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Development of modified transdermal spray formulation of psoralen extract

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ABSTRACT

The present investigation was undertaken to fabricate modified transport psoralen transdermal spray using ethyl cellulose as film-forming polymer and to carry out in vitro characterization of an optimized formulation. Formulated Topical spray was evaluated by pH, clarity of solution, spray pattern, area covered by each spray, dermal adhesion of film, flexibility of film, water washability of film, leakage from container, average weight of formulation per metered dose (or per spray), drug content per spray, film formation time, in vitro drug transport, drug content uniformity after 10th spray, 15th spray, 30th spray, 40th spray and viscosity. Batch F1 containing ethyl cellulose (4%) and polyethylene glycol (0.25%) was found to give good clarity of solution, evaporation rate, spray pattern, and tackiness of the film. Diffusion studies of the optimized formulation through the semipermeable membrane showed the release of drug to the extent 50% over a period of 6 hours. Stability studies were conducted as per ICH guidelines and indicated that formulation was stable. Skin irritation studies were performed using rat as an animal model. The results obtained show that the transdermal spray can be a promising and innovative therapeutic system for the transdermal administration of psoralen.

Keywords: psoralen, *psoralea corylifolia* seeds, transdermal spray, ethyl cellulose, factorial design.

INTRODUCTION

Leucoderma is the most common chronic depigmentation disorder or hypopigmentation (*1*). It includes the loss of functioning melanocytes which causes the appearance of white patches on the skin (*2*). The most common treatment in leucoderma is the use of topical or oral psoralens followed by exposure to UVA radiation in the region of 280-315 nm wavelengths (*3*). A thin coat of 0.01% to 0.1% methoxsalen ointment is applied to leucoderma skin. After 30 min, the skin is exposed to 0.12J/cm^2 to 0.25 J/cm^2 UVA with increments of 0.12J/cm^2 weekly (*4*). Psoralen is an isomer of furanocoumarin obtained from the fruits of *Psoralea corylifolia* Linn. (Family-Leguminosae). It is a photosensitizing agent. *Psoralea corylifolia* (Bakuchi) contains furanocourmarins like psoralen (*5*). Psoralen stimulates skin to produce melanin pigment when exposed to sunlight. The seeds of *psoralea corylifolia* are believed to facilitate amino acid transport across the intestinal mucosa by acting as a photo-sensitizer for the initiation of erythema on the spots of leucoderma. Furanocourmarins initate the transformation of DOPA to melanin under the influence of UV Light or sunlight. Epidermal tissues contains free sulphydryl groups (*6*). These sulphydryl group bind the free copper ions which are required for the function of tyrosinase (*7*). Psoralea corylifolia (Bakuchi) helps in the release of SH-group bound copper as free copper, which activates the tyrosinase activity for melanin synthesis (*URL. 1*).

As per the literature survey the topical treatment containing psoralen (*Psoralea corylifolia* seeds) for leucoderma are mainly creams, ointments and oil (*URL. 1, URL. 2, URL. 3, URL. 4)*. Therefore the objective of the study was to

develop an optimized formulation in the form of a topical spray containing psoralen (*Psoralea corylifolia* seed) for the treatment of leucoderma. The aim of the present research work was to develop patient-friendly modified transport transdermal spray of psoralen using ethyl cellulose as film-forming polymer. A 3^2 full factorial design was employed for optimization. Developed Topical spray was evaluated for pH, clarity of solution, spray pattern, area covered by each spray, dermal adhesion of film, flexibility of film, water washability of film, leakage from container, average weight of formulation per per spray, drug content per spray, film formation time, in vitro drug transport, drug content uniformity after 10^{th} spray, 15^{th} spray, 30^{th} spray, 40^{th} spray and viscosity.

MATERIALS AND METHODS

Materials:

Psoralea corylifolia seeds was kindly obtained as a Gift sample from Aimil pharmaceuticals Pvt. Ltd Delhi, India. Psoralen marker was purchased from Kumaon Chemicals Pvt .Ltd Uttrakhand, India. α-naphthol was procured from S.D. fine chemicals Pvt. Ltd Mumbai, India. Sodium hydroxide, Concentrated Hydrochloric Acid, Fehling's A solution, Fehling's B solution were obtained from Nice chemicals Pvt. Ltd Kochi, India. Sodium carbonate was obtained from Loba chemicals Pvt. Ltd Mumbai, India.

