



Formulation, Optimization and Evaluation of Mometasone Furoate Gel

*R. P. Patel, G. I. Patel, R. Kamani¹

¹Department of Pharmaceutics, S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat vidyanagar, Kherva, Mehsana-Gozaria Highway, PIN-390 001, Gujarat, India.

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ABSTRACT

The objective of this work was to develop the semi-solid topical gel formulations of mometasone furoate and study in vitro skin penetration through rat skin. The concentration of carbopol 940, ethanol and propylene glycol in gel formulation was optimized by Box-Behnken statistical screening design. The concentration of carbopol 940 (X_1), concentration of ethanol (X_2) and concentration of propylene glycol (X_3) were selected as independent variables. The flux (j), total amount released at 8 hours (Q_8) and enhancement Ratio (ER) were selected as dependent variables. All gel formulations showed good results for different parameters like drug content, pH, viscosity, and spreadibility. A marked effect of independent variables (concentration of carbopol 940, ethanol and Propylene Glycol) on mometasone furoate permeation was observed when it was incorporated gel formulations. The values of J , ER and Q_8 were strongly dependent on the independent variables.

Keywords: Mometasone furoate, Topical Gel, Box-Behnken statistical screening design, *In vitro* skin permeation.

INTRODUCTION

The development of topical drug delivery systems designed to have systemic effects appears to be very advisable and beneficial for a number of drugs on account of the several advantages that transdermal delivery offers over conventional routes of drug administration [1-3]. However, due to the relative impermeability of the stratum corneum, which provides the principal resistance to per-cutaneous absorption, extensive preformulation studies are generally necessary in order to optimize both the release of the drug from the topical vehicle and skin permeation. Therefore, in recent years the interest of researchers has been particularly focused on the study of the effectiveness of a large variety of chemical substances as possible enhancers of both dermal and transdermal drug transport [4-6].

Psoriasis is a chronic, non-contagious autoimmune disease which affects the skin and joints. It commonly causes red scaly patches to appear on the skin. The scaly patches caused by psoriasis, called psoriatic plaques, are areas of inflammation and excessive skin production. Bath solutions and moisturizers help soothe affected skin and reduce the dryness which accompanies the build-up of skin on psoriatic plaques. Medicated creams and ointments applied directly to psoriatic plaques can help reduce inflammation, remove built-up scale, reduce skin turn over, and clear affected skin of plaques.

Mometasone Furoate (MOF) is medium-potency topical corticosteroid that depresses formation, release, and activity of endogenous mediators of inflammation, including prostaglandins, kinins, histamine, liposomal enzymes, and complements system; modifies body's immune response. MOF had time to C_{max} ranged from about 1 to 2.5 h. V_d is 152 L. The in vitro protein binding was 98% to 99%. [7]. Primarily and extensively metabolized in the liver by the CYP3A4 isozyme to multiple metabolites. [8]. Terminal $t_{1/2}$ is about 5 h. Excretion up to 7 days is primarily in the feces (74%) and, to a lesser amount, in the urine (8%).

The present work was an attempt to develop topical formulations of MOF that are safe and can deliver the drug locally in an effective concentration for its effect. The effectiveness of the gel formulation would be expected to depend on the concentration of polymer, solvent and co-solvent in the gel formulations. The current work was designed to investigate the effects of the variables listed above on the formulation of the MOF as a topical drug delivery system.

2. MATERIALS AND METHODS

2.1 Materials

MOF sample was gifted by Yash Medicare Pvt. Ltd., Himatnagar, INDIA. Carbopol 940 was generous gift from Maan Pharmaceuticals Ltd., Mehsana, INDIA. Propylene glycol was received from Linclon Pharmaceutical Ltd., Ahmedabad, INDIA. All other chemicals and solvents were of analytical grade.

2.2 Preparation of MOF Gel Formulations

The gelling agent used in the preparation of the formulations was Carbopol 940. The alcoholic and/or co-solvent mixture comprised varying amounts of propylene glycol and ethanol. The gels were prepared with varying amount of the Carbopol 940 polymer. The

*Corresponding author.

