Abstract

Mycophenolate mofetil (MMF), the morpholinoethyl ester of mycophenolic acid is an immunosuppressive agent used to prevent organ rejection after kidney and heart transplant. The drug seems to be effective in dermal diseases mainly psoriasis. However, till date it can be only be administered using systemic route which is often associated with side effects such as nausea, leucopenia, sepsis and diarrhea. The aim of the present study was to develop microemulsion based hydrogel for topical delivery of mycophenolate mofetil and to investigate its in vitro release and its potential in treating psoriatic inflammation using imiquimod induced skin inflammation animal model. Pseudoternary phase diagrams were constructed and on the basis of microemulsion existence range, various formulations were developed using oleic acid, Tween80, propylene glycol and distilled water as oil phase, surfactant, cosurfactant and aqueous phase, respectively. The selected formulations were subjected to physical stability studies and consequently to various physicochemical characterization. The optimized formulation (F2) consisting of 6.06% v/v of oleic acid 36.36% v/v of Tween80 and 18.18% v/v of propylene glycol has shown a globule size of 124 nm, refractive index 1.421, zeta potential -34.35 ±0.051 mV, pH 5.9 and conductivity value of 104 µS cm⁻¹. The permeability of drug from microemulsion after 24 h was observed to be 69.52%. Carbopol 940 was used to convert microemulsion into microemulsion based hydrogel to improve its viscosity for topical administration and was characterized. The histopathological studies performed on mice skin revealed that the treated skin showed complete clearance of hyperkeratotic plaques and a significant reduction in the area of parakeratosis. The results indicated that the formulated gel may be a promising vehicle for topical delivery of mycophenolate mofetil for the treatment of psoriasis.

Keywords: Microemulsion, mycophenolate mofetil, psoriasis, topical drug delivery

Introduction

Psoriasis is a chronic inflammatory skin disease of unknown etiology characterized by epidermal hyperproliferation, inflammation and altered keratinization (1). Different treatments are available for psoriasis and among this topical therapy are most commonly used in majority of patients. However, efficacy of topical therapy has been a major concern due to skin changes that occur in psoriasis such as imbalanced skin lipids, excessive growth and aberrant differentiation and skin sensitivity. Apart from this, skin rigidization occurs as normal moisturizing factors like water are absent in psoriatic skin, posing a stiff challenge in designing a novel topical delivery system (2).

The barrier nature of skin made it difficult for most of drugs to penetrate into or through it. Many strategies have been employed to enhance dermal and transdermal drug delivery. The common method employed to improve drug permeation involves the use of permeation enhancers and percutaneous penetration enhancers.
enhancers such as organic solvents like ethanol or fatty acids. However, these compounds often lead to generation of skin irritation (3). Other method involves the use of ultrasound and iontophoresis but these methods are not commonly used due to requirement of qualified staff for its application (4). Recently, attempts have been made for the topical delivery of various drugs employing novel colloidal carriers such as microemulsions, nanoemulsions and liposomes.

Microemulsions can be considered as ideal liquid vehicles for drug delivery as they possess most of requirements for this including thermodynamic stability, ease of formulation, low viscosity and high solubilization capacity. Due to small droplet size and large amount of inner phase in microemulsions, the surface area and density of microemulsion droplets are assumed to be very high, providing high concentration gradient and improved drug permeation (5). Microemulsions have been reported to enhance the drug permeation through skin as compared to conventional formulations such as gels and creams (6). Studies have revealed the potential of microemulsion in increasing the skin permeation of various drugs such as triptolide, estradiol, and 8- methoxaslen (7, 8, 9). Microemulsions present advantages over liposomal carriers such as higher stability, low preparation cost, absence of organic solvents and no necessity of intensive sonication (4). The viscosity of microemulsions can also be increased by addition of hydrogels which can prolong their skin retention making them suitable for topical drug delivery (10).

Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid, is a new immunosuppressive compound mainly used in combination of cyclosporine and corticosteroids for the prevention of organ rejection after allogenic heart and kidney transplantations. This substance reversibly blocks the de novo synthesis of guanine nucleotides required for the DNA and RNA synthesis in T and B lymphocytes (11). Recently, reports have been published concerning the use of mycophenolate mofetil for the treatment of several autoimmune and inflammatory skin disorders including psoriasis (12). However the systemic administration in dermal therapy is limited due to several side effects such as nausea, leucopenia, sepsis and diarrhea. So, studies are being conducted for the development of suitable topical formulation of MMF. Studies have revealed that MMF is capable of penetrating into skin (13). Furthermore, the biotransformation of the product into its active form in the skin has also been reported (14). Case studies concerning topical application of MMF in patients with plaque type psoriasis showed same effects as topical corticosteroids in controlling erythema and inflammation indicating that MMF could be a promising alternative in the local treatment of psoriasis. The aim of the present study was to develop and evaluate microemulsion based gel system of MMF for the treatment of psoriasis, which could provide improved drug permeation through skin and improved patient compliance.

Materials and Methods

Material: Mycophenolate mofetil was kindly gifted by Panacea Biotech Ltd. Baddi, India. Oleic acid was purchased from Loba Chemie Pvt. Ltd, Mumbai. Soyabean oil, isopropyl palmitate, isopropyl myristate, Tween80, Tween20 and propylene glycol were purchased from SD Fine Chemicals Ltd, Mumbai. Carbopol 940 was obtained from Qualikems Fine Chemicals Pvt. Ltd., Vadodara. Imiquimod cream (Glenmark Pvt. Ltd., Mumbai) was purchased from market. Distilled water (Rions, India) was used throughout the studies. All other chemicals used were of analytical grade.

Screening of Excipients: The solubility of drug in various oils (isopropyl palmitate, isopropyl myristate, oleic acid and ethyl oleate), surfactants (Tween80 and Tween20) and cosurfactant (propylene glycol) was determined by dissolving excess amount of MMF in 5 ml of each selected oils, surfactants and cosurfactants in stoppered vials separately and mixed using vortex mixer (IKA, Germany). Mixtures were then shaken for 72 h in an isothermal shaker (Narang Scientific

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Works Pvt. Ltd., India) maintained at 37±1°C to achieve equilibrium (15). The equilibrated samples were then removed from the shaker, centrifuged at 5000 rpm for 15 minutes to remove the excess amount of undissolved drug. The supernatant was filtered through a 0.45 µm membrane filter and concentration of MMF was determined in each of the selected oil, surfactant and cosurfactant by Ultraviolet Spectrophotometer (Blue Star AU-2701, India) at λ_max of 250nm after appropriate dilutions with methanol (16).

**Construction of Pseudo-Ternary Phase Diagrams:** Pseudo-ternary phase diagrams were constructed so as to find out the concentration range of components for the existence range of microemulsions, using water titration method (17) at ambient temperature (25°C). Three phase diagrams were prepared with 1:1, 2:1 and 3:1 volume ratios of Tween80 and propylene glycol, respectively. For each phase diagram, oil and specific surfactant/cosurfactant (Smix) ratio were mixed thoroughly in different combinations of oil and Smix (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) so that the maximum ratios could be covered for the study to delineate the boundaries of phases formed precisely in the phase diagrams. Slow titration with distilled water was carried out for each specific ratio of oil and Smix under moderate stirring. After equilibration, the mixtures were assessed visually and determined as being microemulsions, crude emulsions or gels. The highly viscous mixtures that did not show any change in the meniscus after being tilted to an angle of 90° were considered as gels (18).

**Selection of Formulations:** From the phase diagram showing maximum microemulsion area, a number of microemulsions with different formulae were selected covering the entire range of microemulsion occurrence in the phase diagrams with minimum surfactant and water concentrations and these formulations were subjected to various physical stability tests. The composition of selected microemulsion formulations is given in Table 1.

**Physical Stability Studies:** Physical stability tests were performed to overcome the problem of metastable formulations. The selected microemulsions were subjected to centrifugation at 5000 rpm for 30 min and the formulations that did not show any phase separation were taken for the heating and cooling cycles. Six cycles between 4°C (refrigerator temperature) and 45°C, with storage at each temperature of not less than 48 h, were carried out (19). The formulations that were found stable were subjected to a freeze-thaw cycle test. Formulations were kept in deep freezer (Vestfrost, New Delhi, India) at -20° C for 24 h. Then, microemulsions were removed and kept at room temperature for the next 24 h. Three such cycles were repeated.