Methods:

Preperation of Ethanolic Extract of *Psoralea corylifolia* Seeds: Extraction of psoralen from *Psoralea corylifolia* seeds was done by simple soxhalation extraction (*8*).

Phytochemical Screening of Ethanolic Extract of *Psoralea corylifolia* Seeds: Phytochemical screening of ethanolic extract of *Psoralea corylifolia* seeds was performed, in order to confirm the presence and absence of compounds like carbohydrates, gums, mucilage, proteins, amino acids, glycosides, tannins and phenols, volatile oils, fats and oils, steroids, flavonoids and alkaloids in the extract.

Table 1: Composition of Batches as per 3^2 Full Factorial Design.

S. No	Batch code	Ethyl cellulose (%w/v)	Eudragit S100 (%w/v)	Polyethylene glycol 200 (%w/v)	Ethanolic extract solution of *Psoralea corylifolia* seeds (ml)
1	F1	4	-----	0.25	10
2	F2	5	-----	0.25	10
3	F3	6	-----	0.25	10
4	F4	-----	9	0.25	10
5	F5	-----	10	0.25	10
6	F6	-----	11	0.25	10
7	B1	6	11	0.25	10
8	B2	5	11	0.25	10
9	B3	4	11	0.25	10
10	B4	6	10	0.25	10
11	B5	5	10	0.25	10
12	B6	4	10	0.25	10
13	B7	6	9	0.25	10
14	B8	5	9	0.25	10
15	B9	4	9	0.25	10
16	C	-----	-----	------	10

Standardization of an ethanolic extract:

FTIR spectroscopy: FTIR spectroscopy of psoralen marker and ethanolic extract of *Psoralea corylifolia* seeds was performed by using Brukar FTIR and major peaks obtained were interpretated and then compared with the standard peaks of psoralen structure in order to confirm the presence of psoralen in the ethanolic extract.

HPTLC: A method was developed and validated for 3000 ml ethanolic extract solution from 1.5 kg seed powder of *Psoralea corylifolia by* HPTLC (CAMAG marketed by Anchrom Enterprises(I) Pvt.Ltd, Mumbai, India). HPTLC was performed for the quantitative determination of psoralen in the ethanolic extract solution.

Compatibility study of polymers with psoralen by FTIR spectroscopy: FTIR spectroscopy of all the polymers in the ethanolic extract of *Psoralea corylifolia* seeds was performed at 25 ± 2°C, for studying the compatibility of polymers with ethanolic extract solution and also the compatibility of polymers with each other.

Design layout for 3^2 factorial design: A 3^2 full factorial design was used for designing the optimization of the formulation. Compostion of batches from B1-B9 and from F1-F6 and control group was shown in table 1.

Characterization of a developed optimized formulation: Characterization and evaluation of a developed optimized formulation was done on the basis of following parameters:-

1. pH : The pH of the optimized formulation was measured by pH meter.
2. Clarity of solution: The clarity of solution was done by naked eye by analyzing the settling down of suspending particles in the formulation solution.
3. Spray pattern: The spray pattern of an optimized formulation was done by spraying from a mechanical spray bottle from a distance of 15 cm on a paper.
4. Area covered by each spray: Area covered by each spray was calculated by using formula, $A = \pi r^2$.
5. Dermal adhesion of film
6. Flexibility of film: Flexibility of film after evaporation of spray was calculated by spraying on the palm and then closing and opening the palm which results in no cracking and breaking of the film.
7. Water washability of film: Film can be easily washed with water.
8. Leakage from container: Effectiveness of the pump seal and its ability to store the contents of the product was calculated by leak test/ pump seal efficiency test. The filled container under test was placed in the upright position at 30°C for 30 minutes. The containers were weighed before and after the test period. The change in the weight was noted down and leakage rate was calculated.
9. Average weight of formulation per metered dose (or per spray)
10. Drug content per spray: Drug content per spray was calculated by weighing the container before and after each spray.
11. Film formation time: Film formation time was calculated by spraying the formulation on the hand and time taken by the film to drying was noted.
12. In Vitro Drug Transport: The skin rat (6–8 weeks old) in a full-thickness type obtained from already sacrificed rat was mounted in a Franz Diffusion Cell. The surface area of membrane available for drug transport was $1cm^2$. 1 ml of the drug formulation was filled in the donor compartment of the Franz Diffusion Cell and 60 ml of phosphate buffer solution of pH 7.4 was filled in receptor compartments of the franz diffusion cell. Throughout the experiment, the stirring rate and temperature of media was kept at 600 rpm and at 37±2°C using a hot-water jacket (37±2°C). At appropriate intervals (0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360 minutes), 5 ml of the receptor medium was withdrawn and immediately replaced with equal volumes of fresh phosphate buffer solution. The permeated amount of psoralens (*9*) was determined by UV spectrophotometer(SCHIMADZU marketed by Effem Technology Pvt.Ltd, New Delhi, India)
13. Drug Content Uniformity: Drug content uniformity after 10^{th} spray, 15^{th} spray, 30^{th} spray, 40^{th} spray
14. Viscosity: Viscosity of the formulation solution was determined by ostwald's viscometer using following formula:-

