ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and Optimization of Emollient Gel Loaded With Salicylic Acid for the Effective Treatment of Psoriasis.

Nitha Vincent, Ramya Devi D, and BN Vedha Hari*.

Department of Pharmaceutical Technology, School of Chemical and Biotechnology, SASTRA University, Thanjavur-613 401, Tamil Nadu, India.

ABSTRACT

The use of salicylic acid (SA) with higher concentration leads to acidic toxicity and other adverse effects such as dryness, redness and exfoliation in the treatment of psoriasis. The objective was to produce the 2% salicylic acid emollient gel formulation for the immediate release of drug thereby reducing the side effects in psoriasis treatment. The best formulation of plain SA gel was optimized through different evaluation studies and the effect of emu oil as emollient in the enhancement of penetration and release mechanism of SA through skin from emollient gel of optimized formulation were evaluated using *in vitro* and *ex vivo* diffusion studies. 100% of drug release was obtained at the end of 2nd h which confirms the immediate release of SA from emollient gel. Around 76% of drug was permeated through skin and 14.95% of drug could entrap inside the skin which revealed the impact of emollient in the permeation of drug. 2% SA emollient gel formulation was effective to provide immediate release of SA and could reduce the side effects on the skin by permeating all drug components through skin and so it could be consider as a best choice for the treatment of psoriasis.

Keywords: salicylic acid, psoriasis, emollient gel, immediate release.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Psoriasis is a common inflammatory skin disease that may affect 2-3% of the total world population and characterized by patches, plaques and papules, also it is believed that some negative signals produced by immune systems are the main reason for psoriasis. In this case, keratinocytes which are the outermost layer of skin possess shortened life cycle and results in the alteration of desquamation process where the cytokines will come out through lesions of affected patients and as a result, scaling marks appears on the skin [1]. These conditions may negatively affect the patient's quality of life and lead to psychosocial stress. So the treatment should be aimed for the reduction of inflammatory reactions for which the topically applied formulation is most preferred since it offers direct application on the wound or lesion, enhanced targeting to skin tissues and faster healing [2].

Topical corticosteroids are widely used to cure psoriasis due to rapid action, low cost, patient acceptability etc., Salicylic acid (SA) otherwise known as ortho- hydroxybenzoic acid, is commonly used for the treatment of psoriasis. SA is a keratolytic agent which eases the removal of scaly layers of psoriatic skin and helps to become softer. The conventional forms of SA include ointment, face wash and creams. The effective treatment of psoriasis requires prolonged use of SA which causes irritation and become toxic when apply in large amount. Less retaining capacity of all these delivery systems on skin make these formulations inconvenient and less adaptable for topical applications. It is also inferred that the characteristics of delivery systems have much influence in the potency of the drugs used for the treatment [3, 4].

Gel, a semisolid possess high viscosity than other conventional formulations which is enough to adhere on to the skin. Emollients are moisturizing agents commonly used to soften the skin and are a key ingredient in cosmetics and skin care products. Emollient gel is a novel formulation with the addition of emollient intended to reduce the dryness and other inflammatory reactions for psoriatic patience [5]. It was reported that prolonged use many of the emollients available in the market such as mineral oils and petroleum oils are having a lot of draw backs. The introduction of emu oil as emollient for the topical formulation can help to retain the moisture content as well as to minimize the irritation on skin [6, 7] ; also it provides better anti-inflammatory activity and helps the drug to penetrate through stratum corneum. Therefore it would be more effective to develop an emollient gel of SA for the treatment of psoriasis.

MATERIALS AND METHODS

Materials

Salicylic acid was bought from Siso research laboratories pvt. Ltd. (Mumbai, India). Emu oil was obtained from Ganesh emu farms, Trichy, India. HPMC was bought from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), ethanol was bought from Changshu Yangyuan Chemical (China), NaOH was bought from SDFCL (Mumbai, India) and propylene glycol was bought from Chemspure (Chennai, India).

ISSN: 0975-8585

Determination of melting point

Melting point of drug was measured by filling fine powder of drug in the capillary tube sealed at one end and kept in the melting point apparatus. The temperature at which the drug started and end of melt was noted using thermometer which was placed inside the melting point apparatus [8].

