11. Optimized formulations

11.1. Selection of optimized formulations from GRPs, GRGs and GRBs

Upon comparison of these optimized formulations (Table 11.1), optimized pellet formulation is better than the optimized granule formulation because of its greater desirability value, higher percentage release of rifampicin at 6 h and also higher usable yield values. Pellet formulation is also a better formulation when compared to beads as there is a greater loss of drug during preparation of beads which was reflected by the values of very low drug entrapment efficiency. Apart from that the optimized pellets showed a greater desirability value and release of drug at 6 h than the optimized bead formulation.

Table 11.1. Comparison of the optimized formulations of GRPs, GRGs and GRBs

	Release at 6 h (%)	Floating time (h)	Usable yield / Drug entrapment efficiency (%)	Desirability
GRPs	99.12	5.75	93.16	0.680
GRGs	97.31	5.6	86.26	0.410
GRBs	98.26	5.4	71.45	0.369

So for further in vitro dissolution, in vivo gastroretention, ex vivo absorption and stability studies, the optimized pellet formulation was selected.

11.2. In vitro dissolution studies

The dissolution study was performed using a USP type II (paddle type) dissolution apparatus (TCT- 06P, Electrolab, Mumbai, India) at 37±0.5 ^{o}C and a paddle speed of 50 rpm. The dissolution testing of optimized formulation was carried out in 900 ml of simulated gastric fluid. At predetermined time intervals, 1ml of sample was withdrawn replacing with fresh medium and the release of rifampicin analysed at 336 nm using UV-visible spectrophotometer. Using this data studies the kinetic model for drug release was determined for the optimized formulation.

The study was conducted for three different conditions

- Optimized gastroretentive rifampicin pellets alone.
- Optimized gastroretentive rifampicin pellets along with immediate release isoniazid pellets.
- Optimized gastroretentive rifampicin pellets along with delayed release (enteric coated) isoniazid pellets.

11.2.1. In vitro drug release kinetics

Drug release mechanism from the optimized dosage form was analyzed by model dependent method. The dissolution data of the optimized dosage form was fitted to four popular release models (zero-order, first order, Higuchi and Kormeyer-Peppas equations). The order of drug release from matrix systems was studied by using Higuchi equation and Peppas equation (Dash et al., 2010).

11.2.1.1. Zero order kinetics

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation

$$Q_t = Q_0 + K_0 t$$

where, Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time. The data obtained are plotted as cumulative drug release vs. time which would yield a straight line.

11.2.1.2. First order kinetics

$$\log C = \log C_0 - (K_t/2.303)$$

where, C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining *vs*. time which would yield a straight line with a slope of -K/2.303.

11.2.1.3. Higuchi model

Higuchi describes drug release as a diffusion process based on the Fick's law, square root of time dependent. It is described by the following equation

$$Q_t = K_H t^{1/2}$$

Where, Qt is the quantity of drug released at time t and K_H is the Higuchi dissolution constant.

The data obtained are plotted as cumulative percentage drug release vs. square root of time.

11.2.1.4. Korsmeyer-Peppas model

$$M_t / M_\infty = Kt^n$$

where, M_t / M_∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent indicative of release mechanism. The data obtained were plotted as log

cumulative percentage release vs. log time. First 60% of drug release is fitted in this model and the slope indicates the mechanism of drug release (Dash et al., 2010).

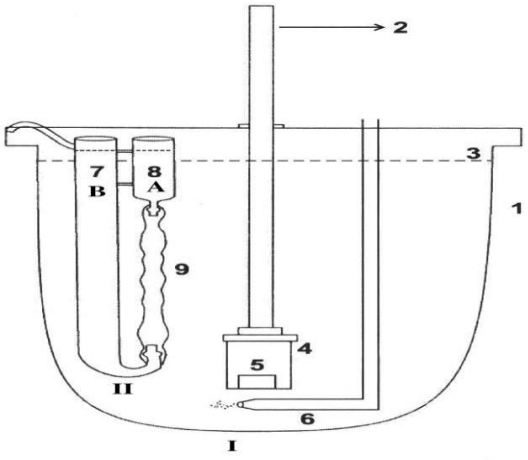
11.3. Ex vivo absorption studies

11.3.1. Isolation of tissue from rat

Permeation study was conducted using rat stomach and small intestine by everted sac technique. Rat was fasted for 12 h and then sacrificed to isolate the stomach and intestine from the animal (n=3). Sacrificed rat stomach and intestine were preserved in krebs solution. The lumen was cleaned by krebs solution. Stomach and intestine was cut into a piece of about 10 cm. Rat stomach and intestine was everted and after eversion the mucosal side is the outer surface and serosal side is the inner surface. Both the ends of the tissue were tied to absorption cell apparatus. The tissue was kept alive with the supply of oxygen by an aerator and buffer solution and the temperature was maintained at 37± 0.5 ºC (Alam et l., 2011).

11.3.2. Continuous dissolution-absorption apparatus design

The ex vivo continuous dissolution–absorption system as depicted in Fig. 11.1 consists of USP dissolution Apparatus I along with a perfusion apparatus holding isolated everted intestine segment.



