Research Article

IMPROVED TOPICAL PREPARATION OF CALCIPOTRIOL IN THE TREATMENT OF PSORIASIS

Meenu Kumari *, Monika Sachdeva, Vijay Agarwal

Raj Kumar Goel Institute of Technology (Pharmacy) 5th Km Stone Delhi Meerut Road, Ghaziabad, U.P., India

*Corresponding Author Email: meenu.prajapati6590@gmail.com

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ABSTRACT

This research project is aimed to prepare improved topical preparation of Calcipotriol in the treatment of psoriasis by incorporation of an anti-inflammatory agent, keratolytic agent, humectants, surfactant, thickener & to perform its evaluation. In these different preparations of lotions sodium carboxymethylcellulose is used as a viscosity-enhancing agent in varying concentrations. This non-steroidal formulation is prepared by adding tea tree oil to avoid side effects associated with steroidal preparation and to avoid the issue of atrophy with long-term use of steroids. Evaluation of all the formulations such as spreadability, viscosity, drug content determination, pH, in-vitro release study, to choose the optimized formulation was carried out. According to ICH guidelines, stability studies were carried out to check the stability (parameters such as appearance, pH, viscosity, drug content) of prepared formulations for 3 months at room temperature. In-vitro drug release study CT5 formulation was carried by dialysis diffusion method. The cumulative% drug release of CT5 formulation in 210minutes was 85.10%. Based on the evaluation, formulation (CT5) was found to be better among all the formulations. The prepared calcipotriol formulation and a commercial formulation of calcipotriol were evaluated for anti-psoriatic activity. In-vivo anti-psoriatic was performed by using the Imiquimod Induced Psoriasis skin model. The results obtained in this study have been concluded that the prepared formulation (CT5) has great potential for topical delivery in the treatment of psoriasis.

Keywords: Calcipotriol, Lotion, Psoriasis, Topical formulation

INTRODUCTION

The word psoriasis is derived from “psora” which is a Greek word that means being “itchy”. The exact origin of psoriasis is not known. This disease is a chronic, autoimmune, anti-inflammatory disorder of the skin which affects 1-3% of the world’s population.¹ Various types of treatments are available for psoriasis, but topical therapy is most commonly used in major patients.² It is characterized by having sharply demarcated scaly, red, coin-sized skin lesions generally on the elbows, knees, scalp, hand & feet. It generally causes excessive growth & differentiation of keratinocytes. Symptoms include itching, irritation, stinging & pain. If the disease affects less than 3% of the body, it is considered mild, & if affecting 3- 10% of the body is considered severe. For diagnosing psoriasis, signs are Koebner Phenomenon & Auspitz sign.³ Topical therapy is used to treat mild to moderate psoriasis while for irresponsive patients, phototherapy & systemic agents are used. Pathogenesis of psoriasis associated with disease including T helper 1 (Th1) & T helper 17 (Th17) cells. Psoriasis is a disease whose pathogenesis mechanism investigated from past decades, plays an important role in immunity. At any age, psoriasis can occur, peaks chances are in the second and third decades of life. Diagnosis is based on the identification of erythematous, scaly plaques for the scalp, the limbs & the trunk.⁴

MATERIALS AND METHODS

Calcipotriol was purchased from Royal enterprises (pharma division) plot no-555, part- III, HSIDC, Barhi, Sonapat-131101.

Tea tree oil was purchased from Rouh Essentials Pvt. Ltd, Delhi, Allantoin has been obtained from Zenivista Meditech Pvt. Ltd. Imiquimod cream procured from Glenmark Pharmaceuticals Ltd. All other chemicals are from Central Drug House Pvt Ltd.

Characterization of calcipotriol

Organoleptic properties

Organoleptic properties of calcipotriol such as color, odor and, appearance were analyzed

Solubility profile

Solubility of calcipotriol determined in different solvents. Solubility of calcipotriol was determined by placing 30ml of each solvent with an excess amount of drug in separate conical flasks. Then, sealed the conical flasks with a rubber cap, fix them in a mechanical shaker. Allow solutions to shake for 24hr (when reaching equilibrium), set the temp. at 25°C. After 24hr, withdraw the sample from conical flasks filter it, diluted & check the absorbance in UV-1700, Shimadzu, Japan.

