MEDDOCS Open Access Publisher

ISSN: 2578-8760

Journal of Nanomedicine

Open Access | Research Article

Formulation and Evaluation of Desonide Loaded Microemulsion Based Gel for Management of Atopic Dermatitis

Kusha Sharma[1]; Bharti Sapra[2]; Shubham Kant[1]; Neena Bedi[1]*

[1]Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, India.

[2]Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India.

***Corresponding Author(s): Neena Bedi**

Assistant Professor, Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, India, 143005.

Tel: +91-9814007358;

Email: neena.pharma@gndu.ac.in

Received: Jan 18, 2021

Accepted: Feb 19, 2021

Published Online: Feb 22, 2021

Journal: Journal of Nanomedicine

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Atopic dermatitis; Desonide; Topical drug delivery; Microemulsion; Rat skin; Photostability.

Abstract

Objective: The aim of the current study was to formulate and evaluate desonide loaded microemulsion gel for effective management of Atopic Dermatitis (AD).

Methods: Microemulsions (MEs) were developed and optimized using D-optimal mixture statistical experimental design. The morphology of ME was studied using transmission electron microscopy. To improve skin retention of formulation, gelling of the optimized ME was carried out using Carbopol 940 (1% *w/w*). The efficacy of the formulation was evaluated on mice with dinitrochloro benzene induced dermatitis.

Results: The ME formulation containing 5% *w/w* oil, 35.643% *w/w* S_{mix}, and 59.357% *w/w* water was selected. The physicochemical characterstics exihibited by developed ME were found to be optimal. *Ex vivo* studies showed 3 fold increase in drug retention from MG in rat skin as compared to commercial formulation. MG resulted in significant reduction ($p<0.05$) in dermatitis score as compared to marketed gel with reduction in neutrophilic infilteration. Transepidermal water loss by application of drug loaded carbopol gel, marketed gel and MG on mice skin was found to be 3.01 ± 0.08 g/m^{2}h, 2.12 ± 0.06 g/m^{2}h and 2.52 ± 0.12 g/m^{2}h, respectively. The significant reduction in transepidermal water loss ($p<0.05$) indicated the potential of MG to minimize steroid associated epidermal barrier impairment due to entrapment of drug in ME droplets.

Conclusion: The overall results elucidated that desonide loaded MG could be a successful carrier system for the treatment of AD.

Cite this article: Sharma K, Sapra B, Kant S, Bedi N. Formulation and Evaluation of Desonide Loaded Microemulsion Based Gel for Management of Atopic Dermatitis. J Nanomed. 2021; 4(1): 1035.

Introduction

Topical corticosteroids (TCs) are widely used for treatment of acute and chronic exacerbations of Atopic Dermatitis (AD) due to their potent immunosuppressive, anti-inflammatory and antihistaminic effects [1]. TCs target the viable epidermis and dermis region where inflammation occurs and are currently available in various conventional formulations such as creams, ointments, lotions and gels [2]. However, due to limitations such as poor permeation, skin irritation and local side effects associated with conventional vehicles for TCs, research is being done in the field of nanocarrier based delivery systems due to their potential role in improving drug permeation and targeted drug delivery [3] .

Desonide is a nonflourinated low-potency TC, widely used in the treatment of various steroid responsive skin diseases [4,5]. It is commercially available in cream, lotion, ointment, gel and more recently in foam form also. Desonide is prescribed for application on sensitive skin due to its mild potency both in adults and children [6]. Although having low potency, several risk factors involving systemic and local side effects have been associated with this drug. Children are more prone to development of systemic side effects which may occur due to higher total body surface area to body weight ratio [7]. This leads to need for development of novel drug delivery systems which are able to minimize dose dependent corticosteroid side effects.

Microemulsion (ME) based systems are widely being developed and evaluated for topical drug delivery due to their small droplet size ensuring close contact with Stratum Corneum (SC), hence promoting the drug delivery into skin. For improving their maintenance on skin, the micromemulsions could be further incorporated into hydrogels [8,9]. The present study was conducted with the objective to improve efficacy of desonide by delivering it to the target site using ME based gel system while reducing the risk of drug associated side effects.

Materials

Desonide was obtained as a gift sample from Zim Laboratories Pvt. Ltd., Mumbai. Ethyl oleate and 2,4- dinitro dichloro benzene was purchased from Loba Chemie Pvt. Ltd., India. Tween80 and Propylene glycol were purchased from Merck Life Science Pvt Ltd., India. Carbopol 940, di-sodium orthophosphate and potassium dihydrogen phosphate were obtained from Himedia Laboratories Pvt. Ltd., India. Acetonitrile and methanol (HPLC Grade) were obtained from Finar Chemicals Pvt. Ltd., India. Marketed desonide gel (0.05% *w/w*) was obtained from a local pharmacy. Triple distilled water (Rions India, Lab Water Systems Pvt. Ltd.) was used throughout the study. All others chemicals used in the study were of analytical grade.

Animals

The animal studies were conducted in accordance with the guidelines and approval of the Institutional Animal Ethical Committee (IAEC) under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Approval no 226/PO/Re/S/2000/CPCSEA. For the studies, BALB/c female mice weighing 20–25 g and male Wistar rats weighing 200–250 g were used. The animals were given free access to a standard rodent diet and water and were acclimatized for two weeks before the experiment.

Methods

HPLC analysis of desonide

HPLC studies were carried out in reversed phase column based high-performance liquid chromatographic method. The HPLC system (Nexera X2, Shimadzu, Japan) was equipped with C-18; 4.6 mm×250 mm; 5 µm analytical column (L-2013, Shimadzu, Japan). The mobile phase used was a mixture of methanol, acetonitrile and water (50:10:40) maintained at pH 5.0 which was filtered using 0.22 µm filter membrane (Millipore, India) and eluted at flow rate of 1.2 ml/min. The samples were analysed by HPLC at wavelength maxima of 250 nm [10].

Microemulsion component screening

The solubility of drug in different oils and surfactants was determined by adding excess amount of pure drug in 3 ml of each selected vehicle in closed vials. The mixtures were vortexed using vortex mixer (Ika, Cole-Parmer, India) for 10 minutes and then kept in an isothermal shaker (Narang Scientific Works Pvt. Ltd., India) at temperature of 37 ± 1ºC for 72 h and rotating speed of 80 strokes per minute to achieve equilibrium. The samples were centrifuged (Laboratory Centrifuge, Remi, India) at 3000 rpm for 10 min and filtered through 0.22 µm membrane filter [11]. The concentration of drug in the filterate was evaluated by HPLC analysis.

The solubilisation capacity of surfactants in the selected oil was determined by adding subsequent aliquots of 5 µl oil to 2.5 ml of aqueous solution of each surfactant (15% *w/w*) under vigorous stirring until solution became turbid. The calculations were done using the following equation [12].

$$\text{Solubility of oil (\% w/w)} = \frac{vd}{Q}.100 \qquad \text{(i)}$$

where, v represents the total volume (ml) of the oil added till turbidity appeared, d is the density of oil (g/ml) and Q is the quantity of surfactant in aqueous surfactant solution.

For the selection of suitable cosurfactant, pseudoternary phase diagrams were constructed at fixed surfactant to cosurfactant ratio (S_{mix}) of 1:1 *w/w*, using the selected oil and surfactant and changing cosurfactants [13]. For this, 5 different cosurfactants were selected i.e. propylene glycol, butanol, isopropyl alcohol, ethanol and methanol. The pseudoternary phase diagrams were prepared by taking different weight ratios of oil and S_{mix} (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) and ME area was calculated for each phase diagram prepared.

Formulation optimization

D-optimal mixture statistical experimental design was applied to optimize formulation variables using software Design-Expert version 7. On the basis of preliminary studies, the percentage range for oil, S_{mix} ratio and water content selected was 5-10% *w/w*, 35-45% *w/w* and 45-60% *w/w* respectively, for investigating experimental trials. The optimal S_{mix} ratio was selected with the aid of pseudoternary phase diagrams (*Figure S1, supporting information*). The response surface methodology involved conducting 16 experimental runs using different combinations of oil, S_{mix} and water and studying their effects on the selected response variables [14]. A group of candidate points were chosen by the software resulting in 11 formulations and five replicates.