Dr. Rakesh P. Patel,
Associate Professor & Head of Pharmaceutics &
Pharmaceutical Technology Department,
S. K. Patel College of Pharmaceutical Education and Research,
Ganpat University, Kherva, Mehsana-Gozaria Highway,
PIN-382711, Gujarat, India.
Tel.: + 91-9879106580
Telefax: +91-2762286082
E-mail: raka_77us@yahoo.com

required amount of Carbopol 940 was added in to water with vigorous stirring and left for overnight for proper dissolving of the polymer. The required amount of Mometasone furoate was dissolved in the alcoholic and/or co-solvent mixtures in a 100 ml beaker containing a magnetic bar placed on a magnetic stirrer/hot plate from Electroquip (DSK instruments). The open end of the beaker was covered with aluminum foil to minimize the evaporation of volatile components of the alcoholic and/or co-solvent mixture. The required amount of Carbopol 940 polymer solution was slowly dispersed in the Mometasone furoate /co-solvent mixture with vigorous mixing at 300 rpm. The beaker was covered with aluminium foil and left mixing for approximately 15 minutes. The mixture was also homogenised with a Virtis Homogenizer for 5 minutes at low speed. After complete addition of the polymer and Proper mixing, the gels were spontaneously formed with the addition of sodium hydroxide 1% solution. The gel was left at room temperature (21 + 3 °C) to cool and set and to allow the air bubbles produced by the mixing to escape from the gel.

2.2.1 Formulation optimization using Box-Behnken statistical design

Designing drug delivery formulations with the minimum number of trials is very crucial for pharmaceutical scientists [9]. In this study, we demonstrate the use of factorial designs to optimize the topical formulation for ibuprofen. Compared with one-factor-at-a-time experiments, a factorial experiment is more efficient in multi-factor optimization. More importantly, although the one-factor-at-a-time experiments can easily miss the optima, the factorial designs will give a combination near the maximum [10].

Design of Experiments (DOE) is a statistical technique that can be used for optimizing such multivariable systems. In recent years, the pharmaceutical industry has used experimental designs more for the optimization of pharmaceutical agents; however, only a few are reported in the literature for the development of dosage forms [11-12].

Box-Behnken statistical screening design was used to statistically optimize the formulation parameters and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the Mometasone furoate gel formulations. Response surface methodologies, such as the Box-Behnken and Central Composite Design (CCD), model possible curvature in the response function. A 3-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models. The Box-Behnken design was specifically selected since it requires fewer runs than a CCD in cases of three or four variables. This cubic design is characterized by set of points lying at the midpoint of each edge of a multidimensional cube and center point replicates (n = 3) whereas the 'missing corners' help the experimenter to avoid the combined factor extremes. This property prevents a potential loss of data in those cases. A design matrix comprising of 15 experimental runs was constructed. The non-linear computer generated model is given as

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y; and X_1 , X_2 and X_3 are the coded levels of independent variables. The

terms X_1 , X_2 and X_3^2 (i = 1, 2 or 3) represent the interaction and quadratic terms, respectively.

The full Equation containing only statistically significant terms is then used for drawing counter plots to visualize the impact of changing variables at a glance. The optimum point may be identified from the plot and replicate trials may be run to verify the prediction of optimum response. For simplicity, it was decided to perform a three variable study at three experimental levels to achieve the set objectives efficiently. The polynomial terms X_1^2 , X_2^2 and X_3^2 are induced to investigate nonlinearity. The polynomial equation can be used to draw conclusion after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative).

Response surface designs were used in this study in order to optimize the topical formulation containing MOF. The statistical analysis was performed using Sigma Plot Software 11.0 version (MYSTAT product manual) with general optimization techniques. A computer optimization technique, based on response surface methodology, has been proven to be a useful approach for selecting pharmaceutical formulations [10,13-15].

A Box-Behnken design was utilized in the present study. In this design three factors were evaluated, each at three levels, and experimental trials were carried out at all fifteen possible combinations. The design layout and coded value of independent factor is shown in Table 1 and Table 2, respectively.

