Original Article

Comparative development and evaluation of topical gel and cream formulations of psoralen

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ABSTRACT: The aim of the present investigation is to develop topical gel and cream formulations of psoralen for enhancing its transport through the skin, with the goal to shorten the delay between drug application and UVA irradiation. In our first studies, oil-in-water (O/W) creams of psoralen (0.05% concentration) were prepared using Apifil (PEG-8 Beeswax) and Plurol Stearique WL 1009 as emulsifying agents and aqueous cream (British Pharmaceutical Codex) as the cream base material. In our second studies, hydroalcoholic transparent gel formulations of this drug in a 0.05% concentration were prepared using hydroxypropylcellulose (HPC) as the gelling agent. The physicochemical compatibility between psoralen and formulation excipients used in the cream and gel formulations was confirmed by using differential scanning calorimetry and Fourier transform infrared spectroscopy. All prepared cream and gel formulations were evaluated for drug content uniformity, viscosity, pH, stability, and limpidity. The release of psoralen from all formulations *via* dialysis through a cellulose membrane into phosphate buffer pH 6.8 at 37°C was studied. The penetration enhancing effect of menthol (0-12.5%, w/w) on the percutaneous flux of psoralen through excised rat epidermis from gel and cream formulations was also investigated. The release profile of psoralen from gel formulations was higher than that from cream formulations. The percutaneous flux and enhancement ratio of psoralen across rat epidermis was significantly enhanced by the addition of menthol in both gel and cream formulations as compared to gel and cream formulations prepared without menthol ($p < 0.05$).

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Keywords: Psoralen, hydroalcoholic gel, cream formulation, *in vitro* skin permiation, vaginal, cosolvents

1. Introduction

Psoralen (*7H*-furo[3,2-g][1]benzopyran-7-one; PSO) is the parent compound of a family of naturally occurring furocoumarins used by plants as phytoalexins to combat attacks from fungi and insects (*1*).

PSO is increasingly used in dermatology for the photochemotherapy of diseases such as vitiligo, psoriasis, mycosis fungoides, atopic eczema and alopecia areata among others. The psoralens are currently employed in dematology (orally or topically), associated with ultraviolet A (UVA) irradiation. The combination of these previous compounds with UVA irradiation is known as PUVA therapy (psoralens plus UVA irradiation) (*2-7*).

According to the British Photodermatology Group, PSO is typically administered per os (1.2 mg/kg), 3 h before UVA irradiation. Topical application of psoralen can be advisable when the number and extension of affected areas are limited because it reduces the systemic side effects (*8*).

Topical and transdermal products are important classes of drug delivery systems, and their use in therapy is becoming more widespread. To be effective, topical dosage forms should conveniently deliver therapeutically useful drug concentrations at target sites (*i.e.*, basal layer of epidermis). Topical cream and gel formulations offer better patient compliance and hence are more acceptable to patients (*9*).

Hydroxypropylcellulose (HPC) is widely used in the pharmaceutical and cosmetic industries to give viscous or gel formulations. HPC possesses several desirable attributes as gelling agent including: high viscosity at low concentrations, stability to heat with negligible batch-to-batch variability, increased stability of formulations and has a pleasant texture. It is unaffected by aging, does not support bacterial or fungal growth, and is nonirritating (*10-12*).

Apifil® (PEG-8 Beeswax) and *Plurol*® *Stearique WL 1009* (polyglyceryl-6-distearate) were selected as O/W emulsifiers. Both of these emulsifiers can be used to formulate creams with varying concentration of oily phase without phase inversion. At higher concentrations, 5% to 15%, they form stable creams with a firm texture and a smooth, glossy appearance. They are particularly well-suited for the emulsification of vegetable oils (up to 15%) or short chain/medium polarity fatty acid esters. They perform well with other fatty acid esters, silicone oils, mineral oils and their substitutes.

The purposes of the present study are (*a*) to formulate an oil-in-water (O/W) cream and hydroalcoholic gel formulations containing psoralen, (*b*) to determine the effect of various additives on *in vitro* release of the drug from these formulations, and (*c*) to investigate the influence of permeation enhancers on drug permeation through rat skin.

2. Materials and Methods

2.1. *Materials*

PSO powder (purity: 90%) was procured from Yucca Laboratories, Bombay, India and used in the study without further purification. Two emulsifiers were chosen for cream PSO formulation: *Apifil*® (PEG-8 Beeswax) and *Plurol*® *Stearique WL 1009* (polyglyceryl-6-distearate), both were obtained as generous gift samples from GATTEFOSSE, France. Aqueous cream (British Pharmaceutical Codex) was used as the cream base material. HPC was procured from Colorcon Asia Pvt Ltd. (Mumbai, India). Other materials used in the study (2-propanol, propylene glycol, methanol, white soft paraffin, liquid paraffin, phenoxyethanol, potassium dihydrogen phosphate, *etc.*) were of analytical grade and procured from SD Fine Chemicals, India. Double-distilled water was used throughout the study.