Analytical methodology

UV spectroscopy

The ultraviolet absorption spectrum of a solution of calcipotriol in ethanol and phosphate buffer (pH 7.4) was obtained using a UV/VIS spectrophotometer over a wavelength range of 200-400nm. λ_{max} values were determined and plot the calibration curve.

FORMULATION OF LOTION

Calciptriol lotion was prepared by adding tea tree oil & viscosity enhancing agents in variable concentration.

Five different formulations are prepared by varying the concentration of thickener.

Preparation of oil phase

- Weigh accurate quantity of tea tree oil & diluted it with argan oil(1 drop of tea tree oil in 9 drops of argan oil).
- Weigh the required amount of salicylic acid, stearic acid, Cetostearyl alcohol and mix these ingredients in mortar & pestle.
- Triturate above mentioned ingredients in mortar & pestle, to reduce the particle size of ingredients, to prevent the formation of lumps, thus increase the stability of the formulation.
- Add triturated ingredients and diluted tea tree oil in a specific quantity in a 100ml beaker.

Preparation of aqueous phase

- Weigh the required amount of powder ingredients allantoin, aloe powder in a beaker.
- In the above mixture add glycerine as a humectant.
- Then, weigh the viscosity-enhancing agent in another beaker and mix it with water.

In last, put both the phases on for heating until all the ingredients melt, then pour the aqueous phase into the oil phase with rapid stirring.

Then homogenize the mixture in a homogenizer until the mixture converts into the required consistency.

Makeup the final volume of lotion with water.

The Final formulated lotion was neutralized by adding triethanolamine under the stirring to reach the pH suitable for skin(measured using pH meter).

EVALUATION OF LOTION

Spreadability

The spreadability of the lotion formulation was measured by wooden block glass slide apparatus by placing 1gm lotion in between two slides. Place 100gm of weight on the upper slide for 5min to form a slim layer of lotion. After 5min, put specific weight in the pan.⁵

Note the time, required to separate both the slides from each other.

Now, calculate the spreadability by the given formula.

$$S=ML/T$$

Where, S= spreadability, M= weight tide to an upper glass slide, L= length moved glass slide, T= time taken

Viscosity

The Viscosity of the lotion formulation was determined by DV-E Brookfield viscometer by using spindle no.6 at 25rpm and st the temperature at [(25±1) °C]. Note reading when viscometer shows constant reading.^{6,7}

Drug content determination

All lotion preparations were analyzed to determine drug content. Drug content of formulation has been determined by weighing 1gm of formulation in a volumetric flask, then diluted it with 100% methanol up to 50ml. Filter the final solution. Analyze the final solution in UV-vis spectrophotometer at 264nm. Make further dilutions if necessary.⁸

pH

The ph of the 1% lotion formulation was determined by a digital pH meter.⁹

Stability test

For stability study, kept lotion in variable temperature and humidity conditions according to ICH guidelines for a period of 3month, and after 30th day, 60th day, 90th day, evaluate lotion to determine the changes in ph, viscosity, physical appearance, spreadability.¹⁰

In-vitro permeation study

To perform an in-vitro permeation study of the final formulation, the dialysis diffusion method is used.

In this method, a dialysis membrane bag is used, which is cut acc. to the required size to retain formulation then the membrane bag is activated by heating in double-distilled water for 1hr & then in ethanol for the next 1hr. Finally, it was put into PBS(7.4) & allow to soak for 24hr.

Now take, 5gm of formulation and put it into dialysis bag, both ends of the dialysis bag ties with a thread.

In a 100ml beaker, pour phosphate buffer(7.4) & place the prepared dialysis bah in it.

Place the beaker on a magnetic stirrer, set the temperature at 37°C and speed at 50rpm.

At the interval of 5,15,30,45,60,90,120,150,180 & 210min, withdraw 5ml sample and replace it with fresh PBS to maintain sink condition.

