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Development of Isotretinoin Gel for the Treatment of Acne Vulgaris

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ABSTRACT

Many people suffer from one of the most common skin disorders is acne i.e. acne vulgaris. The treatment is aimed to reduce the rate of sebum production, reduce bacterial population in pilosebaceous follicle and reduce inflammation. The mild to moderate forms of acne are generally treated topically while the severe form of acne is treated orally. The androgenic hormones influence sebaceous gland size by stimulating rate of cell division and lipid accumulation. Targeting the sebaceous glands with agents to decrease androgenic activity could be useful in the treatment of dermatological disorders such as acne. Isotretinoin is approved for oral administration for the treatment of cystic acne and may be effective against dermatological disorders as well. Objective of presented work are, Formulation of a topical gel of Isotretinoin so as to deliver the drug percutaneously for the treatment of acne vulgaris where systemic therapy is avoidable. However, Isotretinoin has a poor absorption through intact skin Therefore, incorporation of a penetration enhancer to increase the permeation of the drug through the skin and enhance its therapeutic activity & to study the evaluation parameters of the formulated gel. All the formulations were evaluated for their appearance, homogeneity and texture, % drug content and uniformity, pH, spreadability and in vitro release study through cellophane membrane. It was found that all the formulated gels were homogenous, smooth in texture and elegant in appearance. The results of the drug content were found in acceptable range indicating uniform distribution of drug throughout the base. The pH of the gel formulations was found in the normal pH range of the skin that would not produce any skin irritation. There was no significant change in pH values as a function of time for all formulations. All the formulations were subjected to spreadability test and it was found to be equally good with that of the marketed preparation. The in vitro release of profiles of the prepared Isotretinoin gel was compared with that of the marketed product.

Keywords: Acne vulgaris, Isotretinoin, sebaceous gland, Carbopl 940

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INTRODUCTION

The people suffer from various dermatological disorders and amongst them the most common is acne vulgaris. Various types of treatment and drugs are available for the treatment of acne vulgaris. Depending upon the cause and type of acne various types of drugs are prescribed in the treatment of acne vulgaris. The most common is the application of the topical therapy for its treatment. Isotretinoin is commonly prescribed for the treatment of mild to moderate acne by topical route and in severe forms of acne it is prescribed by the oral route [1].

Unfortunately, oral use of retinoids is associated with significant systemic side effects thus limiting the use of these potent agents to only the severe, recalcitrant forms of acne. The even greater concern is that these compounds are highly teratogenic. Topical application of Isotretinoin can produce dermal concentrations in excess of those produced by therapeutic oral doses of the drug. This approach diminishes side effects associated with systemic therapy, particularly teratogenecity, and allows the drug to be used less restrictively, or with greater safety for mild to moderate acne. However the topical application of the compounds is associated with the problem of photoliability and low solubility of compounds Due to the photoliability there is substantial degradation of the drug on the skin surface [2]. Isotretinoin readily penetrates through the hydrophobic stratum corneum while the lower epidermal and dermal layers are hydrophilic in nature, which limits its solubility and transfer of the drug from stratum corneum [3], [4]. As the main area of target is sebaceous glands, the application of hydrophilic penetration enhancers (wetting agents such as cremophore RH 40) may be useful in decreasing the interfacial tension between polar drug and sebum, providing a more favorable environment for drug partitioning and absorption and can significantly alter the penetration of the drug through the stratum corneum and may have effective drug concentrations in the lower epidermal and dermal layers to treat the acne [5, 6]. Isotretinoin has a short half-life and poor bioavailability when given orally. Also the drug has teratogenic effects. Therefore, an alternative route for drug delivery is required. Percutaneous route offers this advantage.

The topical treatment is done particularly with benzoyl peroxide, retinoids or antibacterials preparations. Benzoyl peroxide has antimicrobial action and keratolytic properties, but these preparations suffer from the most common side effects of bleaching of hair and coloring of clothes. Also, some of these products contain sulfites, which may cause allergic type reactions (anaphylactic reactions). The anti-bacterial agents tend to reduce only the bacterial growth in the follicle and may be used particularly for inflammatory acne. However, the development of resistance by the skin flora is an increasing problem. Due to these problems, topical retinoids have been proved to be an effective alternative to benzoyl peroxides and some dermatologist consider them to be the treatment of choice for mild to moderate comedonal acne [7].

