Pre-formulation studies and Evaluation parameters of Bioadhesive Gel Incorporated Metronidazole Loaded Microspheres

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ABSTRACT
Metronidazole is an antiprotozoal and antibacterial medication used mainly in the treatment of anaerobic bacteria. The preformulation studies were carried out in terms of tests for identification (physical appearance, melting point and IR spectra), solubility profile and quantitative estimation of drug. The absorption maxima ($\lambda_{max}$) of drug were determined by UV visible double beam spectrophotometer (Shimadzu-1800). Accurately weighed 10 mg of metronidazole dissolved in 10ml of phosphate buffer (pH 6.8) to prepare a stock solution of 1000 μg/ml concentration. Drug polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug and drug loaded microspheres using FTIR. The solubility of metronidazole was tested in various solvents. A definite quantity (10mg) of drug was dissolved in 100ml of each investigated solvents at room temperature. Microspheres were prepared by emulsion cross linking method. Particle size was determined by using a Zetasizer nano series 90 (Malvern instruments, UK). The prepared microspheres were collected and weight. The entrapment drug concentration was determined by centrifugation of the microspheres. The microspheres were centrifuged for 20 min at 1000 rpm. The sample the scanning electron microscopy (SEM) analysis was prepared by sprinkling the microspheres on one side of the double adhesive stub. Microspheres, equivalent to 10 mg of Metronidazole, were accurately weighed and transferred to 250 ml conical flask containing 100 ml phosphate buffer (pH 6.8). The flask was kept in an incubator at 37 °C, 1 ml samples withdrawn at regular intervals and, after suitable dilution, the amount of drug released was determined using a spectrophotometer at 227.72 nm.

Keywords: Metronidazole, preformulation, Microspheres, drug released and SEM

1. INTRODUCTION:
Microspheres are controlled release drug delivery system which comprises of drug-containing microparticles or microspheres, between 10 and 500 microns in size (Badran et al., 2015). Non-biodegradable as well as biodegradable materials are used for the preparation of microspheres.

Advantages and Disadvantages of Microspheres (Praveen et al., 2015).

- Microencapsulation converts liquids to solids.
- Provides environmental protection.
- Control release characteristics of drug.
- Microspheres ensure more reproducible drug absorption.

- Dose dumping is decreased.
- Microspheres administer smaller doses that reduce local irritation when compared to single unit dosage forms.

Disadvantages

- Difficulties such as incomplete or discontinuous coating.
- Inadequate stability or shelf life of sensitive pharmaceuticals.
Non reproducible and unstable release characteristics of coated products

Bioadhesive Gels
Gels are defined as a dilute crosslinked system that exhibits no flow in the steady state. Visually gels are mostly liquid, but behave like solids due to a three dimensional cross-linked
network in the liquid. In this manner gels are a dispersion of molecules of a liquid with in a solid. Gel is applied sublingually with the help of blunt cannula and syringe. The gel is only marginally affective in decreasing the anaerobic bacterial count. (Praveen et. al., 2014). Bioadhesive gel incorporated drug loaded microspheres are natural polymeric materials that act as adhesives or synthetic materials that adheres to the biological tissue.

2. DRUG PROFILE
(Rowe et. al., 2009), (Hussain et. al., 1980),
(Higuchi et. al., 1959)
Name: Metronidazole
Chemical Structure:

![Chemical structure of metronidazole](image)

Synonyms: 1-(2-hydroxy-1-ethyl)-2-methyl-5-nitroimidazole
IUPAC Name: 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethan-1-ol
Molecular Formula: C6H9N3O3
Molecular Weight: 207.615
Solubility: Sparingly soluble in phosphate buffer pH 6.8, freely soluble in water, ethyl alcohol, chloroform, slightly soluble in ether, dilute acid (IP 2010).
Protein binding: Less than 20% bound to plasma protein
Partition coefficient: logP 4.02
Physical state: White powder
Odour: Odorless
Melting point: 159-162°C
Packaging and storage: Preserve in tight containers
Drug Category: Antiprotozoal, anti-infective, radiation sensitizing agents

3. POLYMER PROFILE (Rowe et. al., 2009), (Shanmugam et. al., 2005),

3.1 GELATIN

![Chemical structure of Gelatin](image)

Synonyms: Gelfoam, Puragel, Gelfilm
Category: Polymer
IUPAC Name: Gelatin
Chemical formula: C105H151O39N31
Density: 1.3-1.4 g/cm³
Solubility in water: Soluble in hot water
Melting point: Less than 35°C
Colour: White to slightly yellow powder
Stability: Stable, hygroscopic incompatible with strong oxidizing agents
Storage temperature: 2-8°C
Functional Category: Coating agent; film-forming agent; gelling agent; suspending agent; tablet binder; viscosity-increasing agent.

