Investigation of Drug Polymer Compatibility: Formulation and Characterization of Metronidazole Microspheres for Colonic Delivery.

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Investigation of Drug Polymer Compatibility: Formulation and Characterization of Metronidazole Microspheres for Colonic Delivery.

Author(s): Choudhury PK, Murthy PN, Tripathy NK, Panigrahi R, Behera S

Abstract

For the design and development of any novel formulation, assessment of compatibility of drug with excipients by different techniques such as thermal and isothermal stress testing are recommended. During pre-formulation studies common methods like UV-Spectrophotometric methods, FTIR are used for the study of compatibility. In the present investigation drug-excepient compatibility study of was conducted for metronidazole with ethyl cellulose to formulate microspheres by using different ratio of drug: polymer for colonic delivery. The drug and polymer mixtures were stored at 50 °C for 2 weeks. The samples were then characterized using UV Spectrophotometric method, FTIR. The results show that metronidazole was compatible with ethyl cellulose; hence ethyl cellulose can be used for formulation of metronidazole microspheres. Microspheres were prepared by modified Novel Quasiemulsification solvent-diffusion method to study the effect of ethyl cellulose on drug release with different proportions of metronidazole and ethyl cellulose. Prepared microspheres of ethyl cellulose were evaluated for size, morphology, sphericity study, percentage yield, loose surface crystal study, drug content and entrapment efficiency. In vitro drug release study was conducted by buffer change method to mimic Gastro Intestinal environment. The investigations revealed that microspheres prepared with metronidazole: ethyl cellulose ratio (1:2) show only 19.394 ±0.67% drug release in first 5 hours and 46.72 ±0.69% in 12 hours, which prove the potentiality of ethyl cellulose for colonic delivery of drugs.

Keywords: Metronidazole, Compatibility, Polymers, UV Spectrophotometric methods, FTIR, Colonic drug delivery, Quasiemulsification Solvent-diffusion technique

Introduction

Drug excipient compatibility is one of the important parameter to be considered during preformulation studies, which can alter the physicochemical properties and bioavailability of the drugs. To develop effective, safe and stable formulation drug excipient compatibility is an important process and it helps in the selection of right excipient. Despite of the importance of the drug excipient compatibility tests, there is no universally accepted protocol for this purpose. Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule[1]. UV Spectrophotometric method is common analytical techniques used to determine the drug content of most of the drugs.

In the present study, the drug excipient compatibility of the metronidazole with various ethyl cellulose was determined by as a part of an ongoing project on the development of metronidazole microspheres. FTIR and UV Spectrophotometric methods were used for the characterization study. Colonic diseases are important causes of death by protozoal infections in the developing world and even in advanced countries. Hence, in the present study metronidazole (MNZ) was selected as a model drug which has extremely broad spectrum of protozoal and antimicrobial activity. It is clinically effective in colonic diseases, both locally and systemically[2]. Based on the observations, an attempt has been made to develop ethyl cellulose microspheres for metronidazole by modified Novel Quasiemulsification solvent-diffusion method to deliver active molecule to the colonic region, which combines pH-dependent and controlled drug release properties.

Experimental

MATERIALS AND METHODS
M/s Diamond Drugs Pvt Ltd, Howrah, WB, India, generously supplied metronidazole as a gift sample. Ethyl cellulose-LR (EC-LR), Span 80 and Tween 80 were procured from S.D. fine-chem. Ltd., Mumbai, India. Light liquid paraffin was obtained from Loba
Chemie Ltd, Mumbai. All other solvents and reagents were of analytical grade procured from local suppliers.

COMPATIBILITY STUDY

The drug and polymer mixtures of metronidazole with various polymers prepared at different ratio. The drug and ethyl cellulose were individually weighed in a 10 ml glass vial and mixed on a vortex mixer for 2 min. In each of the vials, 10% of the ethanol-water was added and the drug-exceptient blend was further mixed. Each vial was sealed Teflon-lined screw cap and stored at 50°C for 2 weeks. These samples were periodically examined for any change of unusual color change.

