6. Preformulation and preliminary studies

6.1. Preformulation studies

6.1.1. Description/Appearance

Rifampicin and isoniazid were observed visually to check whether it complies with the specifications given in the certificate of analysis supplied by the vendor.

6.1.2. Saturation solubility studies of rifampicin and isoniazid in different buffer solutions

Excess amount of drugs were taken in 5 ml RIA vials containing 3 ml of different buffers. The RIA vials were placed in rotospin and were rotated at 50 rpm for 24 h at room temperature. The solutions were then passed through 0.45 µm membrane. The amount of drug dissolved was analyzed spectrophotometrically with suitable dilutions using UV-visible Spectrophotometer (UV-1601PC, Shimadzu, Japan) at their respective λ_{max} (Baka et al., 2008).

6.1.2.1. Preparation of solutions

- **0.1N Hydrochloric acid (0.1N HCl):** Concentrated hydrochloric acid (8.5 ml) was added to 200 ml of distilled water and the volume made up to 1000 ml (IP 2010).
- **Acetate buffer, pH 4.6:** Sodium acetate (5.4 g) was dissolved in 50 ml of distilled water. To this 2.4 ml of acetic acid was added and the volume was made up to 100 ml with distilled water (IP 2010).
- **Phosphate buffer, pH 6.8:** Sodium hydroxide (0.896 g) and 6.804 g of potassium dihydrogen phosphate was weighed and dissolved in 250 ml of distilled water and the volume was made up to 1000 ml with distilled water (IP 2010).
- **Phosphate buffer, pH 7.4:** Sodium hydroxide (1.54 g) and potassium dihydrogen phosphate (6.804 g) was weighed and dissolved in 250 ml of distilled water and the volume was made up to 1000 ml with distilled water (IP 2010).
- **Simulated gastric fluid (SGF):** Sodium chloride (2.0 g) and purified pepsin (3.2 g, derived from porcine stomach mucosa with an activity of 800 to 2500 units per mg) were dissolved in 7.0 ml of HCl. Sufficient water was added to make 1000 ml (USP 32).
- **Simulated intestinal fluid, (SIF):** Monobasic potassium phosphate (6.8 g) was dissolved in 250 ml of water. To this 77.0 ml 0.2N sodium hydroxide and 500 ml water were added. 10.0 g of pancreatin was added and diluted to 1000 ml with water

and pH of the resulted solution was adjusted with either 0.2N sodium hydroxide or 0.2N HCl solutions to a pH of 6.8±0.1 (USP 32).

6.1.3. Drug-excipient compatibility studies

6.1.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed using a Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded in the region of 4000 to 400 cm^{-1}. In this study, FTIR spectra for the pure drugs and drugs along with the excipients were obtained (Lachman et al., 2009).

The procedure consisted of dispersing a sample in Potassium bromide (1:1 ratio) and then it was compressed into discs by the application of a pressure of 5 tons for 5 min in a hydraulic press. The pellet was then placed in the light path and the spectrum was recorded from 4000 to 400 cm^{-1}.

6.1.3.2. Differential Scanning Calorimetry (DSC)

DSC was performed using DSC-60, Shimadzu, Japan. The instrument consists of the calorimeter (DSC 60), flow controller (FCL 60) and thermal analyzer (TA 60). It was operated by software TA-60 from Shimadzu Corporation, Japan.

The sample was placed in a sealed aluminum pan, and then it was heated under nitrogen flow (30 ml/min) at a scanning rate of 5 °C/min from 30 °C to 300 °C. Reference was the empty aluminum pan without the sample. The heat flow as a function of temperature was measured for both the pure drugs and dug-excipient mixture (Lachman et al., 2009).

6.1.4. Micromeritic properties

6.1.4.1. Angle of repose

It is defined as the maximum angle possible between the surface of a pile of the horizontal powder and the plane. It was determined by using fixed funnel method. The powder mixture was weighed accurately and taken into a funnel. Adjustment was made for the height of the funnel in such a way that the funnel tip just touches the apex of the heap of the powder mixture (Lachman et al., 2009).

The powder mixture then freely flowed onto the surface to form a cone. The height and diameter of the powder cone was measured. Angle of repose was calculated using the following formula.

$$\text{Angle of repose} = \tan^{-1}\left(\frac{\text{Height of the pile}}{\text{Radius of thepile}}\right)$$

6.1.4.2. Carr's index

Carr's index is indirectly related to the relative flow rate, cohesiveness and particle size of a powder. It was calculated by determining the bulk and tapped densities. Carr's index of a powder was calculated using the formula (Lachman et al., 2009).

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

6.1.4.3. Hausner's ratio

This value was calculated by using the values of bulk and tapped densities of powder sample using the given equation (Lachman et al., 2009).

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

6.2. Preliminary Studies Conducted

6.2.1. Formulation and evaluation of rifampicin and isoniazid tablets with improved rifampicin stability

To improve the stability of rifampicin, two formulations were prepared. Formulation I includes rifampicin and isoniazid as immediate release uncoated tablets, formulation II includes rifampicin in uncoated form and isoniazid in enteric coated form. The formulation ingredients of rifampicin and isoniazid tablets are given in the Table 6.1. Eudragit L100 was used as enteric coating polymer and the isoniazid tablets were coated by pan coating technique.

6.2.1.1. Preparation of the formulations

Required weights of the drugs and the excipients (as shown in the Table 6.1) were taken separately into mortar and pestle and were mixed thoroughly by trituration. After adjusting the die cavity in the tablet compression machine with respect to the weight required by the vehicle, the tablets were compressed using direct compression method (Lachman et al., 2009).

Table 6.1. Rifampicin and isoniazid formulations

Ingredients	Rifampicin formulation Quantities (mg)	Isoniazid formulation Quantities (mg)
Rifampicin	600	-
Isoniazid	-	300
Talc	10	6
Magnesium stearate	10	6
SuperTab 11SD	q.s. to 700	q.s. to 400

6.2.1.2. Enteric coating of isoniazid tablets

Isoniazid tablets were enteric coated using eudragit L100 at a concentration of 8% w/w by pan coating technique so that its release is delayed and prevented in the gastric medium (Lachman et al., 2009). The various aspects of the pan coating process are given in the Table 6.2.

Table 6.2. Specifications of pan coating process

Polymer	Eudragit L100
Plasticizer	Glycerol
Solvent system	Acetone : Isopropyl Alcohol (40:60)
Technique	Pan Coating Technique
Atomization air pressure	20 psi
Pan speed (rpm)	30
Pump speed (rpm)	1
Inlet air temperature	40 ^{o}C
Exhaust temperature	40 ^{o}C
Spray gun and tablet bed were maintained at a distance of 10 cm	

6.2.1.3. Evaluation of the tablets

- **Weight Variation:** Rifampicin and isoniazid tablets were weighed and the percentage deviation of each and every tablet from the mean weight was calculated. The procedure for weight variation was followed as per Indian Pharmacopoeia (IP) 2007.
- **Hardness:** The uncoated tablets were placed in the hardness tester (Monsanto), the pressure was applied and the pressure at which the tablets break was noted.
- **Friability:** The prescribed numbers of uncoated tablets were taken, weighed and placed into Roche friabilator and were rotated (100 rpm as per IP 2007) and the final weight of the tablets was noted and the percentage weight loss was computed.
- **Disintegration test:** Required numbers of tablets as per IP 2007 were added into the disintegration test apparatus and the disintegration time was noted.
- **Dissolution test:** Required numbers of tablets according to IP 2007 were added into the dissolution test apparatus (USP II) and the release of the drug rifampicin was analyzed using UV-visible spectrophotometer at 336 nm λ_{max}. These studies were conducted in 0.1 N HCl buffer up to 2 h for both the formulations.

6.2.2. Formulation and evaluation of multiparticulate systems of rifampicin and isoniazid with improved rifampicin stability

Similar to the work done in 6.2.1., two different formulations were manufactured. Formulation-I includes capsules of rifampicin and isoniazid containing immediate release uncoated pellets, formulation-II includes capsules containing uncoated immediate release pellets of rifampicin and enteric coated pellets of isoniazid.

The formulation ingredients of uncoated rifampicin and isoniazid pellets are given in the Table 6.1. As discussed in the section 6.2.1., Eudragit L-100 was used as enteric coating polymer and the isoniazid pellets were coated by pan coating technique (Table 6.2).

6.2.2.1. Preparation of the pellets

The required quantities of the drugs and the excipients (as mentioned in the Table 6.1) were mixed uniformly by triturating them in a mortar and pestle. The pellets were prepared by extrusion spheronization method with optimized speed of 10 and 1000 rpm for extrusion and spheronization respectively (Waldron, 2013). Water was used as granulating liquid and polyvinyl pyrrolidone (5% w/v in granulating liquid) was used as binder.

The wet rifampicin and isoniazid pellets were dried at 50 ^{o}C by tray drying till constant weight. Then these pellets were weighed and transferred into hard gelatin capsule shells of sizes 00 and 0 for rifampicin and isoniazid pellets respectively. The dissolution study was conducted to quantify the release of rifampicin from formulation I.

Based on the requirement, for formulation II isoniazid pellets were enteric coated (Lachman et al., 2009). Similarly dissolution studies were conducted for formulation II and the release of rifampicin was estimated from these formulations.

6.2.2.2. Evaluation of pellets

- **Usable yield:** Pellets that passed through sieve no. 12 and retained on sieve no. 20 was considered as yield value. It was determined by sieving technique (Liberman et al., 1990).
- **Pellet size:** Pellet size of both rifampicin and isoniazid pellets was determined by vernier caliper. 20 pellets were taken from usable yield and the diameter was measured by vernier caliper (Sangeetha et al., 2010).
- **Friability:** The prescribed weight of uncoated pellets was placed into the friabilator and was rotated (100 rpm as per IP 2007) and the final weight of the pellets was noted and the percentage weight loss was computed.