Physicochemical characterization of optimized microemulsion formulation

The average size and Polydispersity Index (PDI) of ME droplets were determined using Particle Size Analyzer Nano Series (Malvern, UK). The pH was measured using digital pH meter (S.D. Fine Chemicals, India). For determination of zeta potential, Zeta Sizer Nano Series (Malvern, UK) was used. All the experiments were performed in triplicate [15]. The structure, morphology and droplet size of ME droplets was analysed using transmission electron microscope (Hitachi- H 7650, Japan) operated at 80 KV.

Formulation of microemulsion gel

Microemulsion Gel (MG) was prepared by dispersing 1% *w/w* Carbopol 940 slowly in optimized ME using mechanical stirrer (Remi Motors Pvt. Ltd., India) followed by neutralization with triethanolamine until gelling took place [16].

***Ex vivo* skin permeation study**

The study was conducted using Franz diffusion cell (HEM-100, Harjee Exports Pvt. Ltd., India) on abdominal skin samples of Wistar rats. For conducting the experiment, diffusion cell with effective diffusion area of 2.303 cm^2 and volume of 24.5 ml was used at temperature and stirring speed maintained at 37 ± 0.5°C and 400 rpm, respectively. The methanolic drug solution, ME, MG and marketed gel containing equal amount of desonide (0.05% *w/w*) were carefully applied onto the donor compartments and the receptor compartments were filled with phosphate buffer pH 7.4 (drug solubility=0.93 mg/ml) to mimic physiological conditions [17]. At predetermined intervals (0, 1, 2, 4, 6, 8, 10, 12 and 24 hr), 1 ml sample was withdrawn from each receptor compartment and drug concentration was determined. The receptor compartment was replenished instantly after each withdrawl with equal volume of fresh phosphate buffer to maintain sink conditions [18].

Drug retention study

The effective skin diffusion area was carefully separated at end of the experiment, scraped gently and washed with methanol. The skin was homogenised into small pieces and vortexed with methanol. To ensure complete drug extraction all tested samples were soaked in methanol at 37°C for 24 h with continuous stirring at 100 rpm. The skin samples were kept in bath sonicator for 1 h and centrifuged at 8000 rpm for 20 min. The supernatant was filtered through 0.22 µm membrane filter and drug concentration was analyzed [19].

Fourier transform infrared spectroscopy

To investigate the interactions of MG with SC, Fourier Transform Infrared (FTIR) spectral studies were conducted. The abdominal skin was carefully excised from Wistar rats and subcutaneous tissue was removed surgically. The skin samples were incubated for 4 h with 0.1% trypsin solution in phosphate buffer (pH 7.4) for complete separation of SC from skin. The SC samples were cut into small circular discs (1.5 cm diameter). Equal volume of sodium chloride solution (0.9% *w/w)* was placed in different conical flasks over which the tissue samples were floated for 3 days for complete hydration. After hydration, FTIR spectra of each disc was recorded after 24 h of application of MG using ATR-FTIR spectrophotometer (Cary 630 FTIR, Agilent Technologies, US) and untreated skin sample was taken as control [20]. The FTIR graphs of desonide and MG were also studied to understand influence of formulation on skin.

Pharmacodynamic studies

Hair of dorsal area of BALB/c female mice were removed carefully using depilatory cream 48 h before the experiment. The animals were divided into six groups (n=6): Group I (Negative control), Group II (2,4- dinitrochloro benzene i.e. DNCB control), Group III (drug loaded MG), Group IV (marketed formulation).

AD was induced using method given by Lee *et al.* with some modifications [21]. Sensitization was caused by applying 0.1 ml of 1% DNCB dissolved in vehicle comprising acetone: Olive oil (4:1) solution on the dorsal skin of mice once daily for continuous three days. No formulation was applied for next 3 days and the mice were fed normally. On the seventh day, 0.1 ml of 0.25% DNCB was applied on the same area once daily for 3 days to induce dermatitis. To evaluate ear swelling, 0.1ml of 0.25% DNCB solution was applied inside the right earlobe of mice while same volume of vehicle was applied in the left ear. The negative control group was treated only with vehicle throughout the experiment. After induction of disease, MG and marketed gel were applied twice a day for 4 weeks on the Group III and IV while the negative control and disease induced (DNCB control) group did not recieve any treatment.

The scratching behavior and dermatitis score was recorded at the end of each week. The scratching score was assessed using previously established method reported by Kim *et al.* [22]. The total dermatitis score was calculated as sum of the individual scores for each of the four signs of AD including erythema, edema, erosion and dryness on a scale of 0 to 3 depending on severity of symptom. The mice were then sacrificed and the ear samples were collected using a biopsy punch of 6 mm diameter. The difference of thickness and weight between left and right ear was noted to determine extent of ear swelling [23].

After pharmacodynamic studies, skin tissues were excised and skin samples were prepared in 10% neutral buffered formalin, embedded in paraffin and cut into 4 µm thick sections. The sections were stained with Hematoxylineosin (HE) to evaluate epidermal hyperplasia and infiltration of immune cells in the dermis and toluidine blue for study of mast cells.

Measurement of serum IgE levels

After the final application, blood samples were collected from retro-obital venous plexus from each group and level of IgE were analysed using chemiluminescence immunoassay.

Determination of transepidermal water loss

The skin hydration and epidermal permeability barrier function was determined by measuring transepidermal water loss [24]. Animals were divided in five groups (n=3) and different formulations i.e. placebo carbopol gel, drug loaded carbopol gel, placebo MG, drug loaded MG and marketed gel were applied on dorsal skin of BALB/c mice twice daily. The transepidermal water loss (TEWL) was measured by Tewameter TM 300 (Courage and Khazaka, Germany) at predetermined time intervals. The laboratory temperature and humidity were maintained at 21 ± 2°C and 50 ± 5% RH, respectively.

Skin irritation study

For the determination of skin irritancy potential, Draize test was employed on Wistar rats [25]. An area (3×3cm) of the dorsal region of each animal was shaved 24 h before the study ensuring no sign of abrasion and animals were divided 6 groups (n=3).

Group I received no treatment and acted as negative control, group II received sodium lauryl sulfate solution (20% *w/v*) topically and acted as positive control. Group III, IV and V received topical treatment of drug loaded carbopol gel, MG and marketed formulation, respectively. The skin irritation score was noted for erythema and edema on scale of 0 to 4 after 24, 48 and 72 h.

Stability studies

The stability of MG was evaluated at different storage conditions i.e. 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH for duration of 3 months. Desonide loaded MGs were packed in 10g aluminium tubes (n=3) and samples were withdrawn at 0, 30, 60, and 90 days and were evaluated for physical and chemical stability [26].

Statistical analysis

The data were reported as mean ± SD of the obtained results. The data were statistically analyzed by using Student t- test and one-way analysis of variance (ANOVA) with the help of computer software Graphpad Prism 7.04 and $p<0.05$ was considered to be statistically significant unless mentioned otherwise.

Results & discussion

Microemulsion component screening

Based on the results of solubility studies, ethyl oleate was selected for preparation of microemulsion formulations (Figure 1a) Ethyl oleate is widely used as emollient leading to its suitability for application on dermatitic skin [27]. Previous literature reported non ionic surfactants to be biocompatible, less toxic and less affected by pH as compared to ionic surfactants [28]. For screening, various non ionic surfactants with high HLB value i.e. Tween 80, Tween 60, Tween 20, Cremophor EL, Transcutol and Labrasol were selected and drug solubility in these surfactants was found to be 19.43 ± 2.34 mg/ml, 16.70 ± 1.82 mg/ml, 15.40 ± 2.31 mg/ml, 6.56 ± 0.34 mg/ml, 12.33 ± 1.28 mg/ml and 18.12 ± 1.13 mg/ml, respectively. It is not necessary that a surfactant with good solubilising potential for drug would have desirable solubility in oil phase. Therefore, solubilisation capacity of all these surfactants in ethyl oleate was estimated (Figure 1b). The solubilization capacity of Tween 80 (19.20 ± 2.01%) was highest followed by Cremophor EL (15.31 ± 0.95%), Tween 60 (13.4 ± 1.10%), Transcutol (12.12 ± 1.05%) and Labrasol (6.12 ± 0.98%). Hence, suitability of Tween80 as surfactant for the present study was confirmed by its high drug solubility as well high solubilisation capacity for ethyl oleate.

