Formulation and Evaluation of Mefloquine Hydrochloride Nanoparticles

Dilip Kumar Gupta¹*, B.K.Razdan² and Meenakshi Bajpai²

¹Raj Kumar Goel Institute of Pharmaceutical Sciences, 5th km stone, Delhi-Meerut Road, Ghaziabad, UP 201003, India, and ²,³Faculty of Pharmacy, Uttarakhand Technical University, Sudhhowala, P.O. Chandanwari, Premnagar, Dehradun-248007, India.

Received August 14, 2013; accepted October 31, 2013

ABSTRACT

The present study deals with the formulation and evaluation of mefloquine hydrochloride nanoparticles. Mefloquine is a blood schizonticidal quinoline compound, which is indicated for the treatment of mild-to-moderate acute malarial infections caused by mefloquine-susceptible multi-resistant strains of *P. falciparum* and *P. vivax*. The purpose of the present work is to minimize the dosing frequency, taste masking toxicity and to improve the therapeutic efficacy by formulating mefloquine HCl nanoparticles. Mefloquine nanoparticles were formulated by emulsion diffusion method using polymer poly(ɛ-caprolactone) with six different formulations. Nanoparticles were characterized by determining its particle size, polydispersity index, drug entrapment efficiency, drug content, particle morphological character and drug release. The particle size ranged between 100 nm to 240 nm. Drug entrapment efficacy was >95%. The *in-vitro* release of nanoparticles were carried out which exhibited a sustained release of mefloquine HCl from nanoparticles up to 24 hrs. The results showed that nanoparticles can be a promising drug delivery system for sustained release of mefloquine HCl.

KEYWORDS: Mefloquine; Nanoparticles; Poly(ɛ-caprolactone); Emulsion diffusion; Zeta potential.

Introduction

Many drug candidates face problems like poor absorption, rapid metabolism and elimination, toxicity due to drug distribution to other tissues, poor drug solubility, and unpredictable bioavailability. These problems need to be resolved so as to make the existing drugs successful for therapy (Jain, 2010). One of the promising strategies to overcome these problems is use of nanotechnology. Unique properties like small size, high surface area, and ease of suspending in liquids, deep access to cells and organelles, variable optical and magnetic properties are offered by nanoparticles as compared to micro or macro particles (Gupta, 2006). Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm (Mohanraj et al., 2006). They consist of macromolecular materials in which the active ingredient is dissolved, entrapped, encapsulated, and adsorbed or chemically attached (Mohanraj et al., 2006). They may be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of materials is dependent on many factors including: (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; and (e) drug release profile desired (Thassu et al., 2007). Mefloquine hydrochloride is a BCS Class I drug (Kasim et al., 2004) based on solubility data in water and the calculated partition coefficients. By contrast (Lindenberg et al., 2004) classified Mefloquine hydrochloride as BCS Class II/IV (borderline), because of the low solubility in the gastrointestinal pH range and the lack of reliable permeability data. Although the mefloquine HCl free base has been conjugated with acids other than hydrochloride, in this text mefloquine will mean mefloquine hydrochloride for the sake of brevity.

The purpose of this research was to minimize the frequency of doses, taste masking, toxicity and to improve the therapeutic efficacy by formulating mefloquine nanoparticles.

Materials and Methods

Chemicals and drugs

Mefloquine HCl was obtained as a gift sample from Aristo Pharmaceutical Pvt. Ltd (Mumbai, India). PVA (Cold) M.W Approx 1, 25,000 CDH Laboratory reagent, Caprol micro-express oil (lot no.: BW1-5 for R&D purposes only) was obtained as gift sample from Abitec Corporation, Poly(ε-caprolactone) diol (MW 09063, mp 36-48°C, d 1.073, batch no.:04216DH) was purchased from Sigma–Aldrich (St Louis, MO63103) USA and All other reagents and chemicals used were of analytical grade.
Methodology

Drug-excipient compatibility study

The DSC analysis was carried out to identify the compatibility between the drug and excipients. The DSC analysis of pure drug, 1:1 physical mixture of drug excipient was carried out using DSC 60, Shimadzu, Japan. Samples (2.8 mg) were accurately weighed into an aluminum pan, which was crimped non-hermetically and heated in sealed aluminum pans at a rate of 10°C/min between 0-300 °C temp ranges under nitrogen atmosphere.