The topical gels formulated with the use carbopols produced relatively stable formulations. Ethanol and isopropyl alcohol were used as vehicles for the gel formulations. The effect of polymer concentration on the drug release was also effectively studied for many

topical formulations. Various evaluation parameters for gel formulations were studied out to show the efficacy and safety of the formulation [8].

Carbopl 980 was found to be superior to other grades of carbopol in optical clarity and rheological properties. Patented literature suggested that topical isotretinoin formulation could be prepared with the use of penetration enhancer in order to increase its penetration through the skin. Also the topical route of administration was found to be therapeutically effective and safe for the treatment of acne vulgaris.

Dosage form selection should include those delivery systems that are non-comedogenic. Gels tend to be most effective as they have faster absorption than creams. Gels containing only water tend to be slow to dry; so the addition of ethyl or isopropyl alcohol to the gel hastens their drying to a film. But some patients may need the less drying lotions or creams for dry or sensitive skin or for use during dry winter weather [9].

MATERIALS & METHODS

Isotretinoin was a gift sample from Navketan Pharma, Aurangabad, and Carbopol 980 & Cremophor RH 40 was a gift sample from Khandelwal Laboratories Thane. & other chemical were procured from vendors.

Experimental

Development of gel formulation

Usually, the concentration of isotretinoin selected for the topical dosage form is either 0.025%w/w or 0.05%w/w. These concentrations of the drug provide sufficient therapeutic action and safety profile required for the treatment of the acne. The 0.025%w/w of drug concentration is generally used when the combination therapy is used for the treatment of the acne. Therefore, for the development of the formulation in present attempt, 0.05%w/w of the drug concentration was used

Carbopols are commonly preferred as gelling agents in dermatological gel formulations because it produces gels having a number of desirable characteristics required for dermatological preparations The gelation mechanism depends on neutralization of carboxylic acid moiety to form a soluble salt. . There are various carbopol grades available for the dermatological formulations such as carbopol 934, carbopol 940, carbopol 971 and many more. Most of carbopol grades have favorable rheological properties for topical applications and they can form gels at concentrations as low as 0.5% and they can be used in a concentration ranging from 0.5% to 2%. For the development of the formulation in present attempt, carbopol 980 was selected as gelling agent because this grade exhibits superior optical clarity compared with other grades of carbopol. Gels were prepared by using 0.5% and 1% concentration of carbopol. Carbopol gels are formed on neutralization between pH 5 and 10 with metal hydroxides or

amines such as diisopropanolamine and triethanolamine. Optimum viscosity and clarity with carbopols is obtained in pH ranging from 6 to 8. So, for the present formulation triethanolamine was selected as neutralizing agent. Triethanolamine can be used in concentration ranging from 0.5% to 1%. The concentration of triethanolamine selected for the present formulation was 0.7%, which gave a pH of about 7.2 to the formulation. However the gelling characteristics are adversely affected by either insufficient neutralization or high pH.

The commonly used organic solvents in the gel formulation are ethanol and isopropyl alcohol. However, they can be used up to certain limits when they are to be used with carbopol as gelling agents. Ethanol (95%) was selected for the development of the present formulation. It may be used in concentration ranging from 5% to 50%. But high concentrations of alcohol may lead to decrease in the rheological properties of formulation. For the present study, 15% of ethanol was selected in the development of formulation, as this amount was sufficient to dissolve the drug. Also ethanol used may play dual role in the formulation. It may act as solvent for the drug and may act as a secondary penetration enhancer by delipidizing the sebum and opening the passage for drug deposition within the follicle.

Various types of hydrophilic penetration enhancers can be used in the topical dermatological preparations such as PEG, PG and various types of chromophore grades. Chemically cremophor is poly oxyl hydrogenated castor oil. Cremophor grades act as solubilizer for the fat-soluble vitamins such as vitamin A, D, E, and K. As isotretinoin is chemically vitamin A derivative, cremophor grades was selected for the preparation of present formulation. Cremophor RH 40 grade was selected because it is a non-ionic solubilizer having HLB value ranging from 14-16 i.e. very hydrophilic in nature. It is stable in alcohol and forms clear solution with alcohol.

The antioxidants may be selected from butylated hydroxy toluene, butylated hydroxy anisole. For the present formulation butylated hydroxy toluene was selected.

