CHAPTER 9

THE INFLUENCE OF POLYMER INCLUSION TO MICROEMULSION FOR DERMAL DELIVERY OF TAZAROTENE
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The influence of polymer inclusion to microemulsion for dermal delivery of tazarotene

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THE INFLUENCE OF POLYMER INCLUSION TO MICROEMULSION FOR DERMAL DELIVERY OF TAZAROTENE

9.1. Experimental Work

9.1.1. Formulation of microemulsion based gel of tazarotene
Various gelling agents namely non-benzene grade Carbopol® polymers (Carbopol® 971P NF, Carbopol® 974P NF and Carbopol® 980P NF) were evaluated for their ability to gel optimized TZR-MEs at different concentrations. The suitable gelling agent was selected on the basis of compatibility with ME structure, feel and ease of spreadability (1). Carbopol® 971P NF was selected as the gel matrix to prepare MBG. Carbopol® 971P NF was slowly mixed with water. The oily phase was obtained by mixing appropriate concentrations of oil, surfactant and cosurfactant. Carbopol® 971P NF was entirely swelled in the water and its pH was adjusted by adding 50% w/w triethanolamine (TEA). MBG was obtained by mixing the swelled gel in water with the oily phase (2).

9.1.2. Characterization of microemulsion based gel of tazarotene
Determination of drug content, spreadability, pH and rheological parameters of microemulsion based gel was done as per procedure described in section 7.1.2.
9.1.3. \textit{In vitro} Skin Permeation Study

It was performed as per the procedure described earlier under section 6.1.5.

9.1.4. Skin retention

It was performed as per the procedure described earlier under section 7.1.4.

9.1.5. Histopathological investigation of skin

It was performed as per the procedure described earlier under section 7.1.5.

9.1.6. Infrared Study

The infrared (IR) spectra of TZR, unloaded ME, optimized TZR-ME and TZR-MBG were taken using an IR spectrophotometer (Spectrum GX FT-IR, Perkin Elmer, Norwalk, CT). The unloaded ME, TZR-ME and TZR-MBG were spread as a thin layer on potassium bromide cell and then scanned between 4000 – 400 cm$^{-1}$. The resulting IR spectra of TZR and plain ME were then compared with TZR-ME and TZR-MBG to detect any possible interaction between the drug and different components used (3).

9.1.7. Skin-irritation testing (Draize patch test)

The irritation potential of the ME based ITN gel in comparison to marketed ITN gel was evaluated by carrying out the Draize patch test on rabbits (4). Animal care and handling throughout the experimental
procedure were performed in accordance to the CPCSEA guidelines. The experimental protocol was approved by the Animal Ethical Committee of University Institute of Chemical Technology. White New Zealand rabbits weighing 2.5–3 kg were obtained from Veterinary College, Anand, India and were acclimatized before the beginning of the study. Animals were divided into four groups ($n = 3$) as follows:

Group 1: No application (Control).

Group 2: Marketed formulation (Tazret® gel containing 0.05% w/w TZR, Glenmark).

Group 3: ME based gel without TZR (Placebo gel).

Group 4: ME based gel containing TZR (0.05%, w/w).

The back of the rabbits were clipped free of hair, 24 h prior to the application of the formulations. Formulations, 0.5 g, were applied on the hair free skin of rabbits by uniform spreading within the area of 4 cm². The skin was observed for any visible change such as erythema (redness) at 24, 48 and 72 h after the application of various formulations. The mean erythemal scores were recorded (ranging from 0 to 4) depending on the degree of erythema as follows: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe erythema (extreme redness) = 4 (5).

9.1.8. Statistical Analysis
It was performed as per the procedure described earlier under section 6.1.6.

9.1.9. Stability Studies
It was performed as per the procedure described earlier under section 6.1.7.
9.2. Results and Discussions