**Preparation and optimization of Mycophenolate mofetil Loaded Microemulsion Systems:** In order to prepare drug loaded microemulsions, mycophenolate mofetil was dissolved in the oily phase containing oleic acid and propylene glycol. Tween80 was solubilized in distilled water. Then the aqueous solution of surfactant was added to the clear oily phase drop by drop under continuous stirring using magnetic stirrer (IKA, Germany) (20). The optimization was carried out by assessing the drug loading capacity of microemulsion systems and the effect of drug loading on globule size of microemulsion (21).

**Characterization of Mycophenolate Mofetil Loaded Microemulsion**

**Particle Size and Polydispersity Index:** The average size and polydispersity index of the microemulsion droplets were determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion using Malvern Nanosizer ZS (Malvern instruments, UK) (22). Light scattering was monitored at 25°C at a 90° angle.

**Refractive Index, Conductivity and pH Measurement:** Refractive index was determined for different microemulsion formulations by using Abbe’s refractometer (Nirmal International, Delhi, India) at 25°C. Conductivity was measured by...
using a digital thermo conductivity meter (Emcee Electronics, Venice, USA). The pH was determined for the optimized microemulsion by using a calibrated digital pH meter (S.D. Fine Chemicals, Mumbai, India) in triplicate at room temperature.

Zeta Potential Measurements: Zeta potential of samples was measured using Beckman Coulter Delsa nanoanalyzer (Beckman Coulter Inc., USA). Samples were diluted with double distilled water prior to analysis and placed in clear disposable zeta cells and the results were recorded. All experiments were performed in triplicate.

Surface Morphology by Transmission Electron Microscopy (TEM): Morphology and structure of the microemulsion were studied using transmission electron microscope (TEM) (Morgagni 268D, FEI, Holland) operating at 70 KV and capable of point-to-point resolution. In order to perform the TEM observations, a drop of microemulsion was placed on carbon-coated copper grid with 2% phosphotungstic acid and observed after drying under electron microscope.

Formulation of Microemulsion Based Gel of MMF: Carbomer 940 was selected as gel matrix for the preparation of microemulsion based hydrogel. 1g of carbopol 940 was dispersed slowly in 100ml of the optimized formulation with the help of an overhead stirrer and the dispersion was then neutralized by dropwise addition of triethanolamine until gelling occurs (10). After neutralization, it was kept in dark for 24 h for complete swelling.

Characterization of Microemulsion Based Gel

Rheological Studies on the Microemulsion Based Gel: The microemulsion based gel system was studied for the rheological behavior at 25±1°C using Anton Paar RheolabQC Rotational Rheometer (Ashland, USA). The viscosity of gel was measured at different shear rates and rheological behavior of the microemulsion gel system was evaluated by constructing rheogram where the shear stress (dyne/cm²) versus shear rate (s⁻¹) was plotted.

In vitro Skin Permeation Studies: In vitro skin permeation studies were performed on a Franz diffusion cell (HEM-100, Harjee Exports Pvt. Ltd., Haryana) with an effective diffusional area of 2.26 cm² and receiver chamber capacity of 22.5 ml using rat abdominal skin (10). The full thickness rat skin was excised from the abdominal region, and the hairs were removed. The subcutaneous tissue was removed surgically and dermis side was wiped with isopropyl alcohol to remove adhering fat. The skin was then washed using distilled water and mounted between donor and receptor compartment of Franz diffusion cell, where stratum corneum side faced the donor compartment and dermal side faced the receptor compartment. 1 ml of microemulsion (F2) and microemulsion based gel containing equivalent amount of drug were applied on the surface of rat skin in the donor compartment. Acetate buffer (pH 5.5) was used as receptor media and the temperature in the receptor compartment was maintained at 37±1°C. The receptor phase was stirred at 100rpm using a magnetic stirrer. At predetermined time intervals (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 12, 24 h); 1 ml sample was collected from the receptor compartment and replaced with fresh receptor solution to maintain the sink conditions. The drug solution served as control. All the collected samples were centrifuged and analyzed for MMF content by UV Spectrophotometer at λmax of 250 nm.