2.2. *Investigation of physicochemical compatibility of drug and polymer*

The physicochemical compatibility between PSO and formulation excipients used in the cream and gel formulations was studied by using differential scanning calorimetry (DSC-Shimadzu 60 with TDA trend line software, Shimadzu Co., Kyoto, Japan) and Fourier transform infrared spectroscopy (FTIR-8300, Shimadzu Co., Kyoto, Japan).

In DSC analysis, the samples were hermetically sealed in flat-bottom aluminum pans, and heated over a temperature range of 35-300°C at a constant increasing rate of 10°C/min in an nitrogen atmosphere (50 mL/min). The thermograms obtained for PSO and physical mixtures of PSO with formulation excipients were compared.

Infrared (IR) spectra were recorded using an FTIR in the wavelength region between 4000 and 400 cm^{-1}. The spectra obtained for PSO and physical mixtures of PSO with formulation excipients were compared.

2.3. *Preparation of formulations*

The composition of different cream and gel formulations with and without permeation enhancer is shown in Tables 1 and 2.

2.3.1. *Oil-in-water cream samples*

All the aqueous phase material and the oil phase ingredients were placed in separate stainless steel containers and heated above 70°C. The water phase then was added to the oil phase with continuous agitation. The semisolid emulsions (O/W) were then cooled to approximately 40°C, and the PSO, previously dissolved in methanol, was incorporated. Other additives included in the formulation were also added at this stage. The batch was mixed to reach ambient temperature. The samples were then kept in airtight aluminum tubes.

2.3.2. *HPC gels*

The HPC powder was added to the required quantity of hot distilled water while being stirred, and the solution was allowed to cool. The methanolic solution of PSO (0.05%, w/v) was dissolved in 2-propanol and propylene glycol and mixed with hydrated HPC with continuous stirring at 37°C until the gel was formed (2 h).

2.4. *Analytical method*

All samples were analyzed for PSO content spectrophotometrically at a wavelength of 246 nm.

2.5. *Physicochemical properties*

2.5.1. *Drug content uniformity*

All samples were analyzed for PSO content prior to diffusion studies. Only samples with PSO contents within 100 ± 10% were used for diffusion studies. Drug content of the cream and gel formulations (1 g) was determined by dissolving an accurately weighed quantity of formulation in about 50 mL of pH 6.8 phosphate buffer containing 20% (v/v) ethanol. These solutions were quantitatively transferred to volumetric flasks and appropriate dilutions were made with the same buffer solution. The resulting solutions were then filtered through 0.45 µm membrane filters before subjecting the solutions to spectrophotometric analysis for PSO at λmax of 246 nm. Drug content was calculated from the linear regression equation obtained

Table 1. Composition of gel and cream formulations

Composition of gel formulations (%, w/w)							
Materials	Code						
	G	G1	G2	G3	G4	G5	G6
PSO	0.05	0.05	0.05	0.05	0.05	0.05	0.05
HPC	1.5	1.5	1.5	1.5	1.5	1.5	1.5
2-Propanol	–	10	15	20	–	–	–
Propylene glycol	–	–	–	–	10	15	20
Methanol	30	20	15	10	20	15	10
Distilled water q.s. to make	100	100	100	100	100	100	100
Composition of cream formulations (%, w/w)							
Materials	Code						
	C	C1	C2	C3	C4	C5	C6
PSO	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Apifil®	–	3	6	9	–	–	–
Plurol®	–	–	–	–	3	6	9
Emulsifying wax	9	6	3	–	6	3	–
White soft paraffin	15	15	15	15	15	15	15
Liquid paraffin	6	6	6	6	6	6	6
Phenoxyethanol	1	1	1	1	1	1	1
Methanol	10	10	10	10	10	10	10
Distilled water q.s. to make	100	100	100	100	100	100	100

Table 2. Composition of gel and cream formulations with permeation enhancer

Composition of gel formulations with permeation enhancer (%, w/w)						
Materials	Code					
	G3	G3M1	G3M2	G3M3	G3M4	G3M5
PSO	0.05	0.05	0.05	0.05	0.05	0.05
HPC	1.5	1.5	1.5	1.5	1.5	1.5
2-Propanol	20	20	20	20	20	20
Propylene glycol	–	–	–	–	–	–
Methanol	10	10	10	10	10	10
Menthol	–	2.5	5.0	7.5	10	12.5
Distilled water q.s. to make	100	100	100	100	100	100
Composition of cream formulations with permeation enhancer (%, w/w)						
Materials	Code					
	C3	C3M1	C3M2	C3M3	C3M4	C3M5
PSO	0.05	0.05	0.05	0.05	0.05	0.05
Apifil®	9	9	9	9	9	9
White soft paraffin	15	15	15	15	15	15
Liquid paraffin	6	6	6	6	6	6
Phenoxyethanol	1	1	1	1	1	1
Methanol	10	10	10	10	10	10
Menthol	–	2.5	5.0	7.5	10	12.5
Distilled water q.s. to make	100	100	100	100	100	100

from the calibration data (*13-15*).

2.5.2. *Viscosity measurements*

A Brookfield Rotational Digital Viscometer DV II RVTDV-II was used to measure the viscosity (in cps) of the cream and gel formulations. The spindle was rotated at 10 rpm. Samples of the gels were allowed to settle over 30 min at the assay temperature (25 ± 1°C) before the measurements were taken.

