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Formulation Optimization and Evaluation of Mometasone Furoate Cream

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ABSTRACT

The aim of the present investigation was to develop topical cream formulations of mometasone furoate and study its permeation through rat skin. The surfactant concentration was optimized using 3^2 full factorial designs. The concentration of Tween 80 (X_1) and concentration of Span 80 (X_2) were selected as independent variables. The flux (j), cumulative amount released at 8 hours (Q_8) and Enhancement ratio (ER) were selected as dependent variables. All cream formulations showed good results for different parameters like drug content, pH, viscosity, and spredibility. The penetration enhancing effect of menthol (0–10% w/w) on the percutaneous flux of mometasone furoate through the excised rat epidermis was also investigated. A marked effect of surfactants (Tween 80 Span 80) concentration on mometasone furoate permeation was observed when it was incorporated cream formulations. The values of J, ER and Q_8 were strongly dependent on the independent variables. The percutaneous flux and enhancement ratio of mometasone furoate across rat epidermis was significantly increased by the addition of menthol to the cream formulations.

Keywords: Mometasone furoate, Topical cream, Factorial design, *In vitro* skin permeation, Permeation enhancer

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 (Kydonieus, 1987; Govil, 1988; Asmussen, 1991). How ever, 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 (Walters, 1989; Walters and Hadgraft, 1993; Hsieh, 1994).

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%. (Smith et al., 1998). Primarily and extensively metabolized in the liver by the CYP3A4 isozyme to multiple metabolites. (Isogai et al., 1993). Terminal $t_{½}$ 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 cream formulation would be expected to depend on the concentration of surfactant and permeation enhancer in the cream 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. White petrolatum, White bees wax, Stearic acid and Methyl peraben were generous gift from Maan Pharmaceuticals Ltd., Mehsana, INDIA. Tween 80, Span 80, Propylene glycol, and Titanium dioxide were received from Linclon Pharmaceutical Ltd., Ahmedabad, INDIA. All other chemicals and solvents were of analytical grade.

2.2 Preparation of MOF Cream Formulations

Preparation of cream was done by emulsifying procedure in which at the same temperature of lipophilic phase and aqueous phase was thoroughly mixed. In the separate vessel lipophilic phase and aqueous phase was prepared. For the lipophilic phase, lipophilic ingredients, white petrolatum, white bees wax and stearic acid was melted in a porcelain dish at 65-75°C. For the aqueous phase, hydrophilic ingredient, water and propylene glycol was mixed and heat at temperature of 65-70°C. MOF was dissolved in the propylene glycol. After melting the lipophilic ingredient, heating was stopped, aqueous phase added slowly drop by drop and mixed on the magnetic stirrer at

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the speed of 600-800 rpm. The other surfactants and titanium dioxide were added when the both phase was mixed. After completion of addition of all ingredients the homogeneous mixing for 2-10 minute with effective cooling was done to achieves better cream formulation.

2.2.1 *Optimization of Surfactant Concentration Using 3^2 Full Factorial Design*

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man-hours and raw materials. Designing drug delivery formulations with the minimum number of trials is very crucial for pharmaceutical scientists (Hamed and Sakr, 2001). Traditionally pharmaceutical formulations after developed by changing one variable at a time approach. The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interactions (Li et al., 2005).

The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y_i) is measured for each trial.

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2}$$

Model is fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant terms. The full Equation, an 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 and X_2^2 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 (Takayama and Nagai, 1989; Giannakou et al., 1995; Agyralides et al., 2004; Li et al., 2005).

A 3^2randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The factors were selected based on preliminary study. The concentration of Tween 80 (X_1) and concentration of Span 80 (X_2) were selected as independent variables. The flux (*j*), cumulative amount released at 8 hours (Q_8) and Enhancement ratio (ER) were selected as dependent variables. The design layout and coded value of independent factor is shown in Table 1 and Table 2, respectively. The formulations of the factorial batches (R1-R9) are shown in Table 3.

