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RESEARCH ARTICLE

FORMULATION DEVELOPMENT AND EVALUATION OF CALCIPOTRIOL AND PREDNICARBATE FOR TOPICAL TREATMENT OF PSORIASIS

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ABSTRACT

Calcipotriol and Prednicarbate are widely used effective treatments for psoriasis vulgaris. Combined therapy is known to be superior to monotherapy, but the drug substance degrades when mixed together. The purpose of the study was to develop a formulation which combines Calcipotriol and Prednicarbate in a single vehicle hereby with optimal drug delivery into the skin. As the two substances are incompatible in aqueous and alcoholic media, different non-aqueous formulations were prepared with suitable solvents and antioxidants. Formulations based on DPPG had no overall effect on skin permeability. However, A 5% polyoxypropylene-15 stearyl ether (PSE) formulation had a marked effect, resulted in a permeation rate comparable to the marketed combination product Diavobet (Calcipotriol and Betamethasone ointment). Thus, by using PSE as solvent, it was possible to combine Calcipotriol and Prednicarbate in a single formulation.

Keywords: Calcipotriol, Prednicarbate, polyoxypropylene-15 stearyl ether, psoriasis vulgaris.

1.0 INTRODUTION:

Psoriasis is one of the most common human skin diseases. It occurs when the immune system mistakes the skin cells as a pathogen and sends out faulty signals that speed up the growth cycle of the skin. It leads to rapid accumulation of skin cells on the skin surface forming a thick silvery surface, dry red patches which may pain. This is a persistent and long-lasting disease affecting 1-3 % of the world's population[1-3.] About 80% of patients with psoriasis are treated topically. [4, 5] Although steroids may work very well at first, psoriasis may become resistant to them over time. Twice a day application of formulation belonging to corticosteroid category and vitamin D analogues is the most commonly used treatment for psoriasis. Formulation of vitamin D should be applied first to the skin, followed by corticosteroid formulation after determined duration. The greatest limitation to the combined monotherapy is the inconvenience of administration[6.] Topical corticosteroids are now often used in combination with topical vitamin D analogs. Topical pharmaceutical composition comprising a combination of vitamin D analogue and topical corticosteroid would likely result in better patient compliance. However, the drugs belonging to corticosteroids and vitamin D3 analogues are stable at different pH values, combination of the same is challenging to manufacture [7.]Calcipotriol is sensitive to oxidizing agents and acidic residues; it reacts with alcohols, and easily undergoes epimerisation processes. Contrary to this, corticosteroids like prednicarbate are stable under acidic conditions, but sensitive to alkaline residues and oxidizing agents. Due to differences in physico-chemical properties and stabilities it has not previously been possible to successfully combine the two drug substances in a single formulation. To explore the effect of simultaneous treatment and to improve patient compliance alleviating the inconvenience of separate applications, a combination formulation consisting Betamethasone dipropionate and Calcipotriol in a single vehicle achieving optimal delivery of both substances into the skin was successfully developed by Leo Pharma[8]. Furthermore, the excellent stability of these two drugs has also been documented [9]. A two-compound ointment containing Calcipotriol and corticosteroid betamethasone dipropionate has been shown to be effective and safe in psoriasis in many of the clinical trials [13-15]. The aim of the present study was to combine the two drug substances, Calcipotriol and Prednicarbate, in a single formulation, and to achieve a skin delivery similar to the marketed product Daivobet ointment which contains calcipotriol and betamethasone, 50mcg and 0.5mg/g.

2.0 MATERIALS:

Calcipotriol Monohydrate was supplied by D.K Pharmachem Pvt. Ltd. and Prednicarbate was provided by Sun Pharma. Propylene Glycol (PG), Butylated Hydroxy toluene (BHT), White Soft Paraffin, Cetyl Alcohol, Light Liquid paraffin, Butylated Hydroxy Anisole (BHA), Vitamin E Acetate were gift samples from Elder Pharmaceuticals Ltd, Navi Mumbai, India. Polyoxypropylene – 15- Stearyl ether (ARLAMOL

PS15E LQ) was obtained from Croda Chemicals Pvt. Ltd, Mumbai. Propylene Glycol Dipelargonate (DPPG) was obtained from Gattefosse , Mumbai.

