shama A. Carriers/vesicles based approaches for penetration enhancement in Transdermal drug delivery. Latest Rev 2010;8:1-15.

How to cite this article: Kumari S, Pathak K. Cavamax W7 composite psoralen ethosomal gel versus cavamax W7 psoralen solid complex gel for topical delivery: A comparative evaluation. Int J Pharma Investig 2013;3:171-82.
Source of Support: Nil. **Conflict of Interest:** None declared.