2.2.2 *Effect of Permeation Enhancer*

After achieving better cream formulation in preliminary trials by optimized concentration of surfactant, further achievement of better cream formulation, optimization of the penetration enhancer also very important.

The addition of permeation-enhancing compounds to topical delivery systems may improve the penetration of drugs by modifying the thermodynamic activity of penetrants (e.g., changes in partitioning tendencies) or by altering the skin barrier properties (e.g., changes in fluidity of extracellular lipids). Menthol, terpenes, etc. may cause a reversible disruption of the lipid domain and promote the formation of new polar channels. The presence of penetration enhancers may also change the thermodynamic activity of the drug in the vehicle and consequently alter its permeability.

L-Menthol is a terpenes compound containing alcohols that has been widely used as skin penetration enhancers for a variety of compounds. Menthol was selected for our studies because it is also a refrigerant agent that induces a strong cooling sensation when applied to the skin and numbs the sensation of pain, for this reason, it may provide an advantage for analgesic topical formulations. Permeation enhancement of menthol could involve its distribution into the intercellular space of stratum corneum and the possible reversible disruption of the intercellular lipid domain. This would increase drug diffusivity. L-Menthol was taken as permeation enhancer in concentration of 1, 5, 10 % in the optimized surfactant formulation to achieve best permeation from the cream formulation. The formulations of the optimization of permeation enhancer concentration batches (R10-R12) are shown in Table 4.

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 cream 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 cream 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 cream formulations. The spindle was rotated at 10 rpm. Samples of the creams were allowed to settle over 30 minutes at the assay temperature (25±1°C) before the measurements were taken.

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= ml/t----------(1)

Table 1: Full Factorial Design Layout

Batch no	X_1 (Concentration of Tween 80)	X_2 (concentration of Span 80)
R 1	-1	-1
R 2	0	-1
R 3	1	-1
R 4	-1	0
R 5	0	0
R 6	1	0
R 7	-1	1
R 8	0	1
R 9	1	1

Table 2: Coded Values for % Concentration of Tween 80 (X1) and Span 80 (X_2)

Coded value	% Concentration of Tween 80 (X_1)	% Concentration of Span 80 (X_2)
-1	1	1
0	2.5	2.5
1	5	5

Table 3: Formulations Using 3^2 Full Factorial Design

Batch no	R1	R2	R3	R4	R5	R6	R7	R8	R9
MOF	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
White petrolatum	52	52	52	52	52	52	52	52	52
White bees wax	3	3	3	3	3	3	3	3	3
Stearic acid	5	5	5	5	5	5	5	5	5
Tween 80	1	1	1	2.5	2.5	2.5	5	5	5
Span 80	1	2.5	5	1	2.5	5	1	2.5	5
Methyl peraben	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol	30	30	30	30	30	30	30	30	30
Titanium dioxide	1	1	1	1	1	1	1	1	1
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

** All the Quantity is in percentage*

Table 4: The Formulations for the Optimization of Permeation Enhancer Concentration Batches (R10-R12)

Batch no	R10	R11	R12
MOF	0.1	0.1	0.1
White petrolatum	52	52	52
White bees wax	3	3	3
Stearic acid	5	5	5
Tween 80	5	5	5
Span 80	2.5	2.5	2.5
Menthol	1	5	10
Methyl peraben	0.5	0.5	0.5
Propylene glycol	30	30	30
Water	q.s.	q.s.	q.s.
Titanium dioxide	1	1	1