Preparation of salicylic acid (SA) gel

2% Salicylic acid gel was prepared with different concentrations of ethanol and polymer, as showed in table no.1. HPMC, water and propylene glycol at required quantity were mixed together using a magnetic stirrer by keeping SA concentration constant in all the formulations. Since ethanol was selected as the optimum solvent for SA, the mixture of SA and ethanol was poured into the polymer solution. The solution was kept under stirring and then the pH was adjusted using 0.1M NaOH and the formulated gel was taken for further analysis.

Table 1: Formulation variables for the topical gel of salicylic acid for psoriatic treatment

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
SA (g)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPMC (g)	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
Ethanol (g)	5	10	15	5	10	15	5	10	15
Propylene glycol (g)	2	2	2	2	2	2	2	2	2
Distilled water (g)	12.1	7.1	2.1	11.6	6.6	1.6	11.1	6.1	1.1
Total weight(g)	20	20	20	20	20	20	20	20	20

Optimization of emollient (emu oil)

The selected gel formulation was taken for optimizing the concentration of emu oil as emollient. Emu oil was taken in varying amount (5, 7, 9 and 11%) and was mixed with the formulation by adding drop wise under continuous stirring using magnetic stirrer, followed by sonication for 10min. Again, these emollient gels were taken to carry out further evaluations.

Physical evaluations

Visual inspections of plain gel and emollient gel were conducted to observe the colour, odour and chances of phase separation, and washability. Easiness in the removal of gel from the skin is known as washability where 100mg of gel was applied on the skin of six volunteers, each person was trained to make scores for easiness in the removal of sample after one min and the scores were based on the situations such as washable and not washable [9].

Homogeneity and grittiness

Homogeneity and grittiness were measured by visual inspection as well as by taking a small quantity of gel and rubbing it on the skin surface. About 100mg of gel was taken and rubbed on the skin surface and presence of gritty particles; lumps or non-uniform flakes were observed [9].

pH measurements

pH was measured to determine the level of irritation caused by the formulation when applied over the skin surface. Highly acidic or alkaline formulations can cause severe irritation, rashes, allergy and pH paper was used to measure pH of all formulations. A drop of sample was allowed to fall on the pH paper and the change in the colour was observed [9].

Viscosity measurements

Brookfield viscometer was used for the measurement of viscosity of plain gel and emollient gel. 25g of gel samples were poured into the sample tube and fixed on the instrument. Viscosity of each sample was measured by dipping the spindle into the gel (spindle no.63 at 200 rpm). The experiment was repeated for three times and the average value was noted.

Spreadability

Spreadability study of samples was performed using glass slide method. Two glass slides with standard dimensions were taken and 100mg of gel was kept on one glass slide and covered using another glass slide. After keeping a time period of 5min, the spreading coefficient of sample was obtained by measuring the diameter of the spread samples. The experiment was repeated for three times and the average was taken and tabulated with standard deviations [10].

Uniformity of drug content

Drug content present in each formulation was determined by UV method.100mg of gel was dissolved in 100ml of phosphate buffer; sonicated for 15min followed by shaking for 2h to dissolve the gel completely. The solution was filtered and the absorbance of each sample was measured using UV-visible spectrophotometer (EL150, Elico) at the λmax of 230nm [11]. The percentage of drug present in the gel formulation was calculated using the formula,

$$\%\ drug\ content = \left(\frac{absorbance\ of\ sample}{absorbance\ of\ standard}\right) \times 100$$

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded for each sample using ATR method and the changes in the appearance of functional groups of pure drug and gel samples were noted to determine the extent of chemical interaction of SA with polymer [12].

In-vitro diffusion of SA from gels

The release profiles of SA were obtained using Franz diffusion cell with a capacity of 15ml, wherein a dialysis membrane was placed between donor and acceptor chamber. 0.5g of gel formulation was placed on the membrane facing toward donor region, and the

acceptor chamber was filled with phosphate buffer of pH 7.4. The temperature inside the chamber was maintained at 37° C; the whole system was kept on a magnetic stirrer and the sample was allowed to stir continuously by placing a magnetic bead in the acceptor chamber. 5ml of sample was withdrawn at periodic time interval and replaced with the same quantity of warm fresh phosphate buffer. The release study was continued for 8 hours and the samples were analysed using UV-visible spectrophotometer method using phosphate buffer as blank [13].

