FORMULATION AND CHARACTERIZATION OF TRIMETAZIDINE HYDROCHLORIDE MICROSPHERES USING DOUBLE EMULSION SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT

The present research work was intended to formulate and characterize Trimetazidine hydrochloride microspheres by a novel W/O/O double emulsion solvent evaporation technique which was designed to release the drug in the colon. Polymers such as Eudragit L 100 and S 100 were employed as encapsulation materials for preparing microspheres. Twelve trial formulations (FT1-FT12) were developed by varying the ratio of polymer combination and drug-polymer. Preformulation studies have been carried out for the drug and excipients. Morphological studies were performed for all prepared microsphere by Scanning Electron Microscopy. Percentage yield, particle size, percent drug content, encapsulation efficiency, in vitro drug release and kinetics were analyzed for all the trial batches (FT1-FT12). From the results, it was observed that, Formulation FT7 with 1:1.5 ratio of Eudragit L & S 100 combination and drug-polymer ratio of 1:2 showed spherical shaped microspheres with a maximum encapsulation efficiency of 80.12% when compared with other batches. The in vitro drug release study inferred that the formulation FT7 showed a maximum release of 97.65% up to 24 hours in pH 6.8 which followed non-fickian zero order release kinetics. From the outcome of results, it was concluded that the Trimetazidine hydrochloride loaded Eudragit microspheres showed a maximum encapsulation efficiency with a colonic drug release of 24 h and can be used as an effective antianginal drug without any haemodynamic effects.

KEYWORDS: Stable Angina Pectoris, Trimetazidine Hydrochloride, Microspheres.
INTRODUCTION

Globally, Ischemic cardiac disease is one of the leading causes of death. Stable angina pectoris represents the initial manifestation of coronary artery disease. From a few decades ago, management of cardiac diseases using novel metabolic agents have been emerged as a promising therapy. Conventional therapy available for patients suffering from Angina are nitrates, beta-blockers, and calcium channel antagonists. All those class of drugs act haemodynamically and impose changes in heart rate, systolic blood pressure and etc.\[1\] Unlike, all the conventional therapies, metabolic agents have a peculiar mode of action with non – haemodynamic effects. Undoubtedly, International literatures demonstrated that Trimetazidine Hydrochloride is the first metabolic anti-ischemic drug and is proven to decrease the severity of anginal attacks by acting directly on human myocardial cells.\[2,3\]

Trimetazidine Hydrochloride (1-[2, 3, 4-trimethoxibenzyl]-piperazine hydrochloride) is a freely water soluble drug with a short biological half-life of 6 hours. Usually 40 – 60 mg of drug is administered orally in three divided doses.\[4\] In the present study, Trimetazidine Hydrochloride was chosen as a model drug for formulating microspheres by a novel W/O/O double emulsion solvent evaporation technique.

Emulsification-Solvent evaporation technique of microencapsulation is the most widely utilized method by Pharmaceutical researchers. In the present world of research, microencapsulation by double emulsion solvent evaporation technique is gaining upward attention. It has been recognized as a promising technology for the drugs especially which are hydrophilic in nature, since the water soluble drugs are less capable of getting encapsulated by the encapsulation materials.\[5\] This research work is aimed to prolong the therapeutic effect by releasing the drug in a sustained manner without any haemodynamic effects in the patients with stable angina pectoris thereby minimizing the frequency of administration and improving patient compliance.

MATERIALS AND METHODS

Trimetazidine Hydrochloride (Active Pharmaceutical Ingredient) was obtained as gift sample from Strides Shasun Ltd., Puducherry, India. Polymers such as Eudragit (L100 & S100) were procured from HiMedia Laboratories Ltd., Mumbai, Other chemicals and solvents used were Analytical Grades procured from different manufacturers.
Formulation of microspheres
Trimetazidine Hydrochloride loaded Eudragit microspheres were prepared by W/O/O Double Emulsion solvent evaporation technique. Twelve Trial formulations were developed by changing the drug – polymer concentration as specified in the Table 1. Drug (equivalent to 60 mg) dissolved in water and added slowly to the polymer solution containing different ratio of Eudragit L & S 100 which was dissolves in the mixture of solvent system acetonitrile & dichloromethane in the ratio of 1:1 and Tween 80 (0.5% w/v) as surfactant with constant stirring for 10 minutes. The resulting W/O primary emulsion was slowly dispersed in the oil processing medium containing light liquid paraffin, Span 20 (0.5% w/v) as surfactant and n-heptane as viscosity retarding agent with constant stirring for 1 hour. After that the microspheres were decanted, washed with n-hexane thrice and air - dried for 12 h.[6,7]