$$v_1 = (p_1)(t_1)(v_2)/(p_2)(t_2)$$

15. Skin irritation study: Skin irritation studies were carried out in the presence of skin of rats/mice. The hairs on the dorsal side of the rats/mice were removed 1 day before performing experiment. The rats/mice were divided into 3 groups (n=6). Group 1 served as a control, group 2 received optimized formulation (1% psoralen) and group 3 received formaldehyde solution (0.8%) as a standard irritant. After 30 minutes of application of optimized formulation and standard irritant, group 2 and group 3 were irradiated with UV rays for 5-10 minutes daily for 7 days. Finally the application sites for erythema and edema will be graded according to visual scoring grade (*10 & 11*).

16. Stability study: Optimized formulation (batch F1) was stored for 2 months for stability testing as per ICH guidelines at three different temperatures i.e at 8 ± 2°C, 25 ± 2°C, 37 ± 2°C away from light (*Bakshi A. et al., 2012*). At the end of second month, the chemical stability of the formulation was assessed by estimation of in vitro drug transport and FTIR spectroscopy and physical stability was assessed by monitoring any change in various tests like pH and clarity of solution. The procedure employed for the study was identical to that described above**.**

RESULTS AND DISCUSSION

Tests conducted for the phytochemical screening of ethanolic extract of *Psoralea corylifolia* seeds, confirms the presence of mucilage, coumarin glycosides, tannins and phenols, flavonoids, alkaloids and volatile oils in the ethanolic extract and absence carbohydrates, gums, proteins, amino acids, fats and oils and steroids in the ethanoilc extract solution. FTIR Spectroscopy of psoralen marker was compared with ethanolic extract which shows that major peaks present in the FTIR spectra of ethanolic extract and psoralen marker were same as shown in table 2 and table 3, thereby confirming the presence of psoralen in the ethanolic extract of *Psoralea corylifolia* seeds.

Table 2: Comparison Between the FTIR spectra of Ethanolic Extract and FTIR spectra of Psoralen Marker