- **Flow properties:** Flow properties like angle of repose, Carr's index and Hausner's ratio were determined. Angle of repose was determined by funnel method. Carr's index and Hausner's ratio were calculated from the bulk and tapped densities of these pellets by using the following equations (Lachman et al., 2009).

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

- **Drug content:** 100 mg of uncoated pellets of these drugs were taken separately and crushed in porcelain mortar and pestle. Then the powder was taken separately into 100 ml volumetric flask and thoroughly mixed with 0.1N HCl. Then the dispersion is filtered through 0.45 µm membrane and sonicated in bath sonicator. The filtrate was diluted and analyzed for their drug contents at 336 nm wavelength for rifampicin and 265 nm for isoniazid respectively using UV-visible spectrophotometer (IP 2007).
- **Dissolution test:** Required numbers of capsules according to IP 2007 were added into the dissolution test apparatus (USP II) and the release of the drug rifampicin was analyzed using UV-visible spectrophotometer for all the formulations. Similar to the dissolution studies of tablets in section 6.2.1.3., these studies were conducted in 0.1 N HCl up to 2 h for formulations I and II.

6.3. Results

6.3.1. Preformulation studies

6.3.1.1. Description /Appearance

Rifampicin was odorless and appeared as red powder which was in compliance with the available literature while isoniazid was colorless, odorless, white powder.

6.3.1.2. Saturation solubility

6.3.1.2.1. Rifampicin

The saturation solubility of rifampicin in different distilled water and different buffer solutions was determined and the results are as shown in Table 6.3. Solubility data shows that rifampicin is more soluble in pH 1.2 HCl buffer than in other buffers and distilled water, owing to its weakly basic nature.

Table 6.3. Saturation solubility of rifampicin in various media

Medium	Concentration (mg/ml)
Distilled water	2.5
pH 1.2 HCl buffer	180
pH 4.6 Acetate buffer	2.3
pH 6.8 Phosphate buffer	2.4
pH 7.4 Phosphate buffer	2.9

6.3.1.2.2. Isoniazid

The saturation solubility of isoniazid in different distilled water and different buffer solutions was determined and the results are as shown in Table 6.4.

Table 6.4. Saturation solubility of isoniazid in various media

Medium	Concentration (mg/ml)
Distilled water	128
pH 1.2 HCl buffer	131
pH 4.6 Acetate buffer	129
pH 6.8 Phosphate buffer	129
pH 7.4 Phosphate buffer	130

6.3.1.3. Differential scanning calorimetry (DSC)

DSC is useful in the investigation of solid state interactions. Thermograms are generated for pure drugs and mixtures of drug and excipients. From the thermograms (Fig. 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8 and 6.9) it is quite clear that there is interaction between the drugs and the excipients as far as the melting point of the drug is concerned.