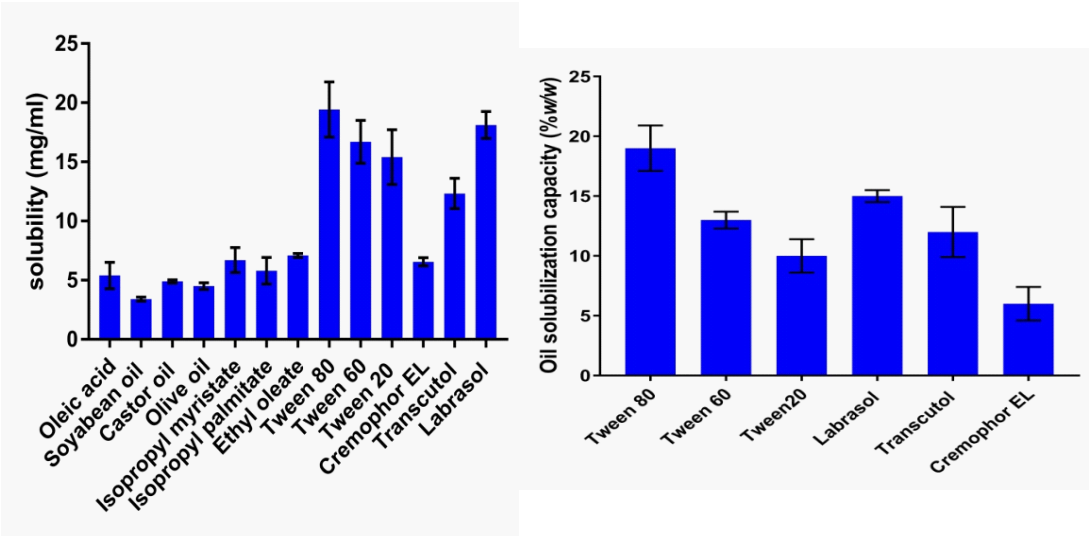

Figure 1: (a) Drug solubility in various components **(b)** Oil solubilisation capacity of surfactants (Values are reported as mean ± SD, n=6)

Alcohols have been reported to increase miscibility of oil and aqueous phases due to its partioning between two phases and thus increasing the fluidity of interface [29]. Therefore, different alcohols were included in cosurfactant screening. The area of ME region was compared at fixed S_{mix} (1:1) using the same surfactant (Tween 80) but replacing the cosurfactant for construction of different phase diagrams. The ME area was found to increase with increase in alkyl chain length of cosurfactant and it followed the order: Propylene glycol > butanol > isopropyl alcohol > ethanol = methanol (Figure S2, *supporting information*). From the results obtained propylene glycol was selected as cosurfactant for further study.

Formulation optimization

The summary of D-optimal mixture design comprising 16 experimental runs is provided in Table 1. The skin retention studies were conducted on Wistar rat skin after 24 h of formulation application. The droplet size (nm) and solubility (mg/ml) were determined 1 h after ME formation to ensure sufficient stabilization of ME system.

Table 1: Experimental runs used for optimizing desonide loaded ME system.

Formulation	Independent variables			Dependent variables		
	Component A: Oil (%w/w)	Component B: S_{mix} (%w/w)	Component C:Water (%w/w)	Y1: Droplet size (nm)	Y2: Solubility (mg/ml)	Y3: Skin retention (µg/cm²)
1	10.000	38.352	51.648	434.2	14.4	14.8
1*	10.000	38.352	51.648	432.1	14.1	14.6
2	8.549	35.000	56.451	275.7	13.5	17.1
2*	8.549	35.000	56.451	278.7	13.8	16.8
3	5.005	35.000	59.995	181.1	9.5	18.2
3*	5.005	35.000	59.995	183.1	9.3	18.5
4	9.996	45.000	45.004	386.8	14.2	16.4
4*	9.996	45.000	45.004	386.2	14.5	16.1
5	5.156	45.000	49.844	163	12.4	17.4
5*	5.156	45.000	49.844	163.6	12.8	18.2
6	5.000	38.692	56.308	165.6	11.5	18.8
7	7.809	37.799	54.392	177.6	12.2	18.4
8	9.513	42.045	48.441	359.9	14.9	15.8
9	7.228	40.647	52.125	211.9	11.4	18.2
10	5.004	40.819	54.176	186.2	10.1	18.4
11	7.643	44.870	47.488	170.2	13.2	18.6

Table 2 provides model summary statistics of the measured responses. For Y1 and Y3, cubic model showed a better fit while for Y2, linear model was selected as the best model based on the higher predicted and adjusted R^2 values and smallest Predicted Residual Error Sum of Squares (PRESS) values as compared to other models. Analysis of Variance (ANOVA) was used to obtain the polynomial equation of the measured responses. ANOVA was performed to evaluate the effect of individual components and interaction of variables on drug solubility in different ME formulations, skin retention and droplet size. A factor is considered to influence the response, if the magnitudes of the effects deviate significantly from zero and the results in $p<0.05$ (Table S1, *supporting information*).

Table 2: Model Summary Statistics of Measured Responses.

Response	Model	SD	R^2	Adjusted R^2	Predicted R^2	PRESS
Y1	Linear	50.81	0.7971	0.7659	0.7185	46556.30
	Quadratic	17.47	0.9816	0.9723	0.9575	7034.83
	Special cubic	18.12	0.9821	0.9702	0.9554	7376.06
	Cubic	5.09	0.9991	0.9976	0.8667	22043.69
Y2	Linear	0.67	0.8868	0.8694	0.8306	8.82
	Quadratic	0.65	0.9190	0.8785	0.8204	9.35
	Special cubic	0.67	0.9226	0.8709	0.7811	11.40
	Cubic	0.46	0.9761	0.9402	-2.3911	176.52
Y3	Linear	0.74	0.7977	0.7666	0.7142	9.94
	Quadratic	0.31	0.9717	0.9575	0.9360	2.22
	Special cubic	0.27	0.9812	0.9687	0.9502	1.73
	Cubic	0.036	0.9998	0.9994	0.9676	1.13

Effect of independent factors on desired responses

The effect of formulation components on the response Y1 to Y2 and Y3 is depicted in 3D contour plots (Figure 2). Equations iii, iv and v represents the mathematical equations of droplet size, solubility and skin retention, respectively.

Droplet size=
+62465.75162A - 1805.83100B + 475.85801 C- 971.43486A B

- 983.59171AC + 25.49443B C + 6.73329ABC - 4.33303AB (A - B)

-3.75363 AC(A-C)+0.40639BC(B-C) ...(iii)

Solubility = +0.85758A+0.093353B+0.044148C ...(iv)

Skin retention=
-476.37131A + 24.66619B-6.81532C + 7.50472AB + 7.17808AC-

0.33821BC-0.046920 ABC + 0.038959 A B(A-B) + 0.024263AC (A-C)-

5.46059E-003BC (B - C) ...(v)

Droplet size: The droplet size varied from 163 to 434.2 nm and lowest droplet size (Y1) was acquired with run 5 (A: 5.156%, B: 45%, C: 49.844%), where the percentage of S_{mix} was highest and percentage of water and oil was low. From equation (iii), it can be observed that increasing the concentration of oil and water lead to increase in the droplet size, although the collective interation of three independent variables resulted in decrease in droplet size.In a study aimed to statistically develop a ME system of itraconazole using D optimal mixture design, Kumar and Shishu reported that at lower level of oil (Capmul 908P), increasing S_{mix} (Tween 40-benzyl acohol) provided least droplet sizes due to better reduction of oil/water interfacial tension, allowing the reduction of ME droplet size [30]. Similarly, in the present study,the contour plot of droplet size (Figure 2a) showed that the droplet size increased with the increase in oil concentration, but this effect was reversed in case of S_{mix}.

Solubility: Solubility of desonide in the formulated MEs was highest when percentage of oil and S_{mix} was high. This is due to the need of higher oil and surfactant concentration to solubilize the drug. The contour plot of solubility and equation (iv) showed that oil content and proportion of S_{mix} has significant effect on drug solubility in MEs.

Skin retention: Several studies reported that skin retention of drug is inversely related to the droplet size of the formulation [31-33]. From equation (v) it was concluded that increasing concentration of oil phase in ME reduced drug retention. The increase in the droplet size due to increased oil proportion might led to reduced penetration of bigger oil droplets into deep layers of skin. Other two components i.e. water and S_{mix} had positive effect on desonide retention in skin. The increased proportion of S_{mix} resulted in reduction of droplet size and thereby the drug permeation was increased (Figure 2c).