3.0 METHODS

3.1 PREPARATION OF FORMULATIONS

Test formulation ointments were prepared by dissolving Calcipotriol in different solvents including Polyoxypropylene – 15- Stearyl ether, Propylene Glycol and Propylene Glycol Dipelargonate and then adding melted ointment base with antioxidant. Both phases were 70^0C. Prednicarbate was suspended in liquid paraffin and added to the mixture. The ointments were continuously stirred to ensure that both the drug substances were homogenously distributed. The test formulations were based on the variation of concentrations of antioxidants and solvents; 5% Light Liquid Paraffin (oint. F1); Combination of BHA, BHT and disodium EDTA (oint F2), 10% Propylene glycol with 0.05%Vitamin E Acetate (oint. F3), 5% PSE and 0.050 % of Vitamin E Acetate (oint. F4); 10% PSE and 0.050 % of Vitamin E Acetate (oint. F5); and 5% DPPG and 0.050% of Vitamin E Acetate (oint. F6), 10 % DPPG and 0.050% of Vitamin E Acetate (oint. F7). The amount of white soft paraffin was varied accordingly. All percentages are weight to weight-ratios (w/w).

Table 1: Formulation details of Calcipotriol and Prednicarbate ointments

Ingredients	Quantity (%w/w)						
	F1	F2	F3	F4	F5	F6	F7
Calcipotriol *	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Prednicarbate	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Light Liquid Paraffin	5.000	5.000	5.000	3.000	3.000	3.000	3.000
Cetyl Alcohol	6.000	6.000	6.000	--	--	--	--
White Soft Paraffin**	79.585	79.585	78.695	91.675	85.675	91.675	86.675
Propylene Glycol	--	9.000	10.000	--	--	--	--
Butylated Hydroxytoluene	--	0.020	---	0.020	0.020	0.020	0.020
Butylated Hydroxyanisole	--	0.020	--	--	--	--	--
Disodium EDTA	--	0.120	--	--	--	--	--
Polyoxypropylene – 15-Stearyl ether (ARLAMOL PS15E LQ)	--	--	--	5.000	10.000	--	--
Propylene Glycol Dipelargonate (DPPG)	--	--	--	--	--	5.000	10.000
Vitamin E Acetate	--	--	0.050	0.050	0.050	0.050	0.050

** added as 52.18 mcg of Calcipotriol Monohydrate equivalent to 50 mcg of Calcipotriol. **Quantity to be adjusted to final 100% w/w based on the equivalency factor of Calcipotriol.*

3.2 PHYSICAL EVALUATION OF OINTMENTS

3.2.1 Appearance:

Appearance of ointment was evaluated by visually checking for clarity and texture.

3.2.2 Spreadability:

Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends on its spreading value. Hence determination of spreadability is very important in evaluating ointment characteristics. Assessment of the spreadability of the prepared formulations were determined individually by measuring the spreading diameter of 1g of ointment between two glass plates (20cm × 20cm) by having a standard weight of 125g on the upper plate[10].

3.2.3. pH:

The pH of the prepared formulations were determined by using digital pH meter

3.2.4. Centrifuge test:

It is a unique tool for the evaluation of accelerated deterioration of ointments. It was determined by using Remi centrifuge in 10 ml-graduated cylinder at 4000 rpm for 10 min[11].

3.2.5 Freeze-thaw cycle study:

This test method is intended to determine the effects of freezing and thawing on the physical parameters of the

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formulation. The ointments were packed in aluminum collapsible tubes and exposed to 10 freeze- thaw Cycles.

The tubes were first exposed to freeze cycle at 2 -8^0 C for a period of 18 hours. After the freeze cycle, tubes were kept at room temperature for 6 hours. Then immediately begin the thaw cycle at a temperature of 40- 45^0 C for a period of 18 hours. After the thaw cycle, tubes were kept at room temperature for 6 hours.

One freeze-thaw cycle is defined as a complete freeze cycle followed by exposure at room temperature and complete thaw cycle followed by exposure at room temperature. The physical observation of ointment was recorded after every cycle.