Dilute the withdrawn sample if necessary and analyzed by U.V spectrophotometer.¹¹

Anti-psoriatic activity

Imiquimod induced mouse model

This study was performed according to the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India) approved by the Institutional animal ethics committee, registration no. 992/PO/RE/S/2006/CPCSEA.

Selection of animals

Healthy Swiss albino mice (40-60gm) get from NIB(national institute of biologicals), Noida used for my research project.

Proper care should be taken of all the animals and were exposed to 12hr light / 12hr dark cycle, according to CPCSEA guidelines, approved by the Institutional animal ethics committee.¹²

Grouping of animals

Group 1{6 animals}: normal (negative control) group. Apply Vaseline on the dorsal skin surface of mice.

Group 2{6 animals}: positive control group. Apply 62.5mg of 5%IMQ cream on shaved back of mice.

Group 3{6 animals}: standard group. Apply IMQ cream than calcipotriol solution(0.005% w/v)

Group 4{6animals}: standard group. Apply IMQ cream then clobetasol propionate & calcipotriol ointment on shaved back of mice.

Group 5{6animals}: sample group. Apply IMQ cream then prepared calcipotriol lotion on shaved back of mice.

Procedure

1. Select animals to perform in vivo study.
2. Then, remove hair from the dorsal surface of mice (3cm×4cm) by applying hair removal cream.
3. After that, leave the animals for half an hour, then apply Vaseline to group 1.
4. Apply 5% IMQ cream to group 2 to group5 with the help of cotton bud on the shaved area of mice.

5. Put each mice in separate cages.
6. Repeat the same procedure for 7-8 days to induce psoriasis.
7. After 7 days, in group 2 measure thickness, once psoriasis is induced sacrifice animal by the spinal dislocation method for histopathological study.
8. After 7 days, apply calcipotriol solution(0.005% w/v) and clobetasol propionate & calcipotriol ointment in group 3 & 4 for 21 days as standard.
9. After 7 days, apply prepared calcipotriol lotion(0.005%) in group 5 for 21 days.
10. After 21 days, mice of group3, 4 & 5 were sacrificed by the spinal dislocation method for further studies.¹³

Scoring severity of skin inflammation

The Antipsoriatic activity of the calcipotriol lotion was evaluated based on PASI (Zero, Slight, Moderate, Very marked) score along with the histopathological examination of mice skin.¹⁴

Table 1: PASI(Psoriasis Area & Severity Index) score

VALUE	0	1	2	3	4
RESULT	None	Slight	Moderate	Marked	Very marked

RESULTS AND DISCUSSION

Characterization of calcipotriol

Physical appearance

The Organoleptic properties of the drug are shown in Table 2. Organoleptic properties obtained practically are similar to the properties in the literature.

Solubility profile

The Solubility of the drug in different solvents is shown in Table 3.

Analytical methodology

The $\lambda_{\max}$ of calcipotriol in ethanol and phosphate buffer pH 7.4 was found to be 266nm & 232nm respectively.

Formulation of calcipotriol lotion formulations

The formulation details of prepared calcipotriol lotions are shown in Table 4.

Physical Evaluation Of Prepared Calcipotriol Lotions

The physical evaluation of prepared lotions such as color, texture, is shown in Table 5.

Spreadability

The spreadability of all lotion formulations was ranging from 7-10cm, shown in Table 6.

Viscosity

The rheological properties such as viscosity can directly affect the drug release from semi-solid formulations. The Viscosity of lotions was determined to ensure the lotion behavior and feel on the skin. The Viscosity of prepared formulations ranged from 2000-6000cps, shown in Table 7.

Drug content

Drug content was determined to determine the calcipotriol drug content in all lotion formulations, the percentage drug content of all lotion formulations were ranged from 95-98%, shown in Table 8.

pH

The pH of all lotion formulations was ranged from 6-8 which is acceptable to apply on skin, avoids skin irritation, shown in Table 9.

Stability study

To determine the expiry date of any formulation, performing a stability study at different environmental conditions is very important, after a specific time check changes in physical appearance, pH, spreadability, viscosity, drug content to determine the stability of the formulation. Results of all lotion formulations are given in Table 10.