The factors were selected based on preliminary study. The concentration of Carbopol 940 (X_1), concentration of Ethanol (X_2) and concentration of Propylene glycol (X_3) were selected as independent variables. The flux (j), total amount released at 8 hours (Q_8) and Enhancement Ratio (ER) were selected as dependent variables. The formulations of the factorial batches (G1-G15) are shown in Table 3.

2.3 Drug content

Approximately 1 gm of topical formulation, equivalent to 1 mg of MOF unless otherwise indicated, from each batch was weighed into a 100 ml flask on a Sartorius electronic balance CP- 225 (Labtronic). Approximately 40 ml of receptor phase was added to the formulation and shaken vigorously until dissolved and dilute up to 100 ml with the same. An aliquot of 1 ml of the solution was accurately transferred with a pipette to 10 ml volumetric flask and made up to volume with receptor phase. The solution was filtered through a filter prior to UV analysis at 249 nm. Individual concentrations were calculated from a standard calibration curve and the average values calculated for each batch. The flask was sealed with a plug to prevent loss of the solvent via evaporation during mixing.

2.4 pH measurements

The pH was measured in each gel formulation, using a pH meter (Systronic, 361-micro pH meter), which was calibrated before each use with buffered solutions at pH 4, 7 and 10. About 20 g of the gel was subjected to pH measurement within 24 hours of manufacture. An average pH reading of three readings was recorded.

2.5 Viscosity measurements

A Brookfield Rotational Digital Viscometer DV II RVTDV-II was used to measure the viscosity (in cps) of the gel formulations. The spindle was rotated at 10 rpm. Samples of the gels were allowed to settle over 30 minutes at the assay temperature (25±1°C) before the measurements were taken.

Table 1: Box-Behnken Design Layout

Batch no	X1	X2	X3
G1	-1	-1	0
G2	1	-1	0
G3	-1	1	0
G4	1	1	0
G5	-1	0	-1
G6	1	0	-1
G7	-1	0	1
G8	1	0	1
G9	0	-1	-1
G10	0	1	-1
G11	0	-1	1
G12	0	1	1
G13	0	0	0
G14	0	0	0
G15	0	0	0

Table 5: Permeation Parameters for Gel Formulations G1 to G15

Batch no	Q ₈	% Released	Flux	ER	R ² Zero order
G1	315.60 ± 1.36	63.12 ± 0.27	15.92 ± 0.37	1	0.992
G2	259.02 ± 0.71	51.80 ± 0.14	13.09 ± 0.06	0.82 ± 0.08	0.989
G3	400.70 ± 1.97	80.14 ± 0.39	20.15 ± 0.03	1.27 ± 0.04	0.993
G4	381.22 ± 1.59	76.24 ± 0.32	19.27 ± 0.18	1.21 ± 0.05	0.992
G5	373.79 ± 0.66	74.75 ± 0.13	18.85 ± 0.03	1.18 ± 0.12	0.992
G6	299.76 ± 0.93	59.95 ± 0.19	15.09 ± 0.57	0.95 ± 0.18	0.993
G7	339.53 ± 0.54	67.90 ± 0.11	17.07 ± 0.06	1.07 ± 0.02	0.992
G8	334.21 ± 1.68	66.84 ± 0.34	16.8 ± 0.15	1.06 ± 0.04	0.991
G9	300.08 ± 0.74	60.01 ± 0.15	15.1 ± 0.52	0.95 ± 0.21	0.993
G10	385.38 ± 1.68	77.07 ± 0.34	19.51 ± 0.18	1.23 ± 0.11	0.993
G11	274.95 ± 0.6	54.99 ± 0.12	13.94 ± 0.26	0.88 ± 0.06	0.994
G12	354.52 ± 0.58	70.90 ± 0.12	17.84 ± 0.09	1.12 ± 0.01	0.993
G13	405.70 ± 1.01	81.14 ± 0.20	20.4 ± 0.55	1.28 ± 0.24	0.994
G14	405.70 ± 1.02	81.14 ± 0.21	20.4 ± 0.56	1.28 ± 0.25	0.994
G15	405.70 ± 1.03	81.14 ± 0.22	20.4 ± 0.57	1.28 ± 0.26	0.994

Table 2: Coded Values for % Concentration of Carbopol 940 (X₁), Ethanol (X₂) and Propylene glycol (X₃).