Preparation and composition of gels

Table 1 Composition of Different Gel Formulations

Sr. No	Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
1	Isotretinoin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
2	Ethanol	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
3	Cremophor RH 40	0.50	1.00	1.50	2.00	0.50	1.00	1.50	2.00
4	Butyl hydroxy	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
5	Carbopol 980	1.00	1.00	1.00	1.00	0.50	0.50	0.50	0.50
7	Triethanolamine	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
8	Distilled water	82.80	82.10	81.60	81.10	83.10	82.60	82.10	81.60
9	Perfume	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Gels were prepared by dispersing the carbopol 980 in sufficient quantity of distilled water, being kept under magnetic stirring until homogeneous dispersion was formed. The dispersion was then neutralized and made viscous by the addition of triethanolamine. A solution of ingredients 1 to 4 from Table 1 was prepared and added to the above mixture. Finally a sufficient quantity of perfume was added to the formulation. The compositions of different gel formations are listed in Table 1. As retinoids are photosensitive, diffusion cells and other glassware's used for handling of retinoids were covered with aluminum foil to minimize photodegradation. Each of these prepared gels was compared for their evaluation characteristics with marketed product Isotroin gel (Cipla), which was considered as reference product.

Evaluation

Physicochemical Evaluation of Gel formulations

Color, Physical Appearance, Homogeneity and texture

The color, physical appearance, homogeneity, and texture of the prepared gels were tested by visual observations. The results obtained are shown in the table 3.

Spreadability

Spreadability of the gel formulations was determined by measuring the spreading diameter of 0.1 g of gel between two horizontal plates (7.0 cm × 2.5 cm) after one min. The standardized weight tied on the upper plate was 25 g. The results obtained are shown in the table 3.

pH

The pH of the gel formulations was determined by using a pH meter. 2 gm of gel was stirred in distilled water until a uniform suspension was formed. The volume was made up to 50ml and the pH of solution was measured. The measurement was performed at 1, 15 and 30 days after preparation to detect any pH fluctuation with time. The pH of the various formulations is mentioned in the table 3.

Drug Content and uniformity

A sample of 0.2 g of gel was weighed into a 50ml conical centrifuge tube. The sample was extracted by addition of 20 ml of acetonitrile followed by vortex mixing for 5 min. An aliquot of 10 ml was transferred to a 20ml-stoppered test tube and subjected to centrifugation at 1,000 rpm for 20 min. An aliquot of 4ml of the supernatant was used to determine the concentration by UV spectrophotometer. For getting unbiased results the gel was collected from different sides of container and subjected to analysis. The concentration of Isotretinoin in

test samples was calculated using the linear regression equation of the calibration curve. The mean % drug content of the various gel formulations is shown the table 3.

In-vitro drug release from gel formulation

The developed formulations were subjected to diffusion studies through cellophane membrane with Keshray Chein cell. Approximately weighed quantity of gel (1gm) was placed on the donor compartment and covered with a piece of aluminium foil to prevent drying. The sampling port was covered with aluminium foil to avoid atmospheric influence. The receptor compartment was filled with ethanol-phosphate buffer pH 7.4 (70:30 v/v). The whole assembly was maintained at 37+ 1º C with constant magnetic stirring. Care was taken that no air bubbles were trapped under the membrane. Aliquots of 3ml were withdrawn at predetermined intervals for a period of 300 minutes and replaced with equal volume of receptor solution to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the penetration data. The sample withdrawn from the receptor compartment was then analyzed by UV spectrophotometer. The concentration of Isotretinoin in test samples was calculated using the linear regression equation of the calibration curve. The Isotretinoin gel (Isotroin) commercially available in the local market was used for comparison. The % cumulative drug release of the various gel formulations are shown table 4.