In-vitro release study

In-vitro release study of final successful formulation(CT5) performs by Dialysis Diffusion Method are shown in Table 10, this show release of lotion formulation up to 8hr. % cumulative drug release is shown in Table 11. Graph of the in-vitro release of CT5 formulation shown in Fig 1.

Anti-psoriatic activity

Psoriasis is induced by imiquimod in 7 days, PASI score was given to each mice before and after treatment with a test, psoriatic control and, standards(marketed formulations).

After histopathological examination of the skin of IMQ induced mice results in

- Increased epidermal thickness
- Hyperproliferation of keratinocytes granulocyte infiltration
- Presence of microabscesses
- Capillary loop dilation
- Parakeratosis

Evaluation of PSI(Psoriasis Severity Index) score of all groups shown in Table 12,13,14 & 15.

Histopathological examination of all groups shown in Figure 2

Table 2: Organoleptic properties of calcipotriol

ORGANOLEPTIC PROPERTIES	RESULT
Color	White
Odour	Odourless
Nature	Crystalline powder

Table 3: Solubility of calcipotriol in different solvents

SOLVENTS	SOLUBILITIES
Water	Practically insoluble
Liquid paraffin	Insoluble
Ethanol	Freely soluble(96%)
Chloroform	Soluble
Propylene glycol	Soluble
DMSO	Soluble
Dimethylformamide	Soluble

Table 4: Formulation details of calcipotriol lotions

	S.No.	Ingredients	Qty(in gms.)				
			CT1	CT2	CT3	CT4	CT5
Drug	01.	Calcipotriol	0.005	0.005	0.005	0.005	0.005
Lipophilic phase	02.	Dil. Tea tree oil	1.2	1.2	1.2	1.2	1.2
	03.	Salicylic acid	5	5	5	5	5
	04.	Stearic acid	0.5	0.5	0.5	0.5	0.5
	05.	Cetostearyl alcohol	5	5	5	5	5
	06.	Triethanolamine					
Hydrophilic phase	07.	Sodium CMC	1	1.5	2	2.5	3
	08.	Allantoin	2	2	2	2	2
	09.	Aloe powder	1	1	1	1	1
	10.	Glycerine	4	4	4	4	4
	11.	Methyl paraben	0.02	0.02	0.02	0.02	0.02
	12.	Propyl paraben	0.02	0.02	0.02	0.02	0.02
	13.	Double distilled water	q.s	q.s	q.s	q.s	q.s

Table 5: Spreadability of prepared calcipotriol lotions

FORMULATION CODE	SPREADIBILITY(cm)
CT1	9.2
CT2	8.9
CT3	8.3
CT4	7.8
CT5	7.5

Table 6: Viscosity of prepared calcipotriol lotions

FORMULATION CODE	VISCOSITY(CPS)
CT1	2600
CT2	2960
CT3	3640
CT4	4854
CT5	5961

Table 7: Drug content of prepared calcipotriol lotions

FORMULATION CODE	DRUG CONTENT(n=3)
CT1	95.30±0.148
CT2	98.06±0.243
CT3	96.19±0.112
CT4	98.59±0.371
CT5	97.24±0.216

Table 8: pH of prepared calcipotriol lotions

FORMULATION CODE	pH
CT1	6.3
CT2	7.2
CT3	6.5
CT4	7.6
CT5	6.8

Table 9: Stability study of prepared calcipotriol lotion

S.no.	Formulations	Months	Viscosity (cps)	pH	Spreadability (cm)
1.	CT1	0	2600	6.3	9.2
		1	2608	6.2	9.3
		2	2712	6.4	9.4
		3	2932	6.3	9.6
2.	CT2	0	2960	7.2	8.9
		1	2987	7.3	8.8
		2	3013	7.5	8.6
		3	3128	7.4	8.8
3.	CT3	0	3640	6.5	8.3
		1	3687	6.6	8.4
		2	3791	6.5	8.6
		3	3965	6.8	8.7
4.	CT4	0	4854	7.6	7.8
		1	4872	7.6	7.7
		2	4898	7.5	7.5
		3	4916	7.7	7.3
5.	CT5	0	5961	6.8	7.5
		1	5969	6.9	7.5
		2	5984	6.8	7.4
		3	5992	6.7	7.3

Table 10: In-vitro release studies by Dialysis diffusion method

Diffusion media	Phosphate buffer 7.4
Membrane	Dialysis
Volume of receptor compartment	100ml
Volume pipette out	5ml
Volume replaced	5ml
Set rpm	50 rpm
Temperature	37°C
$\lambda_{\max}$	264nm

Table 11: In-vitro release study of CT5 formulation

Time	Absorbance	Conc.(μ g)	Conc.(mg)	Dil.factor(1/10)	Conc. $\times$ 100ml	%CDR
5	0.190	4.212	0.00421	0.000421	0.0421	17.32
15	0.268	5.942	0.00591	0.000591	0.0591	24.30
30	0.301	6.674	0.00667	0.000667	0.0667	32.70
45	0.316	7.006	0.00700	0.000700	0.0700	48
60	0.333	7.383	0.00738	0.000738	0.0738	59
90	0.456	10.110	0.00101	0.000101	0.0101	63.33
120	0.548	12.150	0.00121	0.000121	0.0121	68.31
150	0.654	14.501	0.00145	0.000145	0.0145	74.91
180	0.582	12.904	0.00129	0.000129	0.0129	79.50
210	0.608	13.481	0.00134	0.000134	0.0134	85.10

Table 12: Evaluation of redness, erythema, scales in group 2(IMQ induced mice)

Parameters	Days						
	1	2	3	4	5	6	7
Redness	0.6	1.13	1.82	2.4	2.98	3.12	3.56
Erythema	0	0.65	1.2	1.86	2.48	2.76	3.29
Scales	0	0.28	0.54	1.22	1.36	1.51	2.13
Cumulative score	0.6	1.80	3.56	5.48	6.82	7.39	8.98

n = 6

Table 13: Evaluation of PSI (score 0-12) (group 3)

Days	PSI(0-12)
7	8.67
14	6.88
21	4.25

n = 6

Table 14: Evaluation of PSI (score 0-12) (Group 4)

Days	PSI(0-12)
7	6.45
14	4.62
21	1.89

n = 6

Table 15: Evaluation of PSI score (0-12)(Group 5)

Days	PSI score(0-12)
7	4.25
14	2.68
21	0.18

n = 6

Fig 1: In-vitro release of Calcipotriol lotion(CT5)

Fig 2: Histopathological examination a) GROUP - 1 b) GROUP - 2 c) GROUP - 3 d) GROUP - 4 e) GROUP - 5

Fig 3: Photographic images of animal study a)Psoriasis induced b)Test formulation

DISCUSSION

Five different formulations of calcipotriol lotion (CT1,CT2,CT3,CT4,CT5) were formulated by using different concentration of Sodium CMC (thickener). Five lotion formulations were developed with the o/w emulsion method by incorporation the oil phase(tea tree oil, salicylic acid, stearic acid, Cetostearyl alcohol, triethanolamine into the aqueous phase allantoin, aloe powder, glycerine with different concentration of sodium CMC. Prepared calcipotriol lotion was light pink in color, smooth. The pH of all the formulations varies from 6-8 which is suitable for skin. This study includes evaluation of all the five formulations such as organoleptic properties of calcipotriol, viscosity, spreadability, pH, drug content, in-vitro release studies,

in-vivo animal studies. All formulations show viscosity less than 10,000 cps which is required. The lotion was prepared using 3% sodium CMC which shows the best viscosity. The Spreadability of all formulations ranges from 7-10cm. In vitro release study of CT5 formulation has been performed by dialysis diffusion method which shows 85.1% of drug release in 240min. Imiquimod-induced psoriasis model was used to induce psoriasis in swiss albino mice. Psoriasis is induced in all mice by the application of IMQ drug for seven consecutive days which showed the development of erythema, scaling and, thickening of the skin. After examination of the skin of IMQ induced mice results in Increased epidermal thickness, Hyperproliferation of keratinocytes granulocyte infiltration, Presence of microabscesses, Capillary loop dilation, Parakeratosis. The in-

vitro antipsoriatic activity of CT5 formulation is better as compared to control, so this formulation is effective in treating psoriasis.