** All the Quantity is in percentage*

Table 5: Physical Characterization of Topical Cream Formulations of R1 to R9

Batch No	Drug content (%)	pH	Viscosity (cps X 10^3)	Spreadability (gm*cm/sec)
R 1	99.45 ± 0.49	6.6	682 ± 4.24	22.73 ± 1.03
R 2	99.1 ± 0.71	6.8	724 ± 1.41	20.83 ± 0.86
R 3	99.8 ± 0.57	6.5	682 ± 9.90	22.73 ± 1.03
R 4	98.95 ± 0.92	6.5	767 ± 2.83	20 ± 0.80
R 5	99.05 ± 0.92	6.8	709 ± 8.49	20.83 ± 0.86
R 6	100.2 ± 0.42	6.6	684 ± 0.71	21.73 ± 0.94
R 7	99.7 ± 0.57	6.4	787 ± 3.54	20 ± 0.80
R 8	100.1 ± 0.57	6.8	650 ± 11.31	23.8 ± 1.13
R 9	99.1 ± 0.85	6.5	797 ± 7.07	19.23 ± 0.54

Table 6: Permeation Parameters for Cream Formulations R1 to R8

Batch No	*J* (µg $cm^{-2}hr^{-1}$)	*ER*	Q_8 (µg)	% Released	R^2 zero order
R 1	1.84 ± 0.18	1	101.46 ± 1.36	20.29 ± 0.27	0.9823
R 2	1.51 ± 0.26	0.81 ± 0.06	79.59 ± 0.72	15.91 ± 0.14	0.9869
R 3	2.44 ± 0.57	1.31 ± 0.18	121.85 ± 1.97	24.37 ± 0.39	0.9816
R 4	1.47 ± 0.52	0.79 ± 0.21	67.57 ± 1.60	13.51 ± 0.32	0.9885
R 5	1.73 ± 0.37	0.93 ± 0.11	89.28 ± 0.66	17.85 ± 0.13	0.9829
R 6	2.09 ± 0.06	1.14 ± 0.08	122.23 ± 0.94	24.44 ± 0.19	0.9796
R 7	1.04 ± 0.06	0.57 ± 0.02	60.06 ± 0.54	12.01 ± 0.11	0.9889
R 8	2.48 ± 0.03	1.35 ± 0.12	146.95 ± 1.69	29.39 ± 0.34	0.9881
R 9	1.10 ± 0.03	0.6 ± 0.04	65.37 ± 0.75	13.07 ± 0.15	0.9882

Table 7: Permeation Parameters for Cream Formulations

Batch no	*J* (µg $cm^{-2}hr^{-1}$)	*ER*	Q_8 (µg)	% Released	R^2 Zero order
R 8	2.48 ± 0.03	1	146.95 ± 1.69	29.39 ± 0.34	0.988
R 10	4.13 ± 0.15	1.66 ± 0.04	228.21± 0.61	45.64 ± 0.12	0.985
R 11	4.76 ± 0.18	1.92 ± 0.05	264.98 ± 0.58	52.99 ± 0.12	0.992
R 12	5.06 ± 0.55	2.04 ± 0.24	308.48 ±1.01	61.69 ± 0.20	0.988
Market formulation	4.37 ± 0.09	1.76 ± 0.01	256.56 ± 1.03	51.32 ± 0.19	0.998