2.5.3. *pH measurement*

The pH of all samples was measured using a pH meter (361, Systronics, India).

2.5.4. *Stability studies*

All cream and gel samples were stored at 4°C for 48 h and at 40°C for 48 h, respectively, and the physical aspects and homogeneity of the samples were investigated.

2.5.5. *Limpidity studies*

The limpidity of all gel samples was measured spectrophotometrically (transmittance) at 610 nm.

2.5.6. *In vitro skin permeation studies*

The animals used for the preparation of epidermis were male albino rats (150-200 g). All the experiments involving animals were conducted in accordance with institutional guidelines and were approved prior by the Institutional Ethics Committee. The institutional Ethics Committee approved the method of euthanasia. The animals had free access to food and water until used for the study. The care of the rats was in accordance with institutional guidelines. *In vitro* skin permeation studies were performed using a Franz diffusion cell with a receptor compartment capacity of 21 mL and an effective diffusion area of 2.54 cm^2. Dorsal hair was removed with a clipper and the full thickness of skin was surgically removed from each rat. Epidermis was prepared by a heat separation technique (*16*). The entire abdominal skin was soaked in water at 60°C for 60 sec, followed by careful removal of the epidermis. The epidermis was washed with water and used in the *in vitro* permeability studies. The excised rat epidermis was mounted between the donor and receptor compartment of the diffusion cell. One gram of each cream or gel formulations was placed over the skin and covered with paraffin film. The receptor compartment of the diffusion cell was filled with phosphate buffer (pH 6.8) containing 20% (v/v) ethanol (*17*). The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm. Temperature was maintained at 37 ± 0.5°C. Samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically at a wavelength of 246 nm. The concentration of PSO in each sample was determined from a previously calculated standard curve. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal. The cumulative percent amounts of drug permeated per square centimeter of skin were plotted against time.

2.6. *Data analysis and statistics*

The permeation parameters such as flux, permeability coefficient, and enhancement ratio were calculated for prepared cream and gel formulations.

The flux (μg/cm^2·h) of PSO was calculated from the slope of the plot of the cumulative amount of drug permeated per cm^2 of rat epidermal membrane at steady state against time using linear regression analysis (*18,19*).

The steady state permeability coefficient (K_P) of drug crossing rat epidermal membrane was calculated using the following equation (*20*):

$$K_P = \frac{J}{C} \quad (1)$$

where J is the flux and C is the concentration of PSO in donor compartment.

The penetration enhancing effect of menthol was calculated in terms of enhancement ratio (*ER*), and was calculated using the following equation (*21*):

$$ER = \frac{K_P \text{ with penetration enhancer}}{K_P \text{ without penetration enhancer}} \quad (2)$$

The data obtained in this study was subjected to statistical analysis using GraphPad-Prism 3.0 software, for one-way analysis of variance (ANOVA) following Student-Newman-Keuls multiple comparisons test. A P value of less than 0.05 was considered as evidence of a significant difference.

3. Results and Discussion

3.1. *Physicochemical compatibility of drug and polymer*

DSC and FTIR spectra of PSO and its physical mixture with cream and gel formulation polymers are shown in Figures 1 and 2.

The DSC analysis of pure PSO showed a sharp endothermal peak at 168.64°C, corresponding to its melting point (Figure 1). The DSC analysis of the

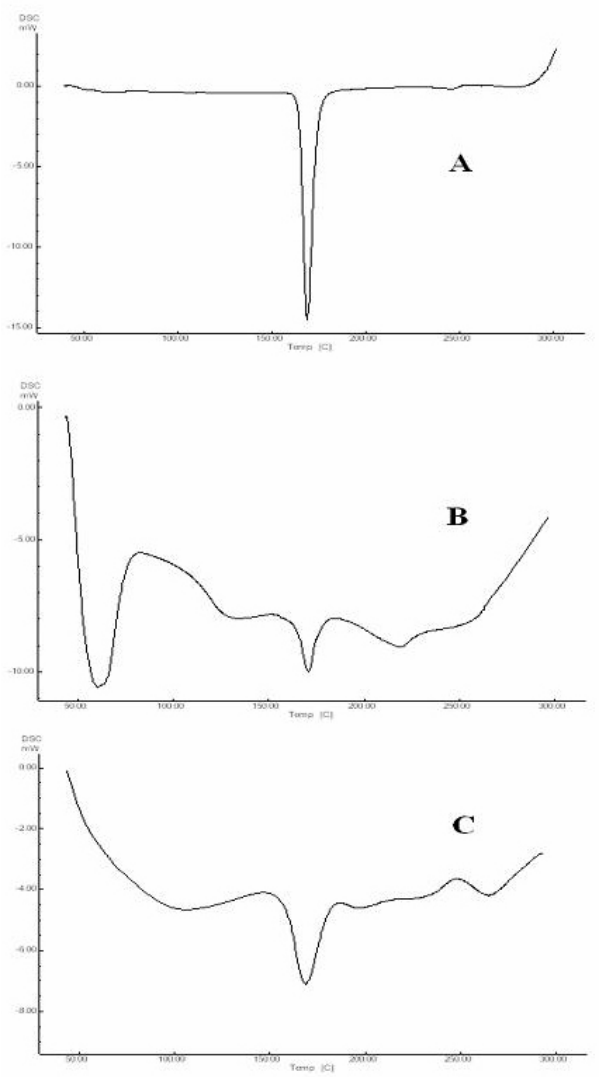

Figure 1. DSC thermograms of PSO (A), and its physical mixture with cream (B) and gel (C) formulation polymers.