3.2 CARBOPOL 934

![Chemical structure of Carbopol](image)

Synonyms: Gelcarb, Carbomer 934, Carbopol 934
Category: Polymers
IUPAC Name: Carbopol
Chemical formula: (CH2)n(CO2H)2
Density: 1.15 g/cm³
Solubility: Soluble in water
Melting point: Less than 35°C
Stability: Stable
Functional Category: Coating agent; film-forming agent; gelling agent; suspending agent; tablet binder; viscosity-increasing agent.
Nonproprietary names: Carbopol  
Category: Biodegradable polymer  
Dissociation constant: \( \text{pK}_a = 6.0 \pm 0.5 \)  
Density: \( 0.2 \text{g/cm}^3 \)  
Glass transition temperature: 100-105°C  
Moisture content: Typical water content is up to 2% w/w. carbopol are hygroscopic and a typical equilibrium moisture content at 25°C and 50% relative humidity is 8–10% w/w.  
Acidity/alkalinity: \( \text{pH} = 2.5–4.0 \)  
Solubility: Swellable in water and glycerin and, after neutralization, in ethanol (95%).

4. OTHER EXCIPIENTS

(Rowe et al., 2009), (Hussain et al., 1980), (Higuchi et al., 1959)  
4.1 Tween 80 Name:- Polysorbate 80

Figure 4: Chemical structure of polysorbate

Molecular Formula: \( \text{C}_{64}\text{H}_{124}\text{O}_{26} \)  
Molecular Weight: 1310gm/mol  
Application: Polysorbate containing 20 units of oxyethylene is hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsion. They can also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspension.

Stability and storage condition:- Polysorbate are stable to electrolytes and week acids and bases; gradual saponification occurs with strong acids and bases.

5. PREFORMULAIION STUDIES

(Gopinath et al., 2011), (Martin et al., 2011) (Patel et al., 2013), (Silverstein et al., 1998)  
Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance that are characterized with the goal of designing optimum drug delivery system. It is an integral part of entire development process. Preformulation studies relates to pharmaceutical and analytical investigations carried out preceding and supporting formulation development efforts of the dosage forms of the drug substance. Before beginning the formal preformulation programs must be considered the following factors must be considered:

- The amount of drug available.  
- The physicochemical properties of the drug already known.  
- Therapeutic category and anticipated dose of the compound.

Physical appearance

The sample of metronidazole was analyzed for physical appearance and compared with the standard. Physical appearances of the received metronidazole complied with pharmacopoeial standards.

Melting point

Melting point was measured by capillary tube method using melting point apparatus. The capillary tube, filled with Metronidazole and a thermometer, to determine the temperature was placed in the apparatus. The temperature was increased gradually until the metronidazole was melted in capillary tube. The temperature (at which the drug was melted) was noted down as melting point.

Preliminary drug excipient compatibility studies

Determination of Absorption maxima (\( \lambda_{\text{max}} \)).

The absorption maxima (\( \lambda_{\text{max}} \)) of drug were determined by UV visible double beam spectrophotometer (Shimadzu-1800). Determination of absorption maxima (\( \lambda_{\text{max}} \)) is necessary to determine exact concentration of
drug in particular media according to their solubility.

**Preparation of 0.2 M NaOH**:- 8.0 ml of NaOH was placed in 1000 ml volumetric flask and volume was made up to 1000 ml.

**Preparation of 0.2 M KH₂PO₄**:- 8.0 ml of KH₂PO₄ was placed in 1000 ml volumetric flask and volume was made up to 1000 ml.

**Preparation of Phosphate buffer 6.8**:- Take 50.0 ml of 0.2 M Potassium dihydrogen phosphate in 200 ml volumetric flask and added 22.4 ml of sodium hydroxide and then added water to volume.

**Determination of absorbance maxima (λ_max) in Phosphate buffer (pH 6.8)**

Accurately weighed 10 mg of metronidazole dissolved in 10ml of phosphate buffer (pH 6.8) to prepare a stock solution of 1000 μg/ml concentration. From above stock solution 1ml was placed in a 100ml volumetric flask and volume made up with phosphate buffer (pH 6.8) yielding a solution of 10 μg/ml concentration.