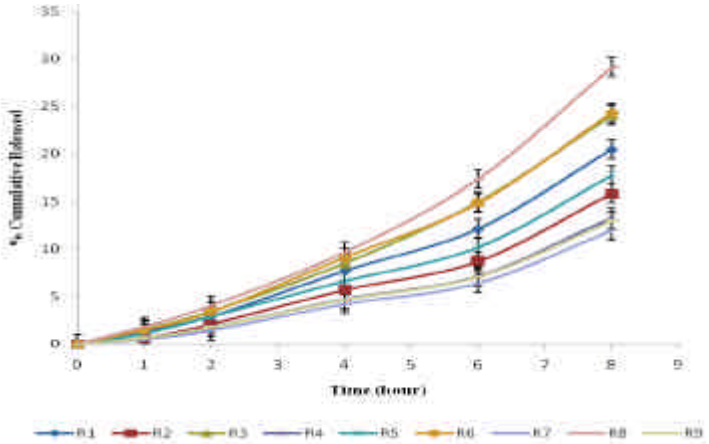


***Figure 1: In vitro* Release Profile of Cream Formulations R1 to R8**

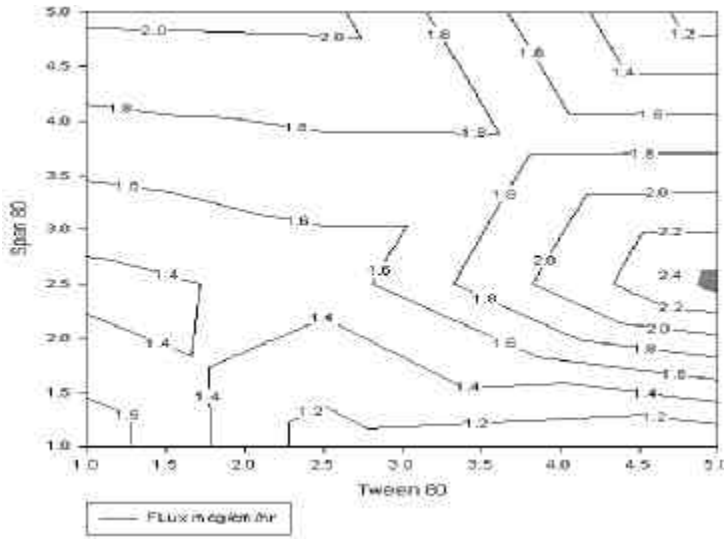


Figure 2: Counter Plot of Concentration of Tween 80 (X_1) and Concentration of Span 80 (X_2) Versus Flux (*J*)

Figure 3: Counter Plot of Concentration of Tween 80 (X_1) and Concentration of Span 80 (X_2) Versus Enhancement Ratio (*ER*).

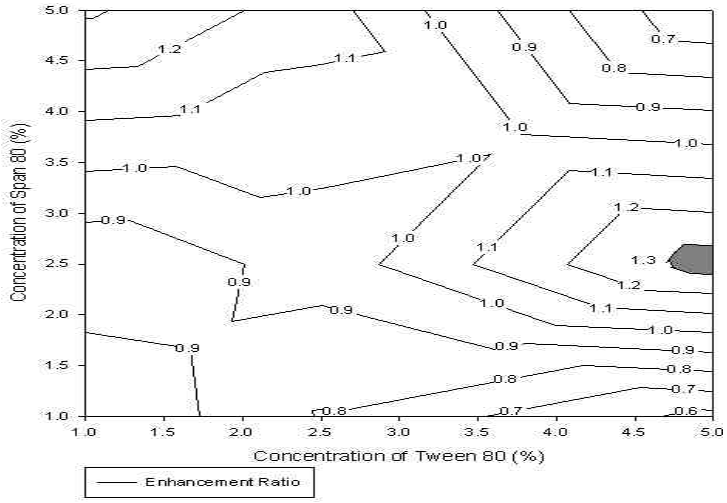
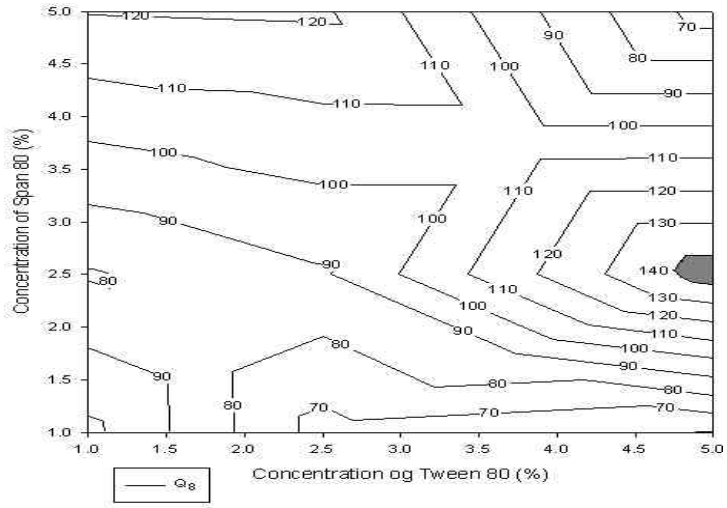