CONCLUSION

Five lotion formulations were developed with the o/w emulsion method by incorporation the oil phase(Tea tree oil, salicylic acid, Stearic acid, Cetostearyl Alcohol, Triethanolamine into the aqueous phase(Allantoin, Aloe powder, Glycerine with different concentration of sodium CMC. From all the formulations, it was concluded that calcipotriol lotion with 3% Sodium CMC was considered as the best formulation amongst all formulations. It showed good anti-psoriatic activity, as well as good stability.

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REFERENCES

1. Su Yu-Han , Fang Jia-You . Drug delivery and formulations for the topical treatment of psoriasis. Expert Opinion on Drug Delivery 2008;5(2):235-249.
2. Katare OP, Raza K, Singh B, Dogra S . Novel drug delivery systems in topical treatment of psoriasis : Rigors and vigors. Indian Journal of Dermatology, Venereology, and Leprology 2010;76(6):612-21.
3. Hwang, S. T., Nijsten, T., & Elder, J. T. Recent Highlights in Psoriasis Research. Journal of Investigative Dermatology 2017;137(3), 550–556.
4. Segaerts S, Rat Duvold L. Calcipotriol cream: A review of its use in the management of psoriasis. Journal of Dermatological Treatment 2006; 17: 327–337.
5. Chen, M. X., Alexander, K. S., & Baki, G. Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. Hindawi Publishing Corporation Journal of Pharmaceutics 2016;1-10.
6. Lieberman A. Herbert et al., Pharmaceutical Dosage Forms Rheology of Dispersed Systems. Deem Donald E.Mercel Dekker, Inc.U.S.A., 1998; 367-422.
7. Subrahmanyam CVS. Text Book of Physical Pharmaceutics Rheology. 2nd edition Vallabh Prakashan, Pitampura, Delhi 2000; 235-74.
8. Chang, R.-K., Raw, A., Lionberger, R., & Yu, L. Generic Development of Topical Dermatologic Products: Formulation Development, Process Development, and Testing of Topical Dermatologic Products. The AAPS (American association of pharmaceutical science) Journal 2012; 15(1), 41–52.
9. Namita and Nimisha. Development and evaluation of herbal cosmeceutical for skin care. International Journal of Pharmacological and Biological Sciences 2013; 4(2):86-92.
10. Gyawali R, Paudel N, Shrestha S. Formulation and Evaluation of Antibacterial and Antioxidant Polyherbal Lotion. Journal of Institute of Science and Technology 2016; 21 (1), 148-156 .
11. Zhou, Y., He, C., Chen, K., Ni, J., Cai, Y., Guo, X., & Wu, X. Y. A New Method for Evaluating Actual Drug Release Kinetics of Nanoparticles inside Dialysis Devices via Numerical Deconvolution. Journal of Controlled Release 2016; 243, 11–20.
12. Na Takuathung, M., Wongnoppavich, A., Panthong, A., Khonsung, P., Chiranthanut, N., Soonthornchareonnon, N., & Sireeratawong, S. Antipsoriatic Effects of Wannachawee Recipe on Imiquimod-Induced Psoriasis-Like Dermatitis in BALB/c Mice. Evidence-Based Complementary and Alternative Medicine 2018;1–13.
13. Rather I, K. Bajpai V, Han J and Nam G. Imiquimod-induced psoriasis-like skin inflammation in mouse model. Bangladesh J Pharmacol 2016;11: 849-851.
14. Das K, Deb S, Karanth T. Phytochemical Screening and Metallic Ion Content and Its Impact on the Antipsoriasis Activity of Aqueous Leaf Extracts of *Calendula officinalis* and *Phlebodium decumanum* in an Animal Experiment Model. Turkish Journal of Pharmaceutical Sciences 2019;16(3):292-302.

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