1. Dissolution flask
2. Rotating shaft
3. Dissolution medium
4. Basket
5. Tablet
6. Oxygen tube
7. Tube B
8. Tube A
9. Everted intestine

I. Dissolution–absorption system
II. Absorption (perfusion) apparatus

Fig. 11.1. C**ontinuous dissolution–absorption apparatus design**

In this apparatus, release of the drug from the extended release pellets and permeation of the drug across everted rat intestine/stomach occurs together. 1000 ml volume of dissolution medium was used for the release of rifampicin from the extended release pellets. Temperature of the dissolution medium was maintained at 37±0.5 °C. Absorption system included 2 glass tubes, A and B, which were connected to each other. 'B' tube contained a bent cannula whereas 'A' tube had a straight cannula at their lower ends. Distance between these two cannulae was fixed. The rat stomach and intestinal segments were isolated and everted. Stomach tissue portion was tied using a thread at the ends of A and B tubes as depicted in the Fig. 11.1. Similar procedure was followed even for the intestinal portion. The whole setup was completely immersed into the dissolution vessel (Alam et al., 2011).

11.3.3. Ex vivo absorption study procedure

Sampling was done simultaneously for estimation of the in vitro release of drug and ex vivo permeation of the drug using the setup consisting of dissolution-absorption apparatus. Ex vivo absorption studies were performed on the optimized formulation in triplicates, in a USP dissolution apparatus, Type-II (Paddle method) maintained at 37±0.5 °C using temperature controller (Electrolab 11L temperature controller). The paddles were kept at 50 rpm. The formulation was placed in 1000 ml of simulated gastric fluid. The perfusion cell with rat stomach was placed in dissolution apparatus jar as shown in Fig. 11.2. The absorption cell was filled with 30 ml krebs solution. Aliquots of 5 ml were withdrawn from the dissolution apparatus for every half an hour till 5 h. For every withdrawal 5 ml of fresh sample was replaced into the dissolution flask. Aliquots of 1ml were withdrawn from the absorption cell at every half an hour till 5 h. For every withdrawal 1 ml of fresh sample was replaced into the absorption cell. The samples were estimated spectrophotometerically with suitable dilutions using UV-Visible Spectrophotometer at 336 nm. Same procedure is repeated for the rat intestine except the formulation is placed in simulated intestinal fluid which is present in dissolution apparatus jar. The study was conducted after obtaining approval from the Institutional Animal Ethical Committee (IAEC/KMC/22/2014).

Fig. 11.2. Continuous dissolution–absorption system using everted rat stomach

11.4. In vivo gastroretention studies of optimized formulation

For in vivo gastroretention study tablets of the same composition as that of GRPs of 100 mg weight containing drug, barium sulphate and other excipients as shown in Table 11.2 were prepared by direct compression method. To make the formulation X-ray opaque, incorporation of barium sulphate was necessary. The amount of the X-ray opaque material in these tablets was sufficient to ensure visibility by X-ray, but at the same time this amount of barium sulphate was low enough to enable tablets to float (Jagdale et al., 2009). These tablets were characterized for floating time. The in vivo X-ray imaging study was carried out in New Zealand White male rabbits. Human equivalent dose of rifampicin for rabbit weight was calculated and incorporated in the formulation. The study was conducted under the guidance of a radiologist after obtaining approval from the Institutional Animal Ethical Committee (IAEC/KMC/22/2014).

Table 11.2. Composition of formulation for in vivo gastroretention study

Ingredients	Quantities (mg)
Rifampicin	30
HPMC K100M	3.6
HPMC K4M	3.6
POLYOX WSR 301	7.2
Sodium Bicarbonate	14.2
Barium sulphate	30
Talc	2
Magnesium stearate	2
SuperTab 11SD	7.4

The rabbits weighing 3 kg were housed with free access to food and water. Tablets (with barium sulphate) were administered orally to overnight fasted rabbits with the help of wooden mouth gag and treated with 10 mL water. After administration of the tablets, the rabbits were anaesthetized using ketamine hydrochloride at a dose of 20 mg/kg and at different time intervals (1,2,4,5 and 6 h post-administration of tablets), the rabbits were exposed to abdominal x-ray imaging at 20 mA, 55 kV and 8 mA (Genius-60 Mobile portable unit, Wipro GE) in sleeping position.

11.5. Stability studies

The optimized batch was charged for the accelerated stability studies as per ICH guidelines Q1C (40 ± 2^{o}C/75 ± 5% RH) for a period of 6 months in the stability chambers (Thermolab, Mumbai, India). Amber coloured capsules containing pellets were packed and stored in high

density poly ethylene (HDPE) bottles. The samples were withdrawn at 3 and 6 months and evaluated for the drug content by using RP-HPLC method and the in vitro release study by UV spectroscopy method. The samples were also evaluated for buoyancy and physical parameters like colour change, friability.