Independent variables	Levels used		
	Low (-1)	Medium (0)	High (1)
X ₁ = Carbopol 940 concentration (%)	0.75	1	1.25
X ₂ = Ethanol concentration (%)	20	30	40
X ₃ = Propylene glycol concentration (%)	10	15	20

Table 3: Formulations Using Box-Behnken Design

Batch No	Mometasone Furoate	Carbopol 940	Ethanol	Propylene glycol	Methyl peraben	Water up to
G1	0.1	0.75	20	15	0.5	100
G2	0.1	1.25	20	15	0.5	100
G3	0.1	0.75	40	15	0.5	100
G4	0.1	1.25	40	15	0.5	100
G5	0.1	0.75	30	10	0.5	100
G6	0.1	1.25	30	10	0.5	100
G7	0.1	0.75	30	20	0.5	100
G8	0.1	1.25	30	20	0.5	100
G9	0.1	1	20	10	0.5	100
G10	0.1	1	40	10	0.5	100
G11	0.1	1	20	20	0.5	100
G12	0.1	1	40	20	0.5	100
G13	0.1	1	30	15	0.5	100
G14	0.1	1	30	15	0.5	100
G15	0.1	1	30	15	0.5	100

* All the Quantity is in percentage.

Table 4: Physical Characterization of Topical Gel Formulations of G1 to G15

Batch No	Drug content (%)	pH	Viscosity (cps)	Spreadability (g. cm/sec)
G1	100.8 ± 0.47	7.1	38600 ± 320	26.31 ± 1.38
G2	99.05 ± 0.56	6.9	43500 ± 250	20.83 ± 0.86
G3	99.75 ± 0.43	6.8	36500 ± 310	27.77 ± 1.54
G4	100.3 ± 0.62	7.2	40800 ± 210	23.8 ± 1.13
G5	100.1 ± 0.43	7.0	39400 ± 340	23.8 ± 1.13
G6	99.8 ± 0.89	6.9	45600 ± 180	19.23 ± 0.54
G7	98.98 ± 0.23	7.2	35300 ± 300	29.41 ± 1.73
G8	100.6 ± 0.71	7.1	37400 ± 270	26.31 ± 1.38
G9	99.85 ± 0.52	6.9	45100 ± 210	20 ± 0.80
G10	100.4 ± 0.48	6.8	43000 ± 340	21.73 ± 0.94
G11	98.9 ± 0.23	7.0	36700 ± 310	27.77 ± 1.54
G12	99.4 ± 0.57	7.2	35400 ± 300	29.41 ± 1.73
G13	99.7 ± 0.37	7.1	39200 ± 290	25 ± 1.25
G14	100.3 ± 0.17	6.8	39200 ± 310	25 ± 1.25
G15	100.8 ± 0.28	6.9	39200 ± 330	25 ± 1.25

Figure 1: In vitro Release Profile of Gel Formulations G1 to G15

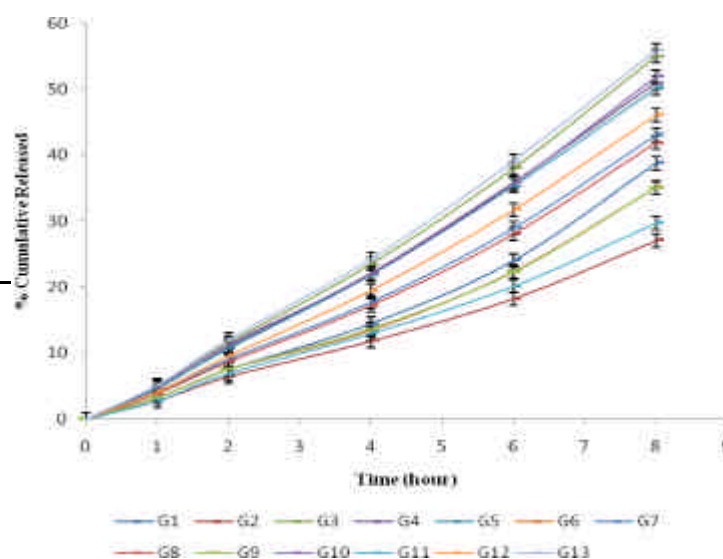


Figure 2: Counter Plot of Concentration of Carbopol 940 (X₁) and Concentration of Ethanol (X₂) Versus flux (J), Cumulative Amount Released at 8 Hours (Q₈) and Enhancement Ratio (ER).