3.2.6 In-Vitro Skin Permeation Study:

Skin from the back of pig ear has proved to have properties similar to those of human skin and has been suggested a good model for human skin permeability[12].Hence, full-thickness skin was removed from the back of pig ears obtained from a local abattoir. The subcutaneous tissues were removed carefully and skin was excised cut into appropriate pieces. The skin samples were mounted in open two-chamber Franz diffusion cell with a recipient volume of 10 ml. The diffusion cells were placed in a thermostated water bath maintaining a temperature of 32^{0}C on the skin surface. A mixture of 0.04 M isotonic phosphate buffer pH 7.4 and 2-propanol (70:30) was used as recipient phase. After a 2 hour equilibrium period, the test formulation was applied to the stratum corneum side of the skin and spread with a tared glass spatula. At appropriate time intervals an exact amount of the recipient phase was withdrawn and replaced by thermostated fluid. The permeation was followed for 24 hours and it was ensured that sink conditions were present. The samples were analyzed by HPLC method.

3.2.7 HPLC Analysis of the Drug Substances:

A reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed for the analysis of calcipotriol and prednicarbate. The concentrations were quantified by simultaneous estimation method using a Waters Alliance 2996 HPLC system with Programmable Auto sampler. The substances were separated on a Kromosil C18, (250 x 4.6mm) 5µ Isocratic illusion method using a detector wave length of 264 nm, injection volume of 50 µl and a flow rate of 1.0 ml/min. The mobile phase consisted of Water and Methanol (150:850 v/v). The retention time was around 5 minutes for prednicarbate and 7.5 minutes for calcipotriol.

3.2.8 *In-vivo* screening of Antipsoriatic potential using oxazolone induced contact dermatitis model: [17]

In-vivo screening of Antipsoriatic potential was carried out using female BALB/c mice (20-25g). The effectiveness of the formulation was studied using oxazolone-induced contact dermatitis animal model. Female BALB/c mice were sensitized by the application of Oxazolone to the abdomen then ears were rechallenged with 20µl of 1% oxazolone in a mixture of acetone and olive oil (4:1) every 3rd day for two weeks to induce severe dermatitis. Treatment was conducted with topical application of formulation F4 once a day to both sides of the ears. Results were compared with the reference. Group without any disease induction served as placebo control while the group with disease induction but no treatment served as positive control for the study. Antipsoriatic activity was evaluated in terms of suppression in the ear thickness which was measured using vernier calipers.

3.2.9 Microbial Evaluation: [18, 19]

3.2.9.1 Total aerobic bacterial count:

The test was performed by pour plate method on the optimized promising formulation F4.10g ointment was added to previously sterilized 90ml of buffered sodium chloride-peptone solution pH 7.0. Pipette 1.0ml of above dilution into two sets (duplicate). Add 15 – 20 ml of sterile Casein soya bean digest agar which has been previously melted and cooled at 45^{0}C to two petri dishes in one set for bacterial count.

3.2.9.2 Total Yeast and Moulds count:

Add 15-20 ml of sterile Sabouraud's Chloramphenicol agar which has been previously melted and cooled at 45^{0}C to two petri dishes in another set for fungal (yeast and mould) count. Incubate the plates for bacterial count at 30 – 35^{0}C for fungal count at 20-25^{0}C for 5 days with negative control. After incubation, observe the plates and count the number of colonies formed.

3.2.9.3 Test for pathogens:

Test was performed to confirm the absence of pathogens i.e. Escherichia coli, staphylococcus aureus, Pseudomonas aeruginosa and Salmonella spp. in the ointment using the relevant medias.

Escherichia Coli: Take 10 g of ointment and add 90ml of buffered Sodium Chloride –peptone solution pH7.0 and use 10ml to inoculate 100ml of Casein Soyabean Digest Broth and incubate at 35- 37^{0}C for 18-48 hours. Shake the container, transfer 1 ml to 100 ml of MacConkey broth and incubate at 43-45^{0}C for 18-24 h. Subculture on plates of Mac Conkey agar at 35- 37^{0}C for 18-72 hours.