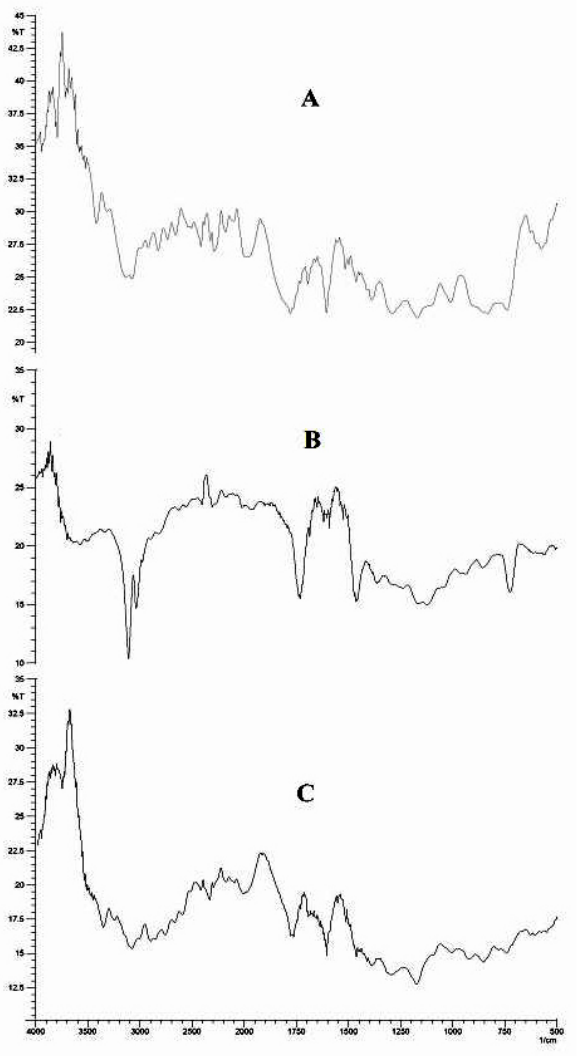

Figure 2. FTIR spectra of PSO (A), and its physical mixture with cream (B) and gel (C) formulation polymers.

physical mixture of PSO and the polymers used in cream and gel formulations revealed melting points of PSO at 167.72°C and 166.34°C, respectively. The negligible change in the melting point of PSO indicated compatibility of PSO with formulation polymers of cream and gel.

The IR spectral analysis of PSO alone showed principal peaks at wavenumbers 3057 (aromatic C-H stretch), 2402, 2284, 2002 cm^{-1} (overtone band), 1787 cm^{-1} (C=O stretching vibration of unsaturated lactones), 1609 cm^{-1} (C=C of ring), 1310 cm^{-1} (C-C(=O)-C stretching vibration), 1189 cm^{-1} (O-C-C band), 1052 cm^{-1} (symmetric C-O-C stretch) and 753 cm^{-1} (out of plane C-H bend), confirming the purity of the drug (Figure 2). In the IR spectra of the physical mixture of PSO with cream and gel formulation excipient the major peaks of PSO were observed at wavenumbers 3077, 2292, 1767, 1596, 1169, and 729 cm^{-1}; and 3054, 2296, 2010, 1776, 1603, 1320, and 752 cm^{-1}, respectively. However, some additional peaks were observed in the physical mixture, possibly because of the presence of polymers. The DSC and IR results suggest that the drug and polymers are compatible. Wade and Weller reported that HPMC, EC, PVP, and other common polymers are popular in controlled- and sustained release formulations because of their compatibility with several drugs (*22*).

3.2. *Physicochemical properties*

3.2.1. *Drug content and viscosity*

All prepared cream formulations of PSO were found to contain 98.7-101.3% of PSO. In gel formulations, increased concentrations of 2-propanol lead to decreased viscosity whereas increased concentrations of propylene glycol lead to increased viscosity of the formulations. In cream formulations, increased concentrations of emulsifying agent lead to increased viscosity of the formulation (Table 3).