Characterization by UV spectrophotometer

The samples on the first day, after 1 week and 2 weeks were withdrawn from storage and analyzed by UV spectrophotometer. The drug content was determined at initial and stored samples in triplicate. An accurately weighed amount of the drug-polymer mixture was taken and suitably dissolved in ethanol. The sample was analyzed after making appropriate dilutions using UV spectrophotometer at 311 nm against blank.

Fourier transforms infrared radiation measurement (FT-IR)

The FT-IR spectra acquired were taken from dried samples. A FT-IR (Shimdu-840-os, Japan) was used for the analysis in the frequency range between 4000 and 400 cm⁻¹, with 4cm⁻¹ resolution.

PREPARATION OF ETHYL CELLULOSE MICROSPHERES

Metronidazole loaded ethyl cellulose (EC) microspheres were prepared by Novel Quasiemulsification solvent-diffusion method[3]. A solution of EC in acetone (2%, 4% and 8%) containing drug (1g) was added to liquid paraffin containing emulgent (Span 80), while stirring, at a speed of 1500 rpm. The emulsion was stirred for 5 to 6 hours at 25°C to 30°C. Subsequently, a suitable amount of petroleum ether was added to the dispersion. The microspheres suspended in liquid paraffin were filtered and collected. The resultant microspheres were washed with water followed by petroleum ether to remove traces of liquid paraffin. The microspheres were dried at ambient temperature and desiccated under vacuum. The batch was coded as ECQ.

Characterization of Microspheres

The prepared microspheres were characterized by evaluating following parameters like % yield, particle size analysis, Determination of Shape and Sphericity, drug content, Drug Entrapment efficiency (DEE %), Loose surface crystal study and In Vitro Drug Release from Microspheres (data disclosed in table 4).

Determination of the Yield of the Microspheres

The yield was calculated using the equation:

\[
\text{Yield of microspheres (%) = \frac{\text{Weight of the microspheres (mg)}}{\text{Drug (mg) + Polymer (mg)}} \times 100}
\]

Particle Size Analysis

Particle size distribution of the microspheres was determined by optical microscopy using calibrated ocular eyepiece[11]. Fifty microspheres were observed and the geometric mean diameter was calculated using the equation:

\[
X_g = 10 \times \left[ \frac{\sum (n_i \times \log X_i)}{N} \right]
\]

Where \(X_g\) is geometric mean diameter, \(n_i\) is no of particles in the range, \(X_i\) is the mid point of range, and \(N\) is total no of particles analyzed.

Determination of Shape and Sphericity

Morphological appearance and surface characteristics of the microspheres were studied by dispersing the microspheres in liquid paraffin and observing under 250X magnification in an optical microscope[11]. The particle shape was measured by computing circularity factor (S). The tracings obtained from optical microscopy were used to calculate area (A) and perimeter (P), which are used to calculate the circularity factor (S) by using the equation[12],

\[
S = \frac{P^2}{(12.56 \times A)}
\]

Determination of drug content[13]

The amount of drug present in the Ethyl cellulose microspheres prepared by quasiemulsion method was determined by a method reported by Thanoo et al (1992). A weighed quantity of the microspheres was extracted with methanol for 24 hr, and drug concentration in supernatant was determined spectrophotometrically at 313.5 nm (UV 1700Shimadzu,Japan).

Drug Entrapment efficiency (DEE %)

Entrapment efficiency of the microspheres was calculated using the formula

\[
\text{DEE\%} = \frac{\text{Practical Drug Loading}}{\text{Theoretical Drug Loading}} \times 100
\]

Loose surface crystal study[14]

Loose surface crystal study was performed to observe
the excess drug present on the surface of microspheres. From each batch 11mg of microspheres were shaken in 100 ml of phosphate buffer, pH 7.4 for 5 minutes and then filtered through Whatman filter paper 41. The amount of drug in the filtrate was determined spectrophotometrically at 319 nm and calculated as percent of total drug content. This estimates the surface entrapment of the drug by the microspheres.