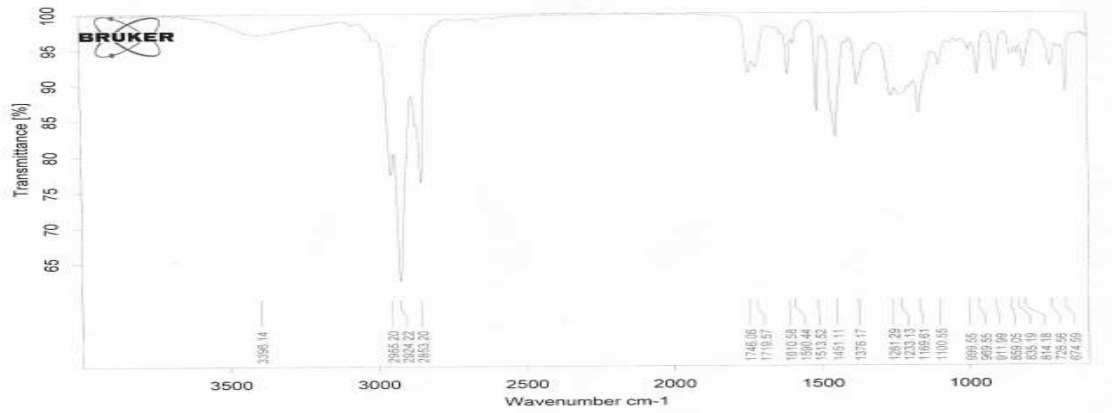
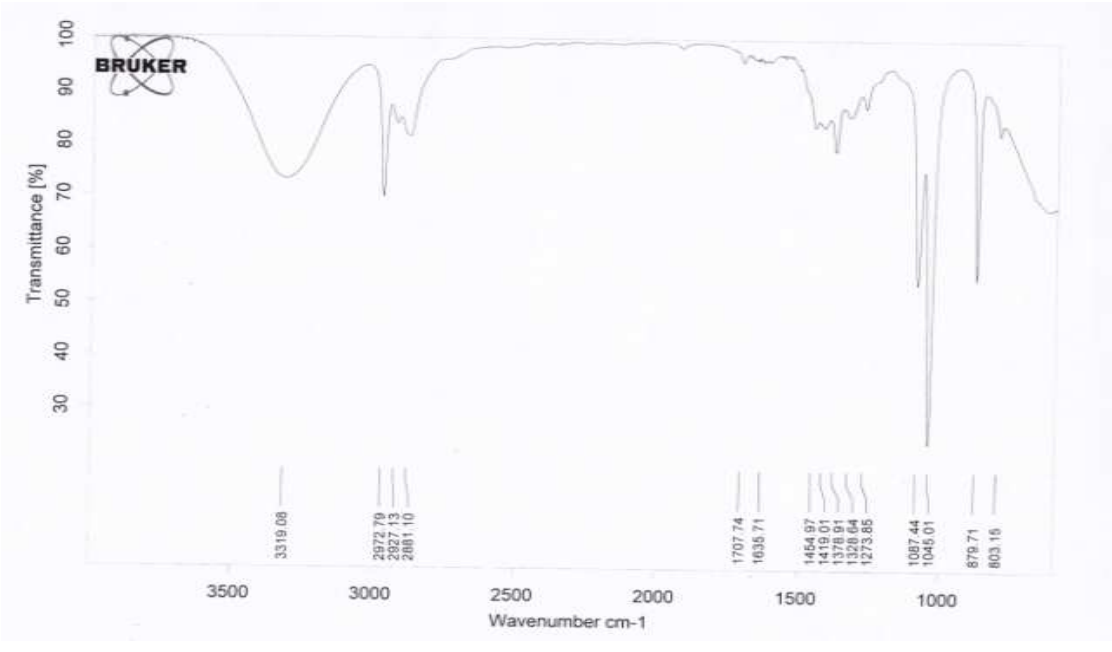
S.No	FTIR Spectra
1	Psoralen marker:- 
2	Ethanolic extract:- 

Table 3: Interpretation of Major Peaks of FTIR Spectra of Ethanolic Extract and FTIR Spectra of Psoralen Marker

S.No	FTIR spectra of psoralen marker	FTIR spectra of ethanolic extract of *psoralea corylifolia* seeds containg psoralen
1	Interpretation of major peaks:- 1).1746.06cm^{-1}, 1719.57cm^{-1} Confirms carbonyl group (C=O) in the structure 2).1100.55cm^{-1}, 1169.61cm^{-1}, 1233.13cm^{-1}, 1261.29cm^{-1} Confirms ether group (C-O-C) in the structure 3).2955.20cm^{-1}, 2924.22cm^{-1}, 2853.20cm^{-1} Confirms an aromatic and vinyl hydrocarbon group (C-H) in the structure 4).1610.58cm^{-1} Confirms C=C group in the structure 1590.44cm^{-1}, 1513.52cm^{-1}, 1451.11cm^{-1} Confirms an aromatic ring in the structure	Interpretation of major peaks:- 1)1635.71cm^{-1}, 1707.74cm^{-1} Confirms carbonyl group (C=O) in the structure 2) 1273.85cm^{-1}, 1087.44cm^{-1}, 1045.01cm^{-1} Confirms ether group (C-O-C) in the structure 3) 2972.79cm^{-1}, 2927.13cm^{-1}, 2881.10cm^{-1} Confirms an aromatic and vinyl hydrocarbon group (C-H) in the structure 4) 1635.71cm^{-1} Confirms C=C group in the structure 1454.97cm^{-1} Confirms an aromatic ring in the structure