Solubility study for oil selection

The solubility of Mefloquine HCl in various oils (Fig: 1) was determined by adding an excess amount of drug to 2 mL of selected oils in 5-mL stopper vials, and mixing using a vortex mixer. The vials were then kept at 25 ± 1.0 °C in an isothermal shaker (Nirmal International, Delhi, India) for 72 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 4000 rpm for 15 minutes. The supernatant was taken and filtered through a 0.45 μm (Rendisk) membrane filter. The concentration of Mefloquine HCl was determined in the oils using UV Spectroscopy at 222 nm (Sheikh et al., 2007).

Method for Preparation

Mefloquine HCl nanocapsules were prepared by emulsion diffusion method described in Fig.1. First mutually saturated aqueous and organic phase were prepared (Delphine et al., 2008). The saturated water contained 8.9% of ethyl acetate and the saturated solvent contained 3% of water. PVA was dissolved in saturated water at 50°C for 2hr. Polyo-caprolactone was dissolved in saturated ethyl acetate at 50 °C and Caprol micro-express oil was added when the solution has come back to room temperature. The resulting organic solution was poured into the aqueous phase and emulsified with lab stirrer devise (Remi Motor Ltd, India) for 45 min at 4000 rpm and sonicated (Bandelin Sonopuls, Germany 2006) for 20 min at 9 cycle/min. After sonication the addition of large volume of water (2times the volume of the emulsion) to the emulsion under gentle stirring with magnetic bar allowed the ethyl acetate to leave the droplets. The organic solvent and part of the water were evaporated under reduced pressure to afford a purified and concentrated suspension. The prepared formulations were stored in cool and dry place. Six different batches were prepared and labeled as MFQ-1 to MFQ-6 with the following composition as shown in Table 1. The turbidity studied of all nanosuspensions is depended on ratio of drugs and excipients.

Evaluation of formulations

Percentage yield

Percentage practical yield is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting material. The percentage yield of prepared nanoparticles was determined by using the formula.

\[ \text{Percentage Yield} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100 \]

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ingredient</th>
<th>MFQ-1</th>
<th>MFQ-2</th>
<th>MFQ-3</th>
<th>MFQ-4</th>
<th>MFQ-5</th>
<th>MFQ-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mefloquine HCl (%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>PVA (%)</td>
<td>0.25</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>Caprol micro oil</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Poly ε-caprolactone</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Water (ml)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl Acetate (ml)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Drug Entrapment Efficiency

The entrapment efficiencies of prepared systems were determined by measuring the concentration of free drug in the dispersion medium. The obtained suspension was centrifuged for 60 min at 10,000 rpm. The supernatant was separated and then filtered through 0.45 μm Millipore (Millipore Filter). The filtrate was diluted using methanol and measured spectrophotometrically at 222 nm (Shimadzu, UV 1700, Japan). The amount of free drug was detected in the filtrate and the amount of incorporated drug was determined as a result of the initial drug minus the free drug. The entrapment efficiency was calculated using the following equation.

\[ \text{Entrapment Efficiency} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100 \]

Where “W initial drug” is the mass of initial drug used and the “W free drug” is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

Particle size characterization

Particle size analysis

In order to analyze particle size, drug loaded nanoparticles were dispersed in deionized water, vortexed for 10 min before sampling. Particle size was determined by laser scattering light using Malvern Laser Analyzer Instruments (Kreuter, 1994).

Zeta potential

Zeta potential is an abbreviation for electrokinetic potential in colloidal systems. Zeta potential is electric potential in the interfacial Double Layer (DL) at the location of the slipping plane versus a point in the bulk fluid away from the interface. Zeta potential is not measurable directly but it can be calculated using theoretical models and an experimentally-determined
electrophoretic mobility or dynamic electrophoretic mobility. Mobility is defined as the velocity of a particle per electric field unit and is measured by applying an electric field to the dispersion of particles and measuring their average velocity. This velocity can be determined by measuring the dropper shift of laser light scattered off the moving particles (Jelvehgari, 2010). The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern instrument, UK). Samples were prepared by diluting with distilled water (pignatello et al., 2006).

**Polydispersity index**

Polydispersity index is a parameter to define the particle size distribution of nanoparticles obtained from photon correlation spectroscopic analysis. It is a dimensionless number extrapolated from the autocorrelation function and range from a value of 0.01 for mono dispersed particles and up to value of 0.5 – 0.7. Sample with very broad size distribution have polydispersity index value > 0.7 (Nithin et al., 2008). The obtained results are shown in Figure 5.