**In Vitro Drug Release from Microspheres**

In vitro drug release studies were carried out using USP dissolution rate test apparatus (basket apparatus, 100 rpm, 37 ± 0.1°C) by buffer change technique. Microspheres bearing MNZ were suspended in simulated gastric fluid (SGF), pH 1.2 (500 ml), for 1 hr. The dissolution media was then replaced with mixture of simulated gastric fluid and simulated intestinal fluid (SIF), pH 4.5 (500 ml) for next two hours, then for next two hours simulated intestinal fluid (SIF), pH 6.8 (500 ml) and the release study was continued further in simulated intestinal fluid (500 ml) pH 7.4.

Samples were withdrawn periodically and compensated with an equal amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance at 319.5 nm using UV spectrophotometer (UV 1700, Shimadzu, Japan).

**Drug Release data model fitting**

The suitability of several equations that are reported in the literature to identify the mechanisms for the release of drug was tested with respect to the release data up to the first 50% drug release. The data were evaluated according to the following equations:

- **Zero order model**
  \[ M_t = M_0 + K_0 t \]

- **Higuchi model**
  \[ M_t = M_0 + K_H t^{0.5} \]

- **Korsmeyer-Peppas model**
  \[ M_t = M_0 + K_n t^n \]

Where \( M_t \) is the amount of drug dissolved in time \( t \), \( M_0 \) is the initial amount of the drug, \( K_0 \) is the Zero order release constant, \( K_H \) is the Higuchi rate constant, \( K_n \) is a release constant and \( n \) is the release exponent that characterizes the mechanism of drug release.

**Results and discussion**

**Drug content estimation**

The drug and polymer mixture was physically observed at different intervals. No characteristic color change was observed. The assay of the drug polymer mixtures were found good. Assay value of 99.12 to 100.1 was observed at initial. Good correlation was observed with the samples of drug polymer mixtures stored at 50 °C for 2 weeks. This clearly indicates the stable nature of the metronidazole with cellulose polymers (Table 1).

**Fourier transforms infrared radiation measurement (FT-IR)**

The characteristic band peaks acquired were taken from the prepared drug-polymer mixtures. The interaction study between drug and polymer was evaluated. The characteristics peak of the drug was found at wave numbers 743.58 cm\(^{-1}\), 1070.53 cm\(^{-1}\), 1160.22 cm\(^{-1}\), 1187.23 cm\(^{-1}\), 1264.38 cm\(^{-1}\) and 1536.35 cm\(^{-1}\), these are almost same as reported in the monograph for metronidazole.

**Formulation and characterization of Microspheres**

Metronidazole (MNZ) loaded ethyl cellulose microspheres were successfully prepared by the Novel Quasiemulsification solvent-diffusion method using light liquid paraffin in the external phase. The effect of drug polymer ratios was analyzed in order to optimize the formulation. It was observed that by changing drug: polymer ratio the shape, size as well as the entrapment efficiency of formulations considerably influenced. The yield of microencapsulation process was increased with increase in ethyl cellulose concentration in the formulations. The microspheres were discrete and fairly spherical in shape while the surface roughness was slightly increased with the incorporation of the drug. Excellent microspheres were produced when the process was carried out with drug: ethyl cellulose ratio 1:2 while the shape of the microspheres was distorted and some of them fused with each other when ethyl cellulose ratio was decreased. The drug particles appeared on the surface of the microspheres when they were prepared with drug: polymer ratio 2:1.