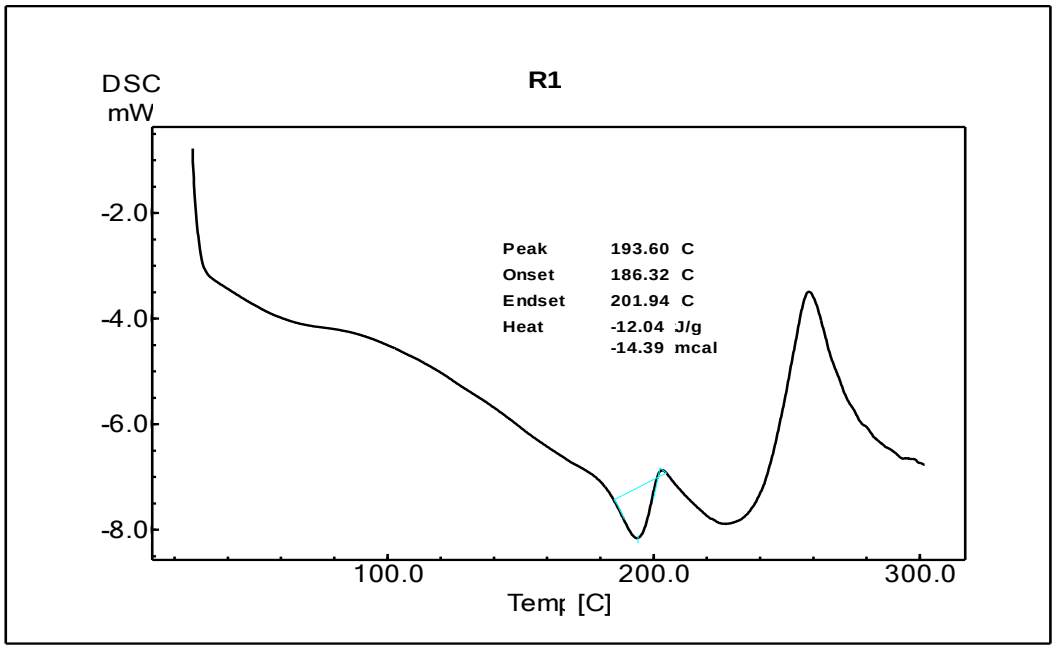

Fig. 6.1. DSC thermogram of rifampicin

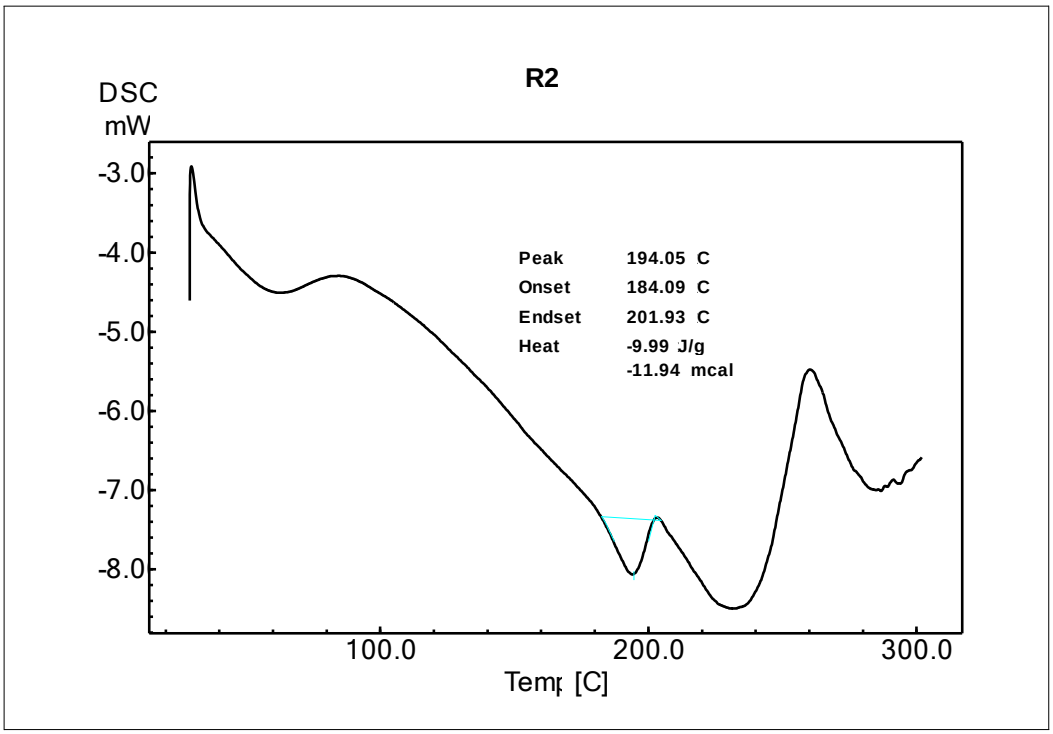

Fig. 6.2. DSC thermogram of rifampicin along with HPMC K100M

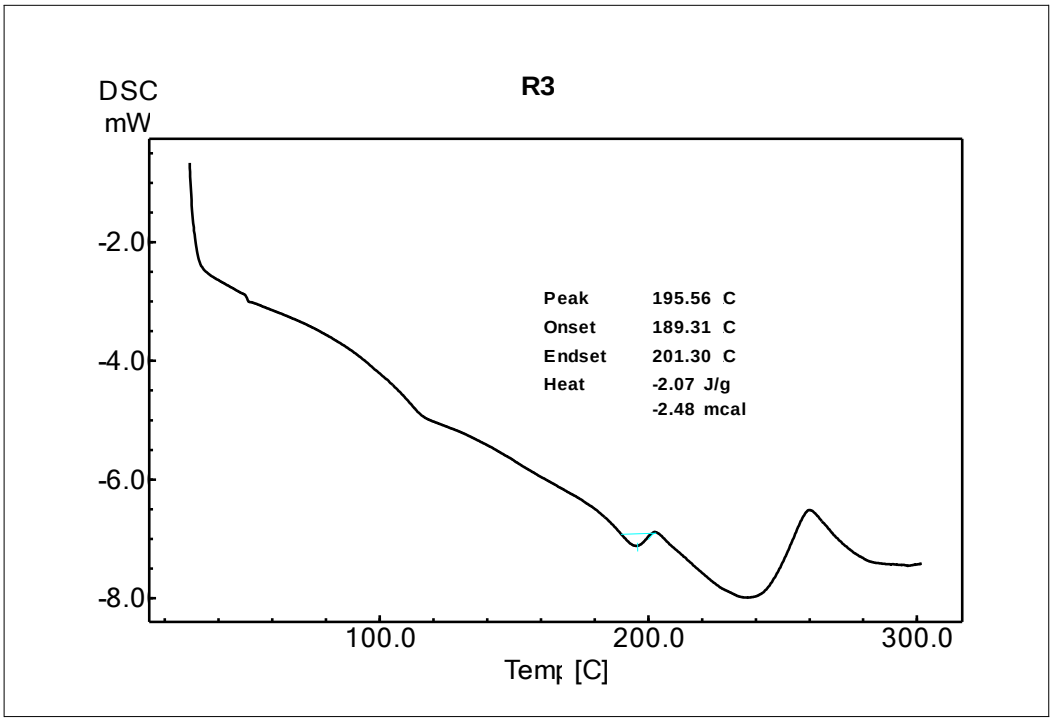

Fig. 6.3. DSC thermogram of rifampicin along with HPMC K4M

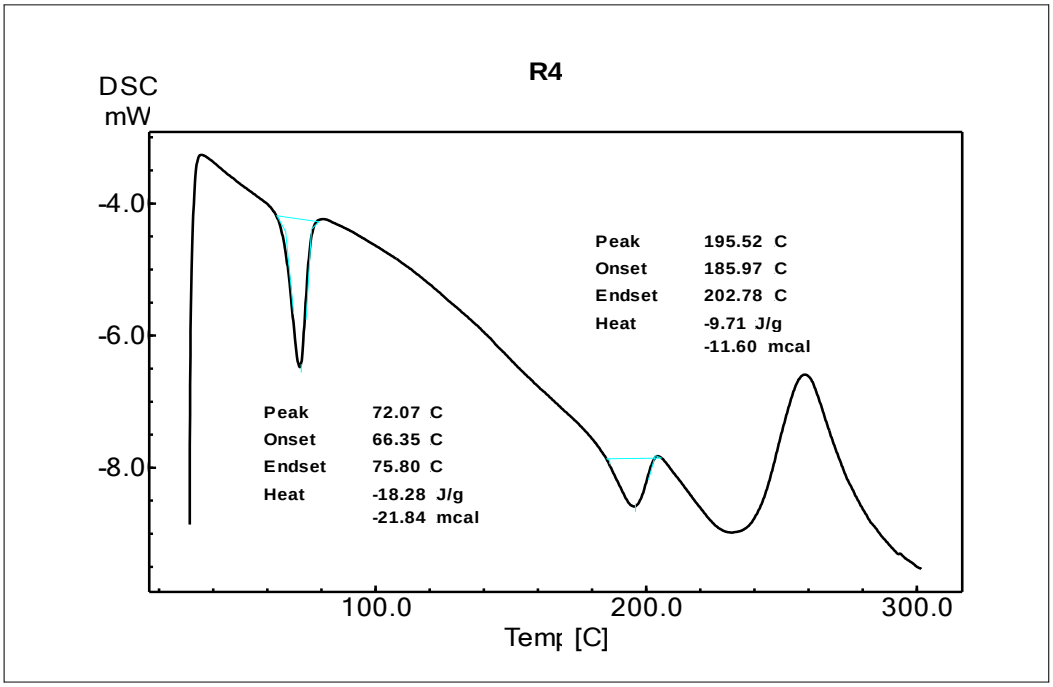

Fig. 6.4. DSC thermogram of rifampicin along with POLYOX WSR 301

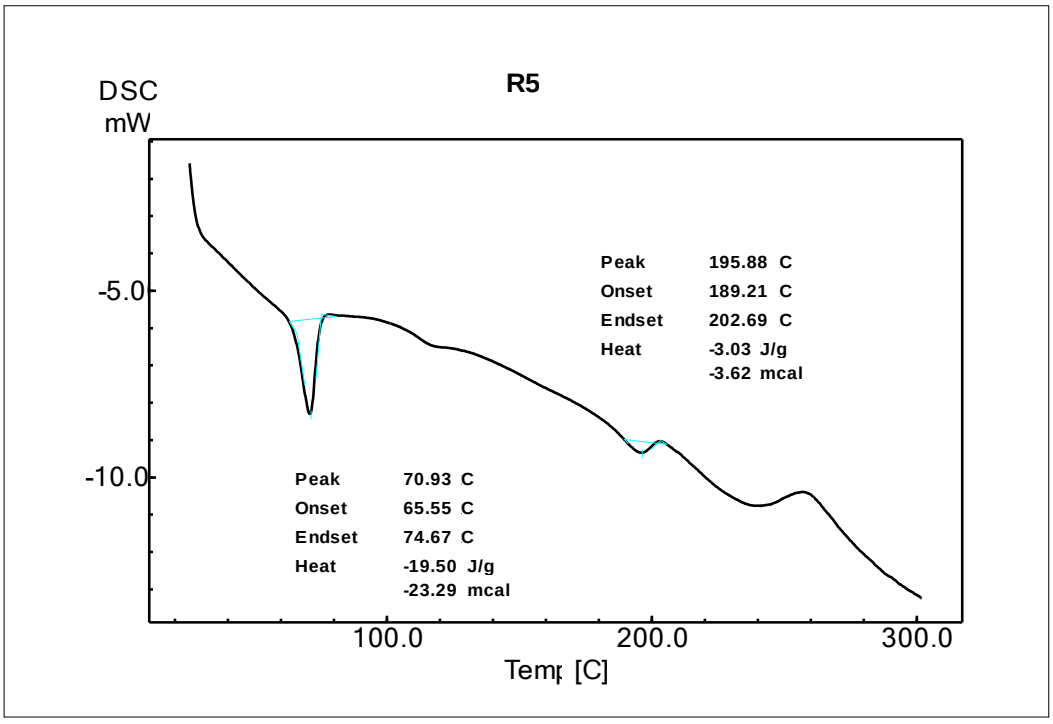

Fig. 6.5. DSC thermogram of rifampicin along with POLYOX WSR 301, HPMC K100M and HPMC K4M

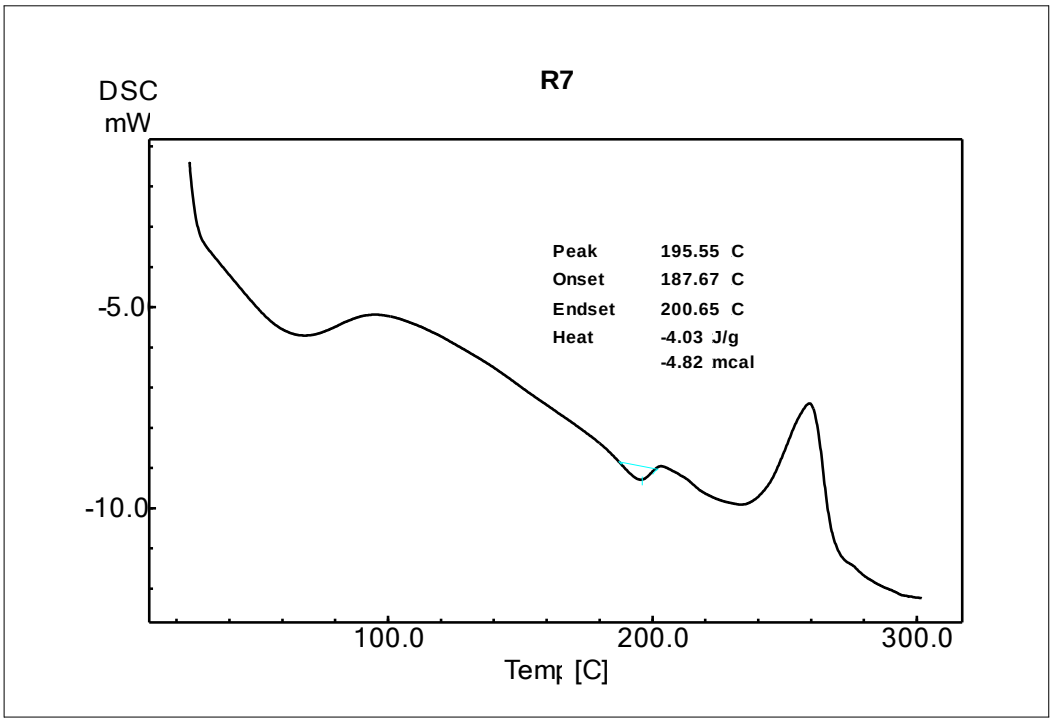

Fig. 6.6. DSC thermogram of rifampicin along with sodium alginate

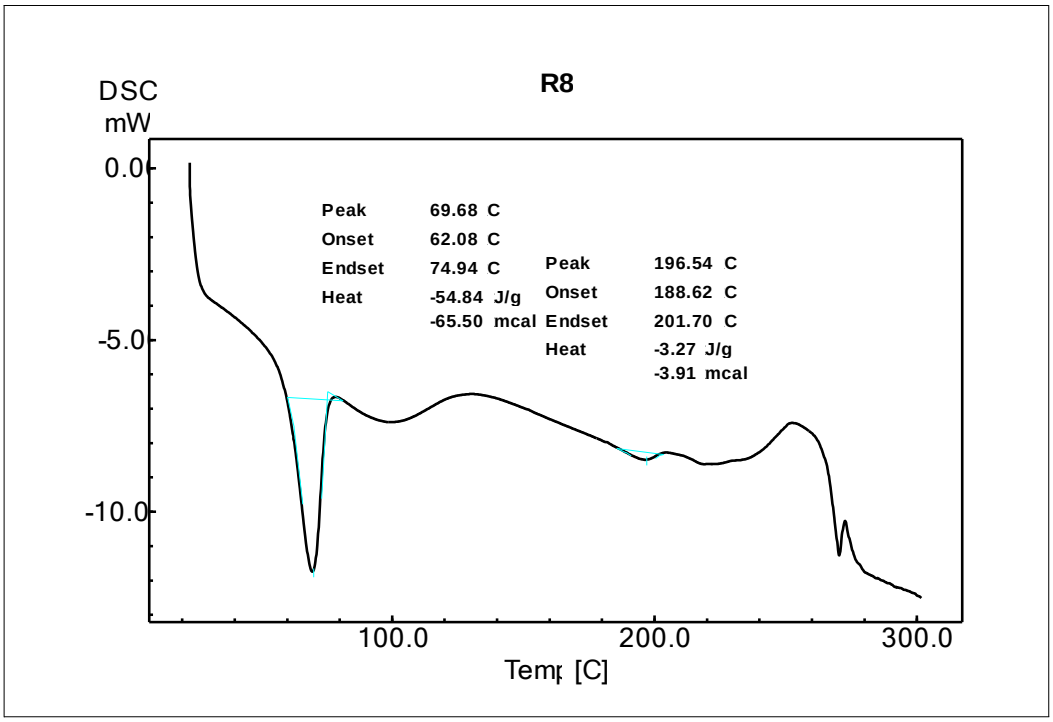

Fig. 6.7. DSC thermogram of rifampicin along with sodium alginate, HPMC K100M and POLYOX WSR 301