From this solution the aliquots of 2-10 ml were withdrawn in a series of 10 ml volumetric flask and diluted to 10 ml with phosphate buffer (pH 6.8). This gave a range of 2, 4, 6, 8, 10 μg/ml. Absorbance was analysed by UV Double beam Spectrophotometer (Shimadzu-1800). Determination of absorption maxima (λ_max) is necessary to determine exact concentration of drug in particular media according to their solubility.

**FTIR Spectroscopy**

Drug polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug and drug loaded microspheres using FTIR. A pellet of approximately 1 mm diameter of metronidazole was prepared by compressing 3-5 mg of the metronidazole with 100-150 mg of potassium bromide in KBr press. The pellet was mounted in IR compartment and scanned between wave number 4000 – 400 cm⁻¹ using a Shimadzu Model 8400 FTIR.

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**Table 1: Bond and their absorption rang (Silverstein et, al., 1998)**

<table>
<thead>
<tr>
<th>BOND</th>
<th>ABSORPTION REGION (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C, C-O, C-N</td>
<td>1300-800</td>
</tr>
<tr>
<td>C=C, C=O, C=N, N=O</td>
<td>1900-1500</td>
</tr>
<tr>
<td>C≡C, C≡N</td>
<td>2300-2200</td>
</tr>
<tr>
<td>C-H, O-H, N-H</td>
<td>3800-2700</td>
</tr>
</tbody>
</table>

**Solubility**

The equilibrium solubility of a compound is defined as the maximum quantity of that substance which can be completely dissolved in a given amount of solvent. Solubility may be defined as the spontaneous interaction of two or more substance to form a homogenous molecular dispersion. The solubility of metronidazole was tested in various solvents. A definite quantity (10mg) of drug was dissolved in 100ml of each investigated solvents at room temperature. The solubility was observed by the visual inspection.

**Table 2: Solubility specifications (IP 2007)**

<table>
<thead>
<tr>
<th>SOLUBILITY</th>
<th>PART OF SOLVENT REQUIRED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very soluble</td>
<td>Less than 1 part solvent needed to dissolve 1 part solute</td>
</tr>
<tr>
<td>Freely soluble</td>
<td>From 1 to 10 parts solvent needed to dissolve 1 part solute</td>
</tr>
<tr>
<td>Soluble</td>
<td>From 10 to 30 parts solvent needed to dissolve 1 part solute</td>
</tr>
<tr>
<td>Sparingly soluble</td>
<td>From 30 to 100 parts solvent needed to dissolve 1 part solute</td>
</tr>
<tr>
<td>Slightly soluble</td>
<td>From 100 to 1000 parts solvent needed to dissolve 1 part solute</td>
</tr>
<tr>
<td>Very slightly soluble</td>
<td>From 1000 to 10,000 parts solvent needed to dissolve 1 part solute</td>
</tr>
<tr>
<td>Practically insoluble</td>
<td>More than 10,000 parts solvent needed to dissolve 1 part solute</td>
</tr>
</tbody>
</table>
EVALUATION OF METRONIDAZOLE MICROSPHERES: (Patel et. al., 2013), (Gangadharappa et. al., 2011), (Ravi et. al., 2016)

Particle size measurement
Particle size was determined by using a Zetasizer nano series 90 (Malvern instruments, UK). The formulation 50mg was dispersed in 50 ml of water in a volumetric flask, mixed thoroughly with stirring, sonicated for 10 minutes and light scattering was monitored at 25°C a 90° angle.

Determine of percentage yield
The prepared microspheres were collected and weight. The measured weight was divided by total amount of all non-volatile components, which were used for the preparation of microspheres. The % yield was calculated following formula

% yield = WRec/weight (drug+ polymer) x 100 ..........(1)

Where, WRec = weight of microspheres recovered

Determination of Entrapment Efficiency and drug loading
The entrapment drug concentration was determined by centrifugation of the microspheres. The microspheres was centrifuged for 20 min at 1000 rpm. The supernatant was collected and filtered after centrifugation after suitable dilution and the drug content was measured UV spectrophotometer at a wavelength of 227.72 nm. The drug entrapment efficiency (EE) and drug loading (DL) in the microspheres were calculated from the following equations:

%EE= (W1-W2)/W1X100 ...................(2)

W1= the wt. of the drug added to the system
W2= the wt. of the drug in the supernatant

%LC= (W1-W2) / (W1-W2+W3) X 100..........(3)

W1= the wt of the drug added to the system
W2= the wt of the drug in the supernatant
W3= the wt of the polymer added to the system

Scanning electron microscopy
The sample the scanning electron microscopy (SEM) analysis was prepared by sprinkling the microspheres on one side of the double adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater. The SEM analysis of the microspheres was carried out using Jeol JSM 5300, Japan. The microspheres were viewed at an accelerating voltage of 15-20 Kv.