Figure 4: Counter Plot of Concentration of Tween 80 (X_1) and Concentration of Span 80 (X_2) Versus Cumulative Amount Released at 8 hours (Q_8)



Figures 5: The Overlapping Counter plot of the Concentration of Tween 80 (X_1) and Concentration of Span 80 (X_2) Versus, *J, ER* and Q_8

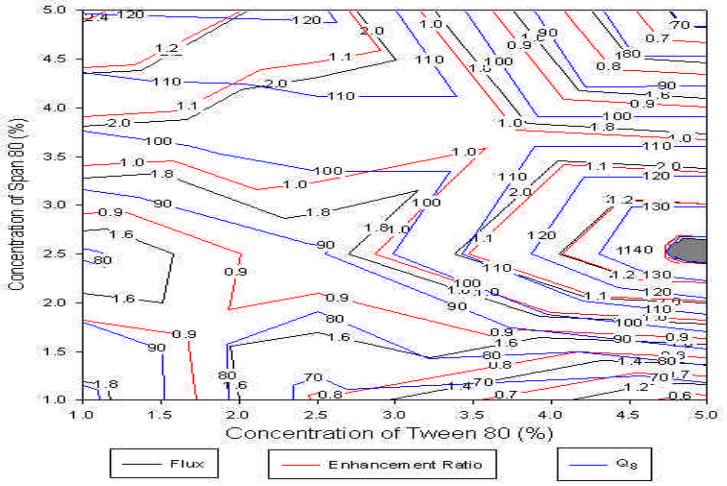
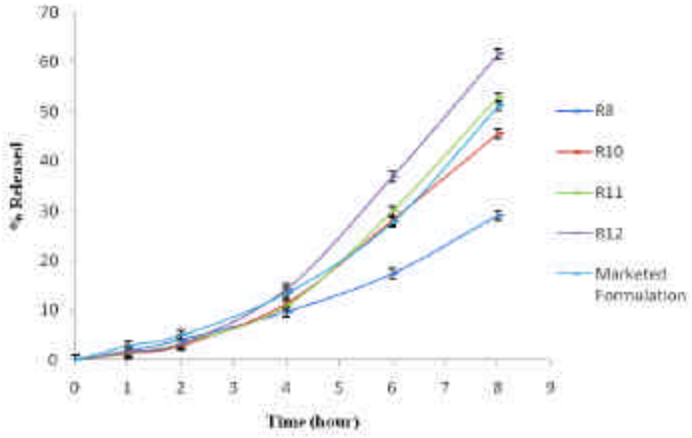


Figure 6: *In vitro* Release Profile of Cream Formulations with Permeation Enhancer and Marketed Formulation.



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 cream formulations were measured through rat skin. The dorsal skin of nude rat was mounted on the Franz diffusion cells (Shah et al., 1999) with a diffusional area of 1.86 cm^2 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 cream 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, Schimadzu 1700, Japan) at a wavelength of 249 nm and the concentration of MOF in each sample was determined from a previously calculated, standart 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

3.1 Optimization of Surfactant Concentration

Physical characterization of topical cream formulations is given in table 5. In all cream 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 showed that all cream formulations have the pH range in between 6.5-6.8. The data on viscosity study indicated that with constant concentration of the Span 80 increase in concentration of the Tween 80, viscosity of formulation was also increased. Whereas increase in the concentration Span 80 with respect to higher concentration of the Tween 80 viscosity was decreased in extent to 3% after than viscosity was again increased. The spreadability was satisfactory higher in the concentration of 5% and

2.5% of Tween 80 and Span 80, respectively. The data demonstrate that both $X_1$and X_2 affect the viscosity and spreadability of the formulations.