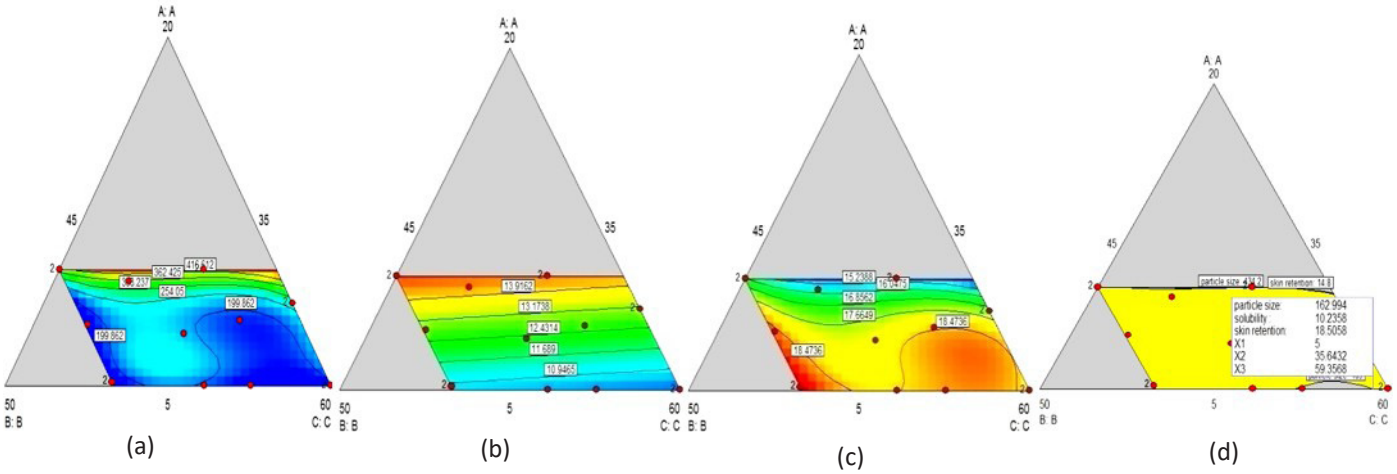

Figure 2: Graphs showing effects of formulation variables on (a) droplet size (b) solubility (c) skin retention and (d) overlay plot of optimized conditions for the desired responses of ME formulation.

Optimization based on desirability function

In this study the dependent responses i.e. drug solubility, droplet size and skin retention were assigned desirable goals and the desirability value lying closer to 1 was selected. Two solutions with highest desirability values i.e. 0.843 and 0.822 were selected and formulations F1 and F2 were prepared (Table 3). In order to ensure the reliability of the mathematical model, the Percentage Prediction Error (PPE) was calculated [34]. The lower values of PPE (<5) indicates robustness and high predictive ability of the experimental model. The formulation F1 showed better correlation between the observed and experimental values with minimal percentage predictionl error (Table 3). Hence, ME formulation with oil, S_{mix} and water concentration of 5%, 35.643% and 59.357% *w/w* respectively, was selected for further study. Figure 2d shows the overlay plot showing the optimized parameters in the design space to obtain desired responses for optimized ME.

Table 3: Comparison of predicted and experimental values of the selected responses for optimized ME formulations.

Formulations	Oil (% *w/w*)	S_{mix} (%*w/w*)	Water (%*w/w*)	Droplet size (nm)	Solubility (mg/ml)	Skin retention (µg/cm²)	Desirability
F1	5.000	35.643	59.357	162.999	10.235	18.505	**0.843**
Experimental value				163.2	10.218	18.411	
Percentage prediction error				-0.184	0.166	0.540	
F2	5.005	35.000	59.995	183.851	10.208	18.205	0.822
Experimental value				189.5	10.216	17.695	
Percentage prediction error				-3.072	-0.078	2.801	

Physicochemical characterization of optimized microemulsion formulation

The average droplet size of optimized ME was 163.2 ± 0.09 nm with PDI of 0.231. The droplet size was found to be within the range of ME systems i.e. 20-200 nm as reported in literature [35]. The close to zero value of PDI indicated that ME droplets were uniform in nature and had narrow size distribution. The higher zeta potential of ME (-32.5 ± 0.6) ensured minimum particle aggregation and stability of the system (Figure S3, *supporting information*). The negative zeta potential of ME indicated steric repulsive forces of hydrocarbon chains of oil phase preventing aggregation with neighbouring oil droplets as stated in previous studies [36]. TEM is an important technique for evaluating the microstructures of droplets of MEs. The droplets of optimized ME appeared as non aggragated and almost spherical in shape. The droplet size was observed to be within the range 100-200 nm which is in concordance with dynamic light scattering study (Figure 3).

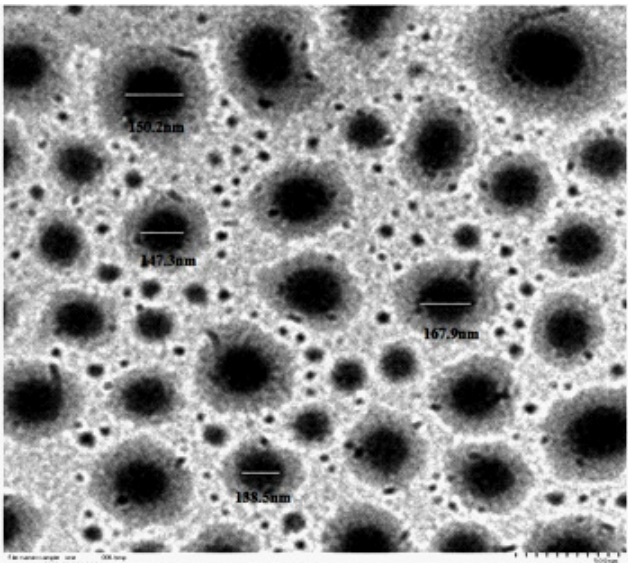

Figure 3: Representative TEM photograph of desonide loaded ME.

***Ex vivo* skin permeation study**

The skin permeation and deposition parameters of the tested formulations are represented in Table 4 and relationship of the amount of drug permeated with respect to time is depicted graphically in Figure 4. The order of drug permeation through different tested formulations was found to be as follows: methanolic drug solution> ME> MG> marketed gel. The higher drug permeation with methanolic drug solution could be due to penetration enhancement properties of methanol along with the evaporative loss of methanol with time leading to increased concentration gradient of desonide in the donor compartment [37].

Both the ME and MG showed significantly higher drug permeation flux values ($p<0.01$) as comapared to marketed gel indicating improved drug delivery through ME based systems. The ME systems were reported to increase skin permeation in several studies. The possible reason for the increased permeation could be the ME components. The role of ethyl oleate in improving drug permeation was reported in a similar study by Chen et al. on MEs consisted 6% ethyl oleate as oil phase. The microemulsions increased the permeation rate of ibuprofen 5.72–30.0 times over the saturated solution mainly due to high concentration gradient of ibuprofen and permeation enhancing ability of ethyl oleate [38]. In another study, ethyl oleate was reported to disrupt fluidity of the stratum corneum and non ionic surfactants were also reported to emulsify sebum thereby increasing the drug permeation through skin [39]. The large amount of inner oil phase and small droplet size of MEs also assists in settling down of droplets in close contact with the skin resulting in enhanced skin permeation [40].

Negi *et al*. reported that addition of Carbopol 934 into ME decreased the permeability of drugs (lidocaine, prilocaine) due to high viscosity of hydrogel than that of ME, accounting for retardation of mobility and consequently, the permeation of drugs [41]. In another study, prilocaine ME showed upto 10 times greater penetration than prilocaine hydrogel [42]. Similarly, in the present study, MG showed slightly lower drug permeation as compared to ME which can be attributed to slow drug diffusion through the gel network.

