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Research Article

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

Amar Kumar, Indu Kanwar, Ashish Masih

School of Pharmaceutical Science, Jaipur National University, Jagatpura, Jaipur, Rajasthan, India

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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 (λ_{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 The flask was kept in an incubator at 37 °C, 1 ml samples withdrawn at regular intervals and, after suitable dilution, the amount of drug released was determined using a spectrophotometer at 227.72 nm.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

*Corresponding author: Amar Kumar | E-mail: amar.87854@gmail.com

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:

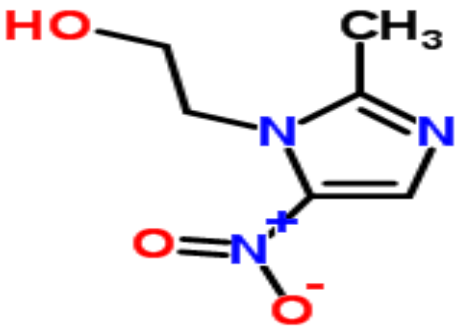

Figure 1:- Chemical structure of metronidazole

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: $C_6H_9N_3O_3$
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

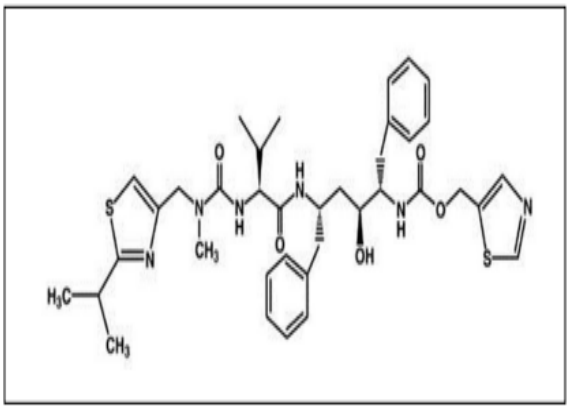

Figure 2:- Chemical structure of Gelatin

Synonyms: Gelfoam, Puragel, Gelfilm
Category: Polymer
IUPAC Name: Gelatin
Chemical formula: $C_{105}H_{151}O_{39}N_{31}$
Density: 1.3-1.4 g/cm^{-3}
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

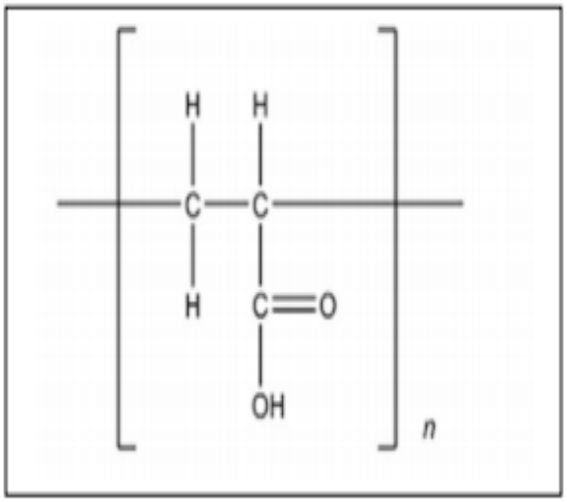

Figure 3:- Chemical structure of Carbopol

Nonproprietary names: Carbopol
Category: Biodegradable polymer
Dissociation constant: pKa =6.0±0.5
Density: 0.2g/cm^3
Glass transition temperature: 100-105^{0}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: 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

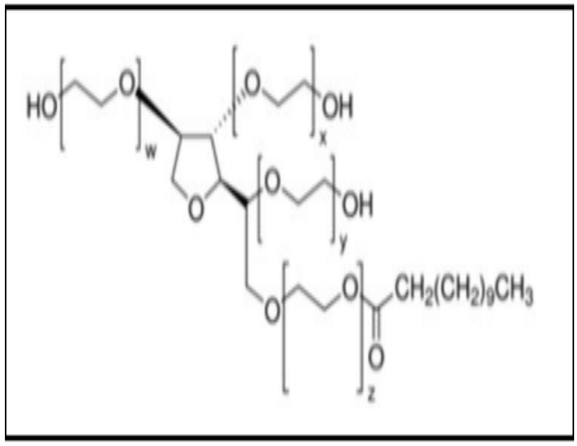

Figure 4:- Chemical structure of polysorbate

Molecular Formula: $C_{64}H_{124}O_{26}$
Molecular Weight: 1310gm/mol
Application: Polysorbate containing 20 units of oxyethylene is hydrophilic nonionic surfactants that are used widely as emulsifying agents in the perpration 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 weeting 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. PREFORMULAION 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 melt) was noted down as melting point.

Preliminary drug excipient compatibility studies

Determination of Absorption maxima (λ_{max}).

The absorption maxima (λ_{max}) of drug were determined by UV visible 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.

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 KH_2PO_4:- 8.0 ml of KH_2PO_4 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.

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

BOND	ABSORPTION REGION (cm^{-1})
C-C, C-O, C-N	1300-800
C=C, C=O, C=N, N=O	1900-1500
C≡C, C≡N	2300-2200
C-H, O-H, N-H	3800-2700

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)

SOLUBILITY	PART OF SOLVENT REQUIRED
Very soluble	Less than 1 part solvent needed to dissolve 1 part solute
Freely soluble	From 1 to 10 parts solvent needed to dissolve 1 part solute
Soluble	From 10 to 30 parts solvent needed to dissolve 1 part solute
Sparingly soluble	From 30to 100 parts solvent needed to dissolve 1 part solute
Slightly soluble	From 100 to 1000 parts solvent needed to dissolve 1 part solute
Very slightly soluble	From 1000 to 10,000 parts solvent needed to dissolve 1 part solute
Practically insoluble	More than 10,000 parts solvent needed to dissolve 1 part solute

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 10minutes 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) × 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

Parameters	Observation	Standard (IP 2010)
Colour	White to brownish cream crystalline	White to brownish cream crystalline
Odour	Odourless	Odourless

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

S No.	Melting point(°C)	Average M.P.(°C)	Standard (°C) I.P. 2010
1.	159	159-162	159-162
2.	159		
3.	161		

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 λ 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 (λ max) of metronidazole in phosphate buffer 6.8 at λ 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).

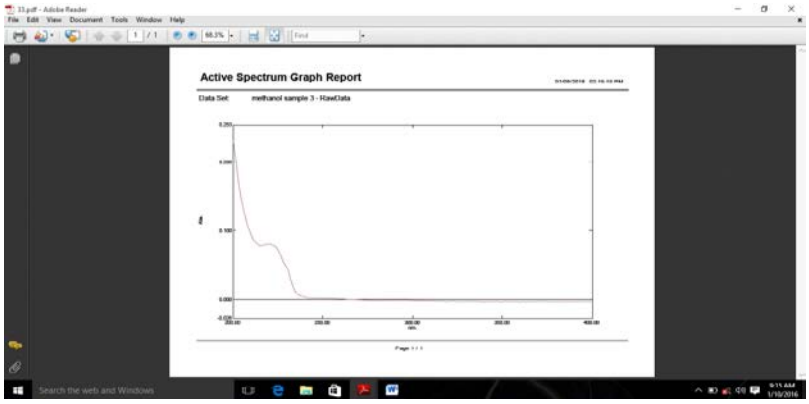

Figure 5: Maximum wavelength of Metronidazole

The λ max of Metronidazole was observed in UV spectrophotometer and the above spectrum suggested that the λ max of Metronidazole was found to be 219.60 nm which provide an appropriate match with the reported λ max 219.60 nm.

Table 5: Absorbance of metronidazole phosphate buffer 6.8 at 219.60 nm

S.No.	Concentration (µg/ml)	Absorbance (nm)	Statistical parameter
1.	2	0.011	Y = 0.0085x + 0.0078 R^2 = 0.9929
2.	4	0.026	
3.	6	0.041	
4.	8	0.058	
5.	10	0.080	

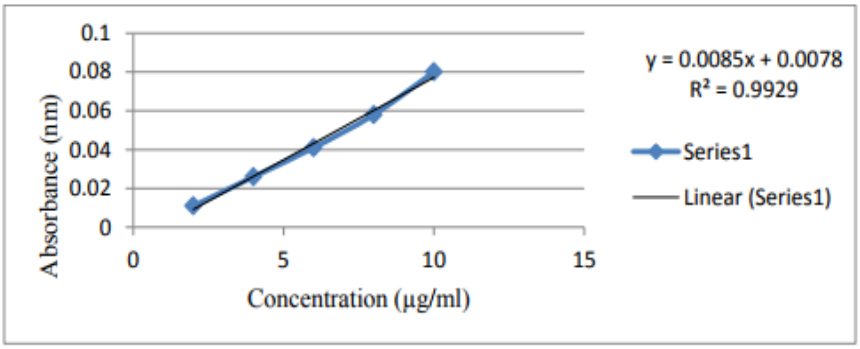

Figure 5: Standard curve of metronidazole in phosphate buffer 6.8 at λ max 219.60 nm (with regression coefficient)

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

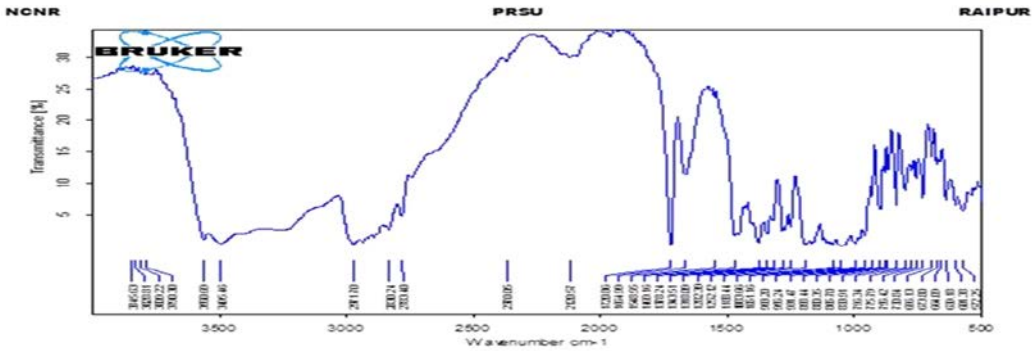

Figure 6: (A) Infra-red spectrum of sample (Metronidazole)

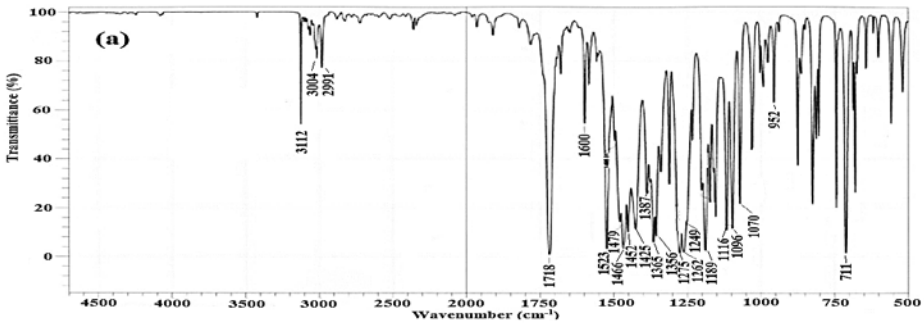

Figure 7: (B) Infra-red spectrum of standard (Metronidazole) (IP 2007)

Table 6: The IR spectrum of standard Metronidazole and sample Metronidazole shows the peaks at different absorbance