Ex-vivo skin permeation study

Goat skin was selected for the permeation study since it has many structural similarities with human skin. The skin was collected from slaughter house and the hair and subcutaneous fat tissues were removed from the skin and washed with distilled water. *Ex vivo* permeation study was carried out using Franz diffusion cell and followed the same methods as described previously for *in-vitro* release and the drug permeated through the skin was calculated using UV- visible spectrophotometer [14].

Determination of drug in the skin layers

After the permeation study, the skin was taken and washed with 5ml of phosphate buffer, and the solution was filtered using 0.22µm membrane filter and then used to find the drug present on surface of the skin, by UV-visible spectrophotometer method. The skin was then cut into small pieces and kept in 10ml of phosphate buffer for 24 hours. Then the mixture was kept for sonication for 10min followed by vortex mixing for 15min and the sample was centrifuged at 7500 rpm. The supernatant was collected to determine the drug retained within the skin layers [15].

Drug release kinetics

The release kinetics of SA was determined by various kinetic models, linear and non-linear kinetic models available which are classified as Zero order, First order, Higuchi model, Korsmeyer-Peppas model, Hixson-Crowell model, Hopfenberg model, Baker-Lonsdale model, Makoid-Banakar model, Weibull model and Gompertz model. Each model follow different rule of kinetic analysis module based on which the drug release mechanism was obtained [16].

Stability studies

Stability studies were conducted for plain and emollient gel formulations, wherein 1g of all the formulations was kept at 4°C and room temperature for a period of 3 months. The samples were analysed at periodic intervals for pH, drug content and physical appearance such as colour, odour, phase separation etc. The stable formulation was selected as the optimized one for further studies [17].

ISSN: 0975-8585

RESULTS AND DISCUSSION

Determination of melting point

Melting point of SA was found to be 160- 190° C and the high value of melting point indicated its physical stability and suitability for formulation development, especially during the heating processes [18].

Physical evaluations

The gel formulations were clear and free from particles and also phase separation was not observed in the gel which indicated the physical stability of the formulation (table 2). Again, all the formulations were found to be homogenous and with a pH range of 7.6. The homogeneity revealed the unique nature of gel with even distribution of all the components and the neutral pH without causing irritation made the gel a better choice for topical application [19].

Table 2: Evaluation studies of SA gel

Formulation code	clarity	odour	Phase separation	washability	homogeneity	Grittiness	pH	Spreadability (cm)	%drug content
F1	Clear	No	No	Washable	Yes	No	7.2	7.6±0.5	96.7±2.6
F2	Clear	No	No	Washable	Yes	No	7	6.5±0.1	105±2.5
F3	Clear	No	No	Washable	Yes	No	7.2	7.6±0.0	109±3.0
F4	Clear	No	No	Washable	Yes	No	6.5	7.6±0.1	105.2±1.0
F5	Clear	No	No	Washable	Yes	No	6.5	6±0.2	104±1.0
F6	Clear	No	No	Washable	Yes	No	7.2	5±0.2	108±0.9
F7	Milky	No	No	Washable	Yes	No	7	4.5±0.1	95±1.5
F8	Milky	No	No	Washable	Yes	No	6.9	4.3±0.3	104±1.8
F9	milky	No	No	Not Washable	Yes	No	6.9	4±0.2	91±2.0

After evaluating the easiness in the removal of emollient gel from skin, it was found that the formulation F9 was very sticky and difficult to remove from skin. The washability was very poor due to high viscosity of gel and also as the concentration of HPMC, emu oil and ethanol increased, the viscosity of emollient gel also increased. Ethanol evaporated fast from the mixture and that resulted in the increase in viscosity. So, F9 was not considered for further studies.

Spreadability

Spreadability coefficient is an important parameter of gel, was found to vary according to the concentration of both HPMC and ethanol. Spreadability data was given in the table no.4 and it was observed that the spreadability was decreased with increase in the HPMC and ethanol concentration. Since ethanol was evaporated from the formulation and due to the presence of high concentration of polymer, the viscosity of the formulation was high and thus spreadability had decreased. Spreading coefficient should be (5-7cm) for the proper spreading of gel on the skin without any loss [10].