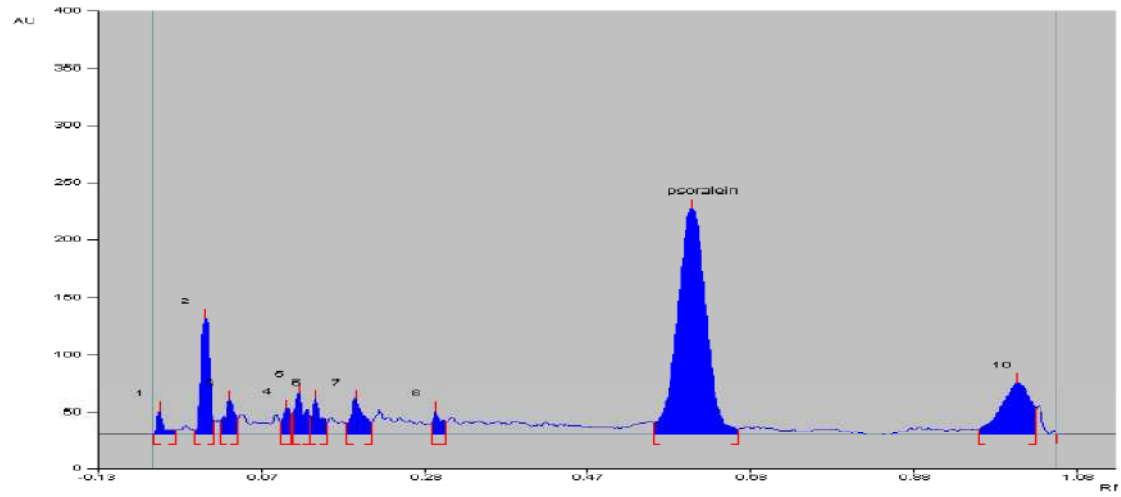


Figure 1: Chromatogram of Psoralen Marker

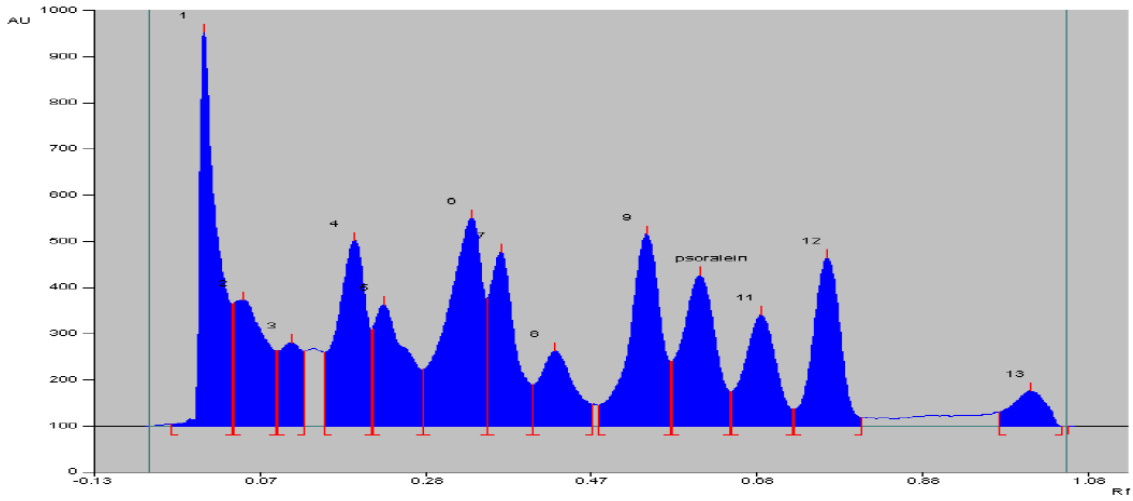


Figure 2: Chromatogram of Ethanolic Extract

Results of the HPTLC include various validation parameters which were shown in table 4. From the chromatogram of psoralen marker as shown in Fig. 1 and from the chromatogram of ethanolic extract as shown in Fig. 2, the concentration of psoralen in the ethanolic extract was observed to be 148.14ng/spot. The volume of spot was 5µl and it was diluted to 10 times, and therefore the concentration of psoralen in the ethanolic extract of *Psoralea corylifolia* seeds was calculated to be 0.3mg/ml. Percentage content of psoralen in ethanolic extract of *Psoralea corylifolia*

seeds was found to be 0.03%. Thus total amount of psoralen present in 3000 ml of ethanolic extract was calculated to be 1 g from the total raw material.