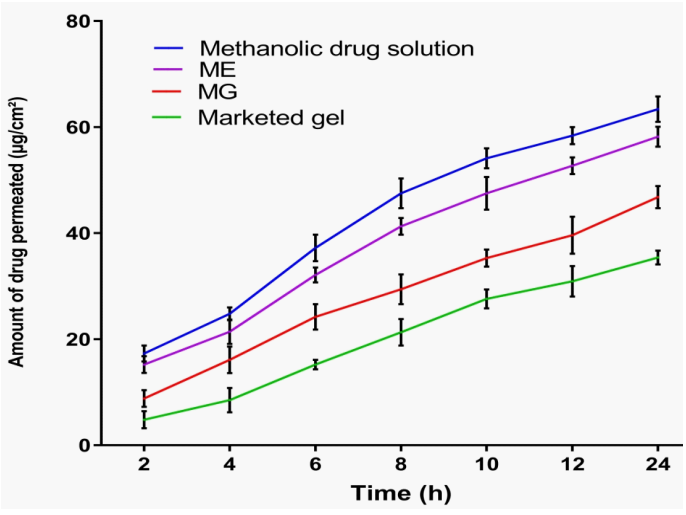

Figure 4: *Ex vivo* permeation profiles of desonide across hairless rat skin from different formulations (Donor dose =0.05% *w/w*). Each point represents mean ± SD, n=3; ME= microemulsion; MG=micoemulsion gel

Drug retention study

The small droplet size of ME resulted in increase of drug accumulation in skin. Both ME and MG resulted in significantly high drug deposition ($p<0.05$) as compared to commercial gel (Table 4). The highest amount of desonide was retained with MG which did not show highest skin permeation rate indicating no direct correlation between drug retention and permeation flux. Maximum drug retention with MG can be related to high viscosity of the gel, converting ME into a highly ordered microstructure and slowing down the migration of drug from the site of application [43]. The results are consistent with a similar study conducted by Tung *et al.* on topical hydrogel containing betamethasone dipropionate ME. They observed a correlation between viscosity of the topical formulation and drug retention in skin. Their study provided comparative data on skin retention of drug using different gelling agents and concluded that polymers imparting high viscosity to gels helped to stabilize the systems and maintain the ME-hydrogels on the skin for longer periods [44]. Enhancement Ratio (ER) of permeation flux and drug deposition from MG with respect to marketed gel was 1.570 and 3.028, respectively. The *ex vivo* studies revealed that MG provided maximum drug retention suggesting its suitability as effective delivery system for desonide in treatment of skin diseases.

Table 4: *Ex vivo* skin permeation and deposition parameters of desonide across rat skin through different formulations.

Parameters	Flux (µg/cm²/h)	Drug deposited (µg)	ER[1]	ER[2]
MG	1.272 ± 0.4	181.1 ± 1.5	1.570	3.028
ME	1.514 ± 0.6	142.4 ± 2.6	1.869	2.381
Methanolic drug sol	2.198 ± 0.5	22.7 ± 1.2	2.713	0.379
Marketed gel	0.810 ± 0.2	59.8 ± 2.2	---	---

ER[1]= Ratio of permeation flux from test formulation to marketed formulation; ER[2]= Ratio of concentration of drug deposited into rat skin from test formulation to marketed formulation (Values are reported as mean ±SD, n=3).

Fourier transform infrared spectroscopy

The spectrum of desonide exhibit peak at 3489 cm^{-1} (NH_2 stretching), 3362 cm^{-1} (OH stretching), 2952 cm^{-1} (CH_3 antisymmetric stretching), 2920 cm^{-1} (CH_2 antisymmetric stretching), 2870 cm^{-1} as well as shoulder correspond to both CH and CH_2 symmetric stretching. The peak at 1707 cm^{-1} correspond to carboxylic acid stretching, 1650 cm^{-1} depicts amide stretching, 1610 cm^{-1} depicts aromatic C=C stretching, 1400-1500 cm^{-1} represents CH bending modes and 1275 cm^{-1}-1100 cm^{-1}shows C-O stretching. MG exhibit peaks at 3340 cm^{-1}(H-O-H stretching) and 1640 cm^{-1} (H-O-H bending) and the presence of drug is evidenced by tracing the peaks at 2929 $cm^{-1}$and shoulder peak ~1710 cm^{-1}.

Topical MEs have been reported to perturbate the lipid bilayer structure of SC which minimizes its barrier function and thus, enhance drug transfer across skin [45]. The FTIR spectra for the untreated skin shows bands at 3280 cm-1 (OH stretching), 2922 cm-1 (CH2 antisymmetric stretching), 2850 cm-1 (CH2 symmetric stretching), 1625 cm-1 (amide I peak), 1543 (amide II peak), absorption peaks observed at wavenumbers 1237 cm-1 and 1088 cm−1 are assigned to the antisymmetric and symmetric stretching of ionized phosphate, respectively. In the spectra of MG treated SC, the CH stretching regions of SC are heavily overlapped with the drug peaks, similarly, amide I regions are overlapped with MG and drug. So, the non-overlapping amide bands were studied to understand the impact of formulation on the skin.The amide bands are recognized as the sensitive markers to understand the protein secondary structure as they are involved in H-bonding and any influence of permeation enhancers would cause a shift in the peak positions [46]. The amide II bands were observed at 1543 cm-1 and 1551 cm-1 in untreated and MG applied SC sample, respectively (Figure 5). This indicates that the developed MG formulation induced protein secondary structure changes leading to enhanced drug permeation.

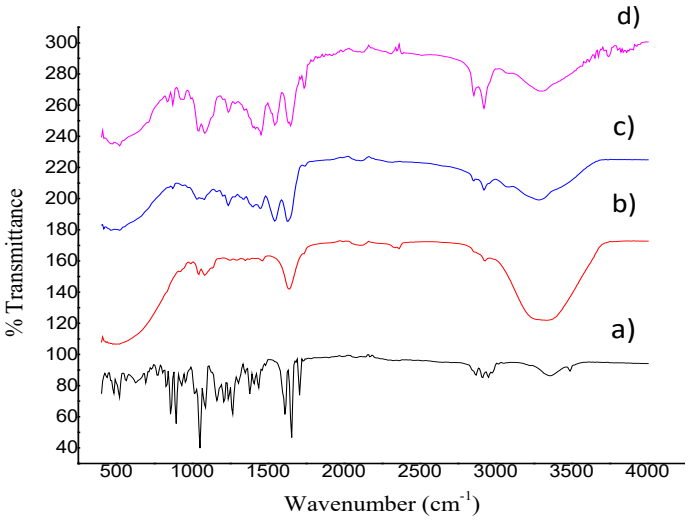

Figure 5: Fourier transform infrared spectrum of **(a)** Desonide **(b)** MG **(c)** Untreated SC **(d)** MG treated SC.

Pharmacodynamic studies

With treatment of DNCB, pruritus, erythema, edema and formation of scabs were observed within ten days indicating induction of dermatitis. After inducing dermatitis, scratching frequency was recorded at the end of every week upto four weeks for each group (Table 5). It was observed that after 4 weeks of topical application of DNCB on mice skin, the rate was gradually increased upto 64.4 ± 1.2 scratches/20 min. In MG treated group, scratching rate was markedly greater as compared to healthy group during the first week (38.1 ± 3.4 scratches/20 min), which was reduced to 14.2 ± 0.8 scratches/20 min after 4 weeks of application. A similar trend was observed in marketed gel treated group. The DNCB control, MG and marketed gel treated mice showed significantly high scratching rate than intact vehicle control mice at the end of the experimental period ($p<0.05$). However, when compared with the DNCB control group, both MG and marketed gel resulted in significant reduction in scratching rate ($p<0.05$). Further, the reduction in scratching behaviour was found to be significantly better in case of MG treated group ($p<0.05$) when compared with marketed gel, indicating improved efficacy of ME based gel system in treatment of AD.

Table 5: Effect of formulations on scratching behavior in mice observed upto 4 weeks.

Days after initial sensitization with DNCB	Control		MG treated	Marketed gel treated
	Negative control	DNCB control		
7	7.8 ± 2.4	52 ± 3.7	38.1 ± 3.4	42.1 ± 5.3
14	8.2 ± 2.8	72.2 ± 8.5	24 ± 4.8	35.5 ± 3.2
21	9.5 ± 3.1	68 ±.7.2	20.8 ± 3.7	29.6 ± 4.8
28	10.4 ± 2.5	64.4 ± 6.5	14.2 ± 2.6	23.4 ± 3.8

Ear swelling is one of the characteristic features of DNCB induced dermatitis. After repeated application for 4 weeks on left ear, the ear swelling was evaluated considering right ear as control for each animal. When compared with the DNCB control group, both MG and marketed gel resulted in significant decrease of the ear swelling and ear thickness ($p<0.05$) (Figure 6a-b). Further, significant reduction ($p<0.05$) in ear swelling was observed when MG treated group was compared with marketed gel.