Table 1: Composition of Trimetazidine Hydrochloride Microspheres

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Ratio of Eudragit L/ S100 combination</th>
<th>Drug - polymer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT1</td>
<td>1:0.5</td>
<td>1:1</td>
</tr>
<tr>
<td>FT2</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>FT3</td>
<td>1:1.5</td>
<td>1:1</td>
</tr>
<tr>
<td>FT4</td>
<td>1:2</td>
<td>1:1</td>
</tr>
<tr>
<td>FT5</td>
<td>1:0.5</td>
<td>1:2</td>
</tr>
<tr>
<td>FT6</td>
<td>1:1</td>
<td>1:2</td>
</tr>
<tr>
<td>FT7</td>
<td>1:1.5</td>
<td>1:2</td>
</tr>
<tr>
<td>FT8</td>
<td>1:2</td>
<td>1:2</td>
</tr>
<tr>
<td>FT9</td>
<td>1:0.5</td>
<td>1:3</td>
</tr>
<tr>
<td>FT10</td>
<td>1:1</td>
<td>1:3</td>
</tr>
<tr>
<td>FT11</td>
<td>1:1.5</td>
<td>1:3</td>
</tr>
<tr>
<td>FT12</td>
<td>1:2</td>
<td>1:3</td>
</tr>
</tbody>
</table>

Characterization of Microspheres

Drug - Excipient Compatibility
Drug - excipient compatibility study was carried out by Fourier Transform Infrared (FTIR) Spectroscopy to find out any interaction between the drug and polymer. The FTIR spectra of the pure drugs, excipients, physical admixtures and the optimized formulation were taken in the range of 4000-400cm⁻¹ using Perkin Elmer FTIR KBr pellet method.[8]

Standardization of Trimetazidine Hydrochloride using Phosphate buffer pH 6.8
100mg of Trimetazidine Hydrochloride was accurately weighed and dissolved in 100ml of Phosphate buffer pH 6.8 to obtain 1mg/ml and from the stock solution 1ml was further diluted to 10 ml to get 100µg/ml. From this, aliquots of 1, 2, 3, 4 and 5 ml of solution were
pipette out and diluted with 10ml of Phosphate buffer pH 6.8 to get the concentrations in the range of 10µg/ml-50µg/ml, the absorbance was measured in UV Spectrophotometer at 269nm.\[9\]

**Morphological studies by Scanning Electron Microscopy (SEM)**

The prepared microspheres were subjected to Scanning Electron Microscopy (SEM) studies. The morphology of microspheres (size and shape) was examined with SEM (Zeiss, Model - EVO 18, Germany) operating at 15kv.\[10\]

**Percentage yield**

Percentage yield of Trimetazidine Hydrochloride microspheres were determined by using the following formula:

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

Practical yield – Amount of microspheres recovered from each batch

Theoretical yield – Amount of starting materials used for the formulating the each batch of microspheres

**Average particle size**

The Average particle size of all the formulations (FT1-FT12) were determined by particle size analyzer (Nano plus common DLS analyzer, USA. version 5.22/3.00).