3.2 *In vitro* skin permeation of Cream Formulations R1 to R8

The effect of surfactants on the permeability of MOF across the rat skin from cream formulations was investigated. The release profile showed the higher release rate was observed in the R8 batch. Permeation parameters for MOF from the cream formulations are shown in Table 6. A marked effect of surfactants, concentration of Tween 80 and concentration of Span 80 on MOF permeation was observed when it was incorporated cream formulations. The cumulative amount permeated at 8 hours (Q_8) of MOF was 146.95 ± 1.69 µg from cream formulation R8. The corresponding flux (J) and enhancement ratio (ER) of MOF was 2.48 ± 0.03 µg $cm^{-2}hr^{-1}$ and 1.35 ± 0.12, respectively for the cream formulation R8.

The statistical analysis of the factorial design batches was performed by multiple linear regression analysis carried out in Microsoft Excel 2007. The values for J, ER and Q_8 for all 9 batches (R1 to R9) are showed in Table 6. The data clearly indicated that the values of J, ER and Q_8 were strongly dependent on the independent variables. The fitted equations relating the J, ER and Q_8 to the transformed factor are shown in following Equations 1, 2 and 3.

$$J = 1.93 - 0.19\,X_1 + 0.21\,X_2 - 0.14\,X_1X_2 - 0.034\,X_1^2 - 0.24\,X_2^2 \quad (1)$$

$$ER = 1.04 - 0.10\,X_1 + 0.11\,X_2 - 0.7\,X_1X_2 - 0.02\,X_1^2 - 0.13\,X_2^2 \quad (2)$$

$$Q_8 = 102.49 - 5.28\,X_1 + 13.05\,X_2 - 3.22\,X_1X_2 + 3.09\,X_1^2 - 15.32\,X_2^2 \quad (3)$$

3.3 *In vitro* skin permeation evaluation of cream 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. The counter plots of concentration of Tween 80 (X_1) and concentration of Span 80 (X_2) versus J, ER and Q_8 ware plotted which was given in figure 2, figure 3 and figure 4, respectively.

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, figure 3 and figure 4 demonstrated the optimized area of the J, ER and Q_8, respectively. For optimization of the batch overlapped counterplot of independent variables versus all dependent variables was plotted. Figures 5 showed the overlapping counter plot of the concentration of Tween 80 (X_1) and concentration of Span 80 (X_2) versus J, ER and Q_8.

The shaded area in the Figure 5 demonstrated the optimized area of the J, ER and Q_8. Figure 5 indicated that Tween 80 (5%) and Span 80 (2.5%) exhibited higher J, ER and Q_8 for the formulations. So for further optimization of the permeation enhancers, batch R8 taken as optimized surfactant batch which have the concentration of Tween 80 and Span 80 was taken 5% and 2.5%, respectively.

3.4 Effect of Permeation Enhancer

A marked effect of menthol on MOF permeation was observed when it was incorporated cream formulations in varying quantities. The Q_8 of MOF were found to be 146.95 ± 1.69, 228.21± 0.61, 264.98 ± 0.58, and 308.48 ±1.01 µg for cream formulations containing 0, 1, 5, and 10 %w/w of menthol, respectively. The corresponding flux values were ranging from 2.48 ± 0.03, 4.13 ± 0.15, 4.76 ± 0.18, and 5.06 ± 0.55 µg cm^{-2} hr^{-1}. As menthol concentration was increased from 0 to 10% w/w, the permeability of MOF was also increased as indicated by an increase in ER (Table 7 and Figure 6).

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 surfactant significantly affects the critical parameters of cream formulation like flux, cumulative amount released at 8 hours and Enhancement ratio. *In vitro* permeation study across rat epidermal membrane showed that menthol enhanced the transdermal absorption of mometasone from cream formulation. The topical cream 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.

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