Staphylococcus aureus: Add 10 g of ointment to 90 ml of buffered sodium chloride peptone solution pH7.0 and use 10ml or the quantity corresponding to 1 g to inoculate 100ml of Casein Soya bean digest broth and incubate at 35- 37^{0}C for 18-48 hrs. Subculture on a plate of Baird –Parker agar and incubate at 35- 37^{0}C for 18-72 hrs

Pseudomanas aeruginosa: Add 10 g of ointment to 90 ml of buffered sodium chloride peptone solution pH7.0 and use 10ml or the quantity corresponding to 1 g to inoculate 100ml of Casein Soya bean digest broth and incubate at 35- 37^{0}C for 18-48 hrs. Subculture on a plate of Cetrimide Agar and incubate at 35- 37^{0}C for 18-72 hrs

Salmonella Spp.: Add 10 g of ointment to 90ml of Casein Soya bean digest broth and incubate 35- 37^{0}C for 18-24 hrs. Transfer 1 ml of the enrichment culture to 10ml of Tetrathionate bile brilliant green broth and incubate at 41- 43^{0}C for 18-24 hrs. Subculture on

Deoxycholate Citrate Agar and Xylose lycin Deoxycholate agar.- Incubate at 35 -37 for 18 -72 hrs.The probable presence of salmonella is indicated by the growth of culture having the following appearance.

Deoxycholate Citrate Agar: Well developed, colourless colonies.

Xylose lycin Deoxycholate agar: Well developed red coloured colonies with or without black centres

4.0 RESULT AND DISCUSSION:

4.1 Physical evaluation of ointments:

Discussion:

Appearance of all formulated ointments was evaluated by visually checking for clarity and texture. White, translucent, homogenous, odourless ointments were obtained.

Formulations complied with the physical evaluation parameters like pH, physical stability, centrifugation, spreadability were found to be acceptable as notified in table 2.

Table 2: Physical parameters observation

Formulation	F1	F2	F3	F4	F5	F6	F7
Spreadability (mm)	62	65	60	65	63	64	60
pH	5.21	5.37	5.0	5.43	5.51	5.62	5.66
Centrifuge test	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation

4.2 Freeze-thaw cycle stability study:

All the formulations (F1 to F7) were exposed to freeze thaw stability and the physical observations after 10^{th} cycle are recorded in Table 3.

Table 3: Physical parameters observation after 10 freeze-thaw cycles

Formulation	F1	F2	F3	F4	F5	F6	F7
pH	5.77	6.22	6.18	5.98	6.03	6.08	6.14
Centrifuge Test	Ointment turned yellowish	No Phase Separation	Ointment turned slightly yellowish	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation

4.3 In-Vitro Skin Permeation Studies:

The chemical analysis of formulations F1 and F3 was not performed as these batches showed significant changes in physical parameters during freeze-thaw Stability

Discussion

The in vitro skin permeation profiles of prednicarbate and calcipotriol in the different test formulations are shown in Fig. 1A and 1B. It is seen that the rate of drug release for both drug substances was found to be related to the solvent systems used in the formulations.

Propylene Glycol (oint. F2) was found to have no enhancing effect itself on either of the drug substances compared with the reference product (Daivobet). Polyoxypropylene – 15- Stearyl Ether (PSE) used in the concentration of 5 % (oint. F4) and 10 % (oint F5) had a marked effect on the cumulated permeated amount of both drug substances whereas formulations containing Propylene Glycol Dipelargonate (DPPG) used in the concentration of 5 % (oint. F6) and 10 % (oint F7) had no significant effect on the rate and extent of permeation.