In-vitro drug release
Microspheres, equivalent to 10 mg of Metronidazole, were accurately weighed and transferred to 250 ml conical flask containing 100 ml phosphate buffer (pH 6.8). The flask was kept in an incubator at 37 °C, 1 ml samples withdrawn at regular intervals and, after suitable dilution, the amount of drug released was determined using a spectrophotometer at 227.72 nm.

Following each sample withdrawal, 1 ml of phosphate buffer was added to the release medium to replenish it. Five minutes before each sampling, the flasks were gently shaken by manually whirling it clockwise (15 revolutions) to minimize any concentration gradient within the release medium. The microspheres were allowed to settle down and clear supernatant medium withdrawn for drug analysis. The sample was filtered and the microspheres collected were transferred to the dissolution flask. Similarly, the release of Metronidazole from gelatin microspheres was determined spectrophotometrically at 227.72 nm.

7. RESULT & DISCUSSION
Physical appearance:
Table 3: Physical appearance of Metronidazole

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observation</th>
<th>Standard (IP 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>White to brownish cream crystalline</td>
<td>White to brownish cream crystalline</td>
</tr>
<tr>
<td>Odour</td>
<td>Odourless</td>
<td>Odourless</td>
</tr>
</tbody>
</table>

Melting point

Another important identification was the melting point study. The melting point of metronidazole was determined by capillary method.

Table 4: Melting point of metronidazole

<table>
<thead>
<tr>
<th>S No.</th>
<th>Melting point(°C)</th>
<th>Average M.P.(°C)</th>
<th>Standard (°C) I.P. 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>159</td>
<td>159-162</td>
<td>159-162</td>
</tr>
<tr>
<td>2.</td>
<td>159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>161</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Melting point of Metronidazole was found to be 159.66°C and the reported melting point was 159-162°C which was in range of specifications. This parameter also was helped to identifying the Metronidazole.

Identification and characterization of Metronidazole

Estimation of \( \lambda \text{ max} \)

Standard curve of Metronidazole in phosphate buffer 6.8

10 mg of accurately weighed metronidazole dissolved in 10ml of phosphate buffer 6.8 to prepare a stock solution of 1000 μg/ml concentration. From above stock solution 1ml was placed in a 100ml volumetric flask and volume made up with phosphate buffer 6.8 yielding a solution of 10 μg/ml concentration. From this solution the aliquots of 2-10 ml were withdrawn in a series of 10 ml volumetric flask and diluted to 10 ml with phosphate buffer 6.8. This gave a range of 2, 4, 6, 8, 10 μg/ml. A straight line with regression coefficient (R²) (0.992) was obtained which indicates that drug follows Beer’s law in the studied concentration range. Absorption maxima (\( \lambda \text{ max} \)) of metronidazole in phosphate buffer 6.8 at \( \lambda \text{ max} \) 219.60 nm were shown in figure (12). Absorbance of metronidazole in phosphate buffer 6.8 at 219.60 nm was shown in table (14). Standard curve of metronidazole in phosphate buffer 6.8 at 219.60 nm (with regression coefficient) are shown in figure (5).

Figure 5: Maximum wavelength of Metronidazole
The $\lambda_{\text{max}}$ of Metronidazole was observed in UV spectrophotometer and the above spectrum suggested that the $\lambda_{\text{max}}$ of Metronidazole was found to be 219.60 nm which provide an appropriate match with the reported $\lambda_{\text{max}}$ 219.60 nm.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance (nm)</th>
<th>Statistical parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>4</td>
<td>0.026</td>
<td>$Y = 0.0085x + 0.0078$</td>
</tr>
<tr>
<td>3.</td>
<td>6</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>8</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>10</td>
<td>0.080</td>
<td></td>
</tr>
</tbody>
</table>

The standard curve of Metronidazole was prepared and the value of regression co-efficient was found to be 0.9929 which showed linear relationship between concentration and absorbance.