After application of MG and marketed gel for 4 weeks on daily basis, the skin appeared to be healed and scabs were observed to be desquamated. Both marketed gel and MG were able to significantly control characterstic dermatitis symptoms. Total dermatitis score was reduced significantly in MG treated group as compared to DNCB control and marketed gel treated group ($p<0.05$) with marked reduction in erythema, edema and erosion (Figure 6c). The reason for enhanced drug action by MG could be the nanosize of droplets leading to better permeation of desonide along with improved skin retention by carbopol gel.

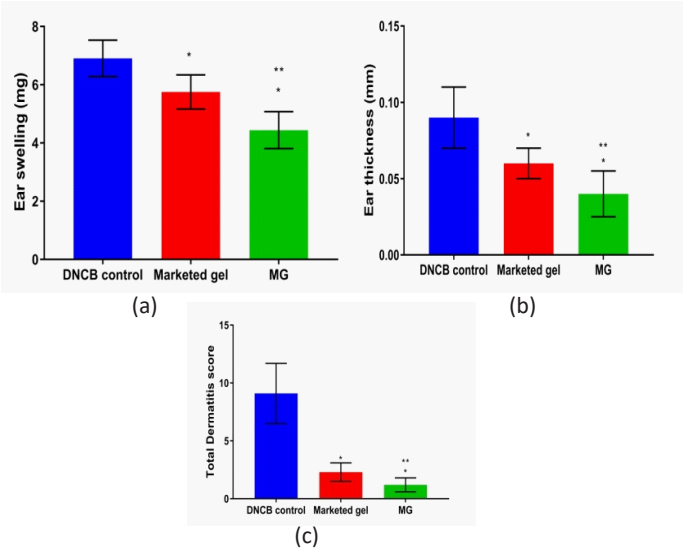

Figure 6: Effect of formulations on **(a)** Ear swelling **(b)** Ear thickness **(c)** Total dermatitis score after 4 weeks of continous treatment on mice skin after sensititized by DNCB. The values for healthy group were considered to be zero for each study.Values are reported as mean ± SD, n=6, *p<0.05 as compared to DNCB control, **p<0.05 as compared to marketed gel. DNCB= 2,4- dinitrochloro benzene; MG=micoemulsion gel.

The microphotographs of histological studies are presented in Figure 7. The vehicle treated skin resembled the normal skin showing well defined epidermal and dermal layers. No inflammatory cells were observed in dermis (Figure 7a). The DNCB control group showed inflammatory changes with presence of eosinophils along with spongiosis as observed by loosening of tissue structure of the dermis and formation of vacoules. MG treated group showed moderate improvement in the epithelial tissue with reduced inflammatory infiltration. Reduction in the inflammatory cell infilteration was also observed in marketed gel treated group as compared to induced group, however loosening of dermis was prominent. The mast cells were found to be increased significantly in DNCB control group. However, a marked reduction in mast cells was observed in MG and marketed gel treated skin samples.

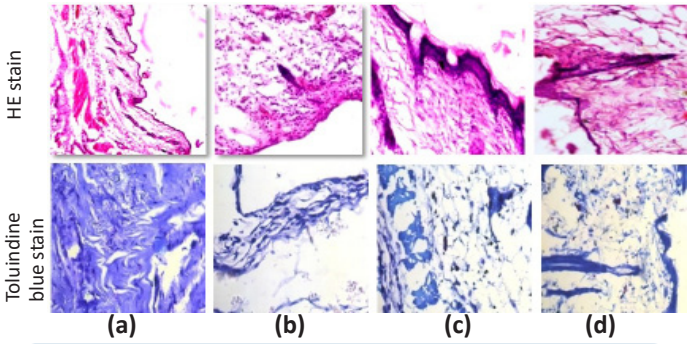

Figure 7: Microphotographs of mice skin samples stained with HE and toluidine blue. **(a)** Normal skin, **(b)** Dermatitis induced, **(c)** MG treated and **(d)** Marketed gel treated skin. (Red and black arrow represent eosinophils and mast cells, respectively).

Measurement of serum IgE levels

Elevation in serum IgE level is often correlated with severity of AD. The serum IgE level increased markedly in dermatitis induced mice. However, serum IgE levels were significantly decreased after topical treatment with MG and marketed gel ($p<0.05$) as compared to DNCB control group. The mean serum IgE level in healthy mice was found to be 410.7 ± 22.5IU/ml. This level was elevated upto 775 ± 31.8 IU/ml after induction of AD in Group II. The values were found to decrease upto 434 ± 28.6 IU/ml and 539 ± 18.4 IU/ml after topical application of MG and marketed gel, respectively. Further, significant reduction by MG was observed ($p<0.05$) when compared with marketed gel indicating its potential in treatment of AD.

Determination of transepidermal water loss

TCs even at low potency have been reported to cause impairement of epidermal function [47]. The most important objective for developing nanocarrier based topical delivery system of desonide was to minimize drug associated local and systemic side effects. In a previous investigation, Sul *et al.* used TEWL studies to determine impairment of epidermal permeability barrier function to investigate the role of multi lamellar emulsion in controlling steroid associated local side effects. The study reported that after 3 days application, increase of TEWL was significant in only the commercial desonide products treated site but not in MLE based desonide products (cream and lotion, 0.05%) treated site as compared to control group [48]. In the current study, significantly high TEWL was observed for both drug loaded carbopol gel (3.01 ± 0.6 g/m^{2}h) and marketed gel (2.52 ± 0.08 g/m^{2}h) containing free form of desonide as compared to drug loaded MG (2.12 ± 0.06 g/m^{2}h) ($p<0.05$). The results suggested that the active ingredient caused disruption of skin barrier function which could be minimized by encapsulation of desonide in ME based gel system. The values of TEWL in animals treated with drug loaded MG (2.12 ± 0.06 g/m^{2}h) were not found to be significantly different from that of the placebo

MG group (2.08 ± 0.08 g/m²h) (p>0.05). This indicates that no skin disruption was caused due to local application of desonide through MG (Figure 8). Moreover, no significant difference in the value of MG treated group (2.12 ± 0.06 g/m²h) and placebo carbopol gel treated group (2.02 ± 0.07 g/m²h) was found (p>0.05), indicating the ability of the developed formulation to reduce drug associated side effects.

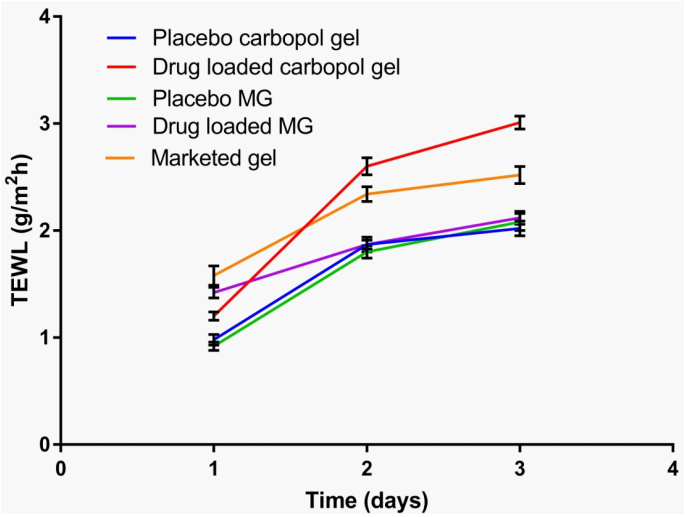

Figure 8: Changes in TEWL of mice dorsal skin following exposure to tested formulations for 72 h.

Skin irritation study

The degree of erythema and edema were assessed on the scale of 0 to 4 where, score of 0 to 0.9 indicates non-irritant nature of the given treatment while score of 1 to 1.9 is considered as mild irritant and a score of 2 to 3 is contemplated as "not safe for topical use". Aqueous SLS solution (20% *w/v*) caused severe erythema indicating its high irritation potential as reported in previous studies [25]. No sign of skin irritancy such as erythema, edema or itching was observed in MG treated animal skin after 24, 48 and 72 h. Marketed formulation resulted in appearance of slight erythema and no edema (Table 6). The results confirmed safety of developed ME system for topical use.

