A NOVEL APPROACH FOR THE USE OF ARTEROLANE MALEATE IN TREATMENT OF MALARIA

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ABSTRACT

Malaria is one of the most prominent life threatening disease inspite of many efforts made to fight against it. There are many clinically effective anti-malarial agents but the major reasons of malaria treatment failure are due resistance development, incorrect dosing, poor drug quality, drug interactions, poor drug absorption and misdiagnosis. Current approach done in this study is to formulate a dosage form which can overcome these drawbacks. Here nanoparticles of Arterolane Maleate was formulated by solvent evaporation method using different concentration of polymers ethylcellulose, eudragit RS 100 and eudragit RL 100 and evaluated for preformulation studies, drug compatibility studies was done using FTIR. Evaluation parameters such as scanning electron microscopy (SEM), particles size analysis, zeta potential and in-vitro dissolution studies were done. The nanoparticles were obtained in the size range of 100 – 200 nm. The evaluation results obtained showed that the formulation F9 having highest polymer ratio showed the best release profile.

KEYWORDS: malaria, nanoparticle, Arterolane Maleate, zeta potential

INTRODUCTION

Malaria is one of the most occurring parasitic diseases around the globe causing 1 to 2 million deaths round the world every year and is considered as a life threatening disease. Chloroquine, amodiaquine, sulphadoxine and pyrimethamine, etc are limited number of clinically effective antimalarial agents but the major problems faced by them are poor bioavailability of the drug and development of resistance against the drug.\footnote{[1,2,3]}
The artemisinins derivatives artesunate (AS), artemether (ARM), arteether (AE) and dihydroartemisinin (DHA) are the most effective anti-malarial drugs known today. Despite these achievements, Artemisinins and its derivatives have low bioavailability, poor pharmacokinetic properties and high cost. Furthermore, in-vitro tolerance was found in South America and South-East Asia. As the drug is obtained from plant source the major drawback with ARTs is to maintain balance between demand and supply.\[4\]

**Arterolane maleate** is a synthetic drug consisting of 1,2,4-trioxolane with a peroxidic pharmacophore. It is a rapidly acting oral antimalarial drug. Arterolane Maleate is highly potent in both in-vitro and in-vivo than other drugs against malarial parasites. The irreversible redox reaction between antimalarial peroxides and heme produces carbon-centred radicals or carbocations that alkylate heme and proteins, leading to perturbation of lipid components of the parasite digestive vacuole.

Arterolane has an elimination half-life (t1/2) between 1 and 3 h. Phase 1 trials were conducted by Medicines for Malaria Venture (MMV), and Ranbaxy Laboratories on the healthy subjects in United Kingdom which proved that Arterolane Maleate having 90% parasitic clearance rate and is well tolerated and has very less side effects. Arterolane has also showed a significant result in phase II and phase III trials conducted to evaluate the antimalarial activity and safety of arterolane to patients with acute uncomplicated *Plasmodium Falciparum* malaria.\[5,6\]

Nanotechnology has proved to be a revolution in recent years in the field of medicines. The particles size ranges from 0 - 1000nm. Due to its small particles size it has significant advantages in diagnosis and treatment of the disease and helps in increasing the bioavailability and reducing toxic effects of the drug. Nanoparticles can also be used for drug targeting.

The aim of the present study was to formulate and evaluate Arterolane Maleate nanoparticles for treatment of malaria. Nanoparticles of Arterolane Maleate were prepared by using solvent evaporation method with the help of Ethylcellulose, Eudragit RS 100 and Eudragit RL 100 polymers. Evaluation parameters such as FTIR, scanning electron microscopy (SEM), Particles size, zeta potential, differential scanning colorimetry (DSC) and in-vitro dissolution studies were done.\[7,8\]
MATERIALS AND METHODS
Gift Arterolane maleate was obtained from china, Ethycellulose and tween 80 from Hi media pvt ltd mumbai, eudragit RS100, Eudragit RL 100 and Polyvinyl alcohol from yarrow chem production mumbai, dichloromethane and n hexane from Loba chemie pvt ltd Mumbai and ethanol from Karnataka state beverages corp ltd.

Preparation of Arterolane Maleate nanoparticles
Nanoparticles will be prepared by Emulsion Solvent Evaporation Method. In this technique nanoparticles will be prepared by dispersing accurately weight quantities of Arterolane and polymers individually (Ethylcellulose, Eudragit RS 100, and RL 100) in the primary phase as a solvent (1: 1 combination of acetone and isopropanol) with continuous stirring at 500 rpm by using magnetic stirrer for 15 min. Sustained released nanoparticles of drug will be prepared by modified hydrophobic emulsion solvent evaporation method (O/O). This primary emulsion will be slowly added to the external secondary oil phase containing span 80 (0.4% v/v) as an emulsifying agent and will be applied to probe sonicator with constant stirring for 2 hours using a four-blade lab stirrer at a speed of 1000 rpm. After complete evaporation, stirring was stopped, the n-hexane (20 ml) will be added to harden the formed nanoparticles, and the mixture will be vacuum filtered to obtain nanoparticles. The resulting nanoparticles will be collected and allowed to dry for 24h at room temperature.\textsuperscript{[9]}

PHYSICOCHEMICAL PROPERTIES OF DRUGS
Solubility
Solubility of Arterolane Maleate was determined in solvents: water, methanol and chloroform. Excess amount of sample were added in 10 ml of solvent with stirring (300 rpm), at temperature 25 ± 0.5°C for 48 h and sonicated using sonicator (Electrolab\textsuperscript{TM}) for 2 h. Samples were filtered through 0.45 μm filters and the solubility was measured.

Melting point
Melting point of Arterolane Maleate was determined by using melting point apparatus (dolphin). The drug was filled in a capillary tube and placed in the apparatus and the melting point was recorded.
Evaluation of micromeritic properties of dried powder of nanoparticles

**Bulk density, tap density, Carr’s index and Hausner’s ratio**

Bulk density and tap density was determined according to following method: A 50 ml glass cylinder was weighed and filled with 30 ml of Arterolane Maleate powder and reweighed. The opening was secured with parafilm. The cylinder was gently reversed once and the powder was carefully levelled without compacting. Bulk volume was determined after one mechanical tap on a tap density tester (DolphinTM). Tap volume was measured after 2000 taps. Each analysis was repeated twice. Values of bulk density and tap density are used to calculate Carr’s index and Hausner’s ratio and are given in table no-1.

\[
\text{Bulk density} = \frac{\text{Weight of the powder}}{\text{Bulk volume}}
\]

\[
\text{Tap density} = \frac{\text{weight of the powder}}{\text{Tapped volume}}
\]

\[
\text{Carr’s Index} = \frac{(\text{Tap density} - \text{bulk density})}{\text{Tap density}} \times 100
\]

\[
\text{Hausner’s ratio} = \frac{\text{Tap density}}{\text{Bulk density}}
\]

**Angle of repose**

Fixed funnel method was used for determination of angle of repose and was calculated by using following formula. The values of angle of repose were as given in table no-1.

\[
\text{Angle of repose} = \tan^{-1}\left(\frac{\text{height}}{\text{radius}}\right)
\]

**AUTHENTICATION OF DRUGS**

**Fourier transforms Infrared spectroscopy (FTIR)**

Fourier transforms Infrared spectroscopy of Arterolane Maleate drug and polymers were recorded using Perkin Elmer (Model Spectrum 1, USA) FTIR system using potassium bromide (KBr) pellet method. Each spectrum was derived from single average scans collected in the region 4000 to 400/500 cm\(^{-1}\). The FTIR spectra of drug and polymers are shown in given in figure 1, 2, 3, 4, 5, 6 and 7 respectively has confirmed the authentication of drugs.
Field Emission Scanning Electron Microscopy (FSEM)\textsuperscript{[13]}

The morphology of samples was carried out using FSEM type II modes S-4800 Hitachi Japan. Surface morphology was analyzed at a working distance of 7-8.8mm and 1.0 kv accelerating voltage.

Particle Size Distribution and Zeta Potential\textsuperscript{[14,15]}

Particle Size Distribution and Zeta Potential were determined in water as a dispersion medium by laser diffraction size analyzer. Malvern Zetasizer (Model 2S200).

Entrapment efficiency Studies\textsuperscript{[16,17]}

5mg of nanoparticles was diluted with 5\% of methanol diluted with water and the absorbance was measured at 223 nm. The amount of Arterolane malate entrapped was determined by subtracting amount of free un-entrapped drug from the total amount of Arterolane malate taken for the preparation. The formula used to calculate entrapment efficiency was given below:

\[
\text{Drug entrapment efficiency} = \frac{\text{mass of drug in nanoparticles}}{\text{mass of drug used in formulation}} \times 100
\]

In-Vitro dissolution studies\textsuperscript{[18,19]}

50 mg equivalent of microspheres were packed in hard gelatin capsules and were subjected to in-vitro dissolution studies using a twin buffer system consisting of 0.1 N HCl and 7.4 pH phosphate buffer using a USP II dissolution apparatus for a period of 24 hours. Samples were withdrawn at regular intervals while substituting the same with fresh buffer. Samples were suitably diluted and tested spectrophotometrically at \(\lambda_{\text{max}}\) of 223nm. The resultant values were taken and cumulative drug release percentage was calculated.

RESULTS

Melting point

Melting point of Arterolane Maleate was determined by using melting point apparatus (dolphin). The melting point was found to be 272.16\(^\circ\) C.

Solubility

From the solubility studies done it has been found that Arterolane Maleate is slightly soluble in water and freely soluble in methanol and chloroform.
Evaluation of granulation Table no.1.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bulk density (gm/cc)</td>
<td>0.544 ± 0.007</td>
</tr>
<tr>
<td>2</td>
<td>Tapped density (gm/cc)</td>
<td>0.0591 ± 0.006</td>
</tr>
<tr>
<td>3</td>
<td>Carr’s Index (%)</td>
<td>7.95 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>Hausner’s Ratio</td>
<td>1.08 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>Angle of Repose (º)</td>
<td>22.23 ± 1.5</td>
</tr>
</tbody>
</table>

Formulation of nanoparticles Table no. 2.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterolane Maleate</td>
<td>50 mg</td>
<td>50 mg</td>
<td>50 mg</td>
<td>50 mg</td>
<td>50 mg</td>
<td>50 mg</td>
<td>50 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>1 gm</td>
<td>2 gm</td>
<td>3 gm</td>
<td>1 gm</td>
<td>1 gm</td>
<td>2 gm</td>
<td>2 gm</td>
<td>3 gm</td>
</tr>
<tr>
<td>Eudragit RS 100</td>
<td>1 gm</td>
<td>1 gm</td>
<td>1 gm</td>
<td>2 gm</td>
<td>2 gm</td>
<td>3 gm</td>
<td>1 gm</td>
<td>2 gm</td>
</tr>
<tr>
<td>Eudragit RL 100</td>
<td>1 gm</td>
<td>1 gm</td>
<td>1 gm</td>
<td>1 gm</td>
<td>2 gm</td>
<td>3 gm</td>
<td>2 gm</td>
<td>1 gm</td>
</tr>
</tbody>
</table>

Fourier transforms Infrared spectroscopy (FTIR)

Fig. no. 1: FTIR of Arterolane Maleate in IP.
Fig no. 2: FTIR of Arterolane Maleate.

Fig no. 3: FTIR of Ethylcellulose.
Fig no. 4: FTIR of Eudragit RL-100.

Fig no. 5: FTIR of Eudragit RS-100.
PHYSICOCHEMICAL CHARACTERISTICS OF NANOPARTICLES

FTIR

Fig no. 6: FTIR of drug and polymer.

Fig no. 7: FTIR of formulation.
SEM OF NANOPARTICLE FORMULATION

Fig no. 8: FESEM of Formulation F1.

Fig no. 9: FSEM of F2.

Fig no. 10: FSEM of F3.
Fig no. 11: FSEM of F4.

Fig no. 12: FSEM of F5.

Zeta potential studies

Fig no. 13: zeta potential results for formulation F3.
ENTRAPMENT EFFICIENCY STUDIES

Table no. 2: Entrapment efficiency.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Entrapment efficiency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>60.56± 0.03</td>
</tr>
<tr>
<td>F2</td>
<td>64.82 ± 0.02</td>
</tr>
<tr>
<td>F3</td>
<td>78.75 ± 0.61</td>
</tr>
<tr>
<td>F4</td>
<td>64.76 ± 0.33</td>
</tr>
<tr>
<td>F5</td>
<td>72.29 ± 0.21</td>
</tr>
<tr>
<td>F6</td>
<td>75.54 ± 0.43</td>
</tr>
<tr>
<td>F7</td>
<td>68.77 ± 0.25</td>
</tr>
<tr>
<td>F8</td>
<td>77.65 ± 0.71</td>
</tr>
<tr>
<td>F9</td>
<td>83.44 ± 0.08</td>
</tr>
</tbody>
</table>

*All the values are expressed as mean ±Standard deviation; n=3
IN-VITRO DISSOLUTION STUDIES

Table no. 4: In-vitro Dissolution studies.

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Time (hrs)</th>
<th>% cumulative release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>26.45±0.07</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>44.75±0.23</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>78.45±0.14</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>84.44±0.23</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>85.31±0.16</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>86.55±0.48</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>86.63±0.23</td>
</tr>
</tbody>
</table>

Fig no. 16: Drug Release Profile for Formulations F1-F4.

Fig no. 17: Drug Release Profile for Formulations F5-F9.
Discussion on the findings of the study.

Table no. 3: Peaks observed in FTIR spectra of Arterolane Maleate.

<table>
<thead>
<tr>
<th>Description</th>
<th>Pure Drug cm⁻¹</th>
<th>Drug + Polymer</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-H</td>
<td>3319.02</td>
<td>3321.05</td>
<td>3291.10</td>
</tr>
<tr>
<td>C-F</td>
<td>1400.12</td>
<td>1405.20</td>
<td>1435.15</td>
</tr>
<tr>
<td>C-O</td>
<td>1349.50</td>
<td>1346.39</td>
<td>1352.41</td>
</tr>
<tr>
<td>N-O</td>
<td>1375.32</td>
<td>1368.28</td>
<td>1370.18</td>
</tr>
</tbody>
</table>

The physicochemical compatibility of the drug and polymers was established through FTIR studies. In the physical mixture of Arterolane Maleate, Ethylcellulose, Eudragit RS-100 and Eudragit RL-100 the major peaks found were 3319.02 (N-H stretch), 1400.12 (C-F stretch), 1349.50 (C-O stretch) and 1375.32 (N-O stretch) wave numbers. The result has indicated that there was no chemical interaction between drug and polymers.

**Particle Morphology by Field Emission Scanning Electron Microscopy (FSEM)**

Fig no- 8, Fig no-9, Fig no-10, Fig no-11 and Fig no-12 shows irregular shape of field emission scanning electron micrographs (FSEM) of preliminary prepared nanoparticles containing Arterolane Maleate prepared by solvent evaporation method. The size of the nanoparticles is ranging from 100-200 nm.

**Particle Size Distribution and Zeta Potential**

The sample of preliminary nanoparticles Arterolane Maleate was sent for analysis of particle size distribution and zeta potential. In which the formulation with the combination of polymers showed the best result within the acceptable range, indicating it to be the better formulation.

**Entrapment ratio**

The percentage entrapment was calculated spectrophotometrically. All the formulations showed various degrees of entrapment. The formulations with eudragit RL 100 showed a better result than that of eudragit RS 100. The combination of both the polymers showed the best set of results. Among that the formulation F9 showed the best entrapment.

**Drug release studies**

The *in-vitro* dissolution studies which were carried out for a period of 24 hours using a twin buffer system showed a minimal drug release during the initial stages for all the formulations. It was observed that as the ratio of the polymer increased in the formulation, the drug release...
was also sustained. The best formulation was found to be F9 which showed a cumulative drug release of $90.95 \pm 0.12\%$ at the end of the study.

**CONCLUSIONS**

The literature review was done till date. Nanoparticles were formulated using solvent evaporation method. The Formulation was done using different polymer ratios. The FTIR results concluded that there are no chemical interactions between drug and polymers. Field emission scanning electron microscopy (FSEM) was done which shows the formation of nanoparticles. The nanoparticles were formed in the size range of 100- 200 nm. Further particle size distribution and zeta potential of nanoparticles were studied and indicated that the combination product with the highest polymer ratio gave the best result of 3.70 mV. Dissolution studies were carried out using a twin buffer system showed a minimal drug release during the initial stages for all the formulations and gradual increase during the later stages of the process. The formulation F9 having the highest polymer ratio on combination showed the best release profile with a cumulative release of $90.95 \pm 0.12\%$ at the end of the study.

**REFERENCES**