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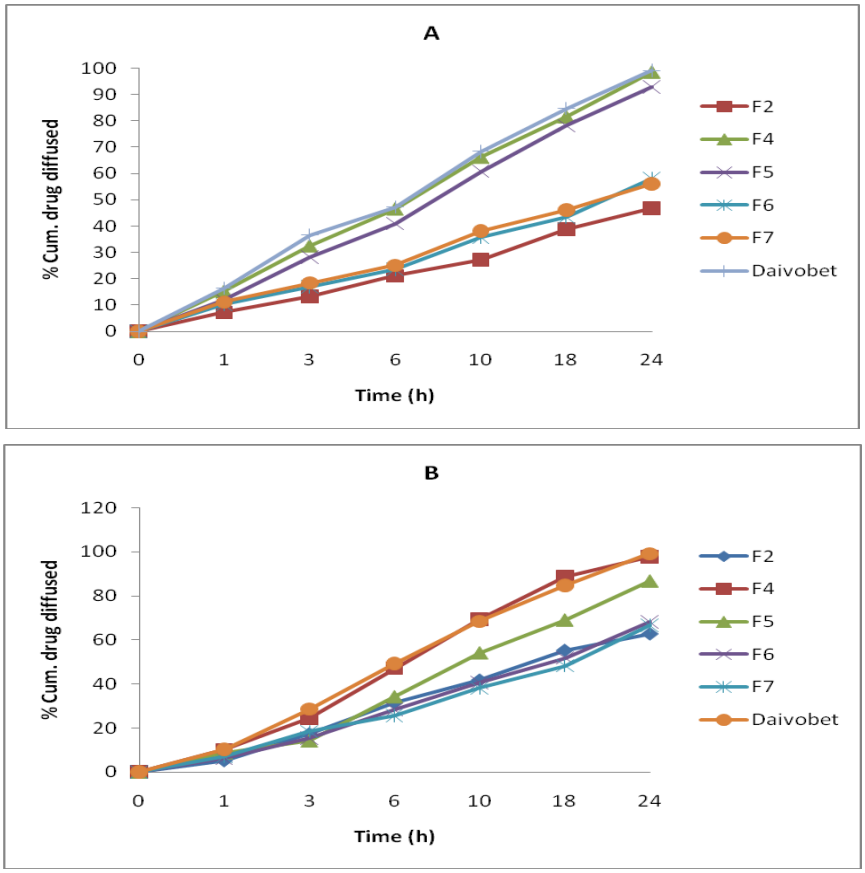

Figure 1: In vitro skin permeation profiles of Prednicarbate (A), and Calcipotriol (B) from the test formulations based on different vehicle components

4.4 HPLC Analysis of the Drug Substances:

Discussion:

The drug content of calcipotriol and Prednicarbate in the ointment formulations F-4 to F-7 were well within the limits whereas the Calcipotriol content of Formulation F2 was found on lower side. This could be due to the oxidation of Calcipotriol as there was no antioxidant used in the formulation F2.

Table 4: Percentage drug contents of Calcipotriol and Prednicarbate in the ointment formulations

Formulation	drug content (%w/w)				
	F2	F4	F5	F6	F7
Calcipotriol	89.54	98.6	99.67	98.41	99.05
Prednicarbate	101.3	99.64	100.10	100.07	98.93

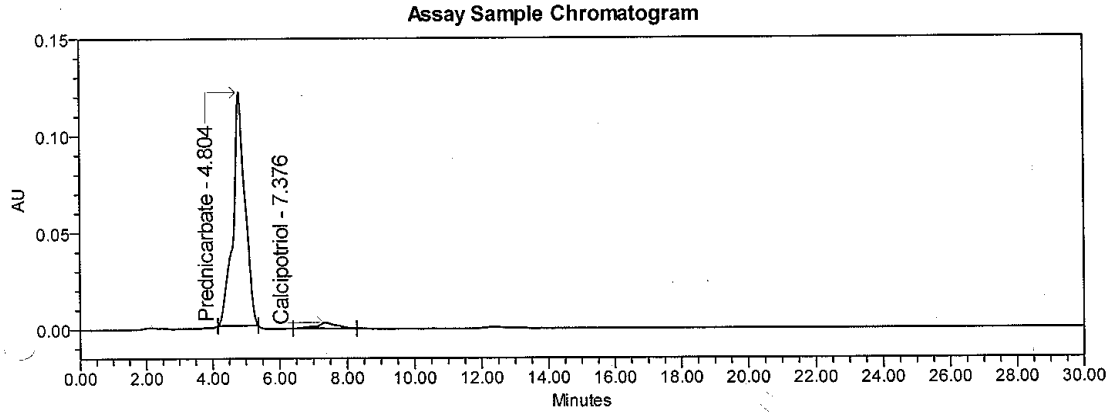

Figure 2: HPLC assay chromatogram showing the peaks of Calcipotriol and Prednicarbate.

4.5 *In-vivo* screening of Antipsoriatic potential using oxazolone induced contact dermatitis model:

Discussion:

Ear thickness is an index of skin inflammation and was measured using a vernier caliper. In vivo efficacy is dependent on the rate at which the drug penetrates the skin and becomes available at the site of action. To verify this, therapeutic efficacy of optimized formulation was evaluated. Reduction in inflammation for group treated with optimized Formulation F4 as well as reference (Diavobet) was significantly higher as compared with the placebo and no-treatment group as shown in figure 3.