The skin permeation rate was calculated from slope of linear plot of cumulative amount
permeated as a function of time. The flux (J) was calculated by using Eq. (i):

\[ J = \frac{dQ}{dt} A \]  

Where, J = Flux (µg h⁻¹ cm⁻²)  
\( \frac{dQ}{dt} = \) Slope obtained from linear curve  
A= Area of diffusion (cm²)  
The permeability coefficient (Kₚ) was calculated by dividing J with initial concentration of drug in the donor cell (C₀) by using Eq. (ii):

\[ K_p = \frac{J}{C_0} \]  

Release Kinetics: In order to investigate the release mechanism of drug delivery system, the data obtained from in vitro drug permeation studies of the optimized microemulsion and microemulsion based hydrogel was fitted into various kinetic models such as zero order, first order, Higuchi and Peppas-Korsemeyer model. Regression analysis was performed for all of these release kinetics models to find out the best fit for drug release from the studied formulations.

In vivo Skin Permeation Studies

For the in vivo studies, BALB/c female mice of 8 to 11 week of age were purchased from Indian Institute of Immunology, Jammu. The animals were kept under standard laboratory conditions at temperature of 25±1°C and relative humidity of 55±5%. The animals were housed in polypropylene cages under standard laboratory conditions with free access to food and water ad libitum. All the experimental procedures in the animal studies were conducted with prior approval of Institutional Animal Ethical Committee and care of laboratory animals were followed at all the times.

The hair on the dorsal area of the animals were removed carefully using depilatory cream 24 h prior to the experiment and the animals were divided into three groups (Healthy group, Psoriasis control and Group treated with MMF loaded microemulsion based gel). Each group contained six animals.

No formulation was applied to the first group. The other two groups received a daily topical dose of 62.5mg of commercially available imiquimod cream on the dorsal area for 7 consecutive days, translating in a daily dose of 3.125 mg of the active compound (24). In the third group, after induction of psoriatic lesions, the animals were treated with the MMF loaded gel formulation for a period of 7 days. The animals were then sacrificed and skin samples were taken for histopathological studies.

Results and Discussion

Solubility Studies and Component Selection: The results of solubility studies of MMF are shown in Figures 1 and 2. The maximum solubility of MMF was found in oleic acid as compared to other oils. The solubility of MMF in oleic acid was 11.505 ± 0.843 mg/ml, which was highest amongst the oils investigated. In the case of surfactants, highest drug solubility was found in Tween80 (8.882±0.601) mg/ml. The solubility of the drug in propylene glycol was found to be 6.242±0.852 mg/ml. Based on the solubility...
studies, it was concluded that oleic acid, Tween80 and propylene glycol could be the most appropriate combination for preparation of microemulsion.

**Pseudo-Ternary Phase Diagrams:**
Pseudo-ternary phase diagrams were constructed separately for each surfactant/cosurfactant \( S_{\text{mix}} \) ratio i.e. 1:1, 2:1 and 3:1 (Fig. 3) so that microemulsion regions could be identified and microemulsion formulation could be optimized. The maximum water solubilization capacity for the microemulsion systems obtained with \( S_{\text{mix}} \) ratio of 1:1 was around 50%. It was also observed that within the formed microemulsion zone, the fluidity of microemulsion was reduced with increasing water content. The gel systems were observed when the surfactant concentration was greater than 60% and the water content in the system was in the range of 25-60%. The gel structure was broken down upon further dilution with water before transformation into coarse emulsion. This behavior could be attributed to the fact that the content below 25% is insufficient to hydrate the polyoxyethylene groups of Tween80 which are critical for the swelling of surfactant chains to demonstrate the gel structure. Accordingly, water content more than 60% will increase the distance between the polyethylene groups and destabilize the gel structure leading to breaking of the swollen gel (25).

As the surfactant ratio was increased in the \( S_{\text{mix}} \) ratio to 2:1, a higher microemulsion region was observed with maximum water solubilizing capacity of 62.2%, perhaps due to further reduction of the interfacial tension and increased fluidity of the interface, thereby increasing entropy of the system. When the \( S_{\text{mix}} \) ratio of 3:1 was studied, it was found that microemulsion region decreased slightly with maximum percentage of water phase of 45%, which may have been due to decreased concentration of cosurfactant.

**Thermodynamic Stability Studies:** It is the thermostability that differentiates nano- or microemulsions from emulsions that have kinetic stability and eventually undergoes phase-separation. Microemulsions are formed at a particular concentration of oil, surfactant and water, which makes them stable and not subject to phase separation, creaming or cracking (22). Thus, the formulations were tested for their thermodynamic stability by using centrifugation

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**Fig. 3:** Pseudoternary phase diagrams developed using the aqueous titration method indicating microemulsion region of oleic acid (oil), Tween 80 (surfactant), Propylene glycol (cosurfactant) at different \( S_{\text{mix}} \) ratios indicated in Group (a) to Group (c)
studies, a heating-cooling cycle, and a freeze-thaw cycle (22). After performing thermodynamic studies, it was observed that all the selected formulations had shown good stability. No phase separation, creaming or cracking was observed. The results of the studies are shown in Table 2.

**Selection of Formulation:** From the tested formulations, optimized formulation was selected on the basis of droplet size, polydispersity index, drug loading capacity and the results obtained are described in Table 3. The logic behind selecting these criteria for optimization is that lower droplet size can result in enhanced permeation as well as provide larger surface area for drug release. Polydispersity index (PDI) is the measure of uniformity of the formulation and PDI value less than 1 is desirable (21). Solubility is also an important criterion for the delivery of a poorly water soluble drug. High drug loading capacity ensures large amount of inner phase in microemulsions. The result showed that the smaller droplet size of microemulsion F2 was obtained due to presence of higher concentration of S\textsubscript{mix} in the microemulsion. The decrease in droplet size with increase in S\textsubscript{mix} concentration can be attributed to solubilisation of the internal phase within a larger number of surfactant micelles. However in case of formulation F1, containing 6.45% oil and 58.05% of S\textsubscript{mix}, the average droplet was found to be increased significantly up to 135 nm, which can be attributed to the expansion of oil droplets of microemulsion by increased amount of oil. Based on the results obtained, the formulation F2 was selected for further studies.

**Physicochemical Characterization of Drug Loaded Microemulsion:** Refractive index is the net value of the components of microemulsion and indicates isotropic nature of formulation. The mean value of refractive index for the formulation F2 was found to be 1.421±0.011. Also the refractive index of drug loaded formulation was determined (1.421±0.006) and compared with that of blank formulation and it was found that there was no significant difference between the values. Therefore, it can be concluded that there were no interactions between microemulsion components and drug.

The specific conductivity of microemulsion F2 was found to be 10\textsuperscript{4} µS cm\textsuperscript{-1} and the pH was found to be 5.9±0.04. The zeta potential of the formulation was found to be -34.35±0.051 mV as shown in Fig. 4. The negative values of microemulsions indicated stability of the formulations. The highly negative zeta potential

![Fig. 4: Zeta potential of MMF loaded microemulsion](image1)

![Fig. 5: TEM micrograph of MMF loaded microemulsion](image2)

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value may be due to the presence of oleic acid at the level of surfactant/cosurfactant film of the microemulsion eliciting an electrostatic repulsion leading to an increase in zeta potential value (26).

Surface morphology of the microemulsion formulation F2 was characterized using transmission electron microscopy (Fig. 5) in which the droplets appeared non-aggregated and spherical in shape.

**Hydrogel-thickened Microemulsion:** Microemulsion gel was prepared using carbopol 940 (1 % w/w). To observe the consistency and homogeneity of the gel, a small quantity of gel was pressed between the thumb and index finger, and it was observed that there were no coarse particles in the optimized gel formulation (17). The spreading of carbopol gel was found to be more uniform and the gel spread in a circular pattern equally on all sides and it almost reached to spreadability diameter of 7.1±0.1 cm upon application of 500 g weight. The pH of the prepared hydrogel thickened microemulsion was found to be 5.5 which is compatible with skin pH.