9.2.1. Formulation of microemulsion based gel of tazarotene

One of the problems associated with the use of MEs for topical drug delivery is the difficulty of using these vehicles on the skin because of their fluidity (6). Hence microemulsion based gel (MBG) of the optimized formulations (ME- Lab CC and ME- IPM) was developed using a suitable polymer capable of modifying the rheological behaviour. Various non-benzene grade Carbopol® polymers (Carbopol® 971P NF, Carbopol® 974P NF and Carbopol® 980P NF) were evaluated for their ability to gel optimized TZR-MEs. Also different concentrations of above mentioned gelling agents were tried. The suitable gelling agent was selected on the basis of compatibility with ME structure, feel and ease of spreadability. When Carbopol® 974P NF was added to ME to prepare the MBG only an ivory-white gel was obtained indicating that the structure was disturbed. The possible reason is that the dehydration of some ingredients such as surfactant and co-surfactant in ME makes the polymer dissociated from hydrated state (7). So it was concluded it was not a suitable gel matrix for this ME system. The incorporation of Carbopol® 980P NF and Carbopol® 971P NF into ME could increase the viscosity and also maintain the ME structure. Further since MBG containing Carbopol® 971P NF had the most appropriate fluidity and spreadability for topical administration, so it was further selected as an optimum gel matrix for the optimized MEs.
Table 40. Composition of microemulsion based gels containing tazarotene.

<table>
<thead>
<tr>
<th>Components</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBG- Lab CC</td>
</tr>
<tr>
<td>Tazarotene (% w/w)</td>
<td>0.05</td>
</tr>
<tr>
<td>Carbopol® 971P NF (% w/w)</td>
<td>2</td>
</tr>
<tr>
<td>Labrafac CC (% w/w)</td>
<td>10</td>
</tr>
<tr>
<td>Isopropyl myristate (% w/w)</td>
<td>-</td>
</tr>
<tr>
<td>Labrasol (% w/w)</td>
<td>7.5</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>7.5</td>
</tr>
<tr>
<td>Capmul MCM</td>
<td>15</td>
</tr>
<tr>
<td>Water (g)</td>
<td>Quantity sufficient to produce 100g of gel</td>
</tr>
</tbody>
</table>

Briefly the MBG of ME containing Lab CC as oil phase (MBG- Lab CC) and ME containing IPM as oil phase (MBG- IPM) was prepared as shown in table 40. The influence of the method of addition of Carbopol® 971P NF on the formation of MBG was investigated. Carbopol® 971P NF was directly added into ME or the aqueous phase of ME, respectively. The following observations were made:

- When Carbopol® 971P NF was directly added to ME, it required much more time to swell in ME than in water (aqueous phase). This might be attributed to the relatively high increase of the viscosity of ME.
- After TEA was added to adjust the pH of ME containing the swollen Carbopol® 971P NF, MBG with a high viscosity was obtained. However, some tiny agglomerates of Carbopol® 971P NF could be observed, since Carbopol® 971P NF did not swell entirely in the ME with a relatively high viscosity.
- A homogenous MBG could be obtained by addition of Carbopol® 971P NF to aqueous phase followed by adjusting pH of swollen gel matrix with TEA and finally mixing the gel matrix with the oily phase of ME.

As per the above observations, we can say that the gel network increased the viscosity of the system having no influence on the spontaneous dispersion of oily phase in aqueous phase or the spontaneous formation of ME. Carbopol® 971P NF as an aqueous gel matrix in continuous phase displayed non-covalent intermolecular associations. These physical interactions could lead to the formation of a three-dimensional gel network and the dispersed oil droplets were reasonably hosted within the meshes of the three-dimensional gel network (8). In conclusion, the order of the addition of Carbopol® 971P NF had no significant influence on the formation of MBG, but might influence the homogenized swelling of Carbopol® 971P NF. In the subsequent study, microemulsion based gels (MBGs) were prepared by mixing the swollen gel matrix with the oily phase at 2% w/w concentration of Carbopol® 971P NF.
9.2.2. Characterization of microemulsion based gel of tazarotene

Table 41. Physicochemical characterization of microemulsion based gels containing tazarotene. (mean ± SEM, n=3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MBG- Lab CC</th>
<th>MBG- IPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug content (%</td>
<td>99.86 ± 0.012</td>
<td>98.49 ± 0.019</td>
</tr>
<tr>
<td>w/w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spreadability (cm)</td>
<td>7.5 ± 0.011</td>
<td>7.1 ± 0.013</td>
</tr>
<tr>
<td>pH</td>
<td>6.8 ± 0.03</td>
<td>6.5 ± 0.06</td>
</tr>
<tr>
<td>Viscosity (cp)</td>
<td>$5.9 \times 10^3 \pm 0.6 \times 10^3$</td>
<td>$6.2 \times 10^3 \pm 0.3 \times 10^3$</td>
</tr>
</tbody>
</table>

The ITN content of the MBG- Lab CC and MBG- IPM was found to 99.86 ± 0.012% w/w of the theoretical value (0.05% w/w).