ISSN: 0975-8585

Viscosity measurement

Viscosity of plain gel and emollient gel were calculated using Brookfield viscometer. All the formulations were containing very low amount of polymer so that viscosity was very less before the addition of emollient which was obtained as 95.6cp. But it was found that the viscosity of SA gel increased with increase in the emollient concentration which was observed in the range of 100cp to 133cp (table no.3).

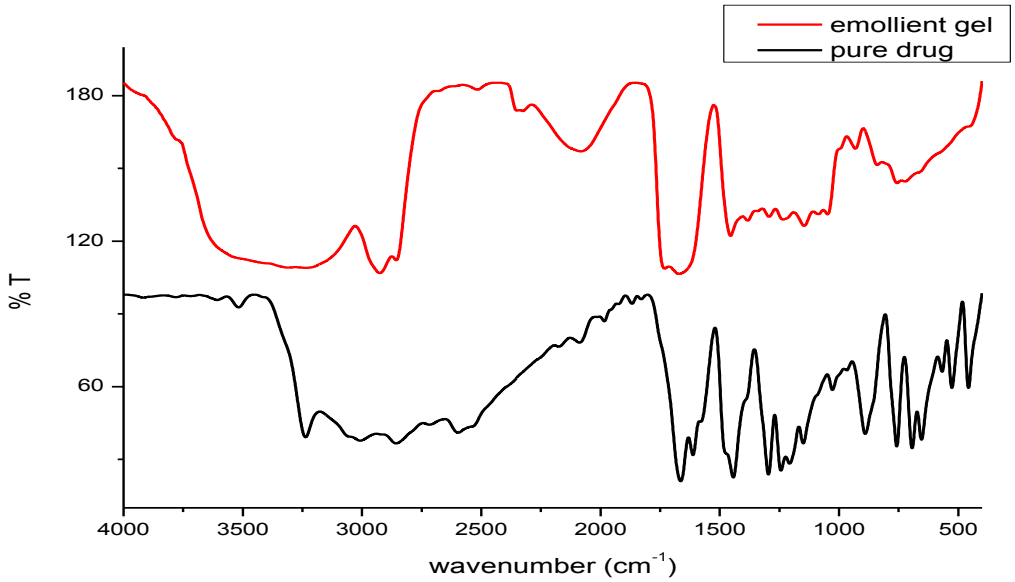
Table 3: viscosity measurement and percentage of SA present in emollient gel

Formulation code	% of emollient	Viscosity (cp)	Drug content (%)
Ef1	5	103±2.0	105±1.0
Ef2	7	112±3.0	98±1.4
Ef3	9	129±1.0	108±0.6
Ef4	11	133±1.0	108±0.9

Fourier transforming infrared spectroscopy (FTIR)

The chemical compatibility between pure SA and the polymer in the gel formulation was evaluated using FTIR method. SA is a carboxylic acid and mainly four peaks of SA could be obtained in the FTIR spectrum and they are C=O stretching, C-O stretching, O-H stretching, and C-H stretching (fig.1). It was obtained that all these peaks were present in the FTIR spectrum of drug as well as emollient gel and shift in the peaks between drug and emollient indicated the H_2 bond interaction and confirmed the absence of any chemical interactions between SA and polymer [20,21].

Figure 1: FTIR spectra of pure SA and emollient gel



Uniformity of drug content

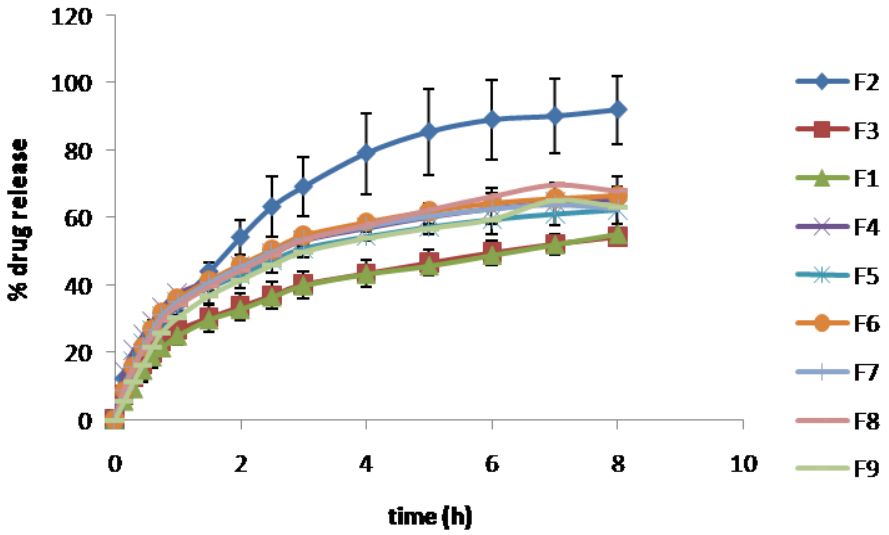
Percentage of SA present in plain gel and emollient gel were noted in table no.2, 3. Drug content present in each formulation was between 95 and 110 (%) and this result revealed that all the formulations consist of SA without any change.