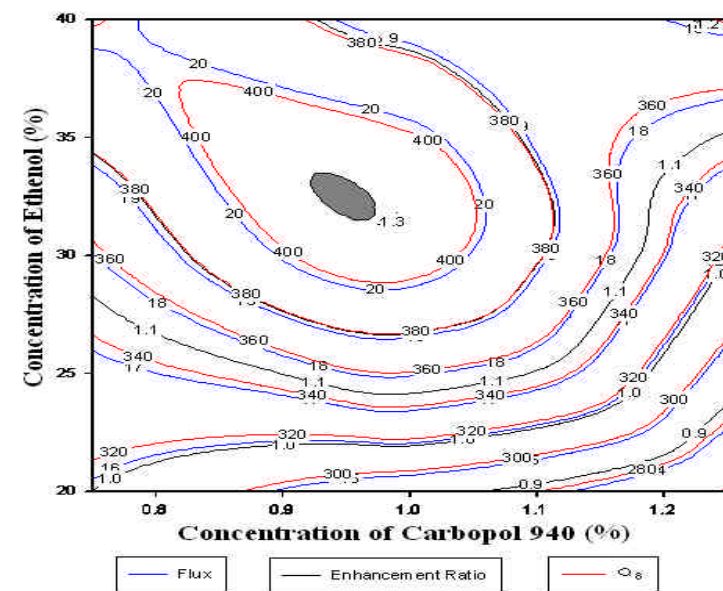


Figure 3: Counter Plot of Concentration of Carbopol 940 (X₁) and Concentration of Propylene Glycol (X₃) Versus Flux (J), Cumulative Amount Released at 8 Hours (Q₈) and Enhancement Ratio (ER).

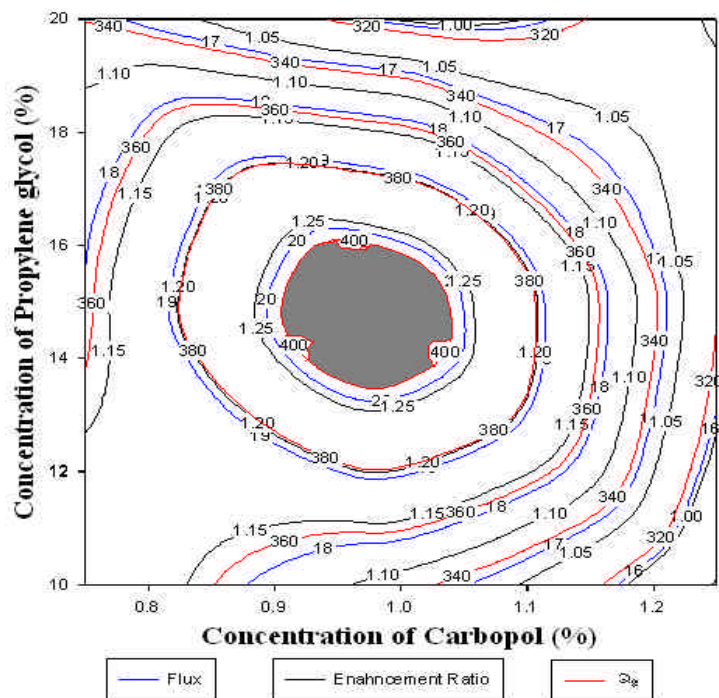
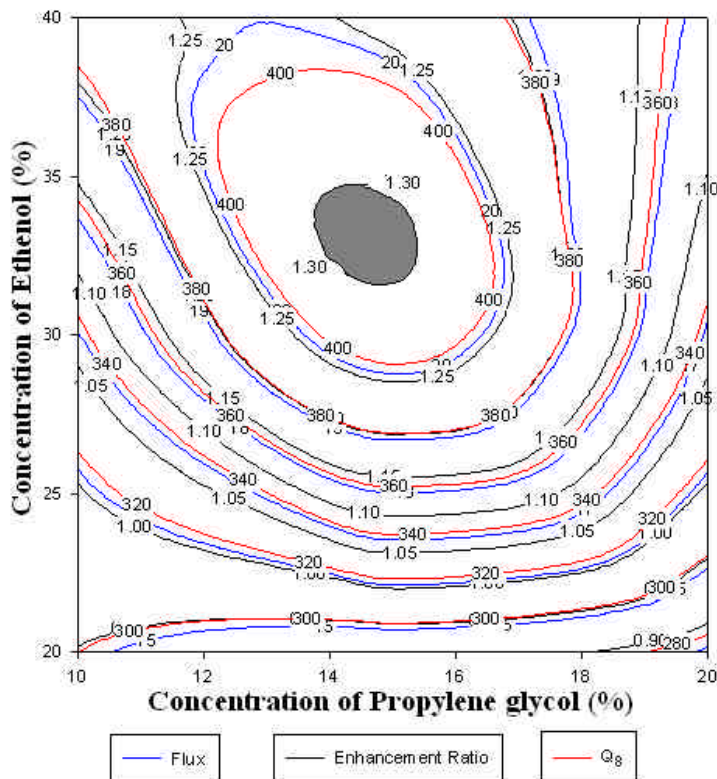


