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FORMULATION AND EVALUTAION OF TOPICAL NANOSPONGE GEL FOR IMMUNOSUPPRESSIVE AGENT

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ABSTRACT

Two new topical immunosuppressive treatments, pimecrolimus and tacrolimus, were developed to provide alternatives to topical corticosteroids without the associated adverse events. They work by inhibiting calcineurin in the skin, which regulates the activity of several transcription factors that control cell division and trigger the early stages of T cell activation. Topical tacrolimus inhibits experimentally induced allergic contact dermatitis, and preliminary studies have suggested that the drug is effective in the treatment of atopic dermatitis.

KEYWORDS: Topical Nanosponge gel, Tachrolimus, Immunosupressive agent.

INTRODUCTION

Atopic dermatitis is a common inflammatory skin disorder that affects 15-20% of children and 1-3% of adults. The social and economic impact of this disorder is considerable, especially in severe cases, with patients experiencing intractable itch, loss of sleep, bleeding from the skin, and interference with most aspects of daily life. Traditionally the treatment of atopic dermatitis has included the frequent use of emollients and the intermittent use of topical corticosteroids to control acute flares. Corticosteroids, although effective, may be associated with several local and systemic adverse events, such as thinning of the skin and adrenal gland suppression.

Topical cyclosporine has been investigated as an alternative treatment in patients with atopic dermatitis and other dermatoses, but these studies have met with little success, presumably because of inadequate penetration of the drug into the skin. One study did show a significant effect of a cyclosporine gel as compared with a placebo gel after two weeks of treatment, but the differences between the dermatitis scores for the study groups were small.

Tacrolimus (FK 506) is an effective and well-tolerated primary immunosuppressant drug used in solid organ transplantation. Although its mode of action is similar to that of cyclosporine, its molecular weight is lower and its potency in inhibiting T-cell activation is 10 to 100 times greater. Moreover, topically applied tacrolimus appears to penetrate the skin sufficiently to effect local immunosuppression.

MATERIAL AND METHOD

Calibration curve of Tacrolimus in Methanol and phosphate buffer pH 6.8 (20:80)

A 0.5 ml standard stock solution of Tacrolimus was transferred to a 10 ml volumetric flask and volume was adjusted to 10 ml with the Methanol and Phosphate buffer pH 6.8 (20:80), the absorbance of the solution was scanned in the range of 200 to 400 nm using UV-visible Spectrophotometer. A 5 µg/ml solution was scanned to know the absorption maxima.

Preparation of stock solution

The stock solution was prepared by accurately weighing 10 mg of Tacrolimus, which was transferred to a 100 ml volumetric flask, and then it was dissolved in 100 ml of the Methanol and phosphate buffer pH 6.8 (20:80) to obtain the standard of 100 µg/ml (stock solution).

Preparation of working sample solution

Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 ml from the stock solution were transferred to 10 ml volumetric flask and the volume was adjusted to 10 ml with the Mthanol and phosphate buffer pH 6.8 (20:80) to obtain 1, 2, 3, 4, 5 µg/ml drug solution. Absorbance of the above solution were taken at 260 nm against the blank solution prepared in the same manner without adding the drug. A graph of absorbance versus concentration was plotted and it was found to be liner over a range of 1 to 5 µg/ml

Drugs – Excipients Interaction Study

Compatibility of Tacrolimus with the used polymers such as Ethyl cellulose & Carbopol 934 and the combination of Ethyl cellulose & was studied by FTIR & DSC.

Calculation of dose of Tacrolimus[22]

Tacrolimus is available as ointment in concentration of 0.1% w/w, which prescribed twice a day. 1 gm ointment = 1 mg Tacrolimus 1 mg Tacrolimus is required for 12 hrs

Thus, 20 mg Tacrolimus is required for preparation of 20 gm nanosponge gel.

Formulation of Tacrolimus Topical Nanosponge gel using 3^2 Factorial design

Factorial design was used in experiments in order to elucidate the effect of different factors or conditions on experimental results. 3^2 randomized full factorial design was used in the present study in preparation of batches of nanosponge gel. In this design 2 independent factors were evaluated, each at 3 levels, and experimental trials were performed for all 9 possible combinations. The Amount of Drug (Tacrolimus) (A) and rotations per minute (B) were chosen as independent variables in 3^2 full factorial design.