Particle size of the microspheres was determined using optical microscopic method. Mean particle size was found to be 21.76 ± 3.33μm in case of microspheres having drug: ethyl cellulose ratio 2:1 while it was significantly increased to 34.8±2.45μm with drug: ethyl cellulose ratio 1:5 (Table 4). The size of the microspheres is controlled by the size of the dispersed droplets of Ethyl cellulose in liquid paraffin. When the concentration of the ethyl cellulose in the formulation was increased, there was increment in the size of dispersed droplets that resulted in the formation of microspheres having bigger particle size.
With increase in the polymer ratio in the formulations the mean particle size of all the formulations increased (Table 4). All the microsphere formulations have the circularity factor nearest to “1” which proves that they are almost spherical in shape (Table 4). The microspheres showed better entrapment efficiency with increase in polymer ratio. Highest entrapment efficiency was observed for the formulation ECQ3 99.29±4.71 (Table 4). As the drug: polymer ratio was increased from 2:1 to 1:2 the surface entrapment of the drug on the microsphere surfaces was decreased which is suitable for the colonic delivery of the drugs and the surface entrapment of drug shows a less amount of drug lose due to the process variables but with further increase in polymer ratio the surface entrapment was increased. It may be due to the dispersion of the drug in polymer layer more evenly rather than entrapped into the polymer layer.

The microspheres were subjected to in-vitro drug release rate studies in SGF (pH 1.2) for 1 hour and in mixture of SGF and SIF (pH 4.5) for the next 2 hours in order to investigate the capability of the formulation to withstand the physiological environment of the stomach and small intestine. The MNZ percent released from the microspheres of drug: ethyl cellulose ratio 1:2 after 12 h studies is 46.72 %. The amount of MNZ released during first 5 h studies was found to be 29.993 ± 1.22 %, 16.342 ± 1.13 %, 19.790 ± 0.48 %, 14.495 ± 0.77 %, 19.394 ± 0.67 % and 10.003 ± 0.88 % for ECQ1, ECQ2, ECQ3, ECQ4, ECQ5, and ECQ6 respectively (Figure. 6) which attests the ability of ethyl cellulose to remain intact in the physiological environment of stomach and small intestine. The little amount of the drug, which is released during 5 h release rate studies, is due to the presence of un-entrapped drug on the surface of the microspheres. The release of the drug was much faster during the 6-12 hour study period. It is due to the fact that during the initial period (0-5 h) the strength of the barrier was too high to be broken and during 6-12 hour period the network was somewhat loosened which facilitated the release of drug (Figure 9.). Basing on all the evaluation parameters studied, like %yield, particle size, sphericity, surface entrapment, entrapment efficiency and in vitro drug release the formulation ECQ3 was found to be the ideal formulation.

Summary and Conclusion

While carrying out drug polymer compatibility study, no characteristic color change was observed during the storage at 50 °C for 2 weeks. Good correlation was observed at initial and after 2 weeks of Isothermal stress testing (IST). This clearly indicates the stable nature of metronidazole with the cellulose polymers used in the preset study. FTIR study of the initial and IST clearly indicates the stable nature of the metronidazole. This study is definitely useful for the preparation of metronidazole microsphere formulations.

The efficacy of the ethyl cellulose was evaluated for colon targeted drug delivery by fabricating it into microspheres. The microspheres of ethyl cellulose prepared by modified quasiemulsion method were capable of providing protection to the drug in the hostile environment of upper gastrointestinal tract and released the drug at the target site. The in vitro drug release studies of ethyl cellulose microspheres revealed that very less amount of the drug was released in the physiological environment of stomach and small intestine. Hence these data attests the potentiality of ethyl cellulose for colon-specific delivery of the drugs.

Acknowledgement(s)

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References


Illustrations

Illustration 1

Formulation composition table

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Amount of drug (mg)</th>
<th>Amount of polymer(mg)</th>
<th>Drug : polymer ratio</th>
<th>Quantity of ethanol(ml)</th>
<th>Quantity of liquid paraffin (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECQ1</td>
<td>1000</td>
<td>500</td>
<td>2:1</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>ECQ2</td>
<td>1000</td>
<td>1000</td>
<td>1:1</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>ECQ3</td>
<td>1000</td>
<td>2000</td>
<td>1:2</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>ECQ4</td>
<td>1000</td>
<td>3000</td>
<td>1:3</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>ECQ5</td>
<td>1000</td>
<td>4000</td>
<td>1:4</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>ECQ6</td>
<td>1000</td>
<td>5000</td>
<td>1:5</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>
Illustration 2

Drug Content estimation

Table 2. Drug content of metronidazole at initial and storage at 50 °C for 2 weeks

