Preparation and evaluation of Radiosensitizing agent Nimorazole in topical emulgel

Chandradeep Singh, Pushpani Sharma, *Trishna Bal, Manik Ghosh, Ram Dubey and Soumyajit Das

Department of Pharmaceutical Sciences & Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

ABSTRACT

The present work is mainly based on the preparation and evaluation of emulgel loaded with nimorazole (NMZ), which is a hypoxic cell radiosensitizing agent. The emulgel formulations prepared were investigated for various tests such as Viscosity, Spreadibility, Extrudability, Drug release, Globules size and Zeta potential. The invitro release studies were done using an egg membrane which showed that the cumulative drug release was 80-92 % within five to eight hours. After conducting the various invitro tests, formulations F3 and F1 were found to give the best results. Skin irritation tests were performed on male wistar rats, which concluded that the formulations developed no irritation on to the site of application on the skin.

Keywords: Emulgel; Nimorazole; Hypoxic; Radiosensitizer; Topical delivery

INTRODUCTION

Several anticancer drugs are available in the market as different topical preparations. Nimorazole (NMZ) which is a nitroimidazole class of drug bearing morpholine moiety in its chemical structure is mainly prescribed for amoebiasis and giardiasis. The drug is under phase III clinical studies and has been enquired to act by mechanism of inducing radiosensitivity among hypoxic cancer cells in supraglottic larynx and pharynx carcinoma (1). Till date there is no marketed topical preparation of nimorazole for cancer treatment. Preparation prepared for topical use are mainly intended to deliver local effects at the site of their application by means of drug penetration through underlying skin layers (2). Both O/W and W/O emulsions are widely used due to their advantages and are employed as vehicles to deliver the drugs to the mucous membrane. Emulsions exhibit elegance to a certain extent and are easily washed off whenever needed. They also have a high tendency to penetrate the skin. Furthermore the formulater can control the viscosity, appearance, and degree of greasiness of cosmetic or dermatological emulsions O/W emulsions are mostly used as water washable drug bases and for cosmetic preparations, On the other hand W/O emulsions are widely utilized for the treatment of dry skin and emollient applications (3). Gels for dermatological use have many beneficial properties such as being thixotropic, greaseless, convenient and easy to apply, easily removable, emollient, easily spreadable, nonreactive with other formulation components, and water-soluble or miscible (3).

Emulgel are emulsions which are gelled by combining with gelling agents. When emulsions and gels are used in combined form it is referred as emulgel (4). These emulgel are stable and serve as better vehicle for both the hydrophilic and hydrophobic drugs. Therefore the resulted Emulgel for dermatological uses have many advantages
such as long shelf life, bio-friendly, greaseless, water miscible, easily spreadable, transparent and attractive appearance (4).

The objective of the current research work is to develop a topical Emulgel formulation of Nimorazole using carpool 940P as gelling agent which can be used as radiosensitizing formulation. Initially, an o/w emulsion is prepared which is then incorporated in the carbopol gel.

MATERIALS AND METHODS

2.1 Drugs and Chemicals
Nimorazole (NMZ) was kindly provided as a gift sample from Centaur Pharmaceuticals Pvt. Ltd. Pune, Maharashtra, and Carbopol 940P was obtained from Loba chemicals Mumbai. Egg membrane was prepared by pouring the egg into 0.1M HCl. All other chemicals used were without any chemical modification and were of analytical grade.

2.2 Preparation of Emulgel
Five different formulations of emulgel were prepared using different combinations of liquid paraffin, Tween 20 and Span 20, taking 1% Carbopol 940P. The oil phase of the emulsion was prepared by dissolving Span 20 in liquid paraffin and aqueous phase was prepared by dissolving Tween 20 in purified water with different combinations as discussed in Table 1. The drug Nimorazole is soluble in water and thus the drug was dissolved in the aqueous phase. As the drug is heat resistant at a temperature of 60 °C, so both oil and drug loaded aqueous phase was heated individually at 60°C and then oil phase was added to the aqueous phase and stirred continuously until it got cooled at room temperature. This process of preparation of drug loaded emulsion was followed for all the formulations. The gel phase of the emulsion was prepared by making 1% of Carbopol 940Psolution in purified water with constant stirring. The pH of the gel was adjusted to 6-6.5 employing triethanolamine. The formed gel was added in 1:1 ratio to the emulsion with gentle stirring to formulate the emulgel.