Figure 4: Counter Plot of Concentration of Carbopol 940 (X₁) and Concentration of Propylene Glycol (X₃) Versus Flux (J), Cumulative Amount Released at 8 Hours (Q₈) and Enhancement Ratio (ER).



2.6 Spreadability measurements

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5 mins. Weight (50gm) was added to the pan. The time required separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability (s).

$$S = \frac{m}{t} \text{ -----(1)}$$

Where m = weight tide to upper slide
 l = length moved on the glass slide
 t = time taken.

2.7 In vitro skin permeation studies

MOF release rates from different gel formulations were measured through rat skin. The dorsal skin of nude rat was mounted on the Franz diffusion cells [16] with a diffusional area of 1.86 cm² and a receiver compartment volume of 20 ml. The receptor compartment contained pH 7.4 phosphate buffer saline and Methanol in ratio of 70:30. To allow the establishment of the ‘sink condition’ and to sustain permeant solubilization, receptor phase was stirred and thermostated at 37±0.5°C during the experiments. 500 mg of each gel formulation was placed with 2 mm thick layer on the diffusion barrier in the donor compartment. At appropriate time intervals samples from receptor compartment were withdrawn and replaced with fresh solution. This dilution of the receiver content was taken into account when evaluating the penetration data. The samples were analyzed spectrophotometrically (UV spectrophotometer, Shimadzu 1700, Japan) at a wavelength of 249 nm and the concentration of MOF in each sample was determined from a previously calculated, standard curve. The total amount of MOF penetrating through the unit membrane surface and diffusing into the receptor medium was calculated and plotted as a function of time.

3. RESULTS & DISCUSSION

Physical characterization of topical gel formulations is given in table 5. In all gel formulations, drug content was found in between 98-102%, which was in the permissible limits and ensured the uniformity of the drug content in the formulation.

The result of pH measurement shows that all gel formulations which were prepared have the pH range in between 6.8-7.2. The data represent that increase with the concentration of Carbopol 940 from 0.75-1.25 % viscosity was increased, when the concentration of ethanol and propylene glycol was taken constant. And with increase in the concentration of ethanol and propylene glycol viscosity was decreased, when the concentration of Carbopol 940 was taken as constant. The viscosity of the all formulations was in between 35000-45000 cps. The spreadability of the all formulations was in between 18-30 gm cm/sec. The data represent that increase with the concentration of Carbopol 940 spreadability was decreased, when the concentration of ethanol and propylene glycol was taken constant. So, the data demonstrate that all independent variables concentration of Carbopol 940 (X₁), concentration of Ethanol (X₂) and concentration of Propylene glycol (X₃) were affect the viscosity and spreadability of the formulations.