11.6. Results and discussion

11.6.1. In vitro drug release kinetics:

The pellets containing a polymeric matrix, on contact with water builds a gel layer around the core which governs the drug release. It is known that the drug release from HPMC and POLYOX matrices is controlled for water soluble drugs by diffusion through the gel layer and for poorly soluble drugs by erosion of the outer polymer chains. The drug release rate kinetics was calculated for optimized formulation using zero order, first order, Higuchi model and Korsmeyer-Peppas model. It can be observed from the kinetic data (as shown in Table 11.3 and Fig. 11.3, 11.4, 11.5 and 11.6) that the prepared floating pellets released the drug by zero order kinetics as it is evident from the linear regression coefficient of 0.9914 compared to the regression coefficient of first order plot. Further, the data when treated using Higuchi diffusion equation and Korsmeyer-Peppas equation to learn about the mechanism of drug released from the pellets, it was observed that the drug release was by diffusion process. The slope of the Korsmeyer-Peppas equation was 1.0356 indicating that the drug release is super case II transport. So it can be concluded that the rifampicin was released from the Tablets by zero order kinetics with super case II transport.

Table 11.3. Dissolution study of Rifampicin GRPs

Time (h)	Percentage cumulative rifampicin release
0	0
0.5	8.82 ± 0.67
1	17.41 ± 0.51
2	37.2 ± 0.74
3	55.6 ± 0.82
4	75.34 ± 0.61
6	99.12 ± 0.84