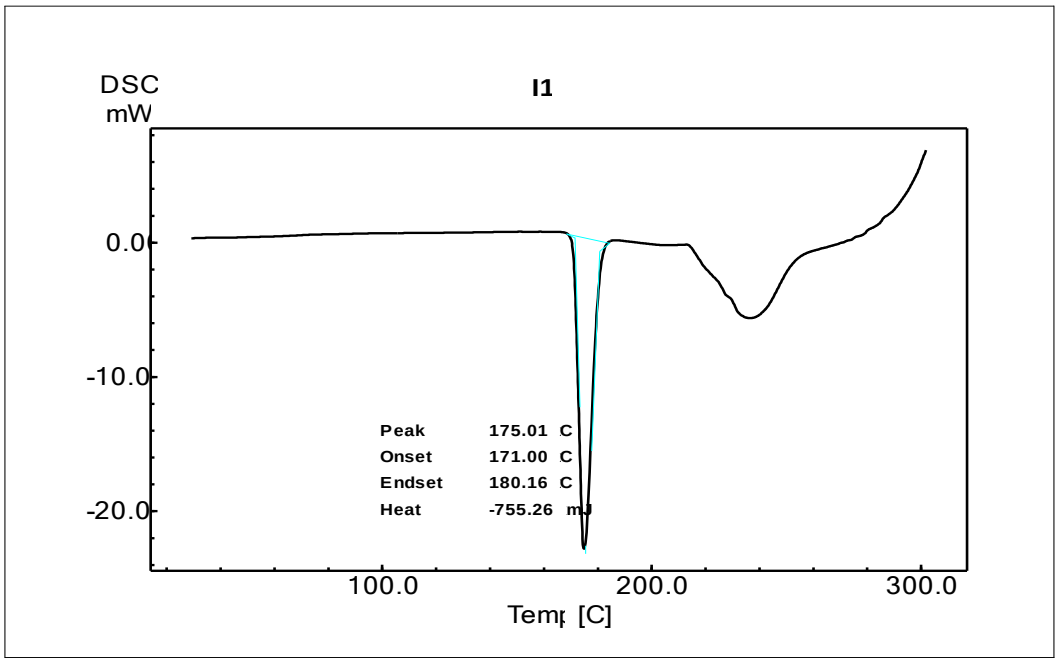

Fig. 6.8. DSC thermogram of isoniazid

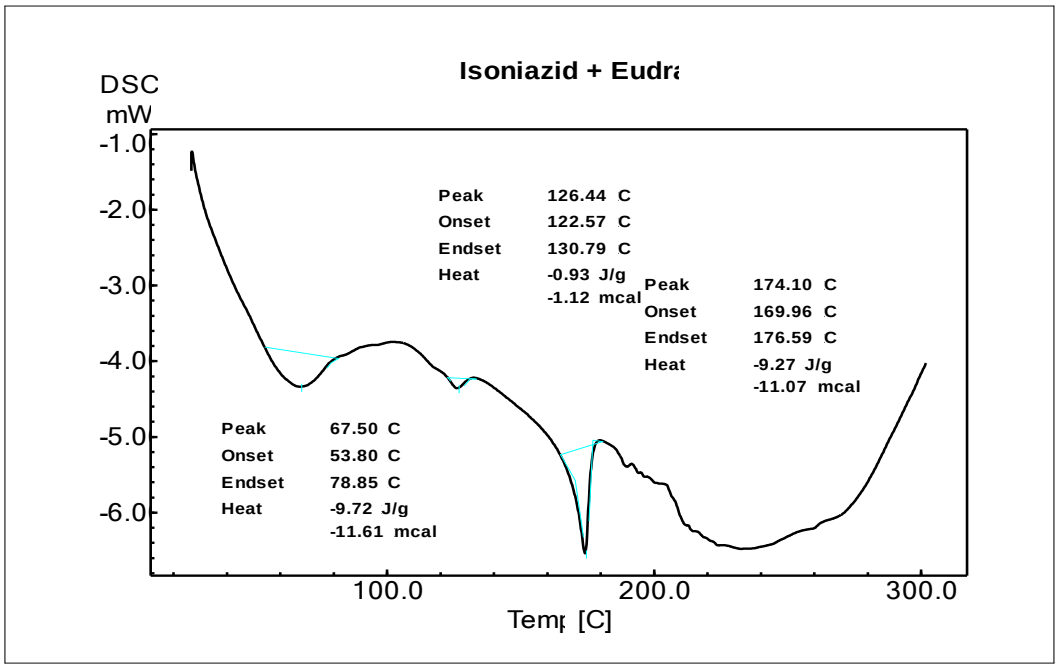

Fig. 6.9. DSC thermogram of isoniazid along with eudragit L100

6.3.1.4. Fourier transform infrared spectroscopy (FTIR)

In the FTIR spectra of rifampicin, the normally sharp band for the NH group was not present, indicating intramolecular bonding between the NH group and the piperazine side chain. The broad band assigned to the ν OH was present; thus, C_1=O chelated with the C_8

hydroxyl group. The presence of the vibration due to the v C=O group explained the hydrogen bonding of the C_{23}-OH to the acetyl group on C_{26}. Summary of FTIR spectrum of rifampicin is given in the Table 6.5. FTIR spectra were also generated for rifampicin along with the polymers. They clearly indicate that there is no interaction between the drug and excipients (Fig. 6.10, 6.11, 6.12, 6.13, 6.14 and 6.15)

Table 6.5. Infrared spectrum data of rifampicin

IR Absorption Band (cm^{-1}) (Experimental)	IR Absorption Band (cm^{-1}) (Literature)	Functional Groups
3416.05 broad peak	3500-3000	-OH stretching
2933.83	2930	-CH3 stretching
2846.1	2820	-CH3O asymmetric stretching
2779.52	2800	-CH3N stretching
1720.56	1715	-C=O acetyl stretching
1647.26	1670	-C=N- asymmetric bending
1560.46	1570	-C=C- stretching
1379.15	1400	-C-N- stretching
1246.06	1255	-C-O-C- acetyl group