Table 3. Drug content, viscosity, limpidity, amount of drug permeated in 3 h (Q_3), and % PSO released for different cream and gel formulations

Formulation code	Drug content[a]	pH	Viscosity η (cps × 10^3)[a]	Limpidity (T%)	Q_3[a] (μg cm^{-2})	% PSO released[a]
C	99.9 ± 3.7	6.8	108 ± 4.66	–	32.20 ± 2.6	16.36 ± 0.7
C1	101.1 ± 3.1	6.7	104 ± 4.87	–	51.10 ± 2.9	25.96 ± 1.2
C2	100.6 ± 3.7	6.8	111 ± 4.72	–	71.67 ± 3.4	36.41 ± 1.4
C3	98.1 ± 3.8	6.7	119 ± 4.65	–	101.82 ± 4.9	51.72 ± 2.4
C4	98.6 ± 3.5	6.7	107 ± 4.31	–	43.25 ± 2.1	21.97 ± 1.2
C5	100.6 ± 3.9	6.9	116 ± 5.06	–	63.57 ± 3.0	32.29 ± 2.8
C6	101.4 ± 4.2	6.9	125 ± 6.87	–	92.97 ± 4.5	47.23 ± 2.0
G	100.2 ± 4.5	6.0	92 ± 3.31	89 ± 3.4	78.50 ± 2.65	39.88 ± 1.5
G1	98.4 ± 4.4	6.1	89 ± 3.34	86 ± 3.1	94.70 ± 2.95	48.11 ± 2.1
G2	99.3 ± 2.1	6.1	79 ± 3.27	83 ±3.2	105.51 ± 4.49	53.60 ± 1.9
G3	101.4 ± 3.9	6.0	71 ± 3.29	81 ± 2.9	115.21 ± 4.94	58.53 ± 2.2
G4	98.3 ± 4.1	5.9	104 ± 3.67	88 ± 3.4	54.70 ± 2.12	27.79 ± 1.1
G5	101.1 ± 3.3	6.0	117 ± 4.52	93 ± 4.2	49.60 ± 2.10	25.20 ± 1.2
G6	99.5 ± 4.3	5.9	129 ± 5.64	96 ± 4.0	41.40 ± 4.51	21.03 ± 0.9

[a] Mean ± SD; n = 3.

3.2.2. *pH measurement*

All gel formulations showed a pH range of 5.9-6.1; however, the O/W creams exhibited a higher pH range, between 6.7 and 6.9.

3.2.3. *Stability studies*

During the study no change in physical aspects and homogeneity was observed in all cream and gel formulations. Moreover, all the formulations also showed no alteration in pH during this study.

3.2.4. *Limpidity studies*

Gels were classified in four categories, from limpid (T% > 80%) to very opaque (T% < 20%). All gel samples prepared were limpid as the T% values were more than 80% for all formulations. Gel formulations containing propylene glycol were more limpid than formulations containing 2-propanol (Table 3).

3.3. *In vitro drug permeation study*

3.3.1. *Cream and gel formulations without permeation enhancer*

3.3.1.1. *Oil-in-water cream samples*

The cumulative amount of PSO released from 0.05% creams were determined and plotted in Figure 3. Each data point represents the mean of 6 determinations. The amount of drug was constant in all different cream formulations.

Substitution of emulsifying wax with increasing concentration of *Apifil*® or *Plurol*® over the range of 3%, 6%, and 9%, exhibited a linear increase in PSO release

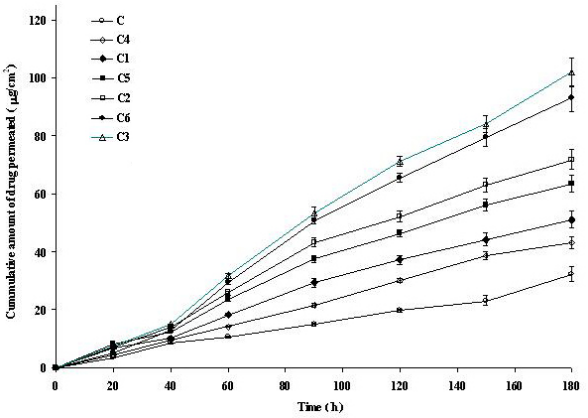

Figure 3. Cumulative amount of PSO permeated from cream formulations containing different concentrations of *Apifil*® or *Plurol*® as emulsifying agent therough rat epidermis (n = 3).

compared to formulations containing only emulsifying wax (9%). Complete substitution of emulsifying wax with *Apifil*® or *Plurol*® showed better release of PSO as compared to partial or no substitution of emulsifying wax with *Apifil*® or *Plurol*®. Among all cream formulations, formulations containing 9% *Apifil*® showed the highest release (101.82 ± 4.9 μg/cm^2) of PSO in 3 h. Hence, this concentration was selected for preparing cream formulations to study the effect of menthol as permeation enhancer.

3.3.1.2. *Gel samples*

Cosolvents were used in various topical formulations to aid the solubilization of the active substances in the vehicle. In this study, 2-propanol and propylene glycol were added as cosolvents to increase the solubility of PSO in the aqueous phase selectively rather than in the micellar portion of the gel. The effect of 2-propanol

on the release of PSO was studied using the gel with 0.05% PSO in 1.5% HPC and varying the 2-propanol concentration (10%, 15% or 20%) (Figure 4). In all of these formulations, 2-propanol concentration (10%, 15% or 20%) was substituted with methanol (20%, 15% or 10%), respectively.