% drug entrapment, particle size and % CDR after 8 hrs were taken as dependent variables. A statistical model incorporating interactive and poly nominal terms was used to evaluate the responses.

$Yi = R0 + R1\ A + R2\ B + R12\ AB + R11\ A^2 + R22\ B^2$

Table 1: 3^2 full factorial design layout for Tacrolimus nanosponge gel Variable levels in coded form.

Formulation code	A (Drug)	B(rpm)
N1	-1	-1
N2	0	-1
N3	+1	-1
N4	-1	0
N5	0	0
N6	+1	0
N7	-1	+1
N8	0	+1
N9	+1	+1

Where Y is the dependent variable, R0 is the arithmetic mean response of the 9 runs and The main effects (A and B) represent the average result of changing 1 factor at a time from its low to high values. The two way interaction terms (AB) show how the response changes when two factors are simultaneously changed.

Table 2: Variables and levels of full factorial design 3^2 for Tacrolimus Nanosponge Gel.

Variables	Low (-1)	Medium (0)	High(+1)
Drug (mg)	50	100	150
rpm	4000	5000	6000

Table 3: Formulation of Topical Tacrolimus Nanosponge Gel.

Batch code	N1	N2	N3	N4	N5	N6	N7	N8	N9
rpm	4000	4000	4000	5000	5000	5000	6000	6000	6000
Drug (mg)	50	100	150	50	100	150	50	100	150
E C (gm)	1	1	1	1	1	1	1	1	1
DCM (ml)	4	4	4	4	4	4	4	4	4
PVA1% (ml)	10	10	10	10	10	10	10	10	10
PVA 0.05% (ml)	50	50	50	50	50	50	50	50	50
Carbopol 934 (mg)	200	200	200	200	200	200	200	200	200
Distilled water (ml)	20	20	20	20	20	20	20	20	20
Triethanol amine	1	1	1	1	1	1	1	1	1
P G (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
N-methyl-2- pyrrolidone (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Preparation of Tacrolimus Nanosponge gel Nanosponge

Nanosponge of Tacrolimus was prepared by Emulsion Solvent Evaporation method Dispersion of drug & ethyl cellulose in to dichloro methane was prepared. Primary emulsion was prepared by adding 10ml 1% w/v poly vinyl alcohol solution at 4000-6000 rpm for 2min in ice bath.

Emulsion was transferred into 50ml 0.5% W/V poly vinyl alcohol solution. Mixture was strirred on magnetic stirrer for 5-6 hrs.

Content was filtered by using watman filter paper. Washed thoroughly with distilled water to remove PVA. The nanosponge were dried at room temperature for 24 hrs.

Gel

The polymer carbopol 934 was initially soaked in water for the gel for 2 hrs & dispersed with agitation by using magnetic stirrer to get smooth dispersion.

Stirring was stopped & dispersion was allowed to stand for 15min to expel entrapped air.

Triethanolamine was added to neutralise the pH.

At this stage nanosponge, propylene glycol & N-methyl-2-pyrrolidone were incorporated as ethanolic solution to the aqueous dispersion.

Evaluation of Formulation

Particle size distribution

Table 4: Particle size distribution of formulation N1 to N9.

Batch Code	Particle Size (nm)
N1	2800-3200
N2	1900-2400
N3	1300-1700
N4	950-990
N5	860-946
N6	795-875
N7	743-775
N8	618-672
N9	520-580

Table 5: Percentage yield of formulation N1 to N9.

Batch code	N1	N2	N3	N4	N5	N6	N7	N8	N9
% Yield	50.14	51.98	50.46	53.27	52.31	56.54	54.07	51.64	53.04
	%	%	%	%	%	%	%	%	%

Table 6: Percentage drug entrapped in formulation N1 to N9.

Batch code	N1	N2	N3	N4	N5	N6	N7	N8	N9
% D	40.21	44.78	48.13	53.62	57.11	60.85	68.34	74.57	80.09
E	%	%	%	%	%	%	%	%	%

Appearance

Nanosponge gel Formulation were transperant and viscous with a smooth and homogeneous appearance.

Table 7: pH of formulation N1 to N9.