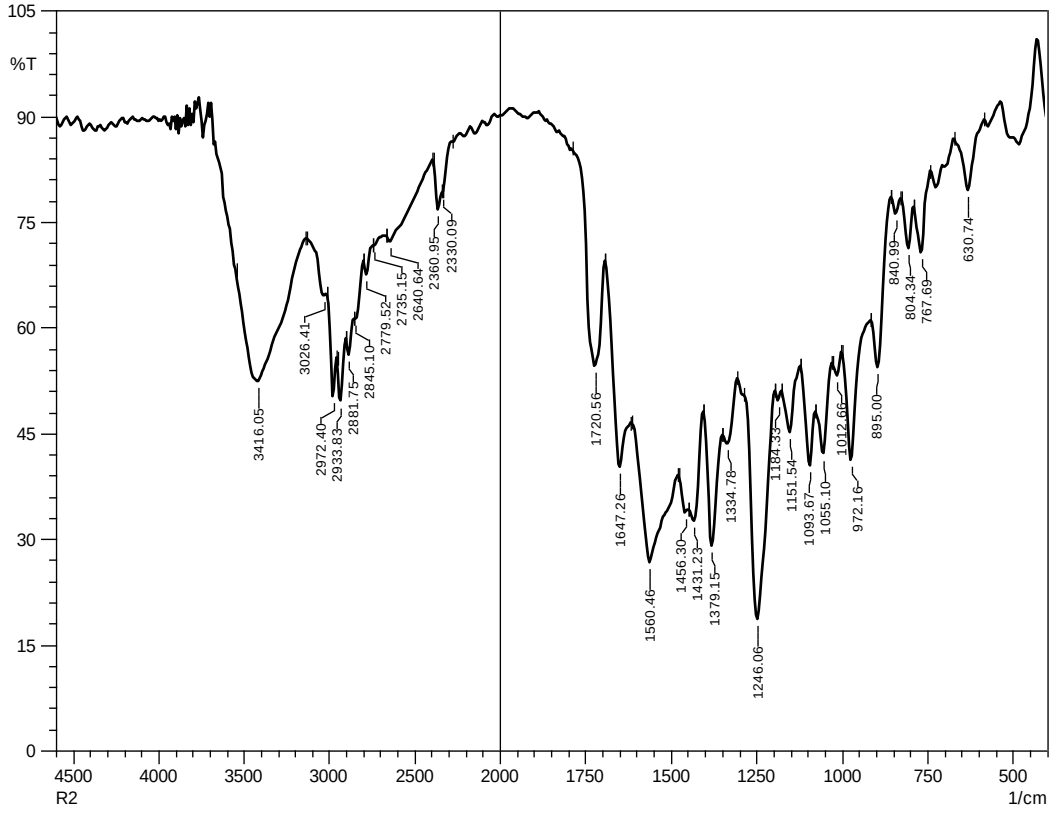

Fig. 6.10. Infrared spectrum of rifampicin

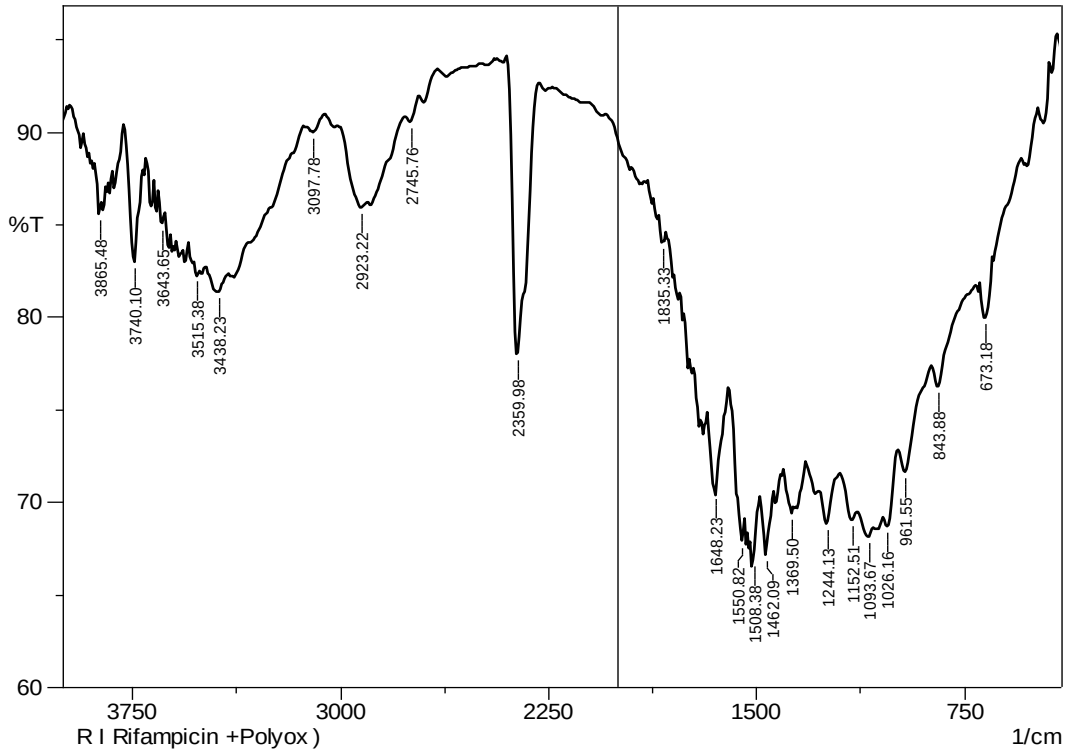

Fig. 6.11. Infrared spectrum of rifampicin along with POLYOX WSR 301

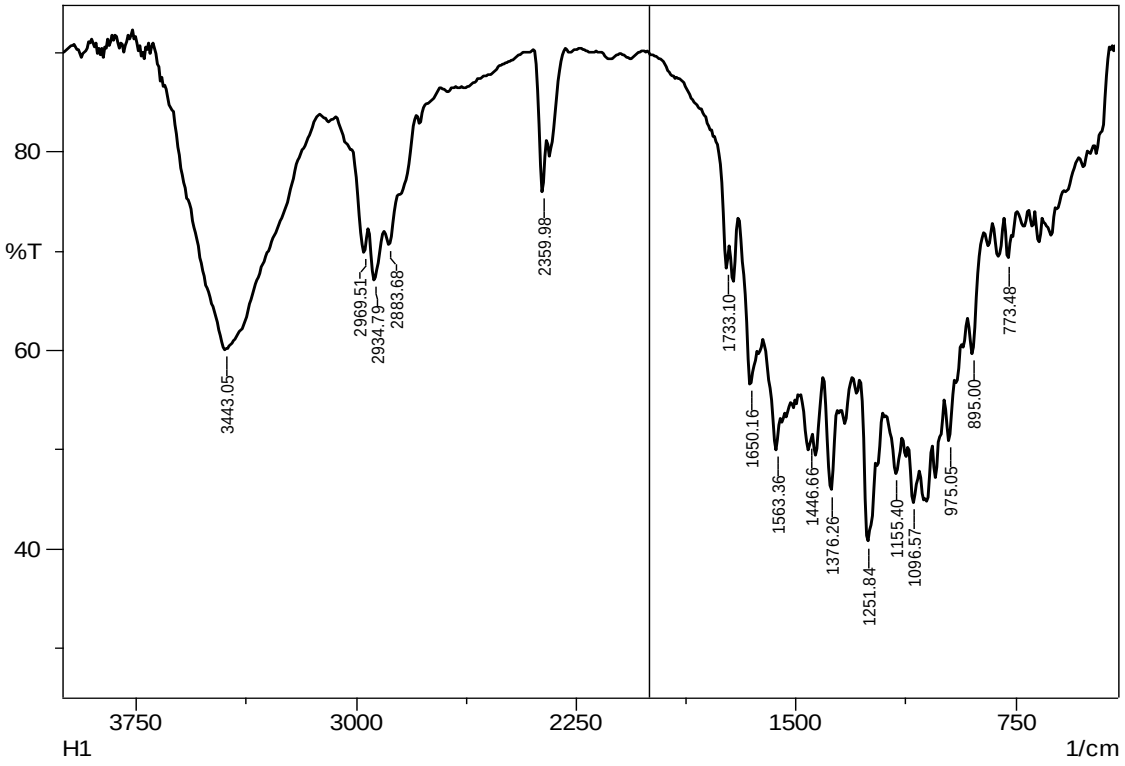

Fig. 6.12. Infrared spectrum of rifampicin along with HPMC

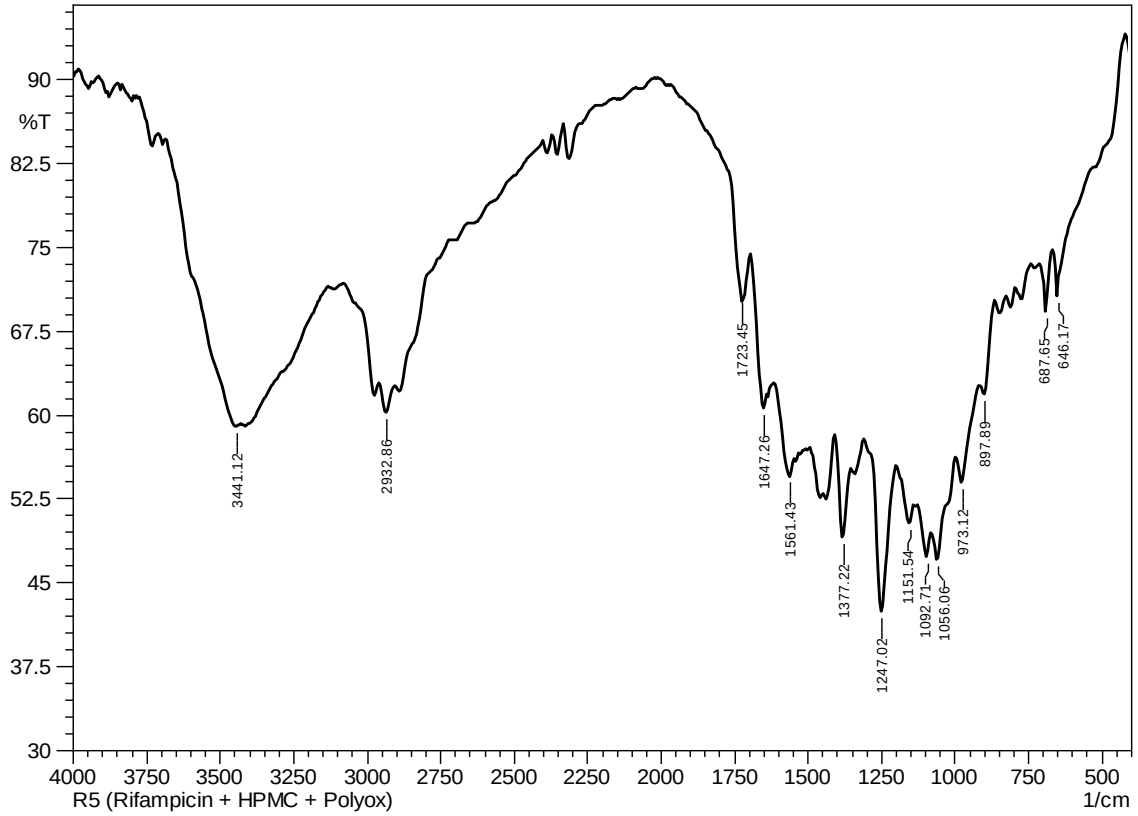

Fig. 6.13. Infrared spectrum of rifampicin along with HPMC and POLYOX WSR 301

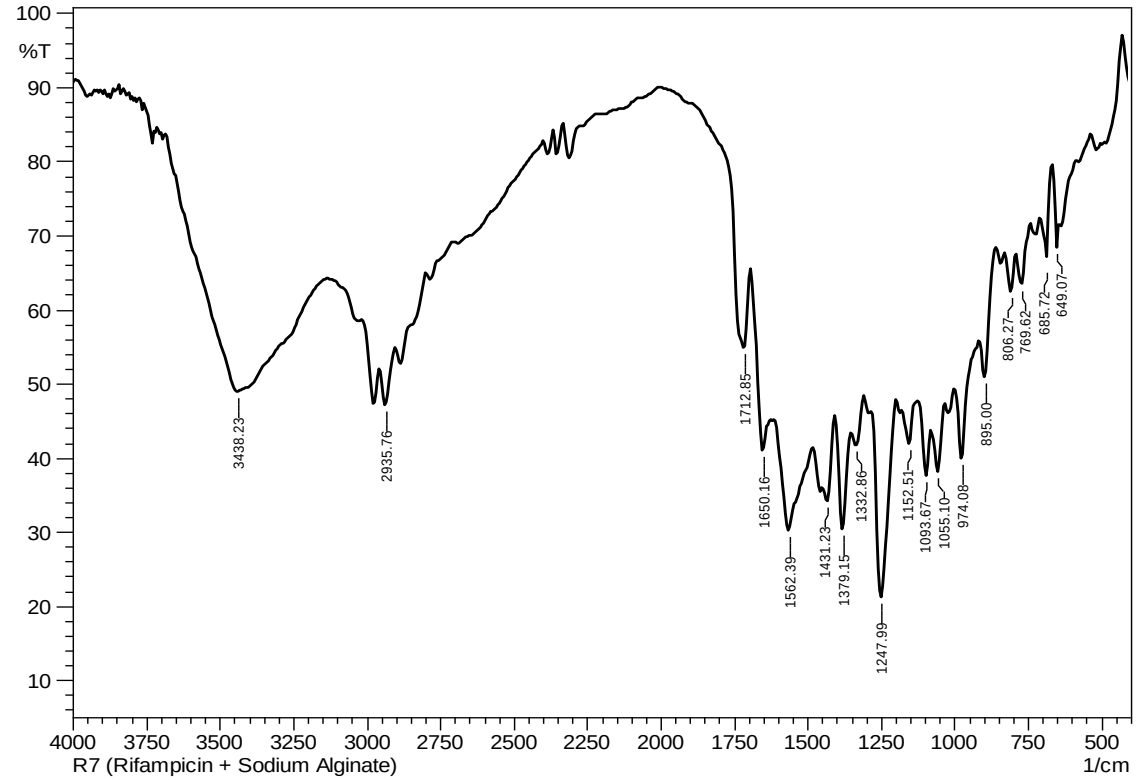

Fig. 6.14. Infrared spectrum of rifampicin along with sodium alginate

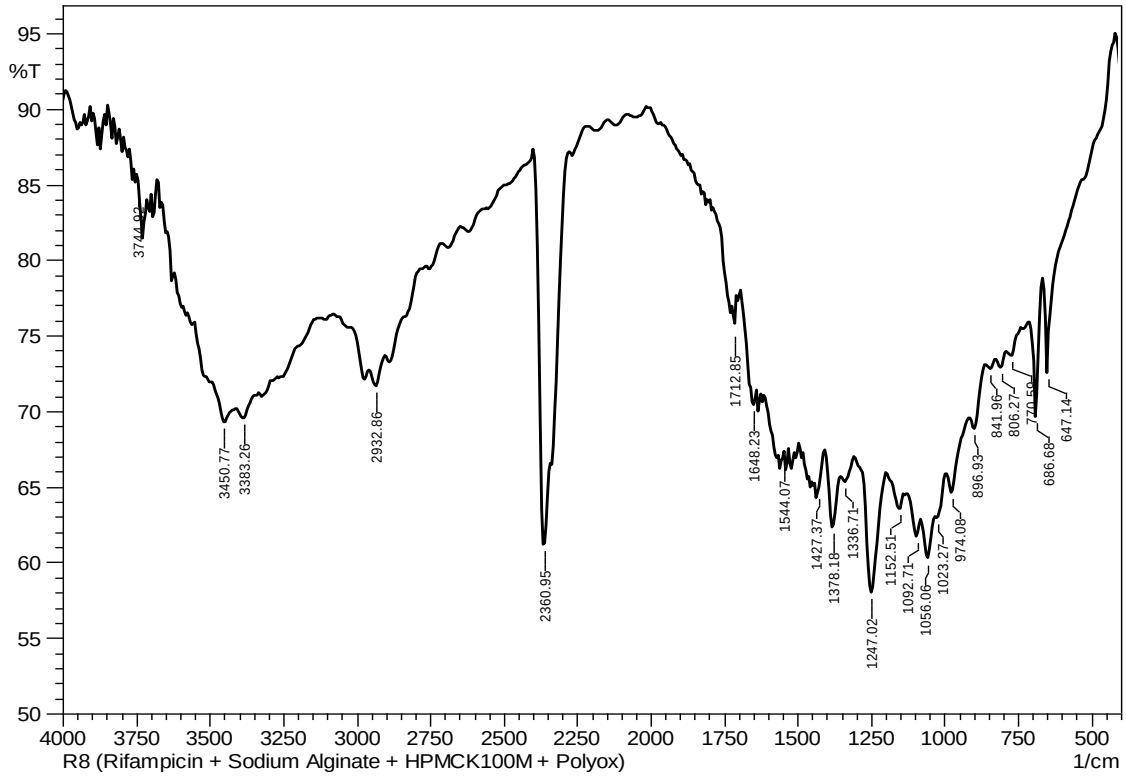

Fig. 6.15. Infrared spectrum of rifampicin along with sodium alginate, HPMC K100M and POLYOX WSR 301

Similarly FTIR spectra were generated for pure isoniazid and also for isoniazid with eudragit L100. The details of pure isoniazid spectrum are given in the Table 6.6. The spectra clearly indicate that there are no interactions between the drug and enteric coating polymer (Fig. 6.16 and 6.17).

Table 6.6. Infrared spectrum data of isoniazid

IR Absorption Band (cm^{-1}) (Experimental)	IR Absorption Band (cm^{-1}) (Literature)	Functional Groups
various peaks 3300.0 - 2800.0	3300.0 - 2800.0	-NH, -NH bonded stretching
1666.55	1670	-C=O stretching
1631.83	1640	-NH3 asymmetric bending
1552.75	1560	Aromatic ring vibration
1494.88	1500	Aromatic ring vibration