RESULT AND DISCUSSION

Analytical Profile

UV Scanning

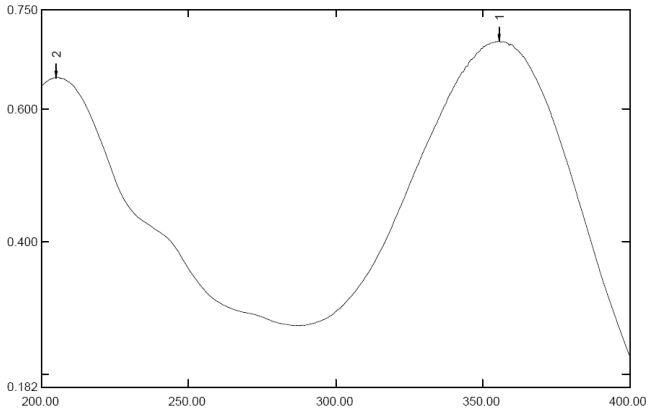

Figure 1 UV Spectrum of Isotretinoin

The scanning of the isotretinoin in the UV Spectrophotometer was performed. The λmax value for the drug was found to be 355 nm, which was found to be in the deviation range of 3% from the reported value of 354 nm. The UV spectrum of the drug is shown in fig. 1.

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Melting point

The melting range of the isotretinoin was found to be 174-176o C.

Interpretation of IR spectra

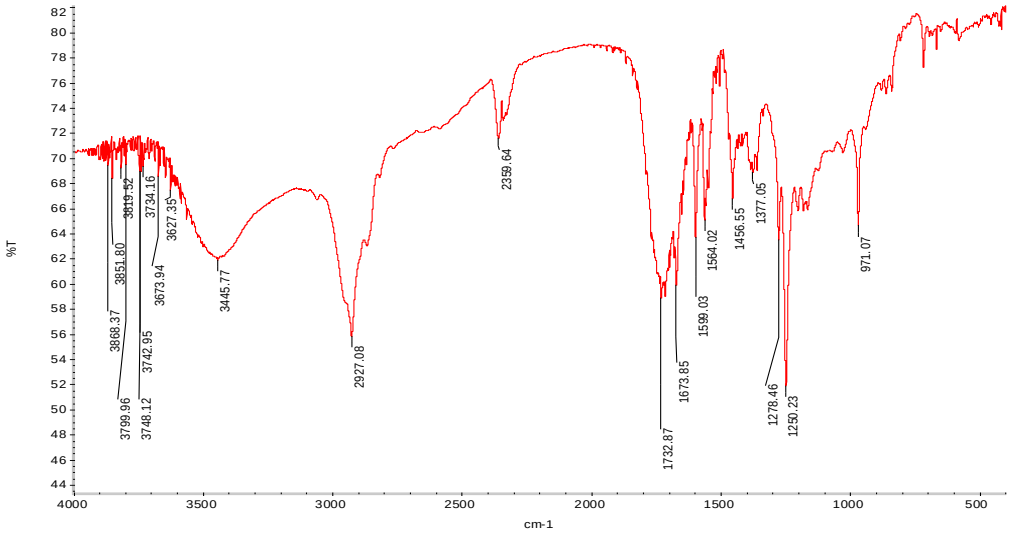

Figure 2 IR Spectrum of Isotretinoin

Table 2 Interpretation of IR spectra

Frequency	Group
3445.77	-OH
2927.08	-CH-
2359.64	-C=C-
1732.87-1673.85	-C=O

The IR spectrum of isotretinoin was recorded from the FTIR with the help of themoelectron software. The spectrum is as shown in fig.2. & its interpretation is shown in table. 2

Evaluation

Physicochemical properties of gel formulation

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Table 3 Evaluation Parameters of Different Gel Formulations

Formulation Code/ Parameters	Color	Physical Appearance	Homogeneity	Texture	Spread ability (gm.mm/min)	pH (Mean ± SD)	% Drug Content
MKT PDT	Yellow	Transparent	Homogeneous	Smooth	24.21	7.22± 0.02	100.99
F1	Yellow	Transparent	Homogeneous	Smooth	24.59	7.16± 0.01	107.16
F2	Yellow	Transparent	Homogeneous	Smooth	24.53	7.18± 0.02	104.93
F3	Yellow	Transparent	Homogeneous	Smooth	24.59	7.10± 0.01	102.88
F4	Yellow	Transparent	Homogeneous	Smooth	24.57	7.20± 0.02	105.27
F5	Yellow	Transparent	Homogeneous	Smooth	24.56	7.36± 0.01	103.05
F6	Yellow	Transparent	Homogeneous	Smooth	24.58	7.39± 0.01	107.33
F7	Yellow	Transparent	Homogeneous	Smooth	24.59	7.33± 0.01	104.07
F8	Yellow	Transparent	Homogeneous	Smooth	24.59	7.35± 0.01	101.16

The physicochemical properties of the gel formulations are shown in table 8. From the results it is clearly evident that all the gel formulations showed good homogeneity, smooth and texture. The physical appearance of the gel formulations was transparent in nature. The physicochemical properties of the prepared gel formulations were in good agreement with those of a marketed product namely Isotroin Gel from Cipla Pharma (table 3).