Batch code	N1	N2	N3	N4	N5	N6	N7	N8	N9
pH	6.2	6.0	5.8	6.1	5.9	6.0	5.8	6.1	6.0

Table 8: Extrudability of formulation N1 to N9.

Batch Code	N1	N2	N3	N4	N5	N6	N7	N8	N9
Extrudability	+++	++	+++	+++	++	++	+++	+++	+++

(+ + + excellent, + + Very good, + Good).

Table 9: Spredability of formulation N1 to N9.

Batch Code	N1	N2	N3	N4	N5	N6	N7	N8	N9
Spredability (cm)	4.1	4.4	4.2	4.0	4.5	4.2	4.3	4.4	4.5

Table 10: Viscosity of formulation N1 to N9.

Batch code	N1	N2	N3	N4	N5	N6	N7	N8	N9
Viscosity (cps)	4220	4223	4221	4219	4222	4221	4220	4223	4220

In-vitro drug release profile

Table 11: % Cumulative drug release data of formulation N1 to N9.

	% CUMULATIVE DRUG RELEASE OF BATCH N1 to N9								
Time (hrs)	**N1**	**N2**	**N3**	**N4**	**N5**	**N6**	**N7**	**N8**	**N9**
0	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
1	1.40	2.35	2.89	4.45	5.04	7.14	8.89	9.91	12.02
2	7.28	8.18	8.92	12.27	13.61	14.96	16.57	18.31	20.26
3	15.04	17.02	17.78	20.26	22.91	24.64	27.49	29.76	32.48
4	22.40	24.56	25.20	29.32	31.79	33.49	35.60	38.01	40.59
5	30.25	31.34	32.15	35.04	37.63	38.11	40.51	43.18	45.61
6	36.39	38.72	39.09	42.11	45.05	47.43	49.83	52.45	55.35
7	45.68	46.51	47.13	54.93	57.41	59.41	61.98	62.98	64.86
8	55.82	56.21	58.93	62.71	67.24	67.24	69.11	71.42	73.69
12	60.23	62.12	63.03	73.16	76.82	76.82	79.95	82.63	84.52

Stability study of optimized batch N9

Stability studies data of the optimized formulation N9 at 40°C±2°C temperature and 75%RH±5%RH.

Table 12: Result of Stability study for optimized Batch N9.

Parameters Evaluated	**Initial**	**After 1 Month**
Appearance	Transperant & Viscous	Transperant & Viscous
pH	6.0 ± 0.04	6.2 ± 0.06
Viscosity (cps)	4220 ± 2	4226 ± 4
% Drug release after 12	84.52%	83.07%

Stability studies showed that formulation was stable and there were no significant change in Appearance, pH, Viscosity and % Drug release after 12 hours under accelerated storage condition.

CONCLUSION

The nanosponge of Tacrolimus with Ethyl cellulose were successfully prepared & incorporated into topical gel. Emulsion solvent evaporation method was preffered for formulation of nanosponge. Carbopol-934 was used as gelling agent. Results of FTIR and DSC shows that there was no significant change has been observed in chemical and physical properties of Tacrolimus and tacrolimus is compatible with excipients. Formulation were optimized using 3^2 full factorial design. Nanosponge were employed for particle size distribution, percentage yield & drug entrapment efficiency. Batch N9 containing 6000 rpm shows smaller particle size 520-580 nm, highest drug entrapment efficiency 80.09%.

Nanosponge gel formulations were employed for pH, viscosity, extrudability, spredability & In-vitro diffusion study. Tacrolimus nanosponges showing diffusion controlled release.

The polymers used in formulation were found to be a efficient carriers for controlled release.

Drug: polymer ratio 1:15 gives maximum drug release 84.52% in phosphate buffer pH 6.8. Kinetic study has been performed for % cumulative release data of N9 batch, which follows Korsmeyer Peppas model with highest R^2 value 0.9885. The results of 3^2 full factorial dsign shows that both independent variables amount of Drug (X1) and Rotating speed (X2) signifficanly affected dependent variables which were % drug entrapped (R1), particle size (R2) & % cumulative release (R3). Scanning electron microscopy, Skin irritation test & Stability study were performed for optimized batch N9. In SEM image it clearly shows porous surface for entrapment of drug molecules. In skin irritation study it dosent cause any edema and erythema. In stability study study there was no signification change observed in properties of nanosponge gel.

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