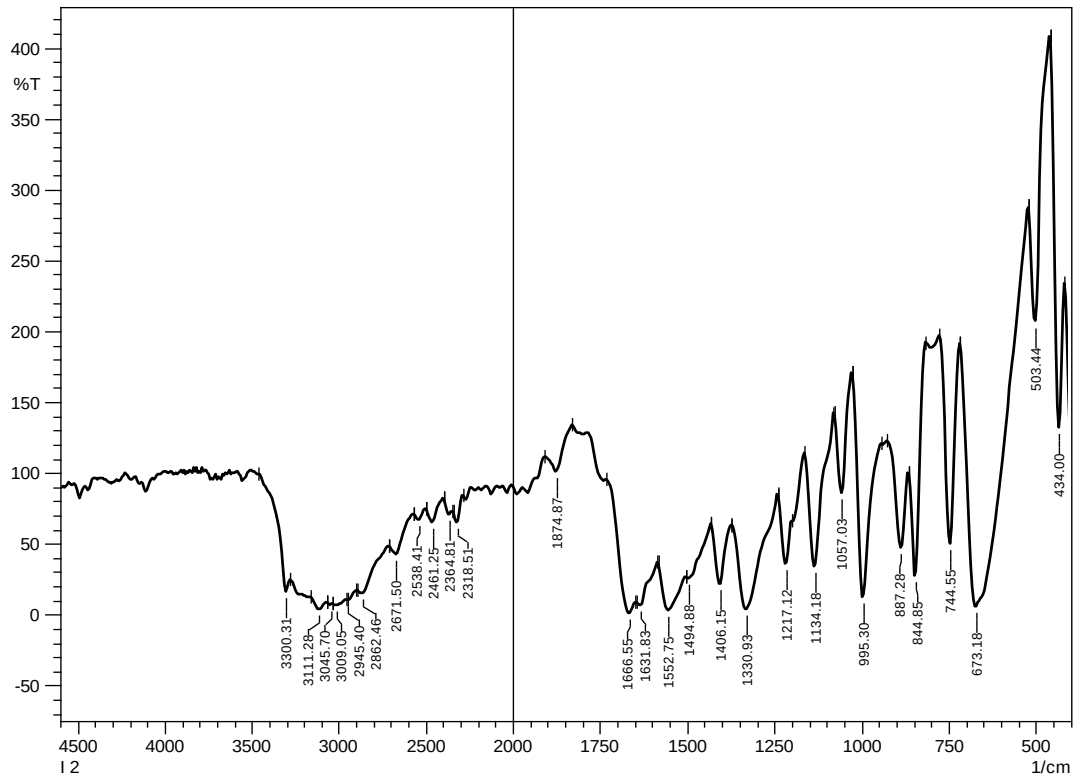

Fig. 6.16. Infrared spectrum of isoniazid

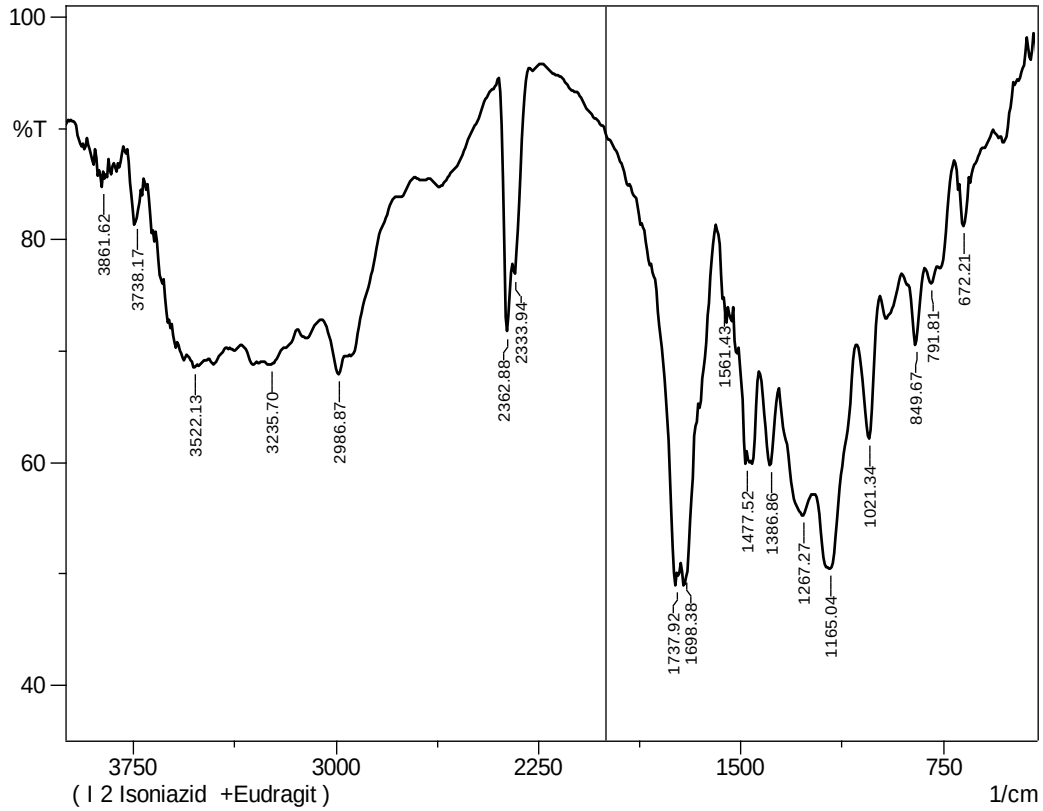

Fig. 6.17. FTIR spectra of isoniazid along with eudragit L100

6.3.1.5. Micromeritic properties

The pure drugs (rifampicin and isoniazid) and the mixtures containing the drug along with their respective excipients were tested for the flow properties. Pure drugs and their mixtures showed poor to passable flow properties with Carr's index values ranging from 20 to 30% and Hausner's ratio ranging from 1.25 to 1.45. These powders showed angle of repose values ranging from 30^o to 40^o which again indicate that these powders have poor flow properties.

6.3.2. Preliminary studies

6.3.2.1. Evaluation of rifampicin and isoniazid tablets with improved rifampicin stability

6.3.2.1.1. Hardness: The average hardness of isoniazid tablets was found to be 4.1 kg/cm^2 whereas it was 4.5 kg/cm^2 for rifampicin tablets.

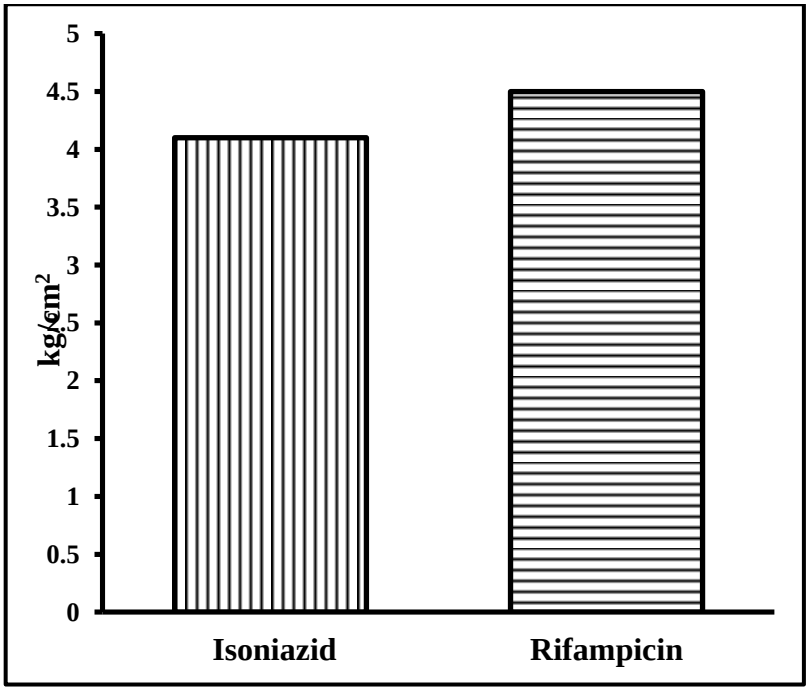

Fig. 6.18. Hardness of rifampicin and isoniazid tablets

6.3.2.1.2. Friability:

The uncoated tablets of both the formulations have passed the IP limits for friability. The results are shown in Fig. 6.19.

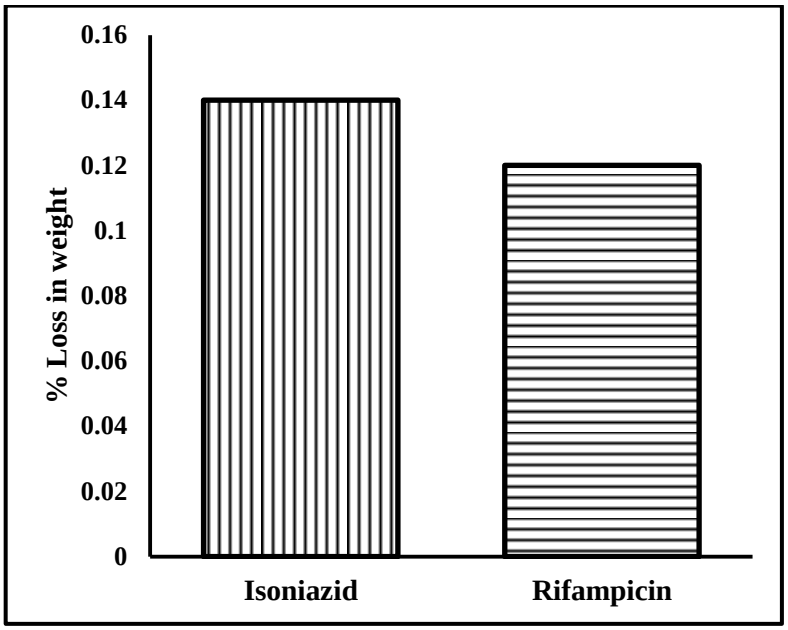

Fig. 6.19. Friability of rifampicin and isoniazid tablets

6.3.2.1.3. Weight variation:

All the tablets were within the limits as per IP 2007 with respect to weight variation test. The results are shown in the Fig. 6.20.

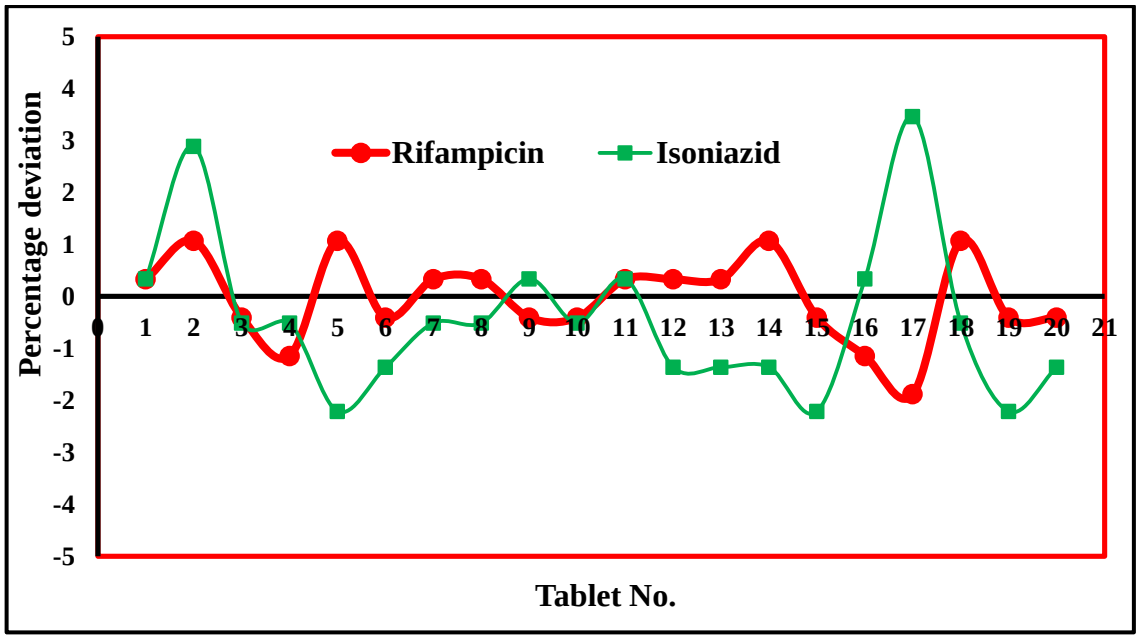

Fig. 6.20. Weight variation test of rifampicin and isoniazid tablets

6.3.2.1.4. Disintegration test: The disintegration time of uncoated tablets of both the drugs of formulation I confirmed to IP 2007 specifications. The enteric coated isoniazid tablet did not disintegrate in 0.1N HCl for 2 h but it disintegrated in phosphate buffer of pH 6.8 in 8 min. The results are shown in Fig. 6.21.