Table 4: Validation Parameters of Ethanolic Extract for Psoralen by HPTLC

S.No	Validation parameters	Result
1	Absorption maxima, λ_{max} (nm)	299
2	Linearity range (ng/spot)	50-150
3	Coefficient of determination (r^2)	0.9976
4	Regression equation	Y=35.79x+129
5	Slope (b)	35.79
6	Intercept (a)	129
7	Limit of detection LOD, (ng/spot)	5
8	Limit of quantitation LOQ, (ng/spot)	20
9	Repeatability (%R.S.D)	Less than 1%
10	Precision (%R.S.D)	Less than 1%
11	Recovery (%R.S.D)	Less than 1%
12	Mobile phase	Toluene : Ethyl acetate (7.5:2.5v/v)
13	Rf value	0.61

Compatibility study done by FTIR spectroscopy of all the polymers in the ethanolic extract of *Psoralea corylifolia* seeds was performed at 25 ± 2°C. It was observed that FTIR Spectroscopy of I).Ethyl cellulose + ethanolic extract, II).Eudragit S 100 + ethanolic extract , III).Polyethylene glycol 200 + ethanolic extract, IV).Ethy cellulose + Eudragit S 100 + Polyethylene glycol + ethanolic extract, shows no major difference in the basic peaks of psoralen although additional peaks are also present because of the presence of polymers. This shows that psoralen present in the ethanolic extract of *Psoralea corylifolia* seeds was compatible with the polymers like Ethyl cellulose, Eudragit S 100, Polyethylene glycol 200.Results of the evaluation parameters for the optimization of a formulation were shown in table 5. Fig. 3 represents the graph between cumulative percentage drug released and time for all batches.

Table 5: Results of the Evaluation Parameters for the optimization of a Formulation

S.No	Batch code	Percentage cumulative drug released	pH	Evaporation time (seconds)	Spray pattern
1	F1	50.17 ± 6.57%	5.5	25.49	Uniform and spherical
2	F2	27.56 ± 0.44%	5.5	29.84	Uniform but non spherical
3	F3	24.01 ± 0.92%	5	32.64	Uniform but non spherical
4	F4	46.57 ± 2.49%	5	73.13	Uniform and spherical
5	F5	16.77 ± 2.43%	4.5	98.35	Uniform but non spherical
6	F6	15.85 ± 2.09%	4.5	113.39	Uniform but non spherical
7	B1	0.75 ± 0.37%	4	87.29	Non uniform and non spherical
8	B2	6.51 ± 0.46%	3	136.50	Non uniform and non spherical
9	B3	20.77 ± 5.56%	3	186.73	Non uniform and non spherical
10	B4	10.62 ± 0.92%	4	205.79	Non uniform and non spherical
11	B5	33.57 ± 2.32%	4	215.49	Non uniform and non spherical
12	B6	45.04 ± 4.83%	4	221.92	Non uniform and non spherical
13	B7	41.99 ± 2.09%	4	183.18	Non uniform and non spherical
14	B8	50.17 ± 6.57%	4	217.70	Non uniform and non spherical
15	B9	52.85 ± 5.17%	4	236.75	Non uniform and non spherical
16	C	58.28 ± 3.63%	6	193.45	Uniform and spherical