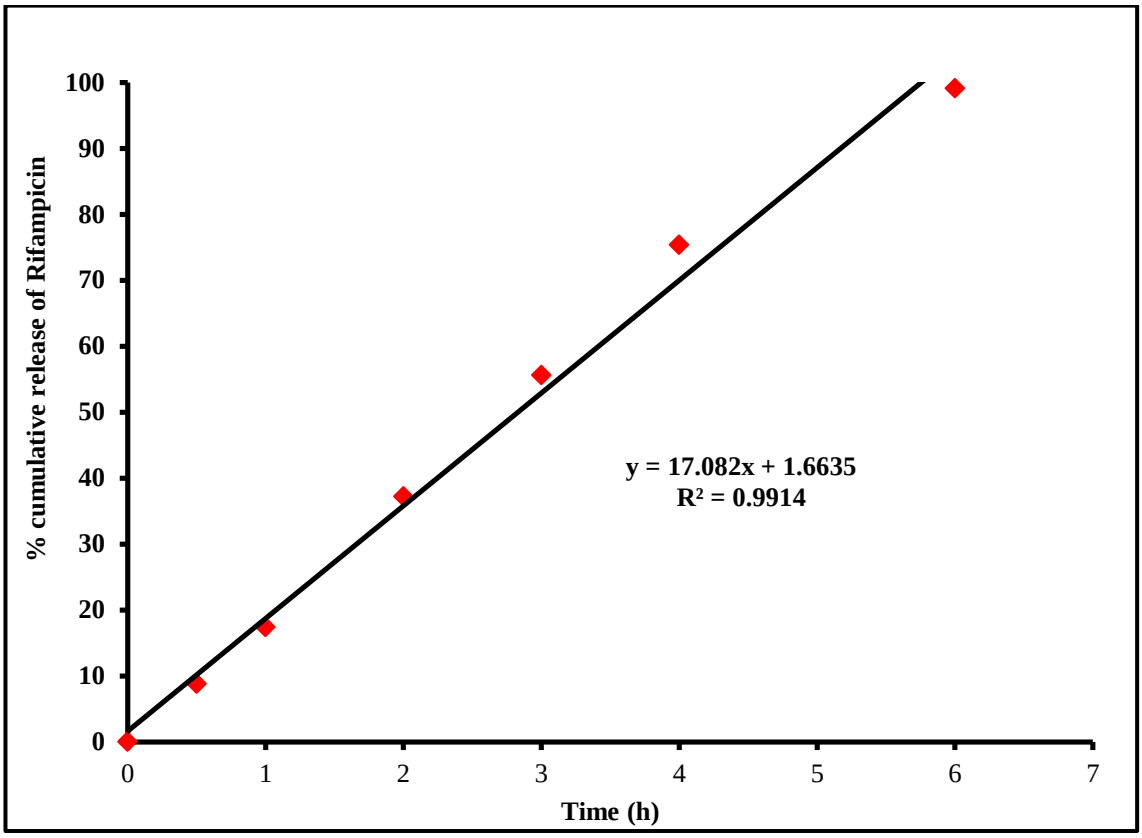


Fig. 11.3. Zero order model for optimized formulation

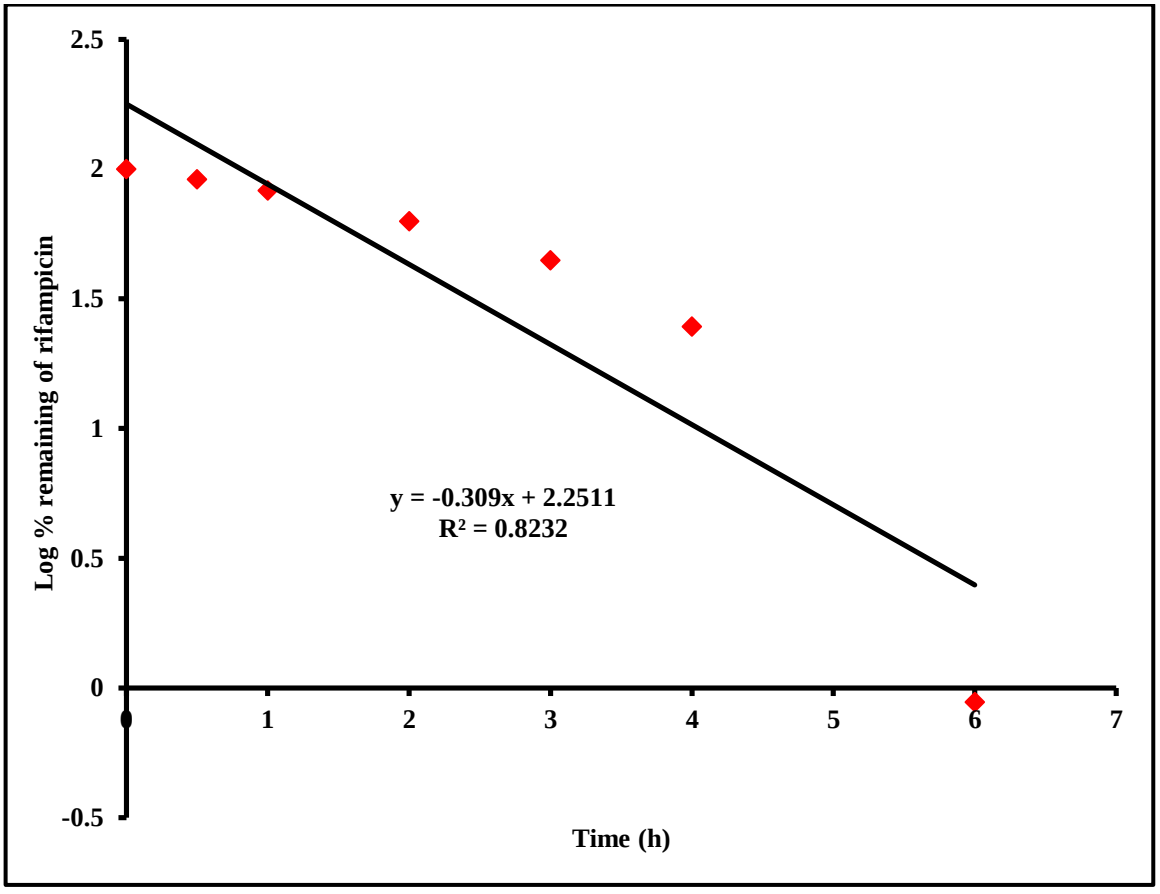


Fig. 11.4. First order model for optimized formulation

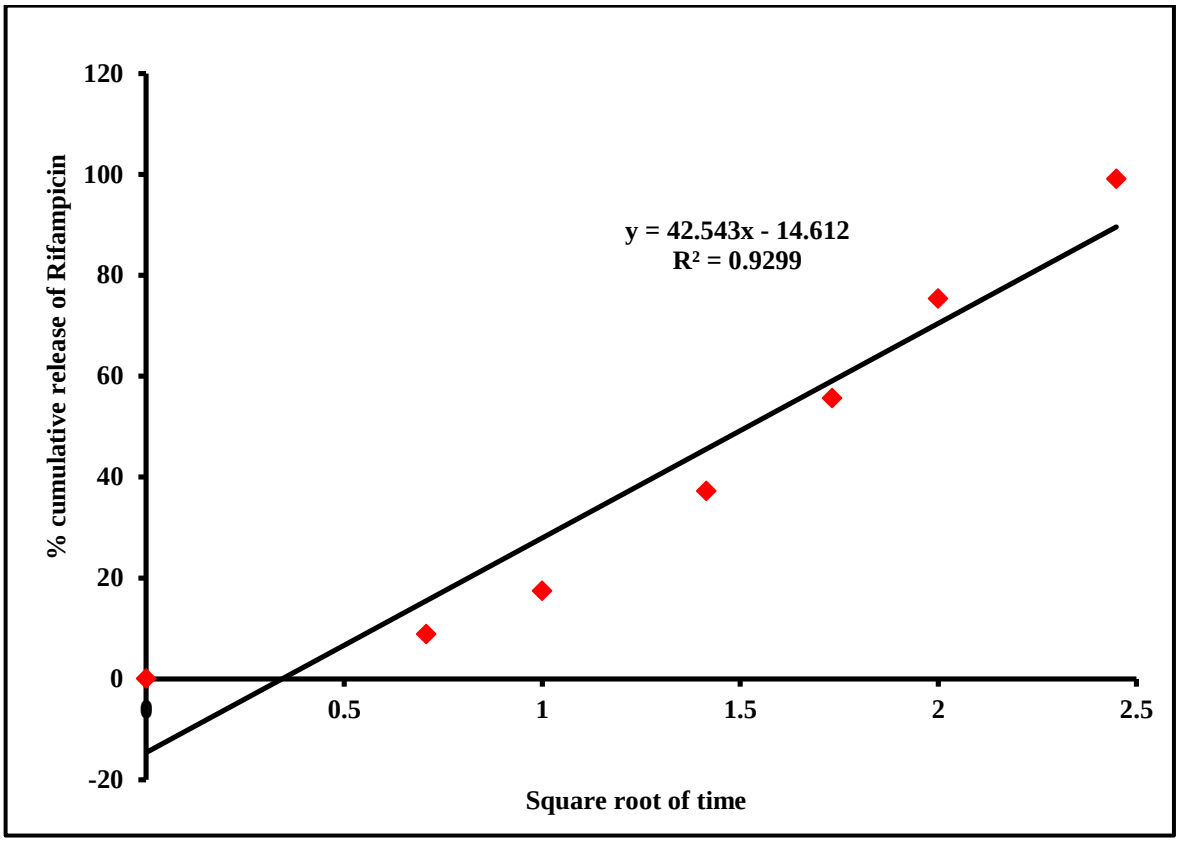


Fig. 11.5. Higuchi model for optimized formulation

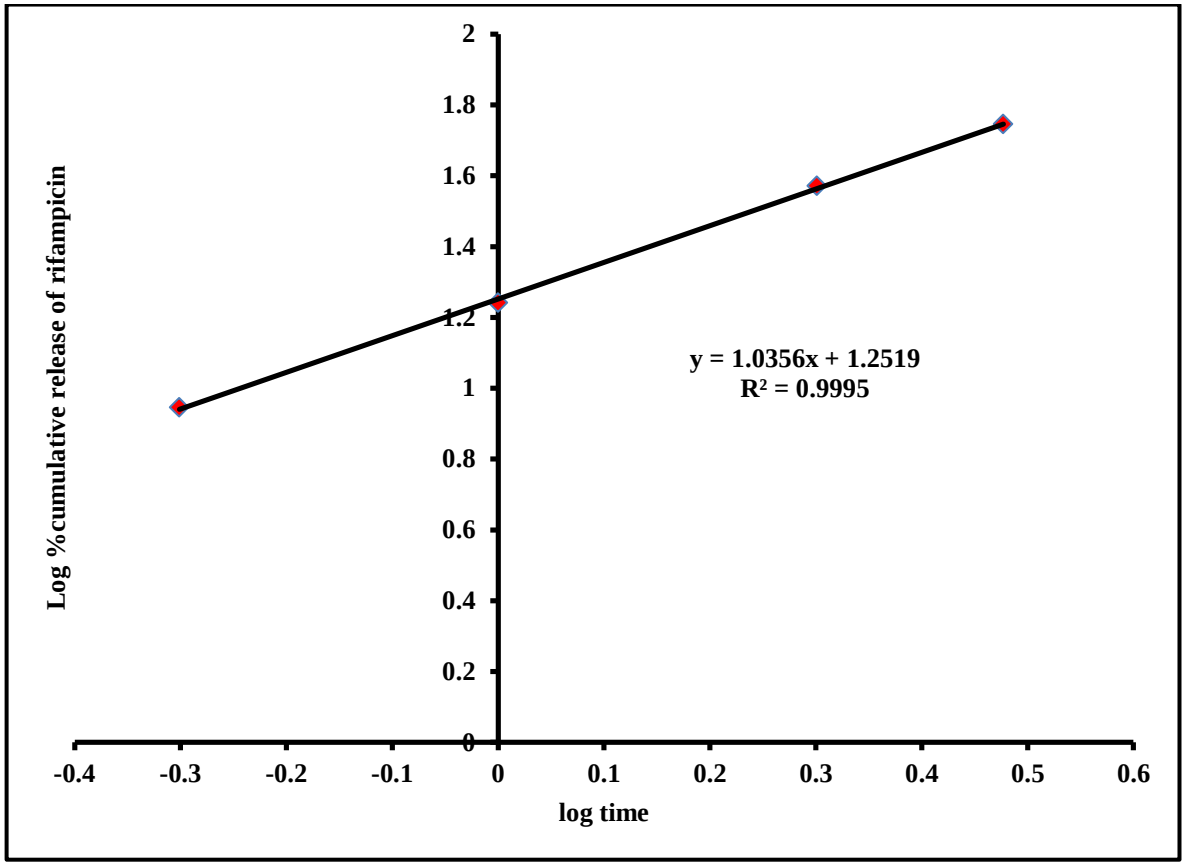


Fig. 11.6. Korsmeyer-Peppas model for optimized formulation

11.6.2. Dissolution study of optimized rifampicin GRPs along with isoniazid pellets

The dissolution study was also conducted for optimized rifampicin pellets in presence of immediate release and delayed release isoniazid pellets. The percentage cumulative release of rifampicin in these two combinations is given in the Table 11.4 and Fig. 11.7. From these results it is very clear that rifampicin interacts with isoniazid in the gastric medium leading to the enhanced degradation of rifampicin. These results also state that this drug-drug interaction can be minimized by formulating rifampicin as gastroretentive formulation and isoniazid as enteric coated formulation.