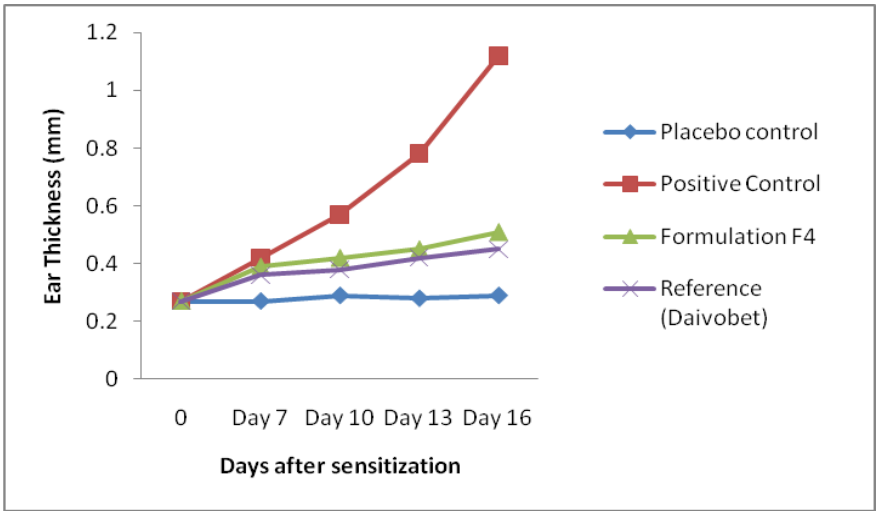

Figure 3: Effect of Calcipotriol and Prednicarbate ointment formulation F4 on the thickness of mouse ear induced by repeated application of Oxazolone.

4.5 Microbial Evaluation:

4.5.1 Total aerobic bacterial count:

Discussion:

The total aerobic microbial count (TAMC) is considered to be equal to the number of colony-forming units (cfu) found using casein- soya bean digest agar. The total yeast and mould count (TYMC) is considered to be equal to the number of cfu found using Sabouraud Chloramphenicol agar. The total viable aerobic count is the sum of the bacterial count and yeast and mould count.

Less than 10 cfu/g bacterial count was observed in optimized formulation F4 after 5 days of incubation.

4.5.2 Test for pathogens:

Discussion:

Growth rate of red, non-mucoid colonies of gram-negative rods indicate the possible presence of E-coli. The product passes if such colonies are not seen or if the confirmatory biochemical tests are negative.

Black colonies of gram – positive cocci surrounded by a clear zone indicate the presence of S. aureus. Confirmation may be effected by suitable biochemical tests such as the coagulase test and the deoxyribonuclease test. The product passes the test if colonies of the type described do not appear on Baird-Parker agar or if the confirmatory biochemical tests are negative.

If no growth of micro organism is detected, the product passes the test. If growth of gram negative rod occurs then they Casein Soya bean digest broth and incubate at 41- 43^{0}C for 18-24 hrs. The product passes the test if no growth occurs at 41- 43^{0}C

The presence of salmonellae is previously confirmed if the deep inoculation but not the surface culture there is a change of colour from red to yellow and usually formation of gas with or without production of hydrogen sulfide in the agar. The product passes the test if colonies of the type do not appear.

No pathogens were detected in the optimized formulation F4.

5.0 CONCLUSION:

After taking trials with different anti-oxidants and solvents at different concentration levels, it was observed that the product with 5 % Polyoxypropylene – 15-Stearyl ether (PSE) and 0.050% of Vitamin E acetate resulted in antipsoriatic potential comparable to the marketed product.

PSE is used as an emollient in cosmetic products and also as a drug solvent in pharmaceuticals. It was found to be a very effective solvent for both drug permeability. PSE is lipophilic in nature hence, when a high concentration is used in the vehicle; the skin vehicle partition coefficient is expected to decrease leading to decrease in the drug permeability. The results demonstrated that the type and the concentration of the solvent had a significant influence on the skin permeability. Formulations based on DPPG had no overall effect on skin permeability whereas the solvent PSE had a marked influence. The study proved it is possible to develop a formulation which combined

calcipotriol and prednicarbate in a single vehicle achieving optimal delivery of both substances into the skin.

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