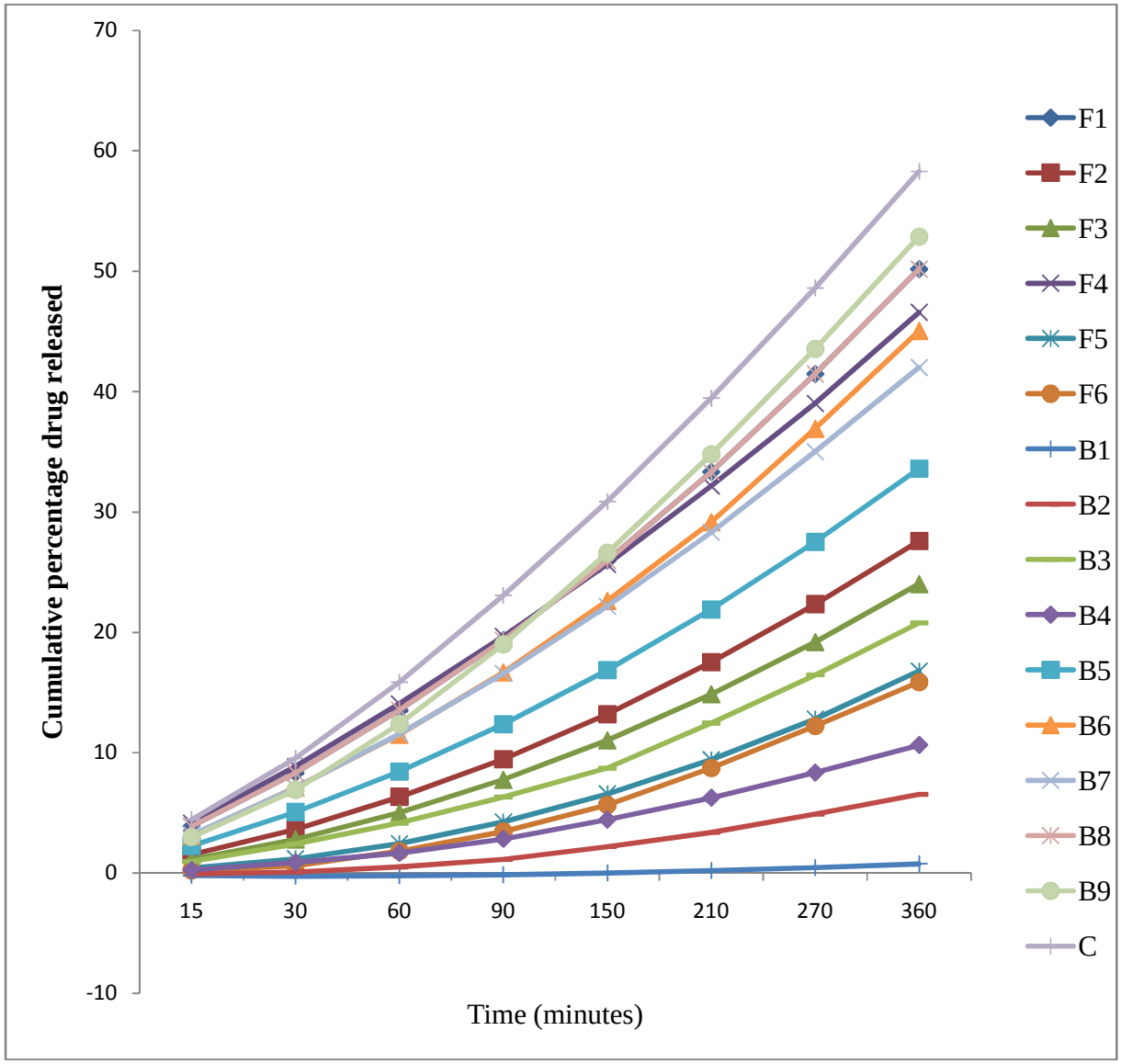


Figure 3: Graph between Cumulative Percentage Drug released and Time for All Batches

Therefore on the basis of cumulative percentage drug released, pH, evaporation time and spray pattern, batch F1 was selected as an optimized batch. An optimized formulation was observed to be a clear solution with pH 5.5 having spray pattern uniform and spherical in shape. An optimized formulation was observed to be having good dermal adhesion, good flexibility and moderate water washability properties. Fig. 4 shows the graph between cumulative percentage drug released and time. In vitro drug transport was observed to be 8.53 $\pm$ 2.15 % per cm^2 area of the rat skin.