**Drug content and Encapsulation efficiency**

The drug content in the prepared microspheres was determined by pulverizing the weighed amount of Trimetazidine Hydrochloride loaded microspheres equivalent to 60mg followed by immersing them in 100ml of pH 6.8 Phosphate buffer with agitating at room temperature for 12 h. After filtration, the drug concentration was determined spectrophotometrically at 269nm. The filtered solution from the empty microspheres (without drug) was taken as blank. All samples were analyzed and from the absorbance value, Percent Drug content (DC) was determined. Encapsulation efficiency (EE) was calculated according to the following formula\[11\]

\[
\text{Encapsulation Efficacy (\%)} = \frac{\text{Actual drug content in microspheres}}{\text{Theoretical drug content}} \times 100
\]
In vitro release profile

The invitro dissolution studies were carried out using USP basket (Type I) apparatus at 100 RPM and 37±0.5°C. Required amount of microspheres equivalent to 60 mg drug was filled in a dialysis bag and placed in the basket containing 900ml of phosphate buffer pH 6.8 (simulated colonic fluid) as dissolution medium and the drug release was observed for 24 hours. 5 ml samples were withdrawn at specified time intervals and replaced immediately with an equal volume of fresh medium. Samples were assayed by using UV-spectrophotometer (Shimadzu 1700, Japan) at 269nm against Phosphate buffer pH 6.8 as blank. From the absorbance values, the cumulative percent drug release were determined.[12]

Drug Release Kinetics

The release mechanism of all the formulations were analyzed mathematically using Zero order, First order, Higuchi and Koresmeyer - Peppas model.[13,14]

Stability studies

The stability studies for the optimized formulation was carried out as per ICH guidelines for the period of six months and were packed in high density polyethylene containers and kept in stability chamber at 40°C ±2°C/75 ± 5% RH.[15]

RESULTS AND DISCUSSION

Drug - excipient Compatibility studies

Fourier Transform Infrared studies (FTIR)

The FTIR study confirmed that there were no characteristic changes of functional peaks and there was no interaction between the drug and the excipients. The FTIR spectrum of the optimized formulation was shown in the Fig.1.

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Fig.1 FTIR Spectrum of optimized formulation of Trimetazidine Hydrochloride Microspheres
EVALUATION OF MICROSPHERES

Surface morphology by Scanning Electron Microscopy (SEM)

Scanning Electron Microscopic studies were performed to examine the surface morphology of all the formulations. The micrographs revealed that, among the 12 formulations, formulation FT7 (Fig.2) showed non-aggregated, discrete, smooth surfaced, spherical shaped microspheres.

Average particle size

The average particle size of all the formulations (FT1-FT12) ranged from 90.15 µm – 258.16 µm. Particle size of all the prepared microspheres were influenced by concentration of polymer which may be due to rise in viscosity effectively at higher polymer concentration resulted in enhancement of interfacial tension and diminished shearing effect.

Percentage yield

The percentage yield of all the formulations (FT1 – FT12) ranged from 55.4% - 79.23% which was increased with increase in concentration of drug polymer ratio from 1:1 to 1:3. This may be due to increase in viscosity of the primary emulsion.

Drug content and Encapsulation efficiency

Irrespective of drug polymer concentration, the drug content of all the formulations (FT1-FT12) were ranged from 90.64 ± 0.112 % to 98.83 ± 0.104 %. Encapsulation efficiency of all the formulations (FT1-FT12) ranged from 44.35 ± 0.532 to 80.12 ± 0.657 % and results were varied according to concentration of polymer used. Increase in drug – polymer concentration
increased the % Encapsulation efficiency, this effect might be due to increase in viscosity of the preparation which caused hindrance for the migration of drug towards the continuous phase and thereby reduction in drug loss by diffusion during the formulation of microspheres. Among the 12 formulations, the formulations FT7 and FT8 showed maximum encapsulation efficiency of 80.12 ± 0.657 % and 79.22 ± 0.458 % respectively.

The results of particle size, percentage yield, and percent drug content and encapsulation efficiency were given in the Table.2

Table: 2. Particle size, Percentage yield, Percent Drug content and Encapsulation Efficiency of Trial formulations (FT1-FT12)