Table 11.4. Dissolution study of rifampicin GRPs along with isoniazid pellets

Time	With uncoated Isoniazid	With enteric coated Isoniazid
0	0	0
0.5	6.54±0.61	8.23±0.52
1	12.21±0.84	16.88±0.46
2	25.69±0.52	35.37±0.69
3	39.56±0.94	50.21±0.75
4	51.18±1.18	67.4±0.83
6	84.02±1.06	98.72±0.81

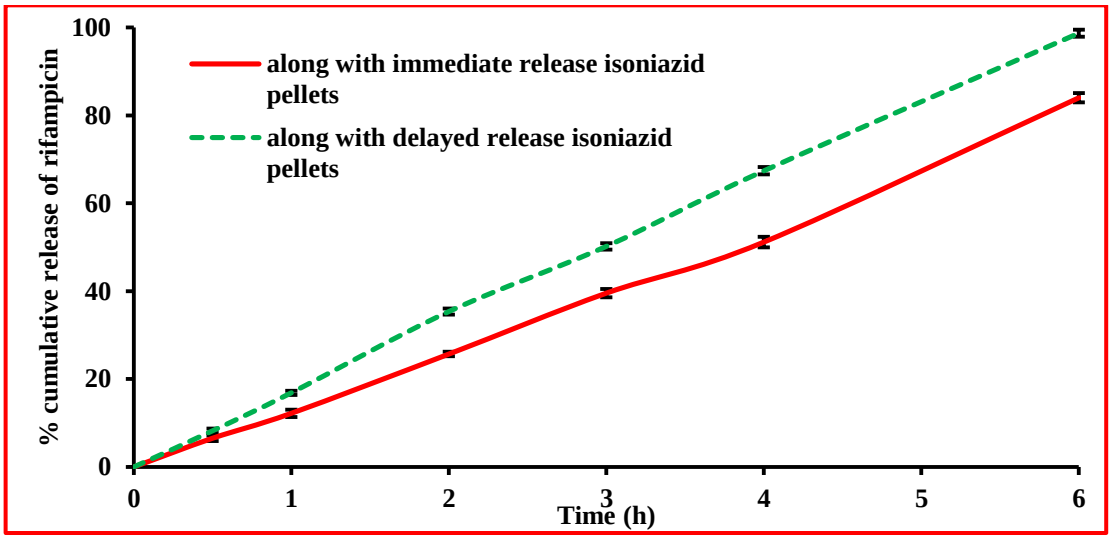


Fig. 11.7. Rifampicin release from optimized GRPs along with isoniazid pellets

11.6.3. Ex vivo studies

Ex vivo studies were performed using rat stomach and intestine. The values obtained from the calculation were used for plotting the graphs fraction of drug dissolved vs. time, fraction of drug absorbed vs. time and fraction of drug dissolved vs. fraction of drug absorbed as shown in Fig. 11.8, 11.9, 11.10, 11.11, 11.12 and 11.13.