<table>
<thead>
<tr>
<th>Time intervals</th>
<th>Drug-EC mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>99.27</td>
</tr>
<tr>
<td>50 °C for 1 week</td>
<td>99.31</td>
</tr>
<tr>
<td>50 °C for 2 weeks</td>
<td>99.43</td>
</tr>
</tbody>
</table>
Illustration 3

UV-Spectrophotometric Characterization

Table 3. UV-Spectrophotometric Characterization

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Pure Drug</th>
<th>Drug-Polymer Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λmax (nm)</td>
<td>Abs</td>
</tr>
<tr>
<td>Initial</td>
<td>311.7</td>
<td>0.610</td>
</tr>
<tr>
<td>After 1 week</td>
<td>311.8</td>
<td>0.611</td>
</tr>
<tr>
<td>After 2 weeks</td>
<td>312.2</td>
<td>0.596</td>
</tr>
</tbody>
</table>
Illustration 4

Evaluation parameters of ethyl cellulose microspheres

Table 4. Evaluation parameters of ethyl cellulose microspheres

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Drug : polymer ratio</th>
<th>Yield (%)</th>
<th>Particle size (µm)</th>
<th>Circularity factor (S)</th>
<th>Loose surface crystal study (surface entrapment)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECQ1</td>
<td>2:1</td>
<td>72.33 ± 3.74</td>
<td>21.76 ± 3.33</td>
<td>1.06 ± 0.030</td>
<td>23.044 ± 3.19</td>
<td>79.02 ± 4.88</td>
</tr>
<tr>
<td>ECQ2</td>
<td>1:1</td>
<td>95.11 ± 2.48</td>
<td>25.84 ± 1.43</td>
<td>1.05 ± 0.005</td>
<td>19.118 ± 3.78</td>
<td>86.96 ± 2.49</td>
</tr>
<tr>
<td>ECQ3</td>
<td>1:2</td>
<td>99.29 ± 4.71</td>
<td>28.36 ± 2.00</td>
<td>1.06 ± 0.025</td>
<td>16.714 ± 4.22</td>
<td>98.796 ± 4.68</td>
</tr>
<tr>
<td>ECQ4</td>
<td>1:3</td>
<td>97.00 ± 3.27</td>
<td>30.2 ± 2.30</td>
<td>1.13 ± 0.018</td>
<td>32.347 ± 4.10</td>
<td>79.02 ± 6.05</td>
</tr>
<tr>
<td>ECQ5</td>
<td>1:4</td>
<td>81.00 ± 2.41</td>
<td>32.3 ± 2.43</td>
<td>1.10 ± 0.011</td>
<td>24.236 ± 4.08</td>
<td>73.35 ± 5.34</td>
</tr>
<tr>
<td>ECQ6</td>
<td>1:5</td>
<td>78.08 ± 3.24</td>
<td>34.8 ± 2.45</td>
<td>1.08 ± 0.026</td>
<td>29.442 ± 4.01</td>
<td>98.47 ± 4.89</td>
</tr>
</tbody>
</table>

Values are expressed as Mean average ± SD (n=3)
Illustration 5

Overlay spectra

Figure 1: Overlay spectra of Metronidazole in ethanol
Illustration 6

Overlay spectra

Figure 4: Overlay spectra at 1st day
Illustration 7

Overlay spectra

Figure 2: Overlay spectra of ethyl cellulose in ethanol
Illustration 8

Overlay spectra

Figure 3: Overlay spectra of mixture in ethanol
Illustration 9

Overlay spectra

Figure 5: Overlay spectra after 1 week
Illustration 10

Overlay Spectra

Figure 6: Overlay spectra after 2nd week
Illustration 11

FTIR Spectra

Figure 7: FTIR analysis of formulation containing ethyl cellulose and metronidazole
Illustration 12

Photomicrographic studies

Figure 8: Photomicrograph of ethyl cellulose microspheres
Illustration 13

In vitro drug release

Figure 9: In vitro drug release profiles of all ethyl cellulose microsphere formulations
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