Spreadability:

Spreadability of gel was evaluated to test the ease of applicability of gels on skin The spreadability of the formulation was between 24.21 to 24.59 which was found to be comparable with the marketed product (table 3).

pH:

The pH of the gel formulations was in the range of 7.16+0.01to 7.39+ 0.01, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time for all formulations (table 3).

% Drug content and uniformity:

The drug content of the gel preparations was found to be uniform among various formulations prepared and was found to range from 100.99 % to 107.33 %. The drug content determination also showed that the drug was uniformly distributed throughout the gel (table 3).

In-vitro Drug release from gel formulation

The experiments for in vitro release of isotretinoin from gel formulations through cellulose membrane were carried out to select appropriate polymer composition for gel

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formulation having suitable consistency for topical application. In vitro release profile of the marketed isotretinoin gel formulation was determined. Then in vitro release profile of the different isotretinoin gel formulations was determined and each of these profiles was compared with that of the marketed gel formulation (table 4).

Table 4 Cumulative % Drug Release from Different Gel Formulations

Time (min.)/ Formulation Code	Cumulative % drug release								
	MP	F1	F2	F3	F4	F5	F6	F7	F8
15	4.596	2.655	4.382	2.655	2.655	4.332	4.482	3.407	3.877
30	6.370	4.953	5.541	3.346	4.347	5.474	5.175	4.779	5.263
45	7.451	6.273	6.859	4.390	5.967	6.722	6.637	5.800	6.115
60	8.569	8.398	7.744	5.470	7.080	7.540	8.520	7.236	7.807
120	11.248	9.995	10.514	7.989	8.452	8.619	11.992	10.838	9.454
180	12.421	10.841	11.542	10.844	9.363	10.037	14.671	11.204	10.020
240	14.807	11.485	12.493	12.271	9.821	12.259	16.989	12.998	10.891
300	16.633	12.232	16.479	13.847	10.072	14.696	19.037	14.081	11.444

The results reveal that the total amount of drug release at 300 minutes for the formulations F1 to F8 was dependent on the concentration of the polymer and the concentration of penetration enhancer used in the formulation. Initially, the gel was prepared with 1% carbopol 980 and 0.5% cremophor RH 40 (F1). The formulation F2 was prepared with 1% carbopol 980 and 1% cremophor RH 40. The in vitro release profiles of both these formulations were compared with that of the marketed product. For the first 45 minutes the formulation F1 followed parallel release pattern as compared to that of the marketed product, though the release was less. At the 60th minute the release was comparable to that of the marketed product. After that the release was significantly decreased. The total cumulative % drug release for formulation F1 was found to be less than that of the marketed product For formulation F2 the release pattern was equal to that of the of the marketed product and the total cumulative % drug release was nearly comparable to that of the marketed product (table 4).

The cumulative % drug release decreased significantly in formulations F3 and F4 Both these formulations comprised of the same concentration of the carbopol i.e. 1% w/w but the amount of cremophor RH 40 in the gel formulation was increased from 1% w/w (formulation F2) to 1.5% w/w (F3) and 2%w/w (F4) respectively; however the total % cumulative drug release was found to be lesser for these formulations than that of the marketed formulation (table 4). The decrease in release may be due to decrease in partitioning of the drug from the base.

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In next formulations, the gels were prepared by decreasing the concentration of carbopol from 1% to 0.5%. The release profile of formulation F5 (0.5% w/w carbopol, cremophor RH 40 0.5%), showed a parallel pattern to that of the marketed product up to 60minutes.However, a decrease in cumulative % drug release was observed after this time, for the formulation F5 was observed. This may be due presence of less concentration of cremophor RH 40 as compared to formulations F6, F7 and F8.

The maximum cumulative % drug release was observed with the formulation F6 (0.5% w/w Carbopol, 1% cremophor RH 40) In formulation F6 at 60th minute the release was equal to that of the marketed product and after 60th minute the release was significantly increased as compared to the marketed product.

In the formulations F7 (0.5% w/w Carbopol, 1.5% cremophor RH 40) and F8 (0.5% w/w Carbopol, 2% cremophor RH 40) the release was significantly decreased as the concentration of cremophor was increased in the formulation. These release patterns were comparable to that of formulation F3 and F4.

The rank order of the various gel formulations based upon their maximum drug release is F6 > F2 > F5 > F7 > F3 > F8>F4. Based on the physicochemical properties and drug release, the formulation F6 was found to be suitable for topical application.

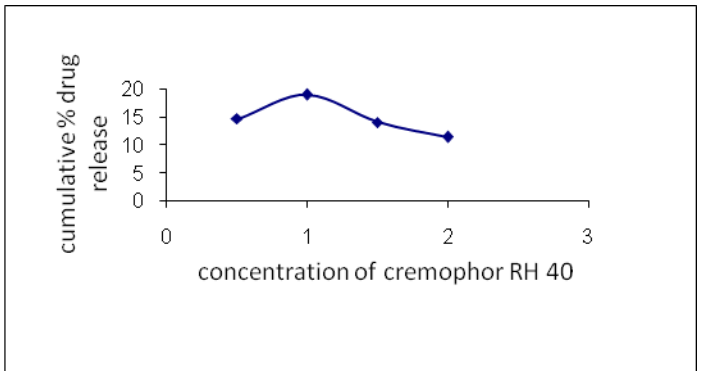

Figure 3 Effect of Cremophor RH 40 on Drug Release with 0.5% w/w Concentration of Carbopol

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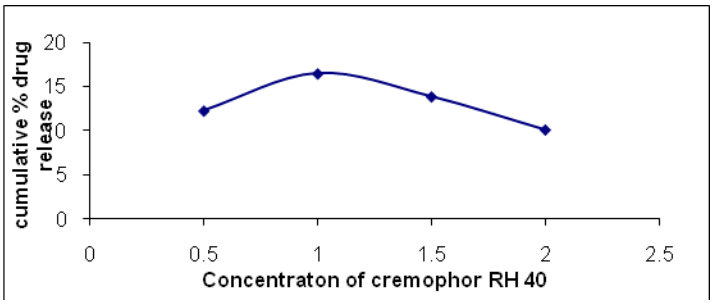

Figure 4 Effect of Cremophor RH 40 on Drug Release with 1% w/w Concentration of Carbopol

In order to further investigate the effect of cremophor RH 40 on the release for supporting the above release order, cumulative % drug release was plotted against concentration of cremophor RH 40 of various formulations comprising 0.5% w/w and 1% w/w carbopol as shown in fig. 3 and 4 respectively. It was observed that on increasing concentration of cremophor RH 40 from 0.5% w/w to 1% w/w there was marked improvement in drug release, which confirmed about its permeation enhancing properties. Surprisingly, on increasing concentration of cremophor RH 40 from 1% w/w to 1.5 % w/w and 2 % w/w there was no further improvement in drug release. Hence, it was evident that 1% w/w concentration of cremophor RH 40 was found to be critical concentration for permeation enhancement. The same finding was observed with cremophor RH 40 at of 1% w/w Carbopol concentration.

REFERENCE

[1] Tortora, G.; Grabowski, S. 8th ed.; Principles of Anatomy and Physiology. Harper Collins Publishers Inc: USA, 1996; pp. 124-132
[2] Lehman, P.A.; et al. The Journal of Investigative Dermatology [online]. 1988; 91:56-61.
[3] Megrab N A, Nahas H M, Balata G F. Saudi Pharmaceutical Journal [online]. 2006; 14(3-4):155-162.
[4] Boch R E, Lutwyche P, North R J, Monk W. 2005.Composotions comprising a Photosensitizer and skin penetration enhancer and their use in photodynamic treatment. US Patent No. 2005009048[1].
[5] Moghimi H, et al. Iranian J Pharm Res 2004;3:17-22.
[6] Prassana Raju Y, et al. Indian J Pharm Sci 2007; 69(2): 269-273.
[7] Lehman P A, et al. The Journal Inv Dermatol 1988; 91: 56-61.
[8] Moffat A C, Osselton D M, Widdop B. Pharmaceutical Press: Great Britain 2004; 1148-1149.
[9] Ellis C, Karch K. Journal American Acad Dermatol 2001; 45: S150-7.