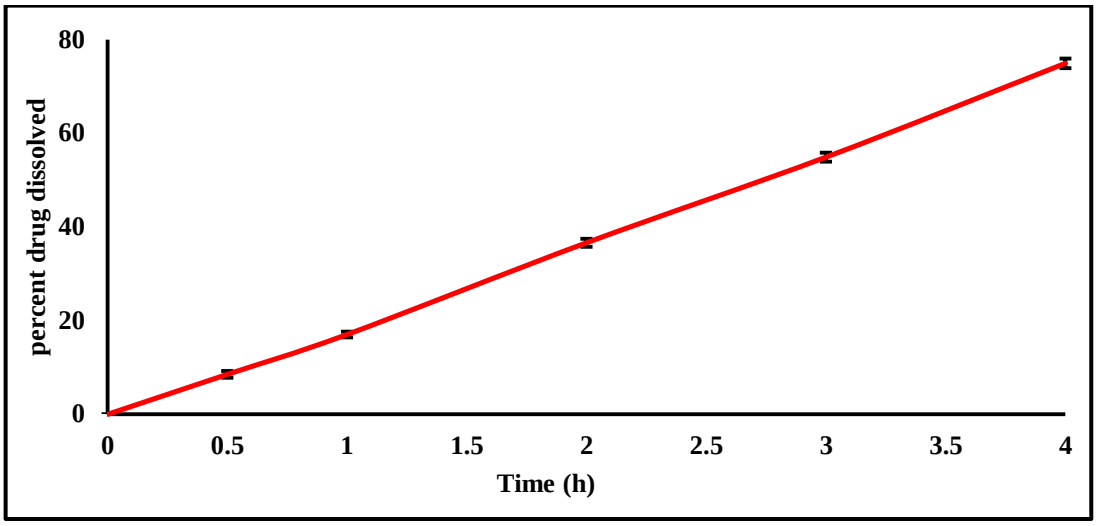


Fig. 11.8. Percentage of drug dissolved in simulated gastric fluid

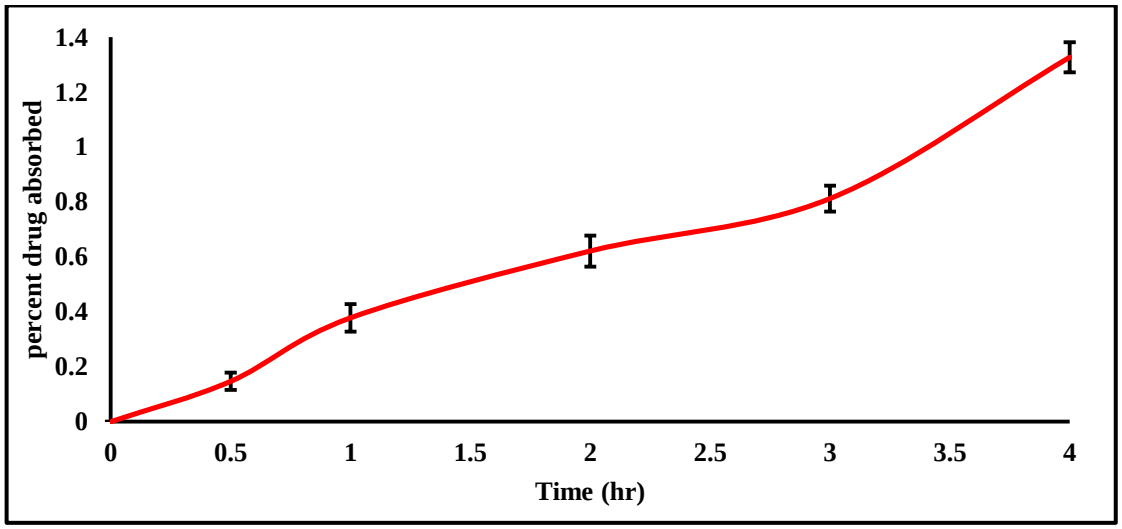


Fig. 11.9. Percentage of drug absorbed through rat stomach

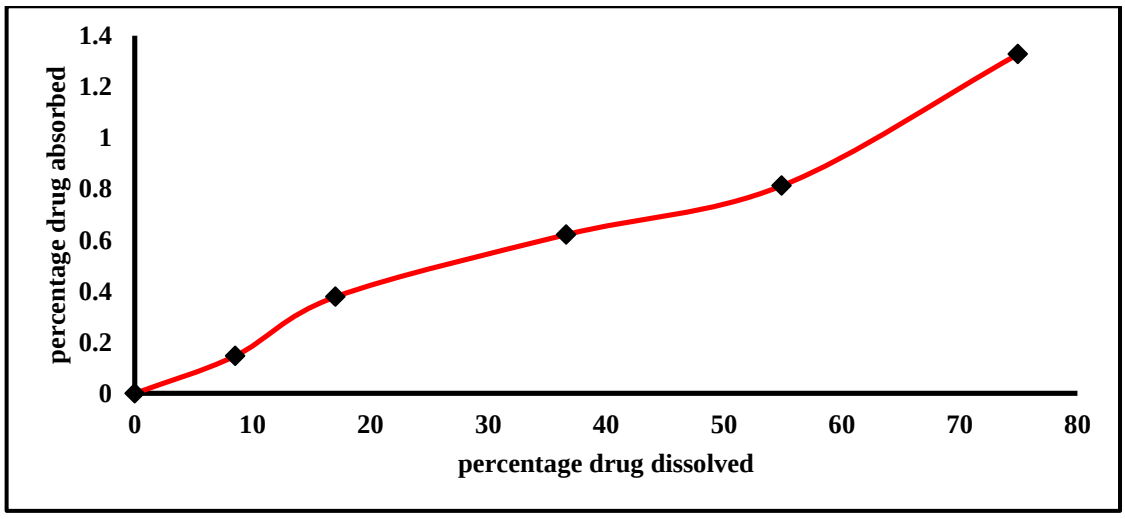


Fig. 11.10. Fraction of drug dissolved vs. fraction of drug absorbed (rat stomach)