Table 6: Mean skin irritation score observed after application of tested formulations at the end of 24, 48 and 72 h.

Treatment	Erythemal scores			Edemal scores		
	24 h	48 h	72 h	24 h	48 h	72 h
Control	0	0	0	0	0	0
SLS solution (20% *w/v*)	1	2	4	1	2	3
Carbopol gel	0	0	1	0	0	1
MG	0	0	0	0	0	0
Marketed gel	0	0	1	0	0	0

Values are reported as mean (n=3)

Stability studies

The physical stability was assessed by observing change in odour, colour, physical appearance, viscosity and droplet size of gel and chemical stability was evaluated by determination of pH and drug content (Table 7). The gel formulations exposed to different stability conditions were found to be homogenous with no change in color intensity or odour throughout the study period. The results of stability studies showed significant degradation at all storage conditions (p<0.05). The rate of degradation was found to increase with increase of temperature. Although the consistency and clarity of gel remained intact throughout the study. MG displayed no signs of phase separation, flocculation, cloudiness or any precipitation. The pH and viscosity exhibited no significant variation at different conditions. The droplet size of MG was found to increase gradually with time period at each stability condition. The droplet size was found to be in the range 177.2-285.4 nm, which is greater than usual droplet size of ME i.e. 20-200 nm. The increased droplet size could be attributed to the increased viscosity of the gel systems as suggested by previous literature [49,50]. A similar trend was reported by Hashem and coresearchers during the stability studies of ME based gel systems of clotrimazole during 6-month storage period [51]. Their study reported gradual growth in the mean droplet size along with increase in drug degradation with the time of storage.

Table 7: Stability study data in respect of pH, viscosity and percentage drug content of drug loaded MG for 3 months at various stability conditions.

Condition	Month	pH	Viscosity (cP)	Drug content (% *w/w*)	Droplet size (nm)
25 ± 2°C / 60± 5% RH	0	5.44 ± 0.05	450.26 ± 0.07	101.2 ± 3.1	177.2 ± 1.8
	1	5.43 ± 0.04	448.14 ± 0.12	99.5 ± 2.1	181.1 ± 2.8
	2	5.41 ± 0.02	445.6 ± 0.82	98.7 ± 0.2	183.2 ± 1.7
	3	5.49 ± 0.08	443.4 ± 0.15	97.5 ± 0.8	211.5 ± 2.4
30 ± 2°C / 65± 5% RH	0	5.42 ± 0.04	446.46 ± 0.91	99.9 ± 2.1	177.8 ± 2.2
	1	5.41 ± 0.02	445.34 ± 0.55	99.6 ± 0.6	186.1 ± 1.5
	2	5.40 ± 0.08	442.15 ± 0.72	97.5 ± 1.2	223.2 ± 3.2
	3	5.48 ± 0.05	440.34 ± 0.11	96.7 ± 1.5	275.0 ± 1.5
40 ± 2°C /75± 5% RH	0	5.45 ± 0.06	448.58 ± 0.73	100.1 ± 1.8	176.5 ± 2.7
	1	5.43 ± 0.07	445.12 ± 0.05	98.5 ± 0.5	212.2 ± 3.2
	2	5.49 ± 0.01	444.65 ± 0.18	96.6 ± 0.9	278.7 ± 2.7
	3	5.48 ± 0.03	441.50 ± 0.26	95.8 ± 1.3	285.4 ± 3.6

(Values are reported as mean ± SD, n=3)

Conclusion

In this study, components of microemulsion were optimized by employing D-optimal mixture statistical design to achieve optimum droplet size, drug loading and drug retention on skin. The optimal ME formulation was selected and further converted into gel using carbopol 940. The average droplet size of optimized ME was 163.2 ± 0.09 nm with PDI of 0.231 and zeta potential of -32.5 ± 0.6 mV indicating uniform particle size distribution and good physical colloidal stability. TEM revealed spherical droplets having nanoparticulate size distribution with neglible coalescence. The final formulation showed physical stability during three months storage at different stability conditions. The marked reduction in transepidermal water loss with desonide loaded microemulsion based gel ensured its potential in minimizing drug associated side effects. The microemulsion gel showed better activity as compared to marketed formulation owning to the characteristics of microemulsion such as small droplet size, permeability enhancing components, improved skin retention and targeted drug delivery. The developed desonide loaded microemulsion gel system resulted in improved drug retention along with increased permeation across skin and found to be safe for topical use in the skin irritation studies.The histopathological studies assured desired efficacy of the formulation. Thus, microemulsion based gel could be a promising formulation in treatment of AD as compared to the conventional vehicles.

Acknowledgements

The authors highly acknowledge the guidance of Dr. Surinderpal Singh (Government Medical College, Amritsar) and Dr. Mridu Manjari (Shri Guru Ram Das Medical College, Amritsar) for providing guidance during histopathological studies. The authors are grateful to University Grants Commission (UGC), Delhi for University with Potential for Excellence (UPE) and Department of Science and Technology (DST), Government of India PURSE, CPEPA and FIST schemes for strengthening infrastructure of Guru Nanak Dev University, Amritsar.

Human and animal rights declaration

No humans were used for studies that are the basis of this research. The reported experiments are in accordance with the standards set forth in the Committee for the Purpose of Control and Supervision of Experiments on Animals under Ministry of Environment, Forests and Climate Change, Government of India.

References

1. Almawi WY, Melemedjian OK. Molecular mechanisms of glucocorticoid antiproliferative effects: antagonism of transcription factors activity by glucocorticoid receptor. Journal of Leukocyte Biology. 2002; 71: 9-15.
2. Darsow U, Lubbe J, Taiib A, Wollenberg A, Seidenari S. Position paper on diagnosis and treatment of atopic dermatitis. Journal of the European Academy of Dermatology and Venereology. 2005; 19: 286-295.
3. Mezei M, Gulasekharam V. Liposomes: A selective drug delivery system for the topical route of administration. Life Sciences. 1980; 26: 1473-1477.
4. Kahane NR, Gelbard CG, Hebert AA. Desonide: A review of formulations, efficacy and safety. Expert Opinion on Investigational Drugs. 2008; 7: 1097-1104.
5. Rosa P, Snovarski Salla AP, Da Silva C, Rolim C, Adams A. Investigation of the Stabilizing Effects of Antioxidants and Benzophenone-3 on Desonide Photostability. AAPS PharmSciTech. 2014; 15: 1155-1162.
6. Freeman S, Howard A, Foley P, Rosen R, Wood G, et al. Efficacy, cutaneous tolerance and cosmetic acceptability of desonide 0.05% lotion (Desowen) versus vehicle in the short - term treatment of facial atopic and seborrheic dermatitis. The Australasian journal of dermatology. 2002; 45: 186-189.
7. Korting HC, Kerscher MJ, Schafer-Kortin M. Topical glucocorticoids with improved benefit/risk ratio: do they exist. Journal of the American Academy of Dermatology. 2007; 27: 87-92.
8. Schwarz JS, Weisspapir MR, Friedman DI. Enhanced transdermal delivery of diazepam by submicron emulsion (SME) creams. Pharmaceutical Research. 1995; 12: 687-692.
9. Chen H, Moub D. Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. International Journal of Pharmaceutics. 2007; 341: 78-84.
10. Braga MP, Dalcin TCS, Rosa, P, Codevilla CF, da Silva CB, et al. Assay and photodegradation kinetics of desonide lotion by an lc-uv stability-indicating method. Journal of Liquid Chromatography & Related Technologies. 2014; 37: 1968-1984.
11. Patel HK, Barot BS, Parejiya PB, Shelat PK, Shukla A. Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo: Ex vivo permeation and skin irritation studies. Colloids and Surfaces B: Biointerfaces. 2013; 102: 86-94.
12. Coneac G, Vlaia V, Olariu I, Mut AM, Anghel DF, et al. Development and evaluation of new microemulsion based hydrogel formulations for topical delivery of fluconazole. AAPS PharmSciTech. 2015; 16: 889-904.
13. Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, et al. Nanoemulsion components screening and selection: a technical note. AAPS PharmSciTech. 2009; 10: 69-76.
14. Holm R, Jensen IH, Sonnergaard J. Optimization of self-microemulsifying drug delivery systems (SMEDDS) using a D-optimal design and the desirability function. Drug Development and Industrial Pharmacy. 2006; 32: 1025-1032.
15. Aboofazeli R, Barlow D, Lawrence MJ. Particle size analysis of concentrated phospholipid microemulsions II: photon correlation spectroscopy. AAPS PharmSciTech. 2000; 2: 1-10.
16. Sharma K, Bedi N. Microemulsion based hydrogel of mycopheolate mofetil for the treatment of psoriasis. Current Trends in Biotechnology and Pharmacy. 2014; 8: 354-371.
17. Chen H, Mou D, Du D, Chang X, Zhu D, et al. Hydrogel- thickened microemulsion for topical administration of drug molecule at an extremely low concentration. International Journal of Pharmaceutics. 2007; 341: 78-84.
18. Chudasama A, Patel V, Nivsarkar M, Vasu K, Shishoo C. Investigation of microemulsion system for transdermal delivery of itraconazole. Journal of Advanced Pharmaceutical Technology and Research. 2011; 2: 30-38.
19. Talaat SM, Elnaggar YSR, Abdalla OY. Lecithin microemulsion lipogels versus conventional gels for skin targeting of terconazole: in vitro, ex vivo, and in vivo investigation. AAPS PharmSciTech. 2019; 20: 1-20.
20. Khurana S, Jain NK, Bedi PMS. Nanoemulsion based gel for transdermal delivery of meloxicam: Physico-chemical, mechanistic investigation. Life Sciences. 2013; 92: 383-392.