In vitro Release Profile of Gel Formulations

The effect of independent variables on the permeability of Mometasone Furoate across the rat skin from gel formulations was investigated. The Release profile of various batches shows that batch G15 was higher release rate the release rate was directly affect on the independent variables of the formulations. Permeation parameters for MOF from the gel formulations are shown in Table 6.

The maximum amount (Q_8) of Mometasone Furoate that permeated during the 8 hr of the study was $405.70 \pm 1.03 \mu\text{g}$ from gel formulation G15. The corresponding flux of Mometasone Furoate was $20.4 \pm 0.57 \mu\text{g cm}^{-2}\text{hr}^{-1}$ for the gel formulation G15. Enhancement ratio was 1.28 ± 0.26 of the batch G15 observed. The zero order R^2 represent that the release profiles were follows zero order linear kinetic model.

The statistical analysis of the factorial design batches was performed by multiple linear regression analysis carried out in Microsoft Excel 2007. The flux (j), Enhancement ratio (ER) and cumulative amount released at 8 hours (Q_8) values for the 15 batches (G1 to G15) showed in Table 5. The data clearly indicated that the values of j , ER and Q_8 were strongly dependent on the independent variables. Permeation parameters for Mometasone Furoate from the gel formulations are shown in Table 5.

The fitted Equations relating the response j , ER and Q_8 to the transformed factor are shown in following Equations 1, 2 and 3.

$$J = 14.18 - 1.02 X_1 + 2.41 X_2 - 0.33 X_3 + 0.46 X_1 X_2 + 0.94 X_1 X_3 - 0.059 X_2 X_3 - 1.45 X_1^2 - 1.93 X_2^2 - 2.05 X_3^2 \text{-----(1)}$$

$$ER = 1.28 - 0.06 X_1 + 1.15 X_2 - 0.02 X_3 + 0.03 X_1 X_2 + 0.05 X_1 X_3 - 0.08 X_2 X_3 - 0.09 X_1^2 - 1.11 X_2^2 - 0.12 X_3^2 \text{----- (2)}$$

$$Q_8 = 405.7 - 19.42 X_1 + 46.52 X_2 - 6.97 X_3 + 9.27 X_1 X_2 + 17.17 X_1 X_3 - 1.43 X_2 X_3 - 29.23 X_1^2 - 37.32 X_2^2 - 39.64 X_3^2 \text{----- (3)}$$

3.3 In vitro skin permeation evaluation of gel formulation by counter plots

For further interpretation of the J , ER and Q_8 , counter plots of the independent variables verses dependent variables were prepared. For fitting the data in to the counter plots, two independent variables choose at a time against all dependent variables. Then, the plots were overlapped for the same independent variables against all dependent variables.

The overlapped counter plot of concentration of Carbopol 940 (X_1) and concentration of ethanol (X_2) versus J , Q_8 and ER was drown which was given in figure 2. The overlapped counter plot of concentration of Carbopol 940 (X_1) and concentration of propylene glycol (X_3) versus J , Q_8 and ER was drown which was given in figure 3. The overlapped counter plot of concentration of ethanol (X_2) and concentration of propylene glycol (X_3) versus J , Q_8 and ER was drown which was given in figure 4.

The counter plots demonstrate that both X_1 and X_2 affect the J , ER and Q_8 of the formulation. The shaded area in the Figure 2, 3, 4 demonstrated the optimize area of the concentration of Carbopol 940 (X_1) and concentration of ethanol (X_2) against versus J , Q_8 and ER, concentration of Carbopol 940 (X_1) and concentration of Propylene glycol (X_3) against versus J , Q_8 and ER, concentration of ethanol (X_2) and concentration of Propylene glycol (X_3) against versus J , Q_8 and ER.