ISSN: 0975-8585

***In-vitro* release of SA**

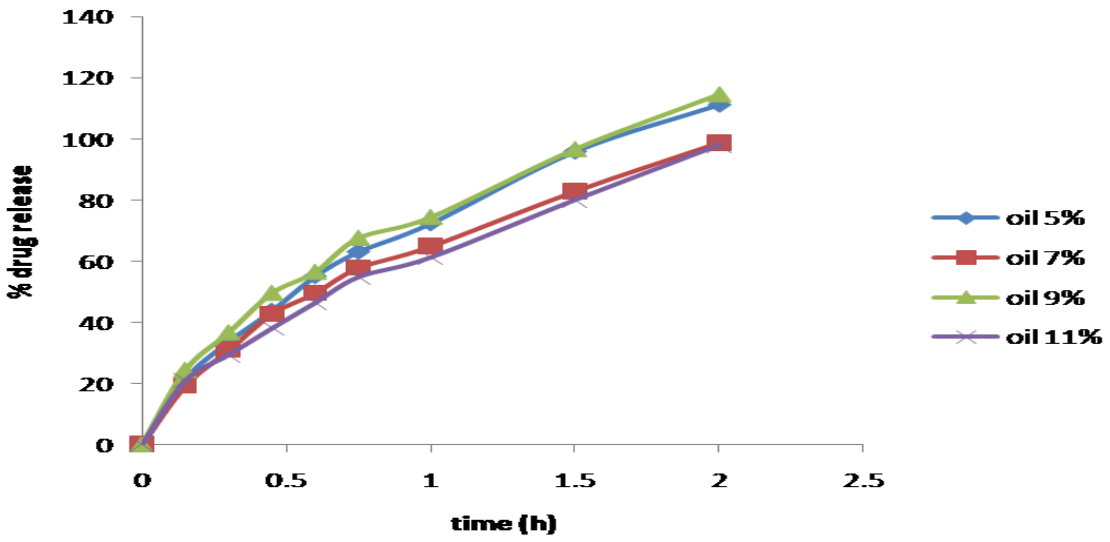
The release profile for each formulation was given in the figure 2. From the data, a significant difference in drug release was observed among the formulations. The release of SA from the gel was mainly influenced by the concentration of HPMC. The cross linking capacity of HPMC was more and SA would be trapped inside these cross links and could provide a prolonged release of drug [22, 23].

Figure 2: Release profiles of SA in phosphate buffer without the presence of emollient



From the release profiles of SA, it was observed that, among all the formulations the highest drug release was shown by F2 *ie* more than 90% and only 70% of drug release was shown by the other formulations at the end of 8th h. So, F2 was selected as the best formulation and different concentration of emollient gels were allowed to carry out the drug release mechanism.

Figure 3: Release profile of SA in phosphate buffer from emollient gel



ISSN: 0975-8585

Emu oil was considered to be a penetration enhancer and after the addition of emollient to the gel, it was observed that the release profiles had reached up to 100% within 2nd hour (fig.3). Since the formulation consisted of less amount of HPMC, the entrapment of drug would be reduced so that most of the drug would be released fast and due to the hydrophobic nature of oil, penetration of SA through the membrane was also rapid.