Over the range of 2-propanol concentrations used, the cumulative amount of PSO increased linearly from 78.50 ± 2.65 μg/cm^2 for gels prepared without 2-propanol to 115.21 ± 4.94 μg/cm^2 for gels containing 20% of 2-propanol at 3 h. The enhanced drug release in the presence of 2-propanol could be due to the decreased viscosity of PSO gel. These results are in agreement with a previous investigation performed by Chi and Jun, 1991 (*23*), who demonstrated that ethanol increased release of ketoprofen from gel formulations due to a decrease in viscosity. Moreover, the relative increase in the release was probably due to the fact that, at these concentrations, 2-propanol augmented the solubility of the drug in the gel, causing an increase in the thermodynamic activity and enhanced permeation.

The effect of various concentrations of propylene glycol on the release of PSO from a gel formulation was also investigated. The concentration of HPC (1.5%) and the drug (0.05%) remained constant; however, the amount of methanol in the vehicle was substituted with an equal volume of propylene glycol (10%, 15%, and 20%) (Figure 4). Over the range of propylene glycol concentrations used, the diffusion coefficient of PSO decreased linearly from 78.50 ± 2.65 μg/cm^2 for the gel without propylene glycol to 41.40 ± 4.51 μg/cm^2 for the gel containing 20% propylene glycol (Table 3). The decrease in drug release from the gel containing propylene glycol might be due to the increase of viscosity of PSO gel. However, the addition of propylene glycol in gel formulations caused improvement of the limpidity of the gels when measured spectrophotometrically at 610 nm. The gel formulation containing 2-propanol (20%) was selected to study the effect of menthol as permeation enhancer.

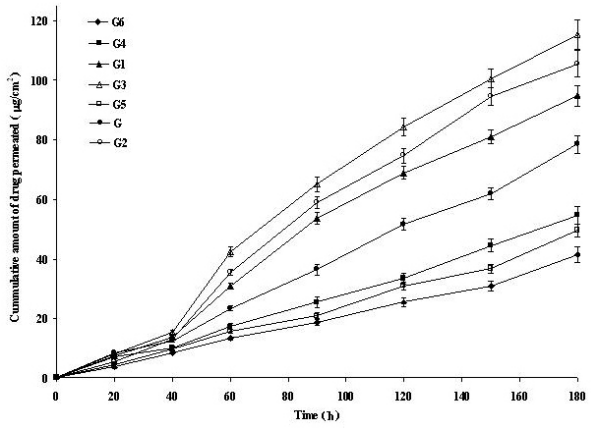

Figure 4. Cumulative amount of PSO permeated from gel formulations containing different concentrations of 2-propanol and propylene glycol as cosolvents therough rat epidermis (*n* = 3).

3.3.2. *Cream and gel formulations with permeation enhancer*

The penetration enhancing effect of menthol on the permeability of PSO across the excised rat epidermis from cream and gel formulations was investigated. Permeation parameters for PSO from the cream and gel formulations are shown in Table 4. The cumulative amount of drug crossing rat epidermis from both cream and gel formulations containing various amounts of menthol is shown in Figures 5 and 6.

The maximum amount of PSO that permeated during the 3 h ($Q3$) of the study was 101.82 ± 4.89 μg·cm^{-2} and 115.21 ± 4.94 μg·cm^{-2} from cream and gel formulations prepared without menthol, respectively (Table 4). The flux was obtained by dividing the

Table 4. Drug content, flux (*J*), permeability coefficient (*K*p), enhancement ratio (*ER*), amount of drug permeated in 3 h (Q_3), % PSO released, and Higuchi R^2 values for the *in vitro* permeation study across rat epidermal membrane from cream and gel formulations of PSO containing selected concentrations of menthol at the end of 3 h

Formulation code	Drug content[a]	*J* (μg·cm$^{-2\text{-}1}$·h^{-1})[a]	*K*p (cm·h^{-1} × 10^3)[a]	*ER*[a]	Q_3[a] (μg·cm^{-2})	% PSO released[a]	R^2 Higuchi
C3	99.9 ± 4.2	35.37 ± 1.4	72.18 ± 3.2	1.00 ± 0.048	101.82 ± 4.9	51.72 ± 2.4	0.9942
C3M1	101.1 ± 4.5	37.49 ± 1.6	76.51 ± 3.7	1.06 ± 0.040	110.40 ± 5.3	56.08 ± 2.9	0.9918
C3M2	98.8 ± 4.0	40.68 ± 1.9	79.76 ± 3.8	1.11 ± 0.042	123.20 ± 6.1	62.58 ± 2.7	0.9947
C3M3	101.5 ± 4.8	43.14 ± 1.8	86.28 ± 4.3	1.20 ± 0.052	134.14 ± 6.4	68.14 ± 3.2	0.9927
C3M4	100.2 ± 4.4	44.93 ± 1.9	89.86 ± 4.4	1.24 ± 0.54	139.60 ± 7.1	70.92 ± 3.4	0.9935
C3M5	99.4 ± 4.3	48.82 ± 2.2	95.72 ± 4.7	1.33 ± 0.048	158.57 ± 6.8*	80.55 ± 3.7	0.9902
G3	100.2 ± 4.1	41.10 ± 1.2	82.2 ± 3.2	1.14 ± 0.034	115.21 ± 4.9	58.53 ± 2.2	0.9872
G3M1	101.7 ± 3.7	45.51 ± 1.7	89.2 ± 3.6	1.24 ± 0.039	137.65 ± 5.4	69.92 ± 3.2	0.9915
G3M2	98.9 ± 3.9	46.65 ± 1.5	95.2 ± 4.2	1.32 ± 0.044	149.21 ± 5.9	75.78 ± 3.9	0.9901
G3M3	100.2 ± 4.5	49.39 ± 2.1	100.7 ± 4.7	1.40 ± 0.051	162.08 ± 6.3	82.33 ± 3.8	0.9945
G3M4	99.6 ± 3.3	51.48 ± 2.4	102.9 ± 4.9	1.43 ± 0.052	173.23 ± 6.8	88.00 ± 4.2	0.9936
G3M5	101.1 ± 3.8	52.59 ± 2.1	107.3 ± 5.1	1.49 ± 0.049	187.69 ± 7.5*	95.30 ± 4.2	0.9957