Gel spreadability is an important parameter. Application of the formulation on inflamed part would be more comfortable if the base spreads easily exhibiting maximum slip and drag. When a weighed quantity of gel was placed in between two glass plates of known weight, it spreaded uniformly to produce a circle, the diameter of which is related to its spreadability; the larger the diameter, the better the spreadability (9). The data in table 41 indicate that the diameter of MBG- Lab CC and MBG- IPM were 7.5 ± 0.011 cm and 7.1 ± 0.013 cm respectively. While the diameter of the marketed gel was found to be 6.7 ± 0.04 cm indicating that the spreadability of MBGs is better than that of conventional gel. This is because of the loose gel matrix nature of MBG formulation due to the presence of oil globules rather than the conventional gel matrix.

The pH of both the gels was in the physiological range. Also the pH near to 7.0 indicated that they could result in less stimulation to skin.
Rheology is less precise but simpler way to identify anisotropic aggregates in the system. In ME, formation of liquid crystalline stage coincides with formation of nonspherical aggregates (cylindrical or lamellar aggregates), which obstructs the flow in the dispersion medium. This produces high yield value (10). MEs being isotropic (spherical) systems offer less resistance to flow and exhibit low viscosity as compared to macroemulsions also. Rheological properties (study of deformation and flow of matter) are required in various pharmaceutical areas. It helps to monitor the effect of vehicles consistency on release of drug from the preparations and subsequent percutaneous absorption. It is also important from the manufacturing point of view. In order for a pharmaceutical or cosmetic product to be spread easily on the skin without running, it must be neither too fluid nor too viscous and show plastic or pseudoplastic rheological behavior (11). The TZR MBGs showed pseudoplastic behavior which facilitates the flow and improves the spreading characteristics of the formulation. The viscosity of MBG-Lab CC and MBG-IPM at 5 rpm was found to be $5.9 \times 10^3 \pm 0.6\times 10^3$ and $6.2 \times 10^3 \pm 0.3\times 10^3$ cp respectively.

9.2.3. In vitro Skin Permeation Study

In order to assess the skin retention and penetration of TZR from MBG, the *invitro* permeation ability through skins and into skins were performed using Franz diffusion cells. The *invitro* permeation of TZR through rat skin from MBGs and marketed gel (Tazret®) was calculated in terms of mean cumulative amount diffused at each sampling time point during time period of 12 hours (Figure 52). The permeation parameters of MBGs and marketed gel are presented in table 42. To understand the mechanism of drug release from these formulations, the data were treated according to zero-order (cumulative amount of drug released v/s time),
first order (log of the cumulative amount of drug released v/s time) and
higuchi (cumulative amount of drug released v/s square root of time)
equation. Moreover the plot of cumulative amount of drug released v/s
square root of time showed a linear relationship for both MBGs and
marketed gel indicating that TZR permeation followed Higuchi model
(Table 43).

The permeation parameters of the tested ME formulations containing Lab
CC as oil phase with various compositions are presented in table 36. A
steady increase of TZR in the receptor chambers with time was observed.
The permeation profiles of TZR through rat skin from MBG- Lab CC,
MBG- IPM and marketed gel are shown in figure 51. The cumulative
amount of TZR that had permeated through excised rat skin (µg/cm²) was
plotted as a function of time (hours). The steady state flux (Jss) was
obtained from the linear portion (2-8 hours) of the graph. From table 42 it
can be said that highest flux was obtained from MBG- Lab CC (0.0413 ±
0.0004 µg/cm²/h) with lag time of 0.182 ± 0.014h and lowest from MBG-
IPM (0.0359 ± 0.0001 µg/cm²/h) with lag time of 0.132 ± 0.021h. The
flux value and lag time of marketed gel was 0.0407 ± 0.0002 µg/cm²/h
and 0.145 ± 0.019 h respectively. Thus the flux value of MBG- Lab CC
was significantly higher than MBG-IPM (p< 0.05). Although the
difference in the flux value of marketed gel and MBG- Lab CC was not
significant (p>0.05). This might be due to presence of surfactant and
cosurfactant in the MBG. They lower the interfacial tension of the
surfactant film resulting in more flexible and dynamic layer (12). The
drug in this energy rich system can diffuse across the flexible interfacial
surfactant film between the phases, a thermodynamic process that
increases partitioning and diffusion into the stratum corneum. Also for a
drug entering the stratum corneum, the intercellular route is known to be
most important. However a participation of follicles and sweat glands has
to be taken into consideration as indicated by several authors. This finding might be attributable to the low surface tension that ensures an excellent contact to the skin (13). Hence it can be said that MBG is capable to enter the skin easily as compared to marketed gel.