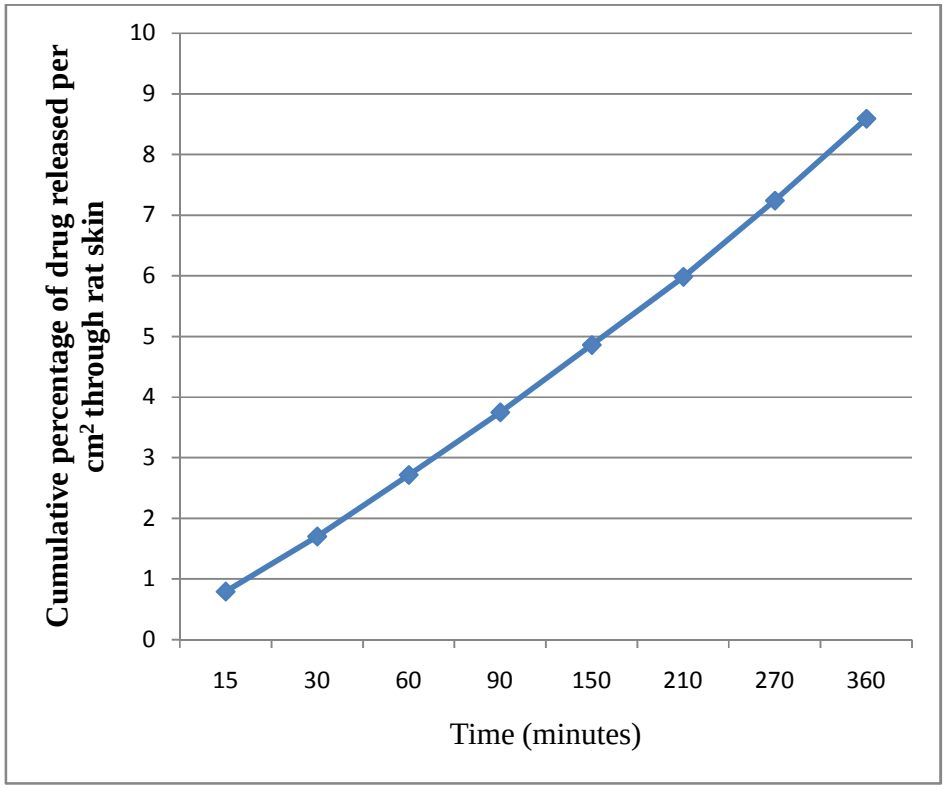


Figure 4: Cumulative percentage Drug release of F1 Formulation

The results of skin irritation study were shown in table 6.

Table 6: Skin Irritation Study

S.No (Rat No)	Negative control		Optimized formulation		Positive control (formaldehyde solution)	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	3	3	4	1
2	0	0	4	2	4	1
3	0	0	4	3	4	1
4	0	0	3	2	4	4
5	0	0	4	1	4	2
6	0	0	3	1	4	4
AVERAGE	0	0	3.5 ± 0.54	2 ± 0.89	4 ± 0	2.16 ± 1.47

Where,
Erythema scale :-0-none, 1-slight, 2-well defined, 3-moderate, 4-scar formation
Edema scale :-0-none, 1-slight, 2-well defined, 3-moderate, 4-scar formation

After the skin irritation study it was observed that the scores in terms of erythema and edema i.e 3.5 ± 0.54 and 2 ± 0.89 respectively, for optimized formulation and the scores in terms of erythema and edema i.e. 4 ± 0 and 2.16 ± 1.47 respectively were greater than 2, therefore the optimized formulation solution when applied on the skin was found to be skin irritant under UV rays.

Stability studies: Optimized formulation (batch F1) was stored for 2 months for stability testing as per ICH guidelines at three different temperatures i.e at 8 ± 2°C, 25 ± 2°C, 37 ± 2°C away from light. Results are as follows:-

1. Chemical stability of the formulation was assessed by estimation of:

a. In vitro drug transport

The cumulative percentage drug released after 6 hours of an optimized formulation was observed to be 8.28 ± 2.16% per cm^2 area of the rat skin, 8.04 ± 1.53% per cm^2 area of the rat skin, 8.45 ± 1.90% per cm^2 area of the rat skin for three different temperatures i.e at 8 ± 2°C, 25 ± 2°C, 37 ± 2°C respectively.

b. FTIR spectroscopy

Results of FTIR spectroscopy of an optimized formulation (batch F1) kept at three different temperatures i.e at 8 ± 2°C, 25 ± 2°C, 37 ± 2°C for two months. It was observed that FTIR Spectroscopy shows no major difference in the basic peaks of psoralen. This shows that an optimized formulation was stable at different temperatures.

2. Physical stability of the formulation was assessed by estimation of:

a. Clarity of solution

An optimized formulation was observed to be a clear solution after two months when kept at three different temperatures i.e at 8 ± 2°C, 25 ± 2°C, 37 ± 2°C.

b. pH

pH of an optimized formulation was observed to be 5.5 after two months when kept at three different temperatures i.e at 8 ± 2°C, 25 ± 2°C, 37 ± 2°C.

Hence after analyzing all the above factors an optimized formulation was found to be stable.

CONCLUSION

An optimized formulation was found to be a clear solution with pH 5.5, having spray pattern uniform and spherical in shape, good dermal adhesion, good flexibility and moderate water washability properties with no leakage in the container. Area covered by each spray was calculated to be 9.003 ± 2.234 cm^2. Average weight of the formulation per metered dose (or per spray) was calculated to be 0.2 ± 0.005 g. Average drug content of the formulation per metered dose was calculated to be 1.5 ± 0.05 mg. Film formation time was calculated to be 144.33 ± 12.01 seconds. In vitro drug transport was observed to be 8.53 ± 2.15 % per cm^2 area of the rat skin. Average drug content uniformity after 10^{th} spray, 15^{th} spray, 30^{th} spray, 40^{th} spray was calculated to be 1.65 ± 0.264 mg. Average viscosity of the formulation was calculated to be 14.57 ± 2.89 cps. Skin irritation studies showed photosensitization property of the formulation. All the data collected from stability studies showed physical and chemical compatibility of ingredients within the formulations.

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