[a] Mean ± SD; $n = 3$; *Significant at $p < 0.05$ when compared with formulation C and G

cumulative amount of drug permeated per cm^2 of the skin with time. Thus, the corresponding flux of PSO was 48.82 ± 2.2 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 52.59 ± 2.1 $\mu g \cdot cm^{-2} \cdot h^{-1}$ for the cream and gel formulations without menthol, respectively.

A marked effect of menthol on PSO permeation was observed when it was incorporated in both types of cream and gel formulations in varying quantities. The cumulative amounts of PSO that permeated over 3 h (*Q*3) were found to have increased ranging from 110.40 ± 5.3 to 158.57 ± 6.8 $\mu g \cdot cm^{-2}$ for PSO cream and 137.65 ± 5.4 to 187.69 ± 7.5 $\mu g \cdot cm^{-2}$ for PSO gel formulations containing 2.5-12.5 % (w/w) of menthol. The corresponding flux values ranged from 37.49 ± 1.6 to 48.82 ± 2.2 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 45.51 ±1.7 to 52.59 ± 2.1 $\mu g \cdot cm^{-2} \cdot h^{-1}$ for PSO cream and gel formulations, respectively. However, a lag period of 40 min was observed in both cream and gel formulations in the permeation of the drug through the stratum corneum (Figures 4 and 5). It may be observed from the results that the flux of drug increased with an increase in concentration of menthol in cream and gel formulations (Table 4).

The increased permeability of PSO with increasing concentrations of menthol in both formulations from 0 to 12.5% (w/w) (Figures 5 and 6) indicated an increase in both the permeability coefficient and enhancement ratio (Table 4). There was a 1.33- and 1.49-fold increase in the permeability of the drug observed from the cream and gel containing 12.5% (w/w) of menthol, respectively. Both the permeability coefficient and enhancement ratio of PSO were increased linearly with all menthol concentrations in both cream and gel formulations (Table 4). When the data were analyzed, the amount of drug permeated fit Higuchi ($r^2 > 0.99$) from 20 min to 3 h with a lag period of about 40 min for both formulation types (Table 4).

The topical cream and gel formulations developed with menthol as permeation enhancer showed good transport of PSO as compared to formulations developed without menthol. Gel formulations of PSO showed good release and transport of PSO as compared to cream formulations. The novel topical gel formulation developed in this study can be used in PUVA therapy to achieve an adequate drug level at the target site at the time of UVA radiation.

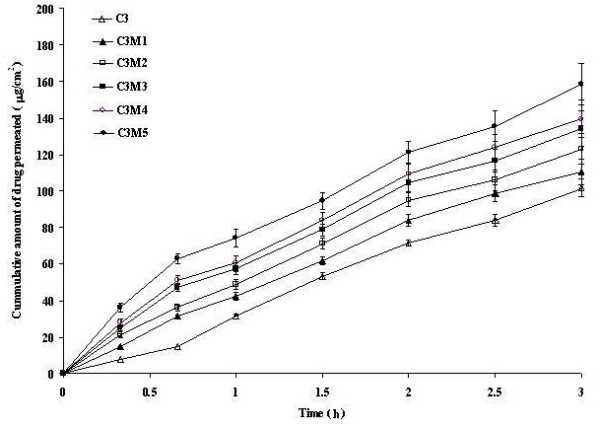

Figure 5. Effect of menthol concentration on permeation of PSO from cream formulations therough rat epidermis (*n* = 3).

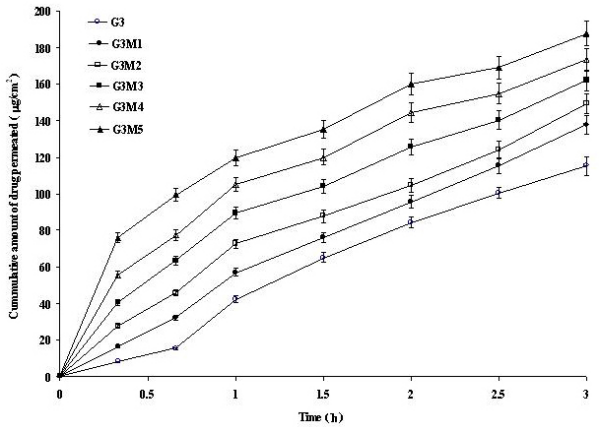

Figure 6. Effect of menthol concentration on permeation of PSO from gel formulations therough rat epidermis (*n* = 3).