21. Lee KS, Jeong ES, Heo SH, Seo JH, Jeong DG, et al. A Novel Model for Human Atopic Dermatitis: Application of Repeated DNCB Patch in BALB/c Mice, in Comparison with NC/Nga Mice. Laboratory Animal Research. 2010; 26: 95-102.

22. Kim CG, Kang M, Lee YH, Min WG, Kim YH, et al. Bathing effects of various seawaters on allergic (atopic) dermatitis-like skin lesions induced by 2,4-dinitrochlorobenzene in hairless mice. Evidence-Based Complementary and Alternative Medicine. 2015; 1-25.

23. Shrotriya SN, Ranpise NS, Vidhate BV. Skin targeting of resveratrol utilizing solid lipid nanoparticle-engrossed gel for chemically induced irritant contact dermatitis. Drug Delivery and Translational Research. 2017; 7: 37-52.

24. Rogiers V. Transepidermal water loss measurements in patch test assessment: the need for standardisation In: Irritant Dermatitis: New Clinical and Experimental Aspects; Elsner P, Maibach HI, Ed.; Current Problems in Dermatology; Basel, Karger, 1995; 23: 152-158.

25. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substance applied to the skin and mucous membranes. Journal of Pharmacology and Experimental Therapeutics. 1944; 82: 377-390.

26. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian Journal of Pharmaceutical Sciences. 2016; 52: 493-507.

27. Rigoni C, Cantu AM, Gelmetti C. Observational clinical study of a new emollient in 26 patients with atopic dermatitis. European. Journal of Pediatric Dermatology. 2018; 28: 218-225.

28. Constantinides PP. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharmaceutical Research. 1995; 12: 1561-1572.

29. Tenjarla S. Microemulsions: an overview and pharmaceutical applications. Critical Reviews in Therapeutic Drug Carrier Systems. 1999; 16: 461-521.

30. Kumar N, Shishu. D-optimal experimental approach for designing topical microemulsion of itraconazole: Characterization and evaluation of antifungal efficacy against a standardized Tineapedis infection model in Wistar rats. European Journal of Pharmaceutical Sciences: Official Journal of The European Federation for Pharmaceutical Sciences. 2015; 67: 97-112.

31. Hua L, Weisan P, Jiayu L, Ying Z. Preparation, evaluation, and NMR characterization of vinpocetine microemulsion for transdermal delivery. Drug Development and Industrial Pharmacy. 2004; 30: 657-666.

32. Ceschel G, Bergamante V, Maffei P, Lombardi Borgia S, Calabrese V, et al. Solubility and transdermal permeation properties of a dehydroepiandrosterone cyclodextrin complex from hydrophilic and lipophilic vehicles. Drug Delivery. 2005; 12: 275-280.

33. Shah KA, Date AA, Joshi MD, Patravale VB. Solid lipid nanoparticles (SLN) of tretinoin: Potential in topical delivery. International Journal of Pharmaceutics. 2007; 345: 163-171.

34. Chouhan P, Saini TR. D-optimal Design and Development of Microemulsion Based Transungual Drug Delivery Formulation of Ciclopirox Olamine for Treatment of Onychomycosis. Indian Journal of Pharmaceutical Sciences. 2016; 78: 498-511.

35. Talegaonkar S, Azeem A, Ahmad FJ, Khar RK, Pathan SA, et al. Microemulsions: a novel approach to enhanced drug delivery. Recent Patents on Drug Delivery & Formulation. 2008; 2: 238-257.

36. Peltola S, Saarinen SP, Kiesavaara J. Microemulsions for topical delivery of estradiol. International Journal of Pharmaceutics. 2003; 254: 99-107.

37. Goates CY, Knutson K. Enhanced permeation of polar compounds through human epidermis I. Permeability and membrane structural changes in the presence of short chain alcohols. Biochimica Et Biophysica Acta. 1994; 1195: 169-179.

38. Chen H, Chang X, Du D, Li J, Xu H, et al. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. International Journal of Pharmaceutics. 2006; 315: 52-58.

39. Javadzadeh Y, Shokri J, Hallaj-Nezhadi S, Hamishehkar H, Nokhodchi, A. Enhancement of percutaneous absorption of finasteride by cosolvents, cosurfactant and surfactants. Pharmaceutical Development and Technology. 2010; 15: 619-625.

40. Ajith S, Rakshit AK. Studies of mixed surfactant microemulsion systems: Brij 35 and Tween 20 and sodium dodecyl sulfate. Journal of Physical Chemistry. 1995; 99: 14778-14783.

41. Negi P, Singh B, Sharma G, Beg S, Raza K, et al. Phospholipid microemulsion-based hydrogel for enhanced topical delivery of lidocaine and prilocaine: QbD-based development and evaluation. Drug Delivery. 2016; 23: 951-967.

42. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterisation and transdermal drug delivery potential of microemulsion systems. Journal of Controlled Release: Official Journal of the Controlled Release Society. 2000; 69: 421-433.

43. Zhang J, Michniak-Kohn B. Investigation of microemulsion microstructures and their relationship to transdermal permeation of model drugs: Ketoprofen, caffeine and lidocaine. International Journal of Drug Delivery. 2011; 421: 34-44.

44. Tung NT, Vu VD, Nguyen PL. DoE-based development, physicochemical characterization, and pharmacological evaluation of a topical hydrogel containing betamethasone dipropionate microemulsion. Colloids and Surfaces. B, Biointerfaces. 2019; 181: 480-488.

45. Lee J, Lee Y, Kim J, Yoon M, Choi YW. Formulation of microemulsion systems for transdermal delivery of aceclofenac. Archives of pharmacal research. 2005; 28: 1097-1102.

46. Van de Weert M, Haris PI, Hennink WE, Crommelin DJ. Fourier transform infrared spectrometric analysis of protein conformation: Effect of sampling method and stress factors. Analytical biochemistry. 2001; 297: 160-169.

47. Kao J, Fluhr JW, Man M, Fowler AJ, Hachem J, et al. Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of epidermal lipid synthesis accounts for functional abnormalities. Journal of Investigative Dermatology. 2003; 120: 456-464.

48. Sul GD, Park HJ, Bae JH, Hong KD, Park BD, et al. Preventive effects of multi-lamellar emulsion on low potency topical steroid induced local adverse effect. Annals of Dermatology. 2013; 25: 5-11.

49. Yilmaz E, Borchert HH. Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema-an in vivo study. International journal of pharmaceutics. 2006; 307: 232-238.

50. Shahin M, Hady SA, Hammad M, Mortada N. Novel jojoba oil based emulsion gel formulations for clotrimazole delivery. AAPS Pharm. Sci. Tech. 2011; 12: 239-247.

51. Eid AM, El-Enshasy HA, Aziz R, Elmarzugi NA. Preparation, characterization and anti-inflammatory activity of Swietenia macrophylla nanoemulgel. Journal of Nanomedicine and Nanotechnology. 2014; 5: 1-10.