**Shape and surface morphology**

Shape and surface morphology of nanoparticles was done by Scanning Electron Microscopy (JSM76360A, JEOIL). SEM has been used to determine surface topography, texture and to examine the morphology of fractured surface. Small volume of nanoparticulate suspension was placed on an electron microscope brass stub. The stubs were placed briefly in a drier and then coated with gold in an ion sputter. Pictures of nanoparticles were taken by random scanning of the stub. The shape and surface morphology of the nanoparticles was determined from the photomicrographs of each batch. Mefloquine HCl nanoparticles have shown smooth and spherical shape with different sizes depending on the ratios of the surfactant and polymer used.

**In-vitro drug release studies**

The in vitro drug release of the formulation was studied by using dialysis membrane and modified apparatus. The dissolution medium was freshly prepared phosphate buffer of pH 7.4. Dialysis membrane (molecular weight cut off ~ 12,000, Hi-media, Mumbai, India), previously soaked overnight in the dissolution medium and was tied to one end. 2ml of formulation was placed and another end was also tied. The dialysis bag containing formulation was placed in 200 ml beaker containing 100ml of phosphate buffer pH 7.4, maintained at 37 °C ± 2 °C. The dissolution medium was stirred at low speed (100 rpm) using magnetic stirrer. Aliquots, each of 5 ml volume were withdrawn at various intervals of time over a period of 24 hr. The aliquots were suitably diluted with dissolution medium and analyzed by UV-Vis Spectrophotometer at 222 nm. The quantity of drug equivalent to 20 mg of Mefloquine HCl was taken for dissolution study.

**Release kinetics**

There are number of kinetic models, which described the overall release of drug (Suvakanta Dash et al., 2010) from the dosage forms. Because qualitative and quantitative changes in a formulation may alter drug release and the use of in-vitro drug dissolution data (Table5) of the Mefloquine HCl release from poly-caprolectone nanocapsules were analyzed to investigate(K. Satish Kumar et al., 2012) the release kinetics.

**Zero order kinetics**

Zero order kinetics model follows the equation as \( (Q/Q_0) = k_0 t \) Where \( k_0 = \) Zero order rate constant, hour\(^{-1}\), \( Q \) = the amount of Mefloquine HCl released, mg; \( Q_0 \) = the amount of Mefloquine HCl initially, mg. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

**First order kinetics**

First order kinetics model follows the equation as: \( \log (Q/Q_0) = -k_1 t/2.303 \) Where \( k_1 = \) first order rate constant, hour\(^{-1}\), \( Q \) = the amount of Mefloquine HCl released, mg; \( Q_0 \) = the amount of Mefloquine HCl initially, mg. The data obtained were plotted as cumulative percentage drug release versus square root of time.

**Higuchi model**

The values of R2 indicated that the Higuchi model was best fitted with the release kinetic data of Mefloquine HCl. Higuchi equation: \( (Q/Q_0) = k_0 t^{1/2} \) Where, \( Q \) = the amount of Mefloquine HCl released, mg; \( Q_0 \) = the amount of Mefloquine HCl initially, mg; \( k_0 = \) Higuchi matrix release kinetics, hour\(^{-1}\). The data obtained were plotted as cumulative percentage drug release versus square root of time.

**Korsmeyer-Peppas model**

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation \( \frac{M_t}{M_i} = k t^n \) Where \( M_t/M_i \) is a fraction of drug released at time \( t \), \( k \) is the release rate constant and \( n \) is the release exponent. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

**Hixson-crowell model**

Hixson and Crowell (1931) recognized that the particle’s regular area is proportional to the cube root of its volume. They derived the equation: \( W_{i}^{1/3} = W_{0}^{1/3} + k t \) where \( W_{i} \) is the initial amount of drug in the pharmaceutical dosage form, \( W_{0} \) is the remaining amount of drug in the pharmaceutical dosage form at time \( t \) and \( k \) (kappa) is a constant incorporating the surface volume relation. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cube root of drug percentage remaining in matrix versus time.
Results and Discussions

Solubility study for oil selection

The solubility of the drug in oils is most important, therefore, the components used in the system should have high solubilization capacity for the drug, ensuring the solubilization of the drug in the resultant dispersion. Results from solubility studies are reported in Figure 1. As seen from the figure, Caprol micro-express and Transcutol showed the highest solubilization capacity for Mefloquine HCl, followed by capmul MCM and Acconon 5005. Thus, for our study we selected Caprol micro-express for the development of the formulation.