4. Conclusions

Topical cream and gel formulations of PSO developed in this study have great potential as an effective and safe way to apply PSO to enhance its transport through skin, with the goal to shorten the delay between drug application and UVA irradiation. The *in vitro* permeation study crossing rat epidermal membranes showed that menthol enhanced the transdermal permeation of PSO from cream and gel drug reservoir systems. Gel formulations showed better permeation of PSO as compared to cream formulations. The topical gel formulations of PSO developed in this study have great utility and are a viable option for effective and controlled management of vitiligo and psoriasis. Further experiments will be conducted in other animal models and based on the results trials may be performed on humans.

References

1. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 12th edition (Budavari, S, ed). Merck & Co., Inc., Whitehouse Station, NJ, 1996; p. 1363.
2. Cardoso CA, Honda NK, Barison A. Simple and rapid determination of psoralens in topic solutions using liquid chromatography. J Pharm Biomed Anal. 2002; 27:217-224.
3. Bettero A, Benassi CA. Determination of bergapten and citropten in perfumes and suntan. J Chromatogr. 1983; 280:167-171.
4. Lehr GJ, Barry TL, Franolic JD, Petzinger G, Scheiner P. LC determination of impurities in methoxsalen drug

substance: isolation and identification of isopimpinellin. as a major impurity by atmospheric pressure chemical ionization LC/MS and NMR. J Pharm Biomed Anal. 2003; 33:627-637.
5. Makki S, Muret P, Said AM, Bassignot P, Humbert P, Agache P, Millet J. Percutaneous absorption of three psoralens commonly used in therapy: Effect of skin occlusion (*in vitro* study). Int J Pharm. 1996; 133:245-252.
6. Cardoso CA, Vilegas W, Honda NK. Rapid determination of furanocoumarins in creams and pomades using SPE and GC. J Pharm Biomed Anal. 2000; 22:203-214.
7. Innocenti G, Piovan A, Filippini R, Caniato R, Cappelletti EM: Quantitative recovery of furocoumarins from *Psoralea bituminosa*. Phytochem Anal. 1997; 8:84-86.
8. British Photodermatology Group. Guidelines for PUVA. Br J Dermatol. 1994; 130:246-255.
9. David AO, Anton HA. Topical Drug Delivery Formulations (David AO, Anton HA, eds). Marcel Dekker, Inc., New York, NY, USA, 1990; p. 1.
10. Macedo T, Block LH, Shukla AJ. Release of tolmetin from carbomer gel systems. Drug Dev Ind Pharm. 1993; 19:887-902.
11. Skiba M, Skiba-Lahiani M, Marchais H, Duclos R, Arnaud P. Stability assessment of ketoconazole in aqueous formulations. Int J Pharm. 2000; 198:1-6.
12. Dittgen M, Durani M, Lehmann K. Acrilyc polymers, a review of pharmaceutical applications. STP Pharma. 1997; 7:403-437.
13. Jones SP, Greenway MJ, Orr NA. The influence of receptor fluid on *in vitro* percutaneous penetration. Int J Pharm. 1989; 53:43-46.
14. Prasad NS. Spectrophotometric estimation of curcumin. Indian Drugs. 1997; 34:227-228.
15. Chignell CF, Bilski P, Reszka KJ, Motten AG, Sik RH, Dahl TA. Spectral and photochemical properties of curcumin. Photochem Photobiol. 1997; 59:295-302.
16. Zhao K, Singh J. *In vitro* percutaneous absorption of propranolol hydrochloride through porcine epidermis by terpenes/ethanol. J Control Release. 1999; 62:359-366.
17. Transdermal and topical drug delivery (Williams A, ed). Pharmaceutical Press, London, UK, 2003; p. 74.
18. Julraht K, Keith AP, James AW. Development of a transdermal delivery device for melotoin *in vitro* study. Drug Dev Ind Pharm. 1995; 21:1377-1387.
19. Ho HO, Chen LC, Lin HM, Sheu MT. Penetration enhancement by menthol combined with a solubilization effect in a solvent system. J Control Release. 1998; 51:301-311.
20. Yamune M, Williams A, Barry B. Terpenes penetration enhancers in propylene glycol/water co-solvent systems: Effectiveness and mechanism of action. J Pharm Pharmacol. 1995; 47:978-989.
21. Williams A, Barry B. Terpenes and the lipid-protein partitioning theory of skin penetration enhancement. Pharm Res. 1991; 8:17-24.
22. Wade A, Weller PJ. Handbook of Pharmaceutical Excipients. American Pharmaceutical Publishing Association. Washington, DC, USA. 1994; pp. 362-366.
23. Chi SC, Jun HW. Release rates of ketoprofen from poloxamer gels in a membraneless diffusion cell. J Pharm Sci. 1991; 80:280-283.

(*Received September 2, 2009; Revised September 22, 2009; Accepted October 3, 2009*)