Table 4: SA release kinetics from gel formulation

models		F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero	R^2	0.5155	0.66	0.3951	0.0196	0.1452	0.2656	0.2124	0.4007	0.4915
	Ko	8.823	15.295	8.877	11.326	10.752	11.497	11.42	11.666	10.716
	SS	2149.27	5071.8	2500.25	5654.46	4801.7	5069.39	5047.24	4361.35	3418.40
First	R^2	0.7503	0.991	0.6709	0.567	0.6077	0.7236	0.6788	0.7989	0.8083
	K_1	0.137	0.399	0.14	0.231	0.205	0.235	0.222	0.235	0.196
	SS	1107.82	134.87	1360.15	2497.20	2203.65	1907.69	2058.45	1463.36	1288.67
Higuchi	R^2	0.9699	0.9767	0.9553	0.8673	0.8923	0.9149	0.8996	0.9534	0.9566
	K_H	21.012	35.99	21.29	27.653	26.157	27.834	27.061	27.995	25.592
	SS	765.54	348.27	184.93	765.54	604.96	587.11	643.29	339.06	291.84
Korsemeyer-Peppas	R^2	0.9829	0.9769	0.9877	0.9862	0.9759	0.9688	0.964	0.9834	0.9707
	K_{KP}	23.219	36.596	24.219	35.224	32.375	33.342	32.843	32.278	28.329
	n	0.43	0.488	0.43	0.326	0.347	0.371	0.362	0.399	0.427
	SS	75.94	343.94	50.66	79.66	135.25	215.30	230.82	121.09	196.78
Hixson-Crowell	R^2	0.6827	0.9689	0.5906	0.4792	0.4792	0.6049	0.5541	0.7011	0.7253
	K_{HC}	0.04	0.111	0.04	0.056	0.056	0.063	0.060	0.063	0.054
	SS	1407.44	463.90	1692.14	2925.62	2925.62	2727.31	2857.33	2175.13	1846.78
Hopfenberg	R^2	0.7502	0.990	0.67	0.5669	0.6075	0.7236	0.6786	0.7988	0.8082
	SS	1108.16	134.99	1360.86	2498.11	2204.80	1908.18	2059.89	1463.96	1289.45
Baker-Lonsdale	R^2	0.985	0.9652	0.981	0.9466	0.9538	0.967	0.9567	0.9874	0.9781
	K_{BL}	0.009	0.035	0.01	0.019	0.016	0.019	0.018	0.019	0.015
	SS	65.24	518.60	77.12	307.92	259.77	227.94	277.32	91.35	147.39
Makoid-Banakar	R^2	**0.992**	**0.998**	**0.995**	**0.9979**	**0.995**	**0.9951**	**0.996**	**0.997**	**0.993**
	K_{MB}	24.47	38.76	25.86	37.33	34.93	36.40	36.19	34.32	30.31
	SS	31.36	23.80	18.69	11.97	25.30	33.80	23.37	18.37	42.32
Weibull	R^2	**0.996**	**0.997**	**0.998**	**0.998**	**0.997**	**0.995**	**0.991**	**0.997**	**0.994**
	α	3.398	2.377	3.206	2.127	2.289	2.186	2.235	2.347	2.652
	SS	14.08	38.43	5.473	11.19	16.77	34.04	51.92	18.22	37.51
Gompertz	R^2	**0.997**	**0.964**	**0.997**	**0.997**	**0.999**	**0.998**	**0.997**	**0.994**	**0.998**
	α	0.894	0.93	1.35	0.985	1.073	1.037	1.058	1.062	1.204
	SS	11.27	529.25	8.981	16.52	4.4854	7.58	15.96	42.14	11.84