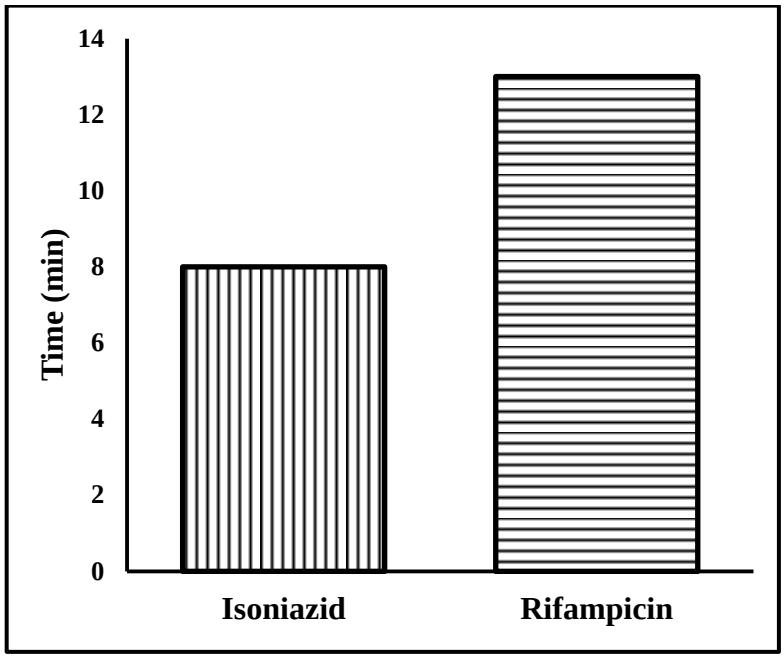

Fig. 6.21. Disintegration test of rifampicin and isoniazid tablets

6.3.2.1.5. Enteric coating of tablets: The weight gain was found to be 10% w/w of core isoniazid tablets.

6.3.2.1.6. Dissolution studies: Dissolution studies were performed for formulation I and II separately in 0.1N HCl. Then the cumulative percentage drug release for rifampicin was calculated from the obtained data. In case of formulation I, the cumulative percentage drug release for rifampicin in 2 h in 0.1N HCl was found to be around 80% whereas formulation released around 91% of rifampicin in 2 h in 0.1N HCl. The results are given in Fig. 6.22. This study clearly indicates that rifampicin interacts with isoniazid in gastric conditions leading to poor stability of rifampicin.

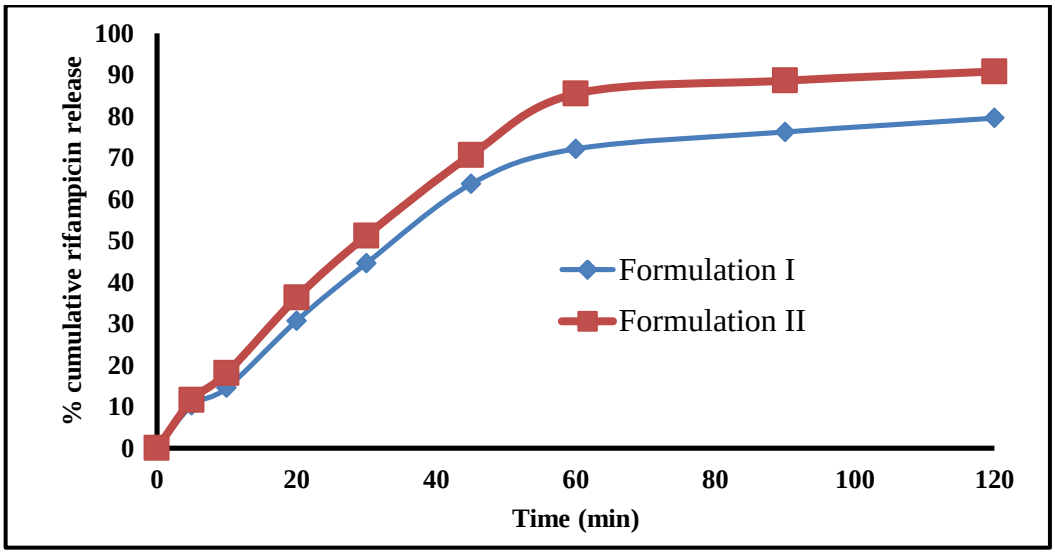

Fig. 6.22. Dissolution study of rifampicin and isoniazid tablets

6.3.2.2. Evaluation of multiparticulate systems of rifampicin and isoniazid with improved rifampicin stability

6.3.2.2.1. Pellet size

The average pellet size for rifampicin and isoniazid pellets was found to be 1042 µm and 1096 µm respectively.

6.3.2.2.2. Usable yield

The usable yield values for rifampicin and isoniazid were found to be 92.48% and 93.32% respectively.

6.3.2.2.3. Friability: The uncoated pellets of both the drugs have passed the IP limits for friability. The results are shown in Fig. 6.23.

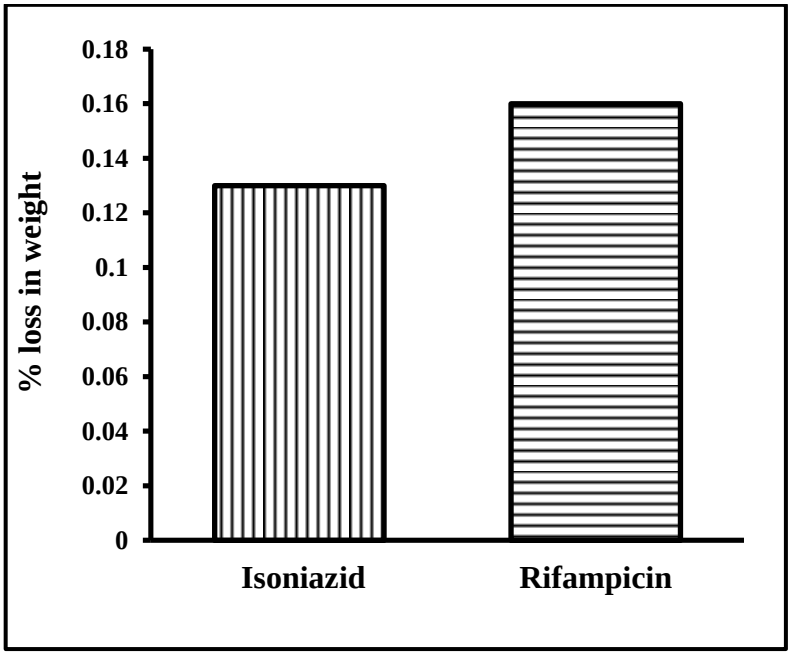

Fig. 6.23. Friability of rifampicin and isoniazid pellets

6.3.2.2.4. Flow properties: The results given in the Table 6.7 indicate that the pellets have good flow properties.

Table 6.7. Flow properties of rifampicin and isoniazid pellets

Flow property	Rifampicin pellets	Isoniazid pellets
Angle of repose	24^{o}	26^{o}
Carr's index	9.67%	10%
Hausner's ratio	1.107	1.111

6.3.2.2.5. Enteric coating of isoniazid pellets: The weight gain was found to be 20% w/w of the core pellets.

6.3.2.2.6. Drug content: The drug content was 98.34% and 98.58% of theoretical yield for rifampicin and isoniazid respectively.

6.3.2.2.7. Dissolution studies: Dissolution studies were performed for formulation I and II separately in 0.1N HCl. Then the cumulative percentage drug release for rifampicin was calculated from the obtained data. In case of formulation I, the cumulative percentage drug release for rifampicin after 2 h in 0.1N HCl was found to be around 81% whereas for formulation II it has shown a phenomenal increase up to 92%. The results are given in Fig. 6.24. These results confirm that the interaction between rifampicin and isoniazid in gastric conditions leading to poor stability of rifampicin. This interaction was minimized by formulating isoniazid as enteric coated delayed release pellets.

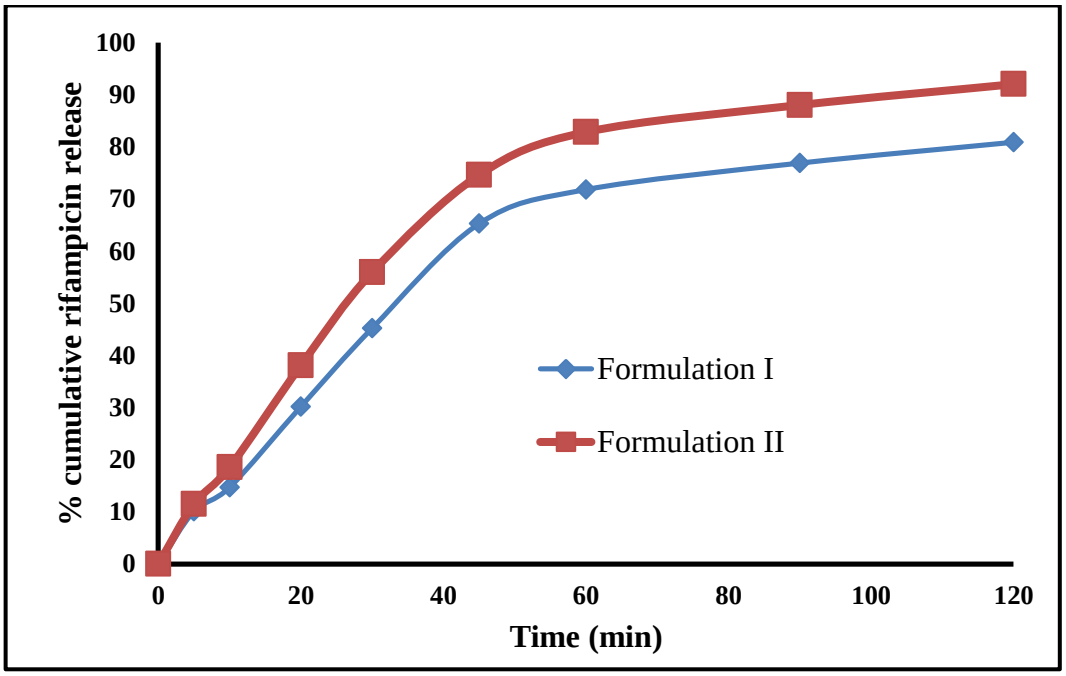

Fig. 6.24. Dissolution study of rifampicin and isoniazid pellets