**FTIR Spectroscopy**

![Figure 6: (A) Infra-red spectrum of sample (Metronidazole)](image)

![Figure 7: (B) Infra-red spectrum of standard (Metronidazole) (IP 2007)](image)
Table 6: The IR spectrum of standard Metronidazole and sample Metronidazole shows the peaks at different absorbance

<table>
<thead>
<tr>
<th>Characteristic absorption</th>
<th>Wave number standard (MTZ)</th>
<th>Wave number sample (MTZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=O asymmetric stretching</td>
<td>1479 cm(^{-1})</td>
<td>1410 cm(^{-1})</td>
</tr>
<tr>
<td>CH(_2) bending</td>
<td>1466-1452 cm(^{-1})</td>
<td>1450 cm(^{-1})</td>
</tr>
<tr>
<td>C-H stretching</td>
<td>2991 cm(^{-1})</td>
<td>3195 cm(^{-1})</td>
</tr>
<tr>
<td>Stretching of C-O</td>
<td>1275-1096 cm(^{-1})</td>
<td>1188.44 cm(^{-1})</td>
</tr>
<tr>
<td>CH(_3) bending</td>
<td>1387 cm(^{-1})</td>
<td>1378 cm(^{-1})</td>
</tr>
<tr>
<td>C=C stretching</td>
<td>1600 cm(^{-1})</td>
<td>1450 cm(^{-1})</td>
</tr>
<tr>
<td>=C-H bending</td>
<td>711 cm(^{-1})</td>
<td>750 cm(^{-1})</td>
</tr>
</tbody>
</table>

The observed peaks in Infra-red spectrum were confirmed the bonds present in the sample Metronidazole and The structure and related functional groups of sample Metronidazole were confirmed on the basis of obtained infra-red spectrum.

**Drug excipients compatibility studies**

Metronidazole and gelatin polymer were mixed in maximum utilized quantity 1:3 and observed for physical as well as IR spectrum to study the interaction between them.

As per IR spectrum and physical appearance, the result shown that there was no evidence of Metronidazole and polymer interactions which allow us to formulate the formulations with this polymer and drug.

**Drug sample (MTZ)**

![Figure 8: (A) FT-IR Spectroscopy of Drug sample (Metronidazole)](image)

![Figure 15: (B) FT-IR Spectroscopy of Drug + polymer (Metronidazole + Gelatin)](image)
Solubility study

Table 14: Solubility profile of Metronidazole in different solvents

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Solvents</th>
<th>Absorbance</th>
<th>Concentration</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethyl alcohol</td>
<td>0.6820</td>
<td>95.402mg/ml</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>1.9515</td>
<td>0.055mg/ml</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>3</td>
<td>Ether</td>
<td>0.9879</td>
<td>27.805mg/ml</td>
<td>Sparingly soluble</td>
</tr>
<tr>
<td>4</td>
<td>Distilled water</td>
<td>3.1498</td>
<td>0.179mg/ml</td>
<td>Very slightly soluble</td>
</tr>
<tr>
<td>5</td>
<td>Dilute acid</td>
<td>1.0046</td>
<td>14.206mg/ml</td>
<td>Slightly soluble</td>
</tr>
</tbody>
</table>

Solubility of Metronidazole in ethyl alcohol was found to be 95.402mg/ml on the basis of absorbance 0.6820 which shows that the Metronidazole is freely soluble in ethyl alcohol due to required solvent amount was more than 10 ml solvent to dissolve 1 gram of solute. Solubility of Metronidazole in chloroform was found to be 0.055mg/ml on the basis of absorbance 1.9515 which shows that the Metronidazole is practically insoluble in chloroform due to required solvent amount was more than 1800 ml solvent to dissolve 1 gram of solute. In ether, the absorbance after 1000 time dilution was 0.9879 which shows the solubility 27.805mg/ml. this result indicated that the Metronidazole was sparingly soluble due to its requirement of more than 30 ml solvent to dissolve 1 gram of solute. Water solubility Metronidazole was 0.1795mg/ml on the basis of absorbance 3.1498 which indicate that the Metronidazole was very slightly soluble and required solvent amount was more than 5000 ml to dissolve 1 gram of solute. Solubility of Metronidazole in dilute acid was found to be 14.206mg/ml on the basis of absorbance 1.0046 which shows that the Metronidazole is slightly soluble in dilute acid due to required solvent amount was more than 2000 ml solvent to dissolve 1 gram of solute.

8. CONCLUSION:

Preformulation study of drug including physical appearance, solubility study, melting point detection, preparation of standard curve using UV spectrophotometer and IR spectroscopy, Surface morphology by SEM. The results are compared to the specific monograph and it complies with IP specification.

REFERENCES: