

## Design and Characterization of Mucoadhesive Microspheres Loaded With Metronidazole for Colon Specific Delivery.

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### Abstract

The purpose of the work was to formulate and evaluate *in vitro* parameters of mucoadhesive Metronidazole microspheres for the potential delivery of the drug to the colon. Prepared by the emulsion-solvent evaporation method using span-80 as an emulsifying agent. The results of the preliminary trials indicate the drug: polymer ratio affected the characteristics of the mucoadhesive microspheres. The mucoadhesive microspheres were prepared in different drug: polymer (Metronidazole: Eudragit RL). Mucoadhesion of microspheres was achieved by coating the microspheres with the 5% Chitosan solution, the resulting mucoadhesive microspheres were filled in the hard gelatin capsule shell followed by coating with Eudragit RL coating solution. *In vitro* drug release study in the three different pH (0.1N HCl, Phosphate buffer pH 6.8 and Phosphate buffer pH 7.4) for 12 hours. The best formulation batch followed Zero order rate release with non Fickian-Diffusion mechanism with 82.77% of drug release at the end of 12 hours.

### Key Words

Metronidazole, Microspheres, Emulsion-solvent evaporation method, Hard gelatin capsule shell and Colon specific delivery.

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### Introduction

Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for systemic delivery of drugs via pharmaceutical products of different dosage form. Oral route is considered most natural, uncomplicated, convenient and safe due to its ease of administration, patient acceptance, and cost-effective manufacturing process.

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000  $\mu\text{m}$  in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it, respectively<sup>1</sup>. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of the

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drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drug to the absorption site achieved by anchoring plant lectins<sup>2</sup>, bacterial adhesions and antibodies, etc. on the surface of the microspheres. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity and urinary and gastrointestinal tract, thus offering the possibilities of localized as well as systemic controlled release of drugs. Application of mucoadhesive microspheres to the mucosal tissues of ocular cavity, gastric and colonic epithelium is used for administration of drugs for localized action. Prolonged release of drugs and a reduction in frequency of drug administration to the ocular cavity can highly improve the patient compliance. The latter advantage can also be obtained for drugs administered intra-nasally due to the reduction in mucociliary clearance of drugs adhering to nasal mucosa. Microspheres prepared with mucoadhesive and bioerodible polymers undergo selective uptake by the M cells of Payer patches in gastrointestinal (GI) mucosa. The type of polymers used to prepare them influences the properties of the mucoadhesive microspheres, based on their surface characteristics, force of mucoadhesion, release pattern of the drug, and clearance. Suitable polymers that can be used to form mucoadhesive microspheres include soluble and insoluble, non-biodegradable and biodegradable polymers. These can be hydrogels or

thermoplastics, homopolymeres, copolymers or blends, natural or synthetic polymers. The oral route of administration has received the most attention for sustained release as well as controlled drug delivery system due to flexibility in dosage form design and minimum the constraints of sterility and potential damage at the site of administration. In past few years there has been considerable research in the field of colonic drug delivery due to following reasons, the local treatment of a variety of bowel diseases, improving the systemic absorption of drugs susceptible to the acidic inactivation or enzymatic digestion in the upper gastrointestinal tract.

## **Materials and Methods**

### **Materials**

Metronidazole was obtained as a gift sample from Unique Pharma Ltd., Gujarat. Eudragit RL was obtained from Micro Labs, Hosur. Chitosan was obtained from Fiesheris institute of technology, Cochin. Span 80, Liquid paraffin (light) was obtained from Loba Chemical. Pvt. Ltd. Mumbai. Petroleum ether, Acetone was obtained from Research labs fine chemicals, Mumbai. All the ingredients used were of analytical grade.

### **Methods**

#### **Preparation of mucoadhesive microspheres by emulsion-solvent evaporation technique<sup>3,4</sup>**

Accurately weighed quantities of the polymers were (Eudragit RL) dissolved in 20 ml of acetone. Weighed quantity of Metronidazole (drug) (previously passed through the

sieve # 150) was then dispersed in the above polymer phase and it was stirred for 2 hours. Then it was emulsified with the 100 ml of liquid paraffin containing 1% w/v of Span 80 with continuous stirring at 800 rpm under a mechanical stirrer. The stirring was continued for 2 hours to ensure complete evaporation of acetone. The microspheres were then separated from liquid paraffin by filtration using Whatmann filter paper No. 44, washed three times with 50 ml of petroleum ether, and air dried for 12 hours. These resultant microspheres were further coated with 5% of Chitosan solution and dried for 12 hours. These mucoadhesive microspheres were filled in the hard gelatin capsule shell and the shell was coated with Eudragit RL solution by dipping and drying method, to exactly target the colon. All the formulations of microspheres were prepared in the same way. The composition of Eudragit RL contains: Eudragit RL (8%), Dibutyl phthalate (2%), in the 80% of methanol solution (Table No.1).

#### **Evaluation Parameters**

##### **Pre-formulation Studies**

##### **Fourier Transform Infrared Spectroscopy**

Compatibility study of drug with the excipients was determined by FTIR Spectroscopy using SHIMADZU-FTIR 410 model. The pellets were prepared at high compaction pressure by using KBr and the ratio of sample to KBr is 1:100. The pellets thus prepared were examined and the spectra of drug and other ingredients in the formulations were compared with that of the original spectra.

##### **Differential Scanning Colorimeter (DSC)<sup>5,6</sup>**

Differential scanning calorimeter is used to measure the specific heat and enthalpies of transition. When a sample undergoes a thermal transition, the power to the heater is adjusted to maintain the temperature, and a signed proportional to the power difference is plotted on the second axis of the recorder is known as thermogram. The area under the resulting curve is direct measure of the heat of transition. Thermograms were obtained by using a differential scanning calorimeter at a heating rate 15°C /min over a temperature range of 0 to 1000°C. The sample was hermetically sealed in an aluminum crucible. Nitrogen gas was purged at the rate of 100 ml/min. For maintaining inert atmospheres.

##### **Evaluation of the Prepared Mucoadhesive Microspheres**

##### **Determination of percentage yield of microspheres<sup>7</sup>**

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below.

$$\% \text{ yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

##### **Microsphere size analysis**

##### **Microsphere size distribution**

Microsphere size determination was done by optical microscopy method. Size distribution plays a very important role in determining the release characteristics of the microspheres.

##### **Angle of repose**

The fractional force in the loose powder can be measured by the angle of repose. This is the maximum angle

possible between the surfaces of the site of the powder to the horizontal plane. Angle of repose was calculated by static method using funnel method. Funnel was kept on triangular stand, which was kept on horizontal plane. The sample was introduced into the funnel, as the pile forms; it reaches to the tip of funnel. The diameter of the pile was noted. The angle of repose ( $\theta$ ) is calculated by the following formula,

$$\theta = \tan^{-1} (h/r)$$

Where,

h = pile height of microspheres;

r = radius of the circular area formed by the microspheres on the ground.

#### **Determination of Bulk density**

The bulk density was determined by 3-tap method. Bulk density is defined as, "the mass of powder divided by the bulk volume". The packing characteristics of the powder play an important role in determining physical properties of product. According to the standard procedure for obtaining bulk density, weighed quantities of prepared microspheres were filled in 10 ml of graduated cylinder the initial volume was noted. After tapping for three times the final volume was noted. The bulk density was calculated as per following formula:

$$D_b = M / V_b$$

Where,

M is the mass of powder

V<sub>b</sub> is the bulk volume of the powder.

#### **Determination of drug content**

Accurately weighed 100 mg microspheres, crushed in glass mortar and pestle and the powdered

microspheres were suspended in 100 ml of phosphate buffer pH 7.4. After 12 hours the solution was filtered and the filtrate was analyzed for the drug content using UV-Visible spectrophotometer at 276.6nm.

#### **Encapsulation efficiency**

Encapsulation efficiency was calculated using the following formula,

$$\text{Encapsulation efficiency} = \left( \frac{\text{Estimated drug content \%}}{\text{Theoretical drug content \%}} \times 100 \right)$$

#### **In vitro wash-off test<sup>8</sup>**

The mucoadhesive property of microspheres was evaluated by an *in vitro* adhesion testing method known as wash-off method. Freshly excised piece of intestinal mucosa (2 x 2 cm) from goat were mounted on to glass slides (3 x 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support, about 25 microspheres were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on to the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given slow, regular up-and-down moment in the test fluid (900 ml of 0.1N HCl/Phosphate buffer pH 6.8 and phosphate buffer pH7.4) at 37 ± 0.5°C. At the end of one hour, and at the hourly intervals up to 5 hours, the machine was stopped and number of microspheres still adhering to tissue was calculated. The studies were carried out in triplicate.

### ***In vitro* dissolution studies<sup>9</sup>**

Dissolution studies were carried out for all the formulations, employing USP XXIII apparatus (Basket method) at  $37 \pm 0.5^\circ\text{C}$  rotated at constant speed of 50 rpm using 900ml of 0.1N HCl as the dissolution medium for first 2 hrs and followed for phosphate buffer pH 6.8 for another 3 hrs and in the phosphate buffer pH 7.4 up to 12 hrs. A sample of microspheres equivalent weight to 477 mg of Metronidazole was used in each test. An aliquot of the sample was periodically with drawn at suitable time interval and the volume was replaced with fresh dissolution medium in order to maintain the sink condition. The sample was analyzed spectrophotometrically at 276.6 nm.

### **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 zero order, first order, Higuchi's, Peppas's and Coefficient of correlation (r) values were calculated for the linear curves by regression analysis of the above plots.

### **Shape and surface characterization<sup>1</sup>**

The shape and surface characterization of microspheres were observed under a Scanning Electron Microscope (SEM). The instrument used for this study was HITACHI-SEM MODEL S-450 Scanning Electron Microscope. The microspheres were mounted directly on to the SEM sample stub, using double-sided sticking tape, and coated with gold film (thickness 200 nm)

under reduced pressure (0.001 torr) and photographed.

## **Results and Discussion**

### **Pre-formulation Studies**

Infrared spectra for pure Metronidazole and for the physical mixture of Metronidazole and all the polymers were determined to check the intactness of the drug in the polymer mixture using SHIMADZU (FTIR 410) by disc method. The following table shows the wave number for the characteristic bands in the IR spectra of pure Metronidazole (Figure No.1, 2 and 3).

### **Differential scanning calorimeter (DSC)**

DSC provides information about all physical properties of sample as Crystalline or Amorphous nature and demonstrates the possible interaction between Drug and other Polymers. The thermal behavior of Metronidazole, Eudragit RL and Chitosan are shown in (Figure No.7), according to thermogram, Metronidazole produced sharp Endothermic peak at  $167.50^\circ\text{C}$  which conformed crystalline form of the drug. DSC curves of the Eudragit RL and Chitosan Exhibited an Endothermic peaks at  $162.7^\circ\text{C}$  and  $164.37^\circ\text{C}$ , which has been attributed to the evaporation of water. The thermogram of the physical mixture of Drug and Polymers showed that there was no interaction between drug and polymers (Figure No.4, 5 and 6).

### **Percentage yield**

The percentage yield of microspheres of all formulations was found in the range of 70.27% to 79.30% which is shown in (Table No.2).

### **Morphology and Particle size**

The microspheres prepared by solvent evaporation method were found to be discreet, spherical, free flowing and it was observed by Scanning Electron Microscopy (SEM) (Figure No. 8). The size of the mucoadhesive microspheres was determined by the optical microscopy method. The microspheres were found to be uniform in size with a size range of 58.9 $\mu$ m to 205.1 $\mu$ m which is shown in (Table No.2). The prepared microspheres were considered more suitable for colon targeting, mucosal retention and penetration which suggesting that the coating was well completed under the present conditions.

### **Micromeritics studies**

The angle of repose was determined by funnel method. The angle of repose was found in the range of 23<sup>0</sup>72' to 25<sup>0</sup>30' which revealed that the microspheres of all the batches (MF<sub>1</sub> to MF<sub>6</sub>) had well flow characteristics and flow rates (Table No.2). The bulk density was in the range of 0.484gm/cm<sup>3</sup> to 0.599gm/cm<sup>3</sup> were shown in (Table No.2).

### **Drug content analysis and Entrapment efficiency**

The drug content values of mucoadhesive microspheres were found in the range of 71.97% to 83.37%, the determination of drug content showed that even if the polymer composition was changed the process was highly efficient to give microspheres having maximum drug loading. (Table No.2). The result indicates that the 20 to 30% of drug leached out of microspheres, however the high drug dose is required for the

treatment of amoebiasis, this drug content was considered acceptable.

### ***In vitro* wash off test for mucoadhesion**

Mucoadhesive Microspheres of Metronidazole exhibited good mucoadhesive properties in the *in vitro* wash off test. The results of wash off test were shown in (Table No.3, 4 and 5). The MF1 formulation has more adhesive strength than others because of small particle size and surface area which is favoured by preparation and evaluation of mucoadhesive microspheres of Indomethacin.

### ***In vitro* drug release studies**

Metronidazole release from the microspheres was studied in 0.1N Hydrochloric acid as a simulated gastric fluid for first 2 hours, for next three hours in the phosphate buffer pH 6.8 as a simulated intestinal fluid and up to 12 hours in phosphate buffer pH 7.4 as a simulated colonic fluid by using USP XXIII basket type dissolution tester. The drug release was retarded by increasing the polymer concentration due to increased viscosity and strength of matrix formed due to Eudragit RL and chitosan. The solubility of Eudragit RL is fully depended on the pH of the medium; it will dissolve at the range of pH 6 to pH 8 so the successful targeting to the colon will be achieved. Chitosan provides good mucoadhesion property for better efficacy of the drug on the colonic mucosa by increasing the colonic transit time due to sticking to the mucous of the colon. *In vitro* drug released at the end of 12 hours showed that MF1 released the 82.77%

of Metronidazole (Table No.6), MF2 released 81.63% of Metronidazole (Table No.6), MF3 released 79.17% of Metronidazole (Table No.6), MF4 released 77.91% of Metronidazole (Table No.6), MF5 released 76.48% of Metronidazole (Table No.6), MF6 released 74.97% of Metronidazole (Table No.6), so the drug release from microspheres was decreased by increasing the polymer concentration because the drug release mainly depends on the composition and amount of the polymer used. Since this finding is in the favour of releasing the Albendazole from the polymeric matrix of Eudragit RL<sup>1</sup> (Figure No.9).

#### **In vitro drug release kinetics**

For all the formulation MF<sub>1</sub> to MF<sub>6</sub> the kinetic drug released data were shown in the (Table No.7), For the first order kinetic the *r* values were found in the range of 0.8421 to 0.8929, For the zero order kinetic the *r* values found in the range of 0.9696 to 0.9822, so all formulations showed the zero order drug release kinetic, among them formulation MF<sub>1</sub> showed best *r* value (0.9822) for the zero order kinetic.

#### **Mechanism of drug release**

In order to understand the complex mechanism of drug release from the mucoadhesive microspheres, the *in vitro* Metronidazole release data were fitted to korsmeyer-peppas's release model and interpretation of *r* values enlightens in understanding the release mechanism from the dosage form. The *r* values thus obtained were ranged from 0.9666 to 0.9849 are shown in (Table No.7). All the

formulations exhibited anomalous (non-fickian transport) diffusion mechanism. The drug release was diffusion controlled as the plot of Higuchi's model was found to be linear ( $r > 0.9778$ ). These formulations are also showed as good '*r*' values of zero order kinetics indicating the Metronidazole release from these mucoadhesive microspheres were by both diffusion and erosion. The formulation MF<sub>1</sub>, Drug: polymer ratio (1:1) was selected as best formulation with 82.77% of drug released at 12 hours.

#### **Conclusion**

In the present work efforts have been made to design and evaluate mucoadhesive microspheres of Metronidazole and the results obtained in the study have been summarized below. All the formulations exhibited anomalous (non-fickian transport) diffusion mechanism and follow zero order kinetic. The formulation MF<sub>1</sub> (Metronidazole-500 mg, Eudragit RL-500 mg, Chitosan 5% w/v solution) was selected as best formulation; with 82.77% of controlled drug release at the end of 12 hours with best mucoadhesion properties, hence such a design can be used for colon targeted drug delivery of Metronidazole to eradicate the parasites from the colonic region. Finally it is concluded that by increasing polymer concentration the drug release from microspheres will be slow. Success of the *in vitro* drug release studies recommends the product for further *in vivo* studies in detail for its viability in clinical practice.

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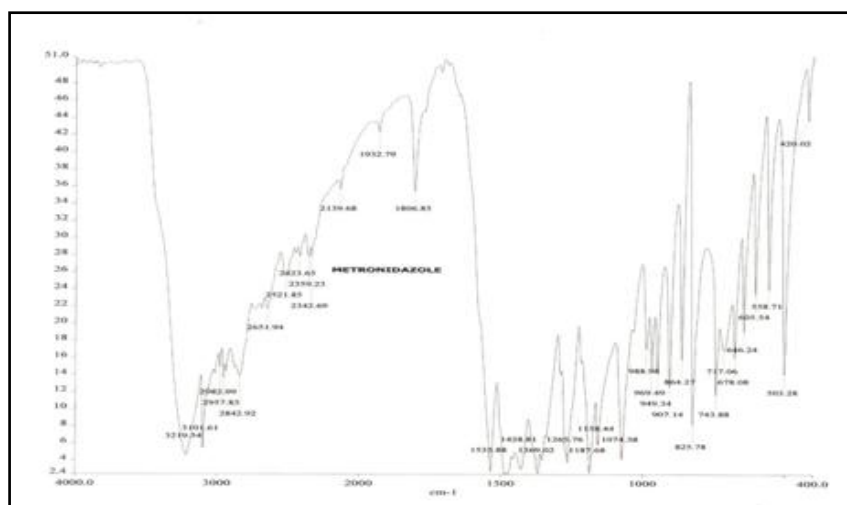


Fig. 1: FT-IR spectra of pure Metronidazole.



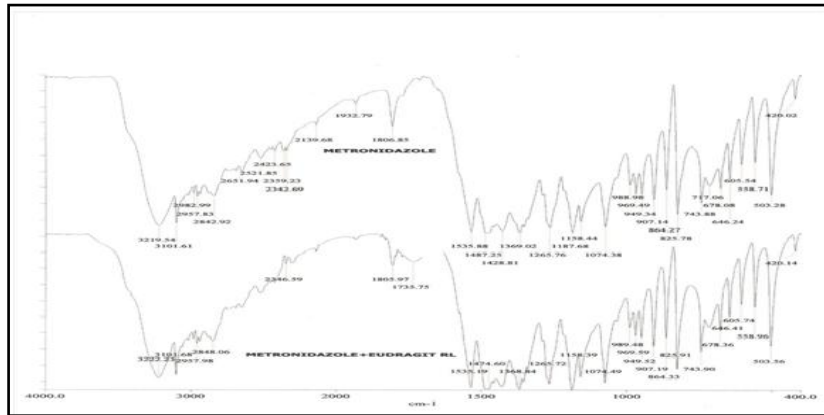


Fig. 2: FT-IR spectra of Metronidazole + Eudragit RL.

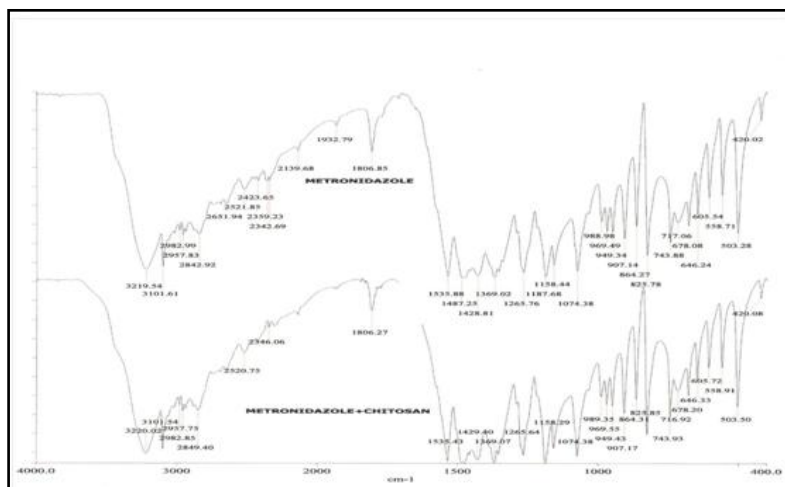


Fig. 3: FT-IR spectra of Metronidazole + Chitosan.

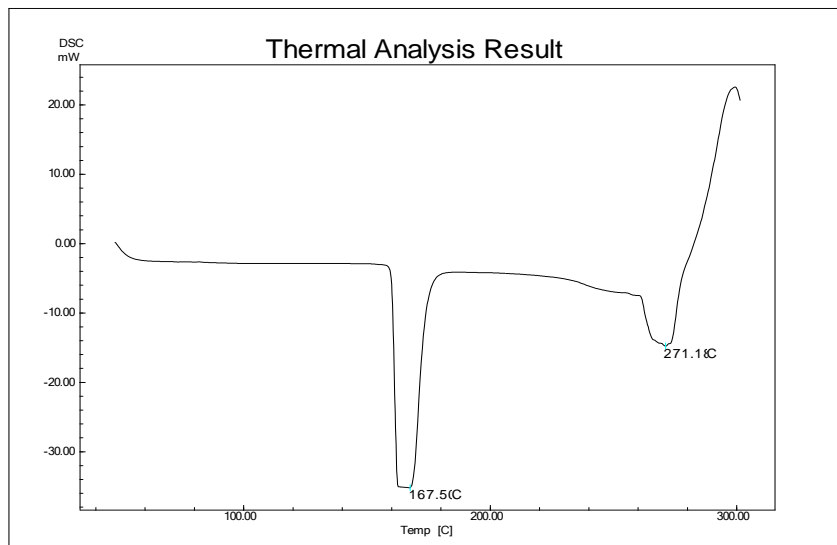
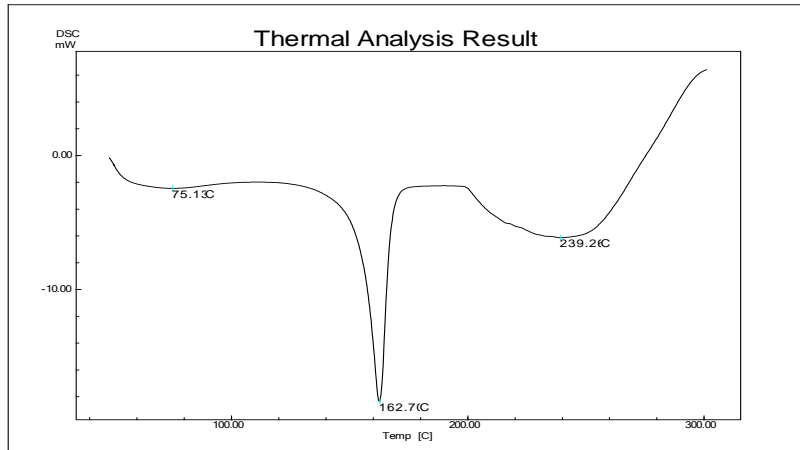
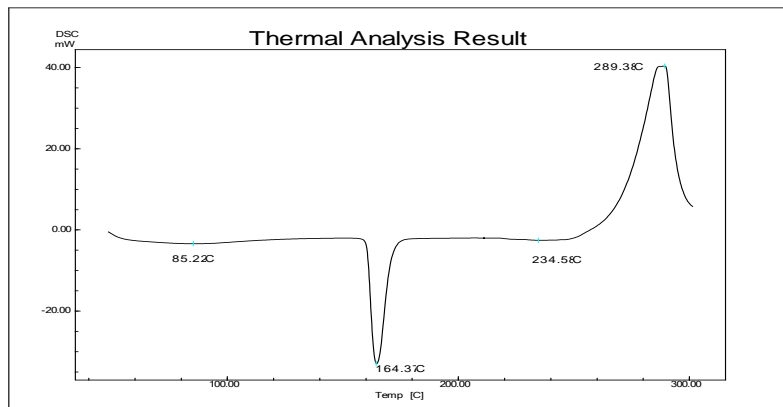


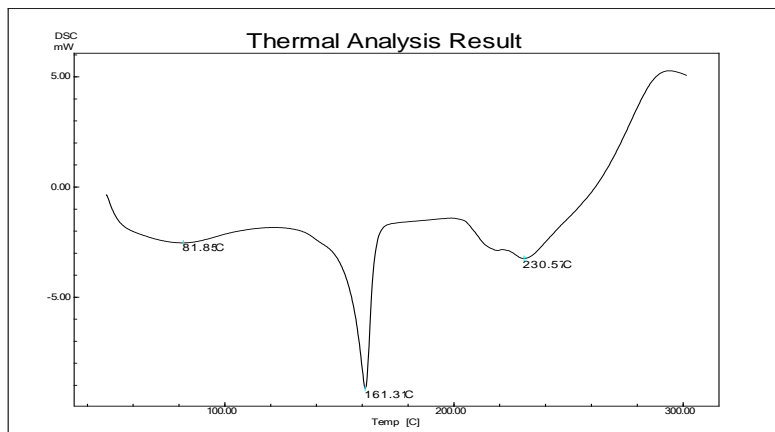
Fig. 4: DSC Spectra for pure drug of Metronidazole.



**Fig. 5:** DSC Spectra for physical mixture of pure drug Metronidazole + Eudragit RL.



**Fig. 6:** DSC Spectra for physical mixture of pure drug Metronidazole + Chitosan.



**Fig. 7:** DSC Spectra for physical mixture of pure drug Metronidazole + Eudragit RL + Chitosan

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