Drug - excipient compatibility study

The pure drug Mefloquine HCl and physical mixture of excipients with drug were studied. The thermo gram of pure drug showed endothermic peak at – 262.36 °C and 268.77 °C either physical mixture of drug and excipient shows peak at 263.15 °C. The obtained DSC thermograms are shown in Figure 2(a) and 2(b).

![Fig. 1. Solubility of mefloquine HCl in different vehicles.](image1)

![Fig. 2. (a) DSC spectrum of pure mefloquine HCl drug (b) DSC spectrum of physical mixture of mefloquine HCl drug + PVA.](image2)
**Percentage yield**

The results of the percentage practical yield are shown in Table 2. Percentage practical yield depends on the concentration of polymer added. It increases with increase in concentration of polymer added to the formulation. Maximum percentage practical yield was found to be 98.375% for formulation.

**TABLE 2**

Percent yield of mefloquine.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation</th>
<th>weight of empty bottle (gm)</th>
<th>Practical Yield (gm)</th>
<th>Theoretical Yield (gm)</th>
<th>% Yield = Practical yield/theoretical yield X 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MFQ-1</td>
<td>16.7730</td>
<td>0.3935</td>
<td>0.4</td>
<td>98.37</td>
</tr>
<tr>
<td>2</td>
<td>MFQ-2</td>
<td>16.2672</td>
<td>0.4475</td>
<td>0.6</td>
<td>74.58</td>
</tr>
<tr>
<td>3</td>
<td>MFQ-3</td>
<td>16.4210</td>
<td>0.6619</td>
<td>1.0</td>
<td>66.19</td>
</tr>
<tr>
<td>4</td>
<td>MFQ-4</td>
<td>17.4905</td>
<td>1.2917</td>
<td>1.4</td>
<td>92.26</td>
</tr>
<tr>
<td>5</td>
<td>MFQ-5</td>
<td>17.1740</td>
<td>1.6076</td>
<td>1.8</td>
<td>89.31</td>
</tr>
<tr>
<td>6</td>
<td>MFQ-6</td>
<td>16.4049</td>
<td>1.8080</td>
<td>2.2</td>
<td>82.18</td>
</tr>
</tbody>
</table>

**Drug entrapment efficiency**

The entrapment efficiency of six batches of mefloquine HCl nanoparticles are recorded in Table 3. As the polymer concentration was increased from 100-500 mg the encapsulation efficiency was increased. The PVA concentration was varied as 0.25%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5%, the encapsulation efficiency was slightly increased. The result indicates the polymer concentration plays a major role in drug entrapment efficiency rather than the PVA concentration. The maximum entrapment efficiency was found in MFQ-4 with 99.34% (Figure 3). The preparation parameters, such as PVA concentration and mefloquine HCl/polyε-caprolactone ratios were modified to obtain nanoparticles with higher entrapment efficiency.

**Particle size analysis**

The size distributions along the volume mean diameter of the nanoparticles were measured by laser scattering light using Malvern Laser Analyzer Instruments. The obtained results are shown in Figure 5. Particle sizes of all six batches are shown in Table 3.

**TABLE 3**

Characterization reports of prepared nanoparticles.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation</th>
<th>% Yield</th>
<th>% Entrapment Efficiency</th>
<th>Z-Average Particle Size</th>
<th>Polydispersity Index</th>
<th>Zeta Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MFQ-1</td>
<td>98.375</td>
<td>95.55</td>
<td>119.8</td>
<td>0.176</td>
<td>37.0</td>
</tr>
<tr>
<td>2</td>
<td>MFQ-2</td>
<td>74.583</td>
<td>97.83</td>
<td>101.7</td>
<td>0.212</td>
<td>43.5</td>
</tr>
<tr>
<td>3</td>
<td>MFQ-3</td>
<td>66.19</td>
<td>98.46</td>
<td>121.0</td>
<td>0.191</td>
<td>23.9</td>
</tr>
<tr>
<td>4</td>
<td>MFQ-4</td>
<td>92.26</td>
<td>99.34</td>
<td>166.4</td>
<td>0.100</td>
<td>25.1</td>
</tr>
<tr>
<td>5</td>
<td>MFQ-5</td>
<td>89.31</td>
<td>98.69</td>
<td>185.1</td>
<td>0.109</td>
<td>27.4</td>
</tr>
<tr>
<td>6</td>
<td>MFQ-6</td>
<td>82.18</td>
<td>97.45</td>
<td>183.2</td>
<td>0.083</td>
<td>16.3</td>
</tr>
</tbody>
</table>