ISSN: 0975-8585

Table 5: SA release kinetics from emollient gel

Models		Ef1	Ef2	Ef3	Ef4
Zero	R^2	0.8570	0.8222	0.8146	0.8547
	k0	64.999	57.944	67.247	56.119
	SS	1419.30	1355.37	1873.18	1054.51
First	R^2	0.9607	0.9865	0.9545	0.9786
	k1	1.434	1.193	1.576	1.099
	SS	389.50	102.63	459.82	155.03
Higuchi	R^2	0.9785	0.9858	0.9881	0.9797
	KH	74.102	66.381	77.110	63.988
	SS	213.35	107.92	120.23	147.22
Korsmeyer-peppas	R^2	0.9985	0.9977	0.9988	0.9983
	kKP	73.288	65.872	76.573	63.312
	n	0.62	0.59	0.582	0.616
	SS	14.99	17.47	17.94	12.32
Hixson-Crowell	R^2	0.9784	0.9849	0.9669	0.9800
	kHC	0.393	0.332	0.426	0.308
	SS	214.44	114.80	334.30	145.35
Hopfenberg	R^2	0.9795	0.9881	0.9669	0.9815
	SS	203.03	90.39	334.29	134.27
Baker-Lonsdale	R^2	0.9042	0.9365	0.9149	0.9268
	kBL	0.143	0.11	0.16	0.099
	SS	951.03	483.96	859.66	531.30
Makoid-Banakar	R^2	**0.9991**	**0.9983**	**0.9983**	**0.9986**
	kMB	80.487	71.51	78.587	59.387
	SS	8.627	13.30	17.37	10.05
Weibull	R^2	**0.9715**	**0.9872**	**0.9602**	**0.9810**
	α	0.770	0.859	0.663	0.979
	SS	282.65	97.31	402.23	138.08
Gompertz	R^2	0.9064	0.9437	0.8984	0.9243
	α	0.263	0.359	0.235	0.400
	SS	928.68	429.42	1026.37	549.73

Ex vivo skin permeation study

Ex- vivo permeation study was conducted using goat skin placed on the Franz diffusion cell. 76% of SA was permeated through skin which indicated the high diffusion coefficient of drug [14]. It was observed that the permeation of drug followed Fick's diffusion theory and so, the main driving force for this mechanism was the concentration difference of SA between the outer and inner layer of skin. Emollient had a great impact on the permeation of drug through skin due to its hydrophobicity and ethanol in the gel formulation itself acted as a penetration enhancer [24].

Determination of drug in the skin layers

After the permeation study of SA, the amount of drug retained on the skin was found to be 5.67% so that the irritation and other side effects due to presence of drug on skin

could be reduced [15]. On the other hand, SA retained in the skin layers was found to be 14.95%, which was higher than the drug present on skin surface. Since more amount of SA was retained in the skin layers, the wastage of drug could be reduced to an extent.

Drug release kinetics

Results of release kinetics of SA from plain gel as well as emollient gel were described in table 4,5. From the results, it was clear that all the formulations follow Makoid-Banakar model, Weibull model, and Gompertz model of release kinetics. The best fit models were selected on the basis of the value of R^2. The Makoid-Banakar model explains the physical interaction between drug and polymer. According to Gompertz model, the drug was released at the maximum rate at the initial stage so that a steep increase can be obtained in the graph and then converges slowly to get highest dissolution of drug [25].

Stability studies

Stability studies were conducted for all the formulations by keeping the samples at 4^oC as well as room temperature for three months of time period. The results obtained for drug content, pH, visual stability etc. were noted in table no.6. After the analysis of stability data, it was found that pH as well as drug content was nearly equal to the initial values but two formulations, namely F1 and F4 were not stable due to the development of phase separation and that would be influenced by the variability of composition of formulation.

Table 6: stability studies at 4°C and at room temperature after a period of one month

Formulation code	Phase separation		pH		Drug content (%)	
	4^oC	Room temp.	4^oC	Room temp.	4^oC	Room temp.
F1	Yes	Yes	6.4±0.3	5.5±0.4	31.2±3	42±3
F2	No	No	7.1±0.1	5.9±0.4	108±2	98±1
F3	No	No	7±0.4	6±0.1	122±0.6	112±1
F4	Yes	No	6.5±0.1	5.8±0.2	49.14±0.9	67±0.4
F5	No	No	6.6±0.3	7.1±0.2	107±2	112±0.5
F6	No	No	7.2±0.2	6.7±0.6	124±2	109±0.8
F7	No	No	6.9±0.2	6.7±0.3	119±1	102±0.4
F8	No	No	6±0.2	5.8±0.2	102±0.5	99±0.7
F9	No	No	5.8±0.4	5.6±0.3	95±0.5	96±0.3

CONCLUSION

In this study, 2% salicylic acid loaded emollient gel was developed for the immediate release drug with the incorporation of emu oil. The permeation of drug through the skin was obtained as 76% with the retention of only 5.67% drug on the skin which could be due to significant effect of emollient in the transdermal delivery of drug by enhancing the penetration of SA. 14.95% of drug was retained into the skin layers for the effective treatment of psoriasis. The release of SA from emollient gel was found to be more than 90% at the end of 2^{nd} h. The emu oil itself had some anti inflammatory activity so that overall effect of emollient gel was increased. So, the 11% emollient gel was considered as the best formulation for the treatment of psoriasis since it possess highest amount of emollient. Thus

the SA loaded emollient gel could be an effective formulation to reduce side effects especially dryness and is very much useful for psoriatic patients.