Characteristic absorption	Wave number standard(MTZ)	Wave number sample (MTZ)
N=O asymmetric stretching	1479 cm^{-1}	1410 cm^{-1}
CH_2 bending	1466-1452 cm^{-1}	1450 cm^{-1}
C-H stretching	2991 cm^{-1}	3195 cm^{-1}
Stretching of C-O	1275-1096 cm^{-1}	1188.44 cm^{-1}
CH_3 bending	1387 cm^{-1}	1378 cm^{-1}
C=C stretching	1600 cm^{-1}	1450 cm^{-1}
=C-H bending	711 cm^{-1}	750 cm^{-1}

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)

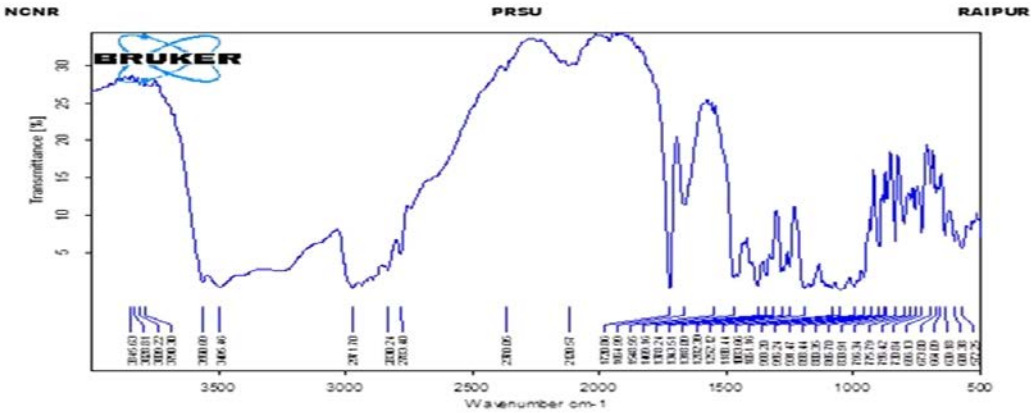

Figure 8: (A) FT-IR Spectroscopy of Drug sample (Metronidazole)

Drug + Polymer (Metronidazole + Gelatin)

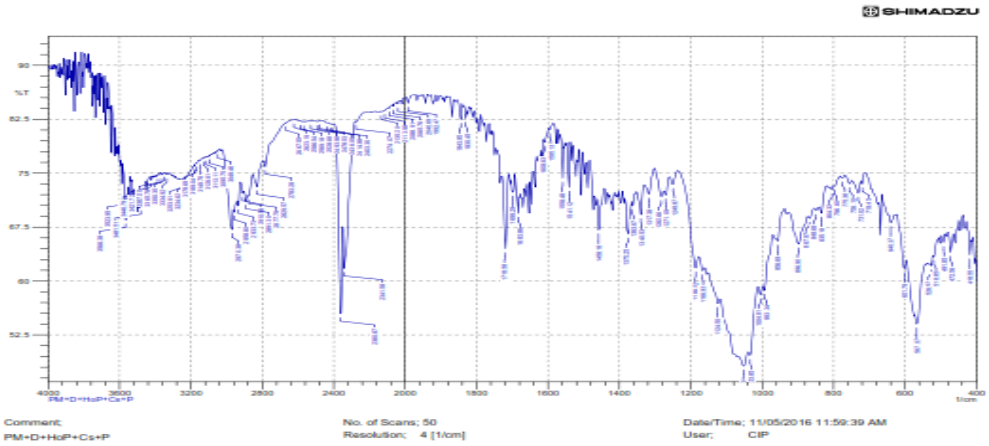

Figure15: (B) FT-IR Spectroscopy of Drug + polymer (Metronidazole + Gelatin)

Solubility study

Table 14: Solubility profile of Metronidazole in different solvents

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

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.

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