2.3 Evaluation of emulgel
2.3.1 Physical evaluation
The formulated emulgel were evaluated as per their physical characteristics viz. color, homogeneity, consistency, phase separation and pH. Table 2(5)

2.4 Rheological study
The viscosity of the formulated emulgel batches were determined using a Bohlin Visco 88 Viscometer, Malvern Instruments Ltd., U.K. The viscometer assembly was held vertically on a thermostatically controlled circulating water bath maintained at 25°C. The formulation whose viscosity was added to a beaker covered with thermostatic jacket. Spindle was allowed to move freely into emulgel to determine the shear stress, shear strain per second as well the viscosity in the Visco 88 software.

2.5 Thixotropic characteristics
To determine the thixotropic behavior, formulations were subjected to different shear rates. Plot of rate of shear against shear rate showed the thixotropic behavior of emulgel with the down curve being shifted to the left of the curve. When shear rate is applied flow start and structure start to break down as the point of contact are disrupted and polymeric chain are aligned and exhibiting shear thinning (6). When the stress was removed, the structure started to reform and there was progressive restoration of consistency. The emulgels had a lower viscosity at any shear rate on the down curve than it had on up curve. The area of loop between up and down curve of rheogram is measure of thixotropic breakdown (6).

2.6 Spreadability
Spreading coefficient was determined using spreadability apparatus suggested by Mutimer. The apparatus consists of two glass slides in which one is fixed in a wooden frame while the other slide is freely movable over the fixed one. The excess amount of gel (approx. 2 gm.) was then sandwiched between the two slides of the spreadability apparatus. Weight of 5 gm. was placed above the two slides for 5 minutes so as to maintain the uniformity of the gel as well as to expel the air between the slides. Measured quantity of weight was placed in the pan attached to the pulley with the help of the hook of the upper movable slide (7). The time (in sec) required by the upper slide to
cover a distance of 7 cm was noted. Shorter the time taken for separation of two slides, indicates better spreadability. Spreading coefficient was calculated using the formula.

\[ S = \frac{M \times L}{T} \]

Where, \( M \) = wt. tied to upper slide
\( L \) = length of glass slides
\( T \) = time taken to separate the slides.

2.7 Extrudability

The prepared emulgel formulations were filled in aluminum collapsible tubes after formulating them. For extrudability of emulgel formulations is based upon the quantity in percentage of emulgel and emulgel extruded from aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of emulgel in 10 seconds. More quantity extruded better is extrudability (8). The Extrudability of the different emulgel formulations are presented in Table no 3.

2.8 Globule size and its distribution in emulgel

Mean globule size in emulgel, poly dispersity index (PDI) and zeta potential were determined by Zeta Sizer (Malvern Instrument 3000HSA, UK)

2.9 Bioadhesive strength measurement

The modified method was used for the measurement of bioadhesive strength. The fresh skin of the goat intestine was tied with two glass slide separately. One was fixed with the wooden piece and the other was tied with the balance on the right hand side. The right and left pans were balanced by adding extra weight on the left hand pan. 1 gm. of emulgel was sandwiched between two glass slides containing skin. The extra weight from the left pan was removed to sandwich both pieces of skin and external pressure was applied to remove the presence of air. The balance was kept in this position for 5 min. Weight was added slowly at 100 mg/min to the left hand pan until the two slides detach. The weight (in gram) required to detach the emulgel is noted to measure the bioadhesive strength (2). The bioadhesive strength was calculated using the following:

\[ \text{Bioadhesive strength} = \frac{\text{Weight required (in g)}}{\text{Area (cm}^2\text{)}} \]

2.10 Photomicroscopy of the formulations

Photomicroscopy of different formulation of emulgel were determined by LEICA DM E Microscope (Meyer Instrument). The optimized formulations were examined to study the globules structure in gel base (6). Emulgel formulation was diluted with water, fixed on the glass slide and examined under microscope of 40X, 100X.

2.11. Analytical evaluation of formulations:

2.11.1. Thermal studies

The thermogram of the optimized formulations (F1 and F3) and the pure drug (NMZ) were determined using DSC (Digital Scanning Calorimetry 60, Shimadzu, Japan) to check any interaction between the drug and the emulgel. Samples of 5mg weight were taken in aluminum crucibles and started heating from 20 – 300°C in nitrogen atmosphere.

2.11.2. FTIR spectroscopy

FT-IR spectroscopy (FT IR- 8400-S, Shimadzu, Japan) was employed to ascertain the compatibility of NMZ with the emulgel base. The spectra of individual drug and the final formulation were compared for confirmation of common peaks (6)

2.12 Drug content

Around 100 mg of different composition of emulgel formulations were dissolved in a suitable solvent and filtered to obtain clear solution. The clear solution was analyzed spectrophotometrically at wavelength of 305 nm. Drug content was determined from the standard curve of NMZ (9). The standard curve of the drug was obtained by preparing standard stock solution of nimorazole by dissolving 10 mg of drug in 100 mL of purified water in a 100 mL volumetric flask having a concentration of 100 µg/mL and measured the absorbance at 305 nm in a UV Spectrophotometer.
2.13. Determination of pH
The pH of different formulations of emulgel were measured by using digital pH meter (Thermo scientific) by dipping the glass electrode into diluted emulgel (10). pH value indicates the suitability of emulgel for topical formulation.

The in vitro drug release studies were carried out using Franz diffusion (FD) cell. The formulations were applied on egg membrane which was prepared by keeping the egg in 0.1M HCl (11). The membrane was stretched between the donor and receptor compartment of the FD cell. The membrane was tied with thread to prevent leakage. The donor compartment was emerged in dissolution vessels which contain 75 mL of phosphate buffer pH 7.4 and maintained at 37°C ± 0.5°C (11). The dissolution media was stirred at 50 rpm and the aliquots of 1 mL were withdrawn at different time intervals. The withdrawn sample was replaced by the equal volume of phosphate buffer. Samples were analyzed spectrophotometrically at 305 nm and concentration of drug was determined from the previous standard curve of nimorazole. The study was recorded for 8 h and cumulative % release and drug release kinetics is reported.

2.15 Skin irritation test (patch test)
A set of six wistar rats issued by the Institute Animal Ethical Committee having approval number PROV/BIT/PH//2014 was used in the study. The emulgel (1 gm.) was applied on the properly shaven skin of rat (1 square inch). After 24 h exposure, the formulation was removed. Any undesirable change i.e. change in skin morphology, erythema/edema were monitored by visual observation (11).

2.16 Stability studies
The prepared formulations were packed in aluminum collapsible tubes (5g) and subjected to stability studies at 5°C, 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH for a period of 3 months. Samples were withdrawn at 15-day time intervals and evaluated for physical appearance, pH, viscosity and drug content (12).

RESULTS AND DISCUSSION

3.1. Physical evaluation
Emulgel formulations were whitish and pale yellow with homogeneous and glossy in appearance. Among all formulations F1 and F3 were showing excellent homogeneous and glossy in appearance. Results were discussed in Table 2.

3.2. Rheological studies
The rheological studies helped to determine the viscosity of formulations and its thixotropic behavior. All the formulations have relatively same viscosity due to same concentration of carbopol 940P employed. F1 and F3 formulations showed slightly high viscosity due to low concentration of liquid paraffin and emulsifying agent. Results were given in Fig.1.

3.3. Thixotropic behavior
The area of loop between up and down curve of rheogram is measure of thixotropic breakdown (6). In formulations F1 and F3, area is more indicating more structure breakdown. Rheograms indicate that all the formulations exhibited pseudoplastic flow as shown in Fig. 1.

3.4. Spreading coefficient
The spreading coefficient of different formulation of emulgel are presented in Table 3 and Fig.2. The results indicates that more higher spreading coefficient, the better is the spreadability of the formulation.

3.5. Extrudability
Extrudability of different formulations of emulgel showed almost similar results but formulation F1 and F3 showed highest extrudability , which also due to same concentration of carbopol content were used. Results are presented in Table 3 and Fig.2a.
3.6. Globule size and its distribution in emulgel
All the formulations of emulgel showed the globules size range from 231 to 283 nm. Among the all, F1 and F3 having lowest particles size while other formulations had larger particles size relatively Table 4. Formulations which are having lower particles size of in nano range and monodispered were stable and will not be converted into macro range particles.

Zeta potential governs the stability of formulations of emulgel. All the formulations of emulgel were showing high negative value of zeta potential more than±61, so all are highly stable. Formulation F1 and F3 having more zeta potential than other formulations. High value of zeta potential indicative electrostatic repulsion between two particles. According to DLVO theory, electric double layer repulsion will stabilize the formulations and aggregation is not expected to occur due the highly negative charge of particles. Results are shown in Table 4.

PDI is measure of particle homogeneity and it varies from 0.0 to 1.0. The polydispersity value are closer to zero means more homogenous are the particles. All the formulations having PDI value below than 1. Formulation F1 and F3 had PDI value near to zero suggesting that the formulations (F1 and F3) were more monodispersed than other formulations of emulgel. Result are shown in Table 4.

3.7. Bioadhesive strength measurement
The bioadhesive strength of different formulations of emulgel has been shown in Table. 5 and Fig. 3. The result explains that the bioadhesive strength of the formulations may be due to the capability of the gelling agent to form hydrogen bond.

3.8. Photomicroscopy of the formulations
Different Emulgel formulations exhibited good consistency and homogeneity. The photo-microscopic evaluations as shown in Fig. 4, showed the presence of spherical globules in the formulations which indicates formation of emulsion in gel base and hence this proved the success of the method employed in preparation of emulgel formulations.

3.9. Analytical evaluation of formulations
3.9.1. Differential Scanning Calorimetry
DSC thermogram of pure drug (Nimorazole) corresponded to its melting point by a sharp endothermic peak as shown in Fig. 5. Pure nimorazole showed a sharp peak at 109.41°C and emulgel formulation showed a peak at 107.04 °C. These two peaks lie between the melting point ranges of pure drug (Nimorazole) and hence absence of major interaction between drug and excipient of emulgel can be concluded.

3.9.2. Compatibility study
FT-IR spectra of the individual drug and the emulgel formulation showed no interaction between drug and excipient and hence it was concluded that the drug is compatible with the excipients used. Results are shown in Fig. 6.

3.10. Drug content and pH determination
Drug content of different formulations of emulgel was found between 90 to 98 %. Formulations F1 and F3 have more drug content than the other formulations. Amount of drug in the emulgel indicates the suitability of the system for high entrapment in internal phase system. Result are shown in Table 6. pH of different formulations was found between 6 to 7. Results are shown in Table 6.

3.11. Invitro drug release studies
Invitro drug release study was done with only two optimized formulations (F1 and F3). Selection of optimized formulation were done by above characterizations viz. low globules size, high zeta potential and low PDI etc. The invitro release profile of optimized formulations are presented in Table 7 and Fig. 7. From the results of invitro diffusion studies, it is concluded that both formulations F1 & F3 shows drug release upto 8 hours, but F3 shows better and increased amount of drug release of 92.33% in 8 hours duration which is beneficial for topical emulgel formulation, whereas in case of F1 shows only 80% drug release.

In order to understand the release kinetics of drug from the formulations, the results were fitted into different kinetic equations such as Zero order, First order, Higuchi, Hixson Crowell and Korsmeyer peppa’s models. The in vitro release data were fitted to Korsmeyer peppa’s release model and interpretation of release exponent value (n) helps us
in understanding the release mechanism from the formulations. The release exponent values thus obtained were from 0.50 to 0.86. Based on these values we concluded that optimized formulation exhibited non-fickian transport. The drug release was diffusion controlled as the Higuchi model were found to be linear (r>0.98). Formulations showed the higher R² value for zero order indicating release profile of drug from the emulgel formulation followed the Zero order and drug release from emulgel were by both diffusion and erosion. Kinetic profile of optimized formulations are presented in Table 8.

3.12. Skin irritation test (Patch test)
Skin test was performed on wistar rat by applying gel on shaven skin. After 24 hours exposure, no any undesirable changes were monitored by visual observation. Results were shown in Fig. 8.

3.13. Stability studies
Accelerated stability studies were performed for optimized formulations of emulgel for 3 months. The samples were analyzed after 15 days for physical appearance and drug content, pH and viscosity. It was found that there was no any change in physical appearance, pH, drug content and viscosity of optimized formulations of emulgel.
Trishna Bal et al

Der Pharmacia Lettre, 2015, 7 (9):132-142

Scholar Research Library
Skin Irritation Study of Emulgel
A: During Application;    B: After 24 hrs.

% Transmittance

Wave number(cm⁻¹)

% Cumulative Drug release

Time (Minutes)
### Table 1: Composition of emulgel formulation

<table>
<thead>
<tr>
<th>Quantity in (%) w/w</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nimorazole</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Liquid Paraffin</td>
<td>4.15</td>
<td>5</td>
<td>5</td>
<td>6.25</td>
<td>7.5</td>
</tr>
<tr>
<td>Span 20</td>
<td>0.60</td>
<td>0.75</td>
<td>0.45</td>
<td>0.60</td>
<td>0.75</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.40</td>
<td>0.5</td>
<td>0.30</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>Carbopol 940P</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 2: Physical parameters of different formulation batches

<table>
<thead>
<tr>
<th>Parameters</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>White</td>
<td>Pale Yellow</td>
<td>Pale Yellow</td>
<td>Yellow</td>
<td>White</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Excellent</td>
<td>Good</td>
<td>Excellent</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Consistency</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Phase separation</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

### Table 3: Spreadability and Extrudability of different formulation batches

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Spreadability Coefficient (in gcm/s) ± S.D*</th>
<th>Extrudability (in g/cm²) ± S.D*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>23 ± 0.07</td>
<td>59.34 ± 0.06</td>
</tr>
<tr>
<td>F2</td>
<td>20.1 ± 0.07</td>
<td>51.13 ± 0.01</td>
</tr>
<tr>
<td>F3</td>
<td>26 ± 0.07</td>
<td>62.51 ± 0.05</td>
</tr>
<tr>
<td>F4</td>
<td>19 ± 0.08</td>
<td>45.55 ± 0.02</td>
</tr>
<tr>
<td>F5</td>
<td>18.2 ± 0.12</td>
<td>49.31 ± 0.03</td>
</tr>
</tbody>
</table>

*Standard deviation mean “n” = 3*

### Table 4: Globule size, Zeta potential and Polydispersity index (PDI)

<table>
<thead>
<tr>
<th>Formulation no.</th>
<th>Globule size (nm)</th>
<th>Zeta potential (mV)</th>
<th>Zeta potential (mV)</th>
<th>PDI (Polydispersity index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>235.5</td>
<td>-75.2</td>
<td>0.206</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>264.1</td>
<td>-72.1</td>
<td>0.235</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>231.3</td>
<td>-76.3</td>
<td>0.198</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>304.1</td>
<td>-64.5</td>
<td>0.345</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>283.2</td>
<td>-68.1</td>
<td>0.541</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Bioadhesive Strength

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bioadhesive strength (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5.39</td>
</tr>
<tr>
<td>F2</td>
<td>4.93</td>
</tr>
<tr>
<td>F3</td>
<td>5.59</td>
</tr>
<tr>
<td>F4</td>
<td>5.32</td>
</tr>
<tr>
<td>F5</td>
<td>4.67</td>
</tr>
</tbody>
</table>

### Table 6: % Drug Content and pH of different Formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Drug Content</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>96.35</td>
<td>6.9</td>
</tr>
<tr>
<td>F2</td>
<td>96.23</td>
<td>7.12</td>
</tr>
<tr>
<td>F3</td>
<td>98.13</td>
<td>6.9</td>
</tr>
<tr>
<td>F4</td>
<td>94.34</td>
<td>7.2</td>
</tr>
<tr>
<td>F5</td>
<td>95.28</td>
<td>7.0</td>
</tr>
</tbody>
</table>

### Table 7: In vitro drug release profile of optimized formulations

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>F1</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>7.718</td>
<td>28.305</td>
</tr>
<tr>
<td>60</td>
<td>10.830</td>
<td>37.403</td>
</tr>
<tr>
<td>120</td>
<td>20.258</td>
<td>46.838</td>
</tr>
<tr>
<td>180</td>
<td>25.170</td>
<td>56.550</td>
</tr>
<tr>
<td>240</td>
<td>41.610</td>
<td>68.685</td>
</tr>
<tr>
<td>300</td>
<td>48.030</td>
<td>79.410</td>
</tr>
<tr>
<td>360</td>
<td>56.153</td>
<td>84.345</td>
</tr>
<tr>
<td>420</td>
<td>68.580</td>
<td>87.435</td>
</tr>
<tr>
<td>480</td>
<td>80.010</td>
<td>92.333</td>
</tr>
</tbody>
</table>
CONCLUSION

Nimorazole, being a radiosensitizer can be used extensively in form of topical drug delivery on the head and neck regions of hypoxic tumors, thereby maintaining highest drug activity and patient compliance. Emulgel has become one of the novel drug delivery due to the rapid onset of drug spreadability over the affected regions in terms with drug permeability through the skin tissues as well as being a stable formulation in oil in water emulsion type bases.

The present study reports the development of an optimized Nimorazole loaded emulgel for topical use before radiotherapy using statistical analysis, which were subjected to various physicochemical test parameters i.e. spreadability, extrudability, rheology, skin irritation test using wistar rats and in-vitro drug release kinetics. During the studies, it has been reported that formulation F1 and F3 were the two optimized formulations having the maximum release of 90-95% in 8 hours. The formulation was stable for 90 days at cool temperature without any change in physical appearance or physicochemical parameters viz. pH, viscosity etc.

Therefore, a stable, rapid, target specific novel delivery system of a hypoxic radiosensitizer nimorazole was designed and formulated employing simple preparation technique using egg membrane, without affecting the conventional routes of administration.

Acknowledgement

The authors sincerely acknowledges Dr. Sanjay Swain, Dr. Sudhir Saw, Mr. Samir, Mr. Rammani, CIF (Central Instrumentation facility) BIT, Mesra, Ranchi, for providing their necessary technical help in analyzing the samples.

REFERENCES