**Zeta potential**

The stability study of the nanoparticles was evaluated by measuring the zeta potential of the nanoparticles by the zeta meter (Table 3). Zeta potential of all formulated nanoparticles was in the range of 16.3 mV to 43.5 mV which indicates moderate stability with no agglomeration. The obtained results are shown in Figure 6.
Characterization of Prepared Nanoparticles

Table 3 and Figure 7 shows that all data are summarized characterization reports of prepared nanoparticles such as percentage yield, percentage entrapment efficacy, average particles size, zeta potential and polydispersity index (PDI).

**Shape and surface morphology**

Shape and surface morphology of nanoparticles was studied by Scanning Electron Microscopy (SEM) (JSM-T330A, JEOL). SEM photographs of all formulations were shown in Figure 8(a) and 8(b). Mefloquine HCl nanoparticles have shown smooth and spherical shape with different sizes depending on the ratios of the surfactant and polymer used.

**In-vitro drug release studies**

The obtained release profile data of mefloquine HCl from nanocapsules until 24 hr after dispersion are shown in Figure 9 and Table 4. As evident from the graph, the curve of dissolution release profile indicates that, with the increase in the polymer ratio release of mefloquine HCl from the nanocapsules decreases. In the third hour i.e. in the alkaline medium, the concentration of the drug released from the nanocapsules increased and reached a maximum. The initial burst release could be related to the surface drug as well as small size of the nanocapsules with increased surface area.

**TABLE 4**

Comparative cumulative percent drug release.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Time (hr)</th>
<th>CUMULATIVE % DRUG RELEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MFQ-1</td>
<td>MFQ-2</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>24.0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>37.0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>56.0</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>59.0</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>63.0</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>72.0</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>80.0</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>92.0</td>
</tr>
</tbody>
</table>

**Fig. 5.** Average particle size distribution report.

**Fig. 6.** Zeta potential report.
Fig. 7. Summary data of average particle size, zeta potential and PDI.

Fig. 8.(a) & (b) SEM Photomicrograph of mefloquine HCl nanoparticles formulation.

Fig. 9.Comparative dissolution study.
Kinetics of drug release

In order to understand the mechanism and kinetic of drug release, the drug release data of the in-vitro dissolution study were analyzed with various kinetic model like Drug release kinetics from nanocapsules was analyzed by using Zero – order kinetics (Fig.10), first order kinetics (Fig.11), Higuchi (Fig.12), Korsmeyer-Peppas model (Fig.13) and Hixson Crowell model (Fig.14) and Coefficient of correlation (r) values were calculated for the liner curves by regression analysis of the above plots. It was difficult to predict exact fit model for the nanocapsules but as the $R^2$ value for Higuchi model and Korsmeyer-Peppas models was highest it can be proposed that the prepared mefloquine HCl nanocapsules followed the release kinetics for diffusion model. The best fit model obtained from this data was shown in Table 5.
Conclusions

The result of the present investigation proposes a novel formulation of Mefloquine HCl nanoparticles by emulsion diffusion method. It was found that nanoparticles with desirable particle size and high entrapment efficiency can be produced by adjusting the process parameters. The particle size and drug entrapment as well as the drug release kinetics can be optimally controlled.

Acknowledgements

The authors would like to thank R.K.G.I.T College of Pharmacy, Ghaziabad (U.P.) for providing the facility for nanoparticles formulation and development. And also thank to Manager-QC Aristo Pharmaceutical Ltd, Raisen (M.P.) for providing gift sample of Mefloquine HCl and also thankful to Uttarakhand Technical University, Dehradun.


---

Address correspondence to: Dilip Kumar Gupta, Faculty of pharmacy, Uttarakhand Technical University, Sudhhowala, P.O.Chandanwari, Premnagar, Dehradun-248007, India.

E-mail: kumarjai24sep@gmail.com