The statistical comparison of flux value of MBG- Lab CC and its ME revealed that the release of drug from viscosized ME was lower as compared to fluid ME (p<0.05). The same result was also obtained on comparing MBG- IPM and its ME with higher flux value in gel than that of its ME (p<0.05). This might be attributed to the gel formation in the ME by addition of Carbopol® 971P NF that will increase its viscosity, transform the microstructure of ME to lamellar or a highly ordered microstructure and further decrease the permeation in the skin (14). Also the movement of droplets in case of MBG would be limited further limiting the diffusion of TZR dissolved in the droplets and slowing down its releasing rates. Therefore it can be said that MBG might have an excellent ability for sustained release as compared to other formulations.
Figure 51. *In vitro* permeation profiles of tazarotene through rat skin from marketed gel and microemulsion based gels (MBG-Lab CC & MBG-IPM). (mean ± SEM, n = 3)
Table 43. The modeling parameters of isotretinoin from marketed gel and microemulsion based gel. (ITN-MBG). (mean ± SEM, n = 3)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R^2</td>
<td>R^2</td>
<td>R^2</td>
</tr>
<tr>
<td>Marketed Gel</td>
<td>0.9380</td>
<td>0.9384</td>
<td>0.9927</td>
</tr>
<tr>
<td>MBG-Lab CC</td>
<td>0.9261</td>
<td>0.9266</td>
<td>0.9942</td>
</tr>
<tr>
<td>MBG-IPM</td>
<td>0.9410</td>
<td>0.9413</td>
<td>0.9894</td>
</tr>
</tbody>
</table>
9.2.4. Skin retention

As shown in table 42 the highest amount of TZR was retained in skin from MBG- Lab CC (47.33 ± 0.82 μg, 9.5% of applied dose) and lowest from marketed gel (35.00 ± 1.73 μg, 7% of applied dose). The accumulative amount of TZR in skin from MBG- IPM (39.67 ± 1.20 μg, 8% of applied dose) was higher as compared to marketed gel (p> 0.05) inspite of low cumulative amount of TZR at 12 h from MBG- IPM as revealed by skin permeation studies. It was believed that the static droplets of MBG could closely contact with the skin due to adhesiveness of polymer and a large amount of inner IPM might penetrate into stratum corneum due to small diameter of droplets (15).

The MBG- Lab CC could significantly (P < 0.05) increase the accumulative uptake of TZR in skin compared to the marketed formulation. Also on statistical comparison it was found that both MBGs could increase the skin uptake of TZR as compared to their respective ME formulations although not statistically significant (p>0.05). ME have been shown to improve the dermal localization of several topical therapeutic agents. This was one of the reasons to employ ME approach for topical delivery of TZR as its epidermal localization is highly desirable for enhancing the treatment of skin diseases such as acne. Thus MBG- Lab CC having appropriate physicochemical properties, higher skin permeation and uptake (as compared to marketed gel) was considered as optimized formulation and used for further studies.
9.2.5. Histopathological investigation of skin

The rat skin is a multilayered organ with many histological layers. The histology of excised rat skin in control and treated with optimized ME, MBG and marketed gel is shown in figure 52 (A)-(D). The microscopic observations indicate that the optimized ME (ITN ME), MBG (MBG-Lab CC) and marketed gel has no significant effect on the microscopic structure of the skin. The surface epithelium lining and the granular cellular structure of the skin were totally intact. No major changes in the ultra structure of skin morphology could be seen and the epithelial cells appeared mostly unchanged.

![Figure 52. Light microscopic photographs of in vitro histological study. A) Control B) The rat skin treated with optimized microemulsion (TZR ME). C) The rat skin treated with microemulsion based gel (MBG-Lab CC). D) The rat skin treated with marketed gel.](image-url)
9.2.6. Infrared Study

The infrared spectra of TZR pure powder, plain ME, TZR loaded ME and MBG are as shown in the figure 53 (A) — (D). TZR spectrum shows absorption bands at 3055 cm\(^{-1}\) due to aromatic C–H stretching and at 2908 cm\(^{-1}\) and 2961 cm\(^{-1}\) due to aliphatic C–H stretching. A absorption band at 2201 cm\(^{-1}\) corresponds to C=C stretching of the di-substituted alkyne and prominent absorption band at 1719 cm\(^{-1}\) related to C=O stretching of ester. Band at 1585 cm\(^{-1}\) is due to aromatic C–C stretching. Absorption bands at 1365 cm\(^{-1}\) and at 1448 cm\(^{-1}\) are cue to aliphatic C-H bending. Other bands at 823 cm\(^{-1}\) and 777 cm\(^{-1}\) corresponds to aromatic C-H bending. The IR spectrum of TZR loaded ME and MBG is entirely different from TZR powder, while it closely resembles the spectrum of plain ME. The characteristic bands of TZR either have disappeared or few bands of TZR reduced in intensity probably due to the restriction inside the formulation matrix. In IR spectrum of TZR loaded ME and MBG no additional peak was observed, which emphasized the absence of any possible interaction between the drug and formulation components used.
Figure 53. Infra red spectra of (A) Tazarotene (TZR), (B) Unloaded microemulsion (C) Tazoretene loaded microemulsion (TZR-ME) and (D) TZR loaded microemulsion based gel (TZR-MBG).
9.2.7. Skin-irritation testing (Draize patch test)

One of the major disadvantages associated with the TZR therapy is skin irritation (erythema), which strongly limits its utility and acceptability by the patients. Ideally, the delivery system of TZR should be able to diminish or abolish these erythematic episodes. However, most of the currently marketed conventional dosage forms such as creams, lotions and gels are not able to reduce the irritation caused by topical application of ITN. It was hypothesized that encapsulation of TZR in ME would reduce the skin irritation (16). The results obtained from the primary skin irritation studies are listed in table 44 and the actual photographs are depicted in figure 54 (A)–(D). Draize patch test is a reliable method and the results obtained from this study can be linked to that obtained in humans (4). The skin-irritation studies indicated that MBG containing TZR did not show any sign of skin irritation as compared to moderate erythema shown by marketed TZR formulation (Tazret® gel) after 72 h of application (Table 44). Thus, ME based gel demonstrated advantage over marketed formulation in improving the skin tolerability of TZR indicating their potential in improving patient acceptance and topical delivery of TZR. Also due to appropriate physicochemical properties, high skin permeation ability, higher accumulative uptake in skin and improved skin tolerability it was assumed that the MBG might be suitable for short contact therapy of TZR also. However this needs to be further confirmed by appropriate studies on humans.
Table 44. Mean erythemal scores for various tazarotene formulations obtained at the end of 24, 48 and 72 hours.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Erythmeal score (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h</td>
</tr>
<tr>
<td>Control (Group 1)</td>
<td>0</td>
</tr>
<tr>
<td>Marketed gel (Group 2)</td>
<td>1</td>
</tr>
<tr>
<td>Microemulsion based gel without tazarotene (Group 3)</td>
<td>0</td>
</tr>
<tr>
<td>Microemulsion based gel containing tazarotene (Group 4)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 54. Photographs of skin irritation studies carried out on New Zealand rabbits (A) control (no application); (B) microemulsion based gel without tazarotene; (C) microemulsion based gel containing tazarotene; (D) marketed formulation. Photographs have been taken after 24 h.
9.2.8. Stability Studies

A stability study of the MBG-TZR was carried out under stressed (centrifugation) and unstressed conditions by visual inspection as well as determination of physicochemical parameters such as drug content, pH, spreadability and viscosity. The visual inspection test was carried out for 6 months by drawing sample at weekly interval for the first month and monthly interval for the subsequent months. The MBG showed good shelf stability showing no phase separation or cloudiness for 6 months. It also showed no sign of phase separation under stress when subjected to centrifugation at 5000 x g for 30 minutes. Thus centrifuge tests showed that MBG-TZR had good physical stability The MBG-TZR was found to be stable at room temperature for 6 months with no significant change in the viscosity (6.0 x 10^3 ± 0.8x 10^3 cp), spreadability (7.3 ± 0.06 cm), pH (6.7 ± 0.05) and drug content (99.89 ± 0.042 %w/w).

9.3. References