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Particle size (µm)</th>
<th>Yield (%)</th>
<th>*Drug content (%)</th>
<th>*Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT1</td>
<td>92.78</td>
<td>55.40</td>
<td>90.64 ± 0.211</td>
<td>53.68 ± 0.204</td>
</tr>
<tr>
<td>FT2</td>
<td>114.60</td>
<td>57.24</td>
<td>95.65 ± 0.234</td>
<td>59.32 ± 0.122</td>
</tr>
<tr>
<td>FT3</td>
<td>203.16</td>
<td>63.20</td>
<td>97.33 ± 0.189</td>
<td>66.14 ± 0.296</td>
</tr>
<tr>
<td>FT4</td>
<td>228.20</td>
<td>68.72</td>
<td>92.20 ± 0.156</td>
<td>72.13 ± 0.207</td>
</tr>
<tr>
<td>FT5</td>
<td>104.08</td>
<td>70.80</td>
<td>96.83 ± 0.201</td>
<td>69.32 ± 0.467</td>
</tr>
<tr>
<td>FT6</td>
<td>129.43</td>
<td>71.23</td>
<td>97.22 ± 0.265</td>
<td>71.83 ± 0.187</td>
</tr>
<tr>
<td>FT7</td>
<td>144.56</td>
<td>78.80</td>
<td>98.83 ± 0.128</td>
<td>80.12 ± 0.657</td>
</tr>
<tr>
<td>FT8</td>
<td>160.50</td>
<td>79.23</td>
<td>95.43 ± 0.231</td>
<td>79.22 ± 0.282</td>
</tr>
<tr>
<td>FT9</td>
<td>90.15</td>
<td>69.45</td>
<td>96.22 ± 0.192</td>
<td>44.35 ± 0.532</td>
</tr>
<tr>
<td>FT10</td>
<td>230.87</td>
<td>73.40</td>
<td>93.12 ± 0.202</td>
<td>59.24 ± 0.266</td>
</tr>
<tr>
<td>FT11</td>
<td>244.66</td>
<td>75.53</td>
<td>94.87 ± 0.134</td>
<td>63.77 ± 0.239</td>
</tr>
<tr>
<td>FT12</td>
<td>258.16</td>
<td>77.29</td>
<td>92.05 ± 0.108</td>
<td>68.12 ± 0.301</td>
</tr>
</tbody>
</table>

*All the values are expressed as Mean ± S.D, n=3.

**In vitro** release profile

From the results, it was noticed that *in vitro* drug release pattern of all the formulations depend on the change in drug – polymer concentration. Among the 12 formulations, formulations FT1 – FT4 with a drug – polymer ratio of 1:1 released up to 12 h. The formulations FT 5 - FT8 with drug – polymer ratio of 1:2 showed a sustained release of drug from 20 to 24 hours. In that, the formulation FT7 with 1:1.5 ratio Eudragit L & S 100 and drug- polymer concentration of 1:2 released 97.65 ± 0.622 % of drug at the end of 24 h. Further increase in drug polymer ratio to 1:3, the formulations FT9 - FT12 showed no significant increase in drug release at the end of 24 h. This may be occurred due to system saturation. The results of cumulative percentage drug release of the all the formulations were depicted in the Fig.3, 4, 5.
Fig. 3 *In vitro* drug release pattern of the microsphere formulations (FT1-FT4)

Fig. 4 *In vitro* drug release pattern of the microsphere formulations (FT5-FT8)

Fig. 5: *In vitro* drug release pattern of the microsphere formulations (FT9-FT12)
Release kinetics
The Cumulative percentage release data of all the batches obtained were fitted to various kinetic equations to determine the mechanism of drug release. The optimized formulation FT7 was best fitted to zero order kinetic equation with r² value of 0.986. Further Korsmeyer–peppas model showed a good linearity of 0.953 with n value of 0.942 which implies that the formulation follows non-fickian zero order kinetics.

Stability study
Results of stability studies for the optimized formulation FT7 inferred that there was no significant changes when stored at 40°C ± 2°C/75%±5% RH after 6 months.

CONCLUSION
From the results obtained, it was concluded that as a hydrophilic drug, Trimetazidine Hydrochloride was found to be a suitable candidate for formulating as microspheres by double emulsion technique. Among the 12 formulations of Trimetazidine Hydrochloride loaded Eudragit microspheres, formulation FT7 showed a maximum encapsulation efficiency and sustained drug release upto 24 h with non-fickian zero order release kinetics. Thus, the colon targeted Trimetazidine Hydrochloride microspheres was successfully formulated and can be used as monotherapy or in combination with other anti-anginal drugs for the long term treatment of stable Angina pectoris.

REFERENCES