AKNOWLEDGEMENT

The authors are thankful to the SASTRA University, Thanjavur, Tamil Nadu for providing the facilities to complete the research.

REFERENCES

[1] Carrascosa JM, et al. Actas Dermosifiliogr 2009;100: 190-200.
[2] Tsen-Fang Tsai, et al. J Dermatol Sci 2011; 63: 154–163.
[3] Miriam Canavese, Fiorella Altruda, Thomas Ruzicka, Ju¨ rgen Schauber. J Dermatol Sci 2010; 58: 171–176.
[4] Sudhir Bharadwaj, Gupta GD, Sharma VK. Journal of Chemical, Biological and Physical Sciences 2012; 2: 856-867.
[5] Steven B Hoath, Vivek Narendran. Semin Neonatol 2000; 5: 289–296.
[6] Elisha DO Roberson, Anne M Bowcock. Trends Gen 2010; 26: 415–423.
[7] Tarl W Prow, et al. Adv Drug Deliv Rev 2011; 63: 470–491.
[8] Hernanz JM, Brufau KC, Herreram Shital Uttarwar E. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012; 3: 1103-1118.
[9] Rachit Khullar, Deepinder Kumar, Nimrata Seth, Seema Saini. Saudi Pharm J 2012; 20: 63–67.
[10] Tikshdeep Chauhan, Parashar B, Sonia Arora. Int J Pharm Chem Sci 2013; 2: 72-81.
[11] Mohammed Haneefa KP, Shahima Hanan K, Saraswathi R, Guru Prasad Mohanta, Chandini Nayar. Asian Pacific J Trop Med 2010; 988-992.
[12] Shu-Tuan Yeh, Hao-Ting Wang, Hua-Yang Liao, Shun-Lian Su, Che-Chen Chang, Hung-Chan Kao, Bor-Shiunn Lee. Dental Mater 2011; 27: 187–196.
[13] Ana Flo Sierra, Maria L. Garduno Ramırez, Ana C. Calpena Campmany, Adolfina Ruiz Martınez, Beatriz Clares Naveros. J Dermatol Sci 2013; 69: 202–214.
[14] Poonam Verma, Kamla Pathak. Nanomedicine: Nanotechnology, Biology, and Medicine 2012; 8: 489–496.
[15] Mona M.A. Abdel-Mottaleb, Dirk Neumann, Alf Lamprecht. Eur J Pharm Biopharm 2011; 79: 36–42.
[16] Hetal K. Patel, Bhavesh S. Barot, Punit B. Parejiya, Pragna K. Shelat, Arunkumar Shukla. Coll Surf B: Biointerf 2013; 102: 86– 94.
[17] Yunqi Wua, Reza Fassihi. Int J Pharm 2005; 290: 1–13.
[18] Abu TM Serajuddin. J Pharm Sci 1999; 88: 1058-1066.
[19] Loganathan V, Jaswanth A, Sulaiman A, Rajaseskaran A, Manimaran S, Kumar Senthil B. Indian J Pharm Sci 2001; 63: 200-204.
[20] Prasanthi Tangula, Vasanth PM, Ramesh T, Ramesh M. Der Pharmacia Lettre 2013; 5: 65-73.
[21] Yolanda K. Jones, Zhonghui Li, Michael M. Johnson, Fabien Josse, Jeanne M. Hossenlopp. IEEE Sens J 2005; 5: 1175-1184.
[22] Thomas A Wilsona, Robert J Nicolosia, Garry Handelmana, Subbiah Yoganathan, Timothy Kotyla, Frank Orthoefer, Paul Binford. Nutr Res 2004; 24: 395–406.
[23] Niklas Ohrner, Mats Martinelle, Anders Mattson. Biotechnol Lett 1992; 14: 263-268.

ISSN: 0975-8585

[24] Zemtsov A, Hosier H. J Cosm Dermatol Sci App 2013; 3:18-21.
[25] Renuka Sharma, Roderick B. Walker, Kamla Pathak. Ind J Pharm Edu Res 2010; 45: 25-31.