**Rheological Studies:** Rheology is an important parameter as it affects the spreadability and adherence of drug. The plot of shear stress versus shear strain was obtained and plot of shear rate versus viscosity was obtained. The

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Table 1. Composition of Selected Formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Oleic acid (%v/v)</th>
<th>Tween 80 (%v/v)</th>
<th>Propylene glycol (%v/v)</th>
<th>Total S_mic. concentration (%v/v)</th>
<th>Water (%v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>6.45</td>
<td>38.70</td>
<td>19.35</td>
<td>58.05</td>
<td>35.48</td>
</tr>
<tr>
<td>F2</td>
<td>6.06</td>
<td>36.36</td>
<td>18.18</td>
<td>54.54</td>
<td>39.39</td>
</tr>
<tr>
<td>F3</td>
<td>5.56</td>
<td>33.33</td>
<td>16.66</td>
<td>49.99</td>
<td>44.44</td>
</tr>
<tr>
<td>F4</td>
<td>5</td>
<td>30</td>
<td>15</td>
<td>45</td>
<td>50</td>
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Table 2. Results of Thermodynamic Stability Studies

<table>
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<tr>
<th>Formulation Code</th>
<th>Heating/Cooling cycle</th>
<th>Centrifuge</th>
<th>Freeze thaw</th>
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<tbody>
<tr>
<td>F1</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>F2</td>
<td>√</td>
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</tr>
<tr>
<td>F3</td>
<td>√</td>
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<td>√</td>
</tr>
<tr>
<td>F4</td>
<td>√</td>
<td>√</td>
<td>√</td>
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</table>

Table 3. Droplet size, polydispersity values and drug loading capacity of microemulsion formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Droplet Size (nm)*</th>
<th>Polydispersity Index</th>
<th>Drug loading capacity (mg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>135 nm±11.59</td>
<td>0.091</td>
<td>17.2±1.27</td>
</tr>
<tr>
<td>F2</td>
<td>124 nm±10.31</td>
<td>0.504</td>
<td>13.60±0.88</td>
</tr>
<tr>
<td>F3</td>
<td>146 nm±15.03</td>
<td>0.128</td>
<td>9.98±0.55</td>
</tr>
<tr>
<td>F4</td>
<td>167 Åm±18.11</td>
<td>0.141</td>
<td>6.93±0.52</td>
</tr>
</tbody>
</table>

*(mean ± S.D., n=3)

Table 4. Permeation data of MMF loaded microemulsion and microemulsion based hydrogel

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Flux (µg/cm²/h)*</th>
<th>Permeability coefficient x 10⁻³ (cm/h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug solutions</td>
<td>0.460±0.18</td>
<td>0.338±0.05</td>
</tr>
<tr>
<td>MMF loaded microemulsions</td>
<td>1.047±0.35</td>
<td>0.769±0.08</td>
</tr>
<tr>
<td>Microemulsion based hydrogels</td>
<td>0.899±0.21</td>
<td>0.661±0.09</td>
</tr>
</tbody>
</table>

*(mean ± S.D., n=3)

Table 5. Kinetic parameters of mycophenolate mofetil released from the microemulsion-based gel

<table>
<thead>
<tr>
<th>Kinetic models</th>
<th>Correlation (r²)</th>
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<td>Zero order</td>
<td>0.928</td>
</tr>
<tr>
<td>First order</td>
<td>0.760</td>
</tr>
<tr>
<td>Korsemeyer-Peppas</td>
<td>0.910</td>
</tr>
<tr>
<td>Higuchi diffusion</td>
<td>0.549</td>
</tr>
</tbody>
</table>

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The flow index of the formulation was calculated by plotting graphs of log shear rate versus log shear stress by applying Ostwald-de Waele equation:

\[ s = K g^n \]  

where, \( s \) is shear stress, \( g \) is shear rate, \( K \) is consistency index and \( n \) is flow index. Flow index (dimensionless) is a measure of the deviation from Newtonian behavior (\( n=1 \)), \( n < 1 \) indicates shear thinning (pseudoplastic behavior) and \( n > 1 \) shear thickening (dilatant behavior). On the basis of rheological studies performed, the formulated gel was said to follow pseudoplastic behavior (27).