4. CONCLUSION

Topical route of application has a great potential as an effective and safe way to administer mometasone for its anti-inflammatory effect in psoriasis. The concentration of polymer, solvent and co-solvent significantly affects the *In vitro* permeation study across rat epidermal membrane and also affect the critical parameters of gel formulation like flux, cumulative amount released at 8 hours and Enhancement ratio. The topical gel formulation of mometasone developed in this study holds the promise for the further *in vivo* studies and can be extrapolated for further development in treatment of psoriasis.

REFERENCES

1. Kydonieus, A.F. Fundamentals of transdermal drug delivery. In: Kydonieus, A.F., Berner, B. (Eds.), *Transdermal Delivery of Drugs*, CRC Press, Boca Raton, FL, 1987; pp. 4-6.
2. Govil, S.K. *Transdermal Drug Delivery Devices*, In Tyle P, ed *Drug delivery devices, Fundamentals and Applications*, New York, Marcel Dekker, 1988 pp. 386-419.
3. Asmussen, B. *Transdermal therapeutic systems: actual state and future developments*. *Methods Find. Exp. Clin. Pharmacol.* 1991; 13: pp. 343-351
4. Walters, K.A. *Penetration enhancers and their use in transdermal therapeutic systems*. In: Hadgraft, J., Guy, R.H. (Eds.), *Transdermal Drug Delivery*, Marcel Dekker, New York, 1989; pp. 197-233.
5. Hadgraft, J.; Williams, D.G.; Allan, g.; in *Pharmaceutical Skin Penetration Enhancement* Walters, K.A., Hadgraft, J. Eds.; Marcel Dekker, New York, 1993; pp. 175-197.
6. Hsieh, D.S. *Drug Permeation Enhancement*, Marcel Dekker, New York, 1994 pp.323-343.
7. Smith CL, Kreutner W: *In vitro* glucocorticoid receptor binding and transcriptional activation by topically active glucocorticoids. *Arzneimittelforschung.* 1998; 48(9): 956-60
8. Isogai M, Shimizu H, Esumi Y, Terasawa T, Okada T, Sugeno K. Binding affinities of MOF and related compounds including its metabolites for the glucocorticoid receptor of rat skin tissue. *J Steroid Biochem Mol Biol.* 1993; 44(2): pp. 141-145.
9. Hamed, E., Sakr, A., 2001. Application of multiple response optimization technique to extended release formulations design. *J. Control. Rel.* 73, 329-338.
10. Li, W., Nadig, D., Rasmussen, H.T., Patel, K., Shah, T., 2005. Sample preparation optimization for assay of active pharmaceutical ingredients in a transdermal drug delivery system using experimental designs. *J. Pharm. Biomed. Anal.* 37, 493-498
11. Rothhäuser B, Kraus G, Schmidt PC. Optimization of an effervescent tablet formulation using a central composite design optimization of an effervescent tablet formulation containing spray dried L-leucine and polyethylene glycol 6000 as lubricants using a central composite design. *Eur J Pharm Biopharm.* 1998;46:85-94.
12. Nazzal S, Nutan M, Palamakula A, Shah R, Zaghoul A, Khan MA. Optimization of a self-nanoemulsified tablet dosage form of Ubiquinone using response surface methodology: effect of formulation ingredients. *Int J Pharm.* 2002;240:103-114
13. Takayama, K., Nagai, T., 1989. Novel computer optimization methodology for pharmaceutical formulations investigated by using sustained-release granules of indomethacin. *Chem. Pharm. Bull.* 37, 160-167.
14. Giannakou, S.A., Dallas, P.P., Rekkas, D.M., Choulis, N.H., 1995. Development and in vitro evaluation of nitrendipine transdermal formulations using experimental design techniques. *Int. J. Pharm.* 125, 7-15.
15. Agyralides, G.G., Dallas, P.P., Rekkas, D.M., 2004. Development and in vitro evaluation of furosemide transdermal formulations using experimental design techniques. *Int. J. Pharm.* 281, 35-43.
16. Shah, V.P., Elkins, J.S., Williams, R.L., 1999. Evaluation of the test system used for in vitro release of drugs for topical dermatological drug products. *Pharm. Dev. Tech.* 4, 377- 385.

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