**Skin Permeation Studies:** To study the influence of formulation on the permeation of mycophenolate mofetil, permeation profile of drug was investigated from drug solutions, MMF loaded microemulsions and microemulsion based hydrogels for a period of 24 h using excised rat abdominal skin on Franz diffusion cell. The drug solutions exhibited only 44.891±2.115%, whereas microemulsion demonstrated 69.524±1.612% of drug permeation in 24 h (Fig. 6). The comparison of cumulative permeation between microemulsion and drug solution showed that MMF loaded microemulsion enhanced the drug permeation significantly (\( p<0.05 \)). The microemulsion based gel showed slightly lower drug permeation of 57.778±1.812% which may be attributed to slow diffusion of drug through the gel network. Besides providing optimum viscosity to microemulsion for topical application, Carbopol in topical gels was found to provide better adhering of the formulation to skin and delayed drug delivery (18).

Apart from the contribution of oleic acid in enhancing drug permeation in skin by disrupting the fluidity of stratum corneum, the surfactant composition might also be responsible for enhanced permeation from microemulsion. The non-ionic surfactants reportedly emulsify the sebum, thereby enhancing the thermodynamic coefficient of the drug, allowing it to penetrate into cells more effectively (29). The other mechanism depends on the possibility of direct drug transfer from the microemulsion droplet to the stratum corneum. The density of droplets and their surface area are assumed to be high due to the small droplet size and large amount of inner oil phase in the microemulsions. Therefore, droplets settle down to close contact with the skin providing high concentration gradient and improved drug permeation (30).

**Release Kinetics:** To study the release kinetics, various kinetic models including zero-order, first-order, Korsemeyer-Peppas and the Higuchi diffusion model were applied. It was found that in vitro release of microemulsion based gel formulation follows zero order (Table 5). It can be attributed to the fact that drug is present in internal phase of microemulsion system. The depletion of drug from the external phase is supplemented by the release of drug from internal phase resulting in sustained drug delivery from the system (31).

**Histopathological Studies:** The photomicrograph of untreated skin (Fig. 7A) showed normal skin with well defined epidermal and dermal layers. Keratin layer was well formed and lied just adjacent to the topmost layer of epidermis and dermis was devoid of any inflammatory cells. Psoriasis induced mice skin closely resembled human plaque type psoriasis with respect to erythema, skin thickening, scaling and epidermal alterations as well as with respect
to inflammatory infiltrate in the epidermal and dermal regions (Fig. 7B). Parakeratosis, hyperkeratosis, elongation of rete ridges with supra papillary thinning of epidermis and presence of Munro’s abscess in parakeratotic layer were observed as characteristic features of psoriatic lesions were observed (32).

After treatment with the MMF loaded gel formulation (Fig. 7C), keratin layer appeared healthy and showed an increase in the thickness of the epidermis. Hyperkeratosis was not observed and the areas of parakeratosis became significantly reduced in comparison to the control. However, inflammatory infiltrate was observed in dermis and munro’s abscess persisted. The results obtained indicated partial recovery of the skin with the application of the formulated MMF loaded microemulsion gel.

Conclusion

In the current study, topical microemulsion gel of mycophenolate mofetil for the treatment of psoriasis was developed which provided enhanced skin permeation of drug and reduced dosing frequency. Different microemulsions were selected from the pseudoternary phase diagrams. The formulation containing 6.06% v/v of oleic acid 36.36% v/v of Tween80 and 18.18% v/v of propylene glycol was considered as optimum formulation having globule size of 124 nm and rate of permeation of 1.047±0.109 µg/cm²/h. The microemulsion was converted into gel using carbopol 940 (1% w/v) so as to improve its viscosity to attain better adherence to skin. The in vitro studies revealed that both the microemulsion and microemulsion gel increased the drug permeation through skin as compared to control solution. The in vivo studies performed on imiquimod induced skin inflammation on mice skin revealed that the application of the drug loaded microemulsion gel for seven consecutive days lead to complete clearance of hyperkeratotic plaques and a significant reduction in the area of parakeratosis. The results indicated that the formulated gel may be a promising vehicle for the treatment of psoriasis. The future perspective may include elaborate stability and clinical studies for developing commercially viable topical microemulsion formulation of mycophenolate mofetil.

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