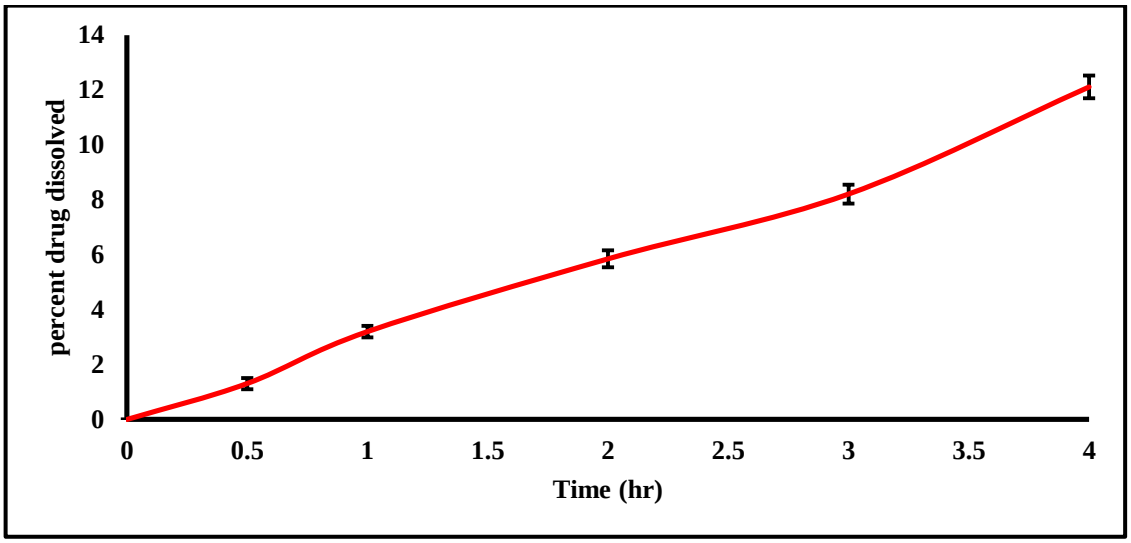


Fig. 11.11. Fraction of drug dissolved in simulated intestinal fluid

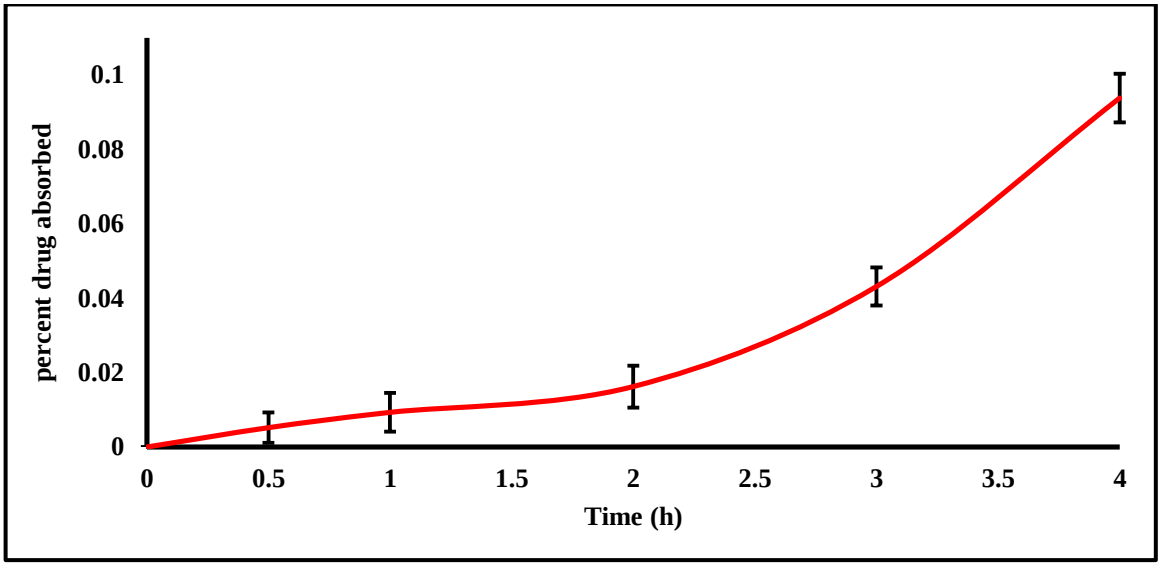


Fig. 11.12. Fraction of drug absorbed through rat intestine

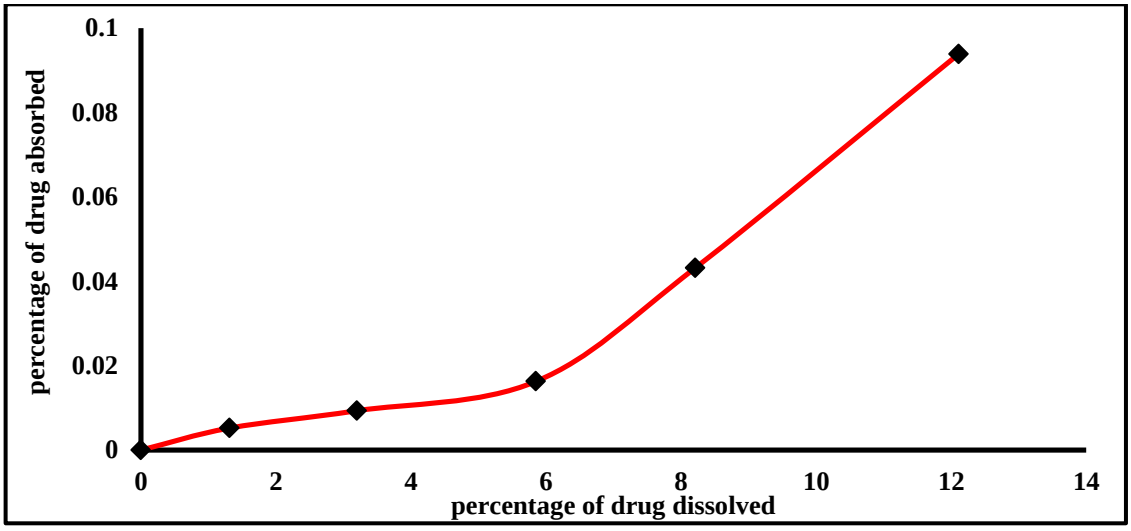


Fig. 11.13. Fraction of drug dissolved vs. fraction of drug absorbed (rat intestine)

The results showed that there was increase in fraction of drug dissolved and fraction of drug absorbed with increase in time. The absorption of dissolved drug was more in stomach at pH 1.2 than in intestine at pH 6.8. This is due to the poor solubility of drug in intestinal pH.

11.6.4. In vivo buoyancy studies

A radiological study was conducted to find out the in vivo floating time for the optimized gastroretentive floating Tablet in the gastric region of rabbits under fasting conditions. It was found that the floating Tablet remained buoyant in gastric contents up to 5 h in all rabbits (n=6) and was indicated by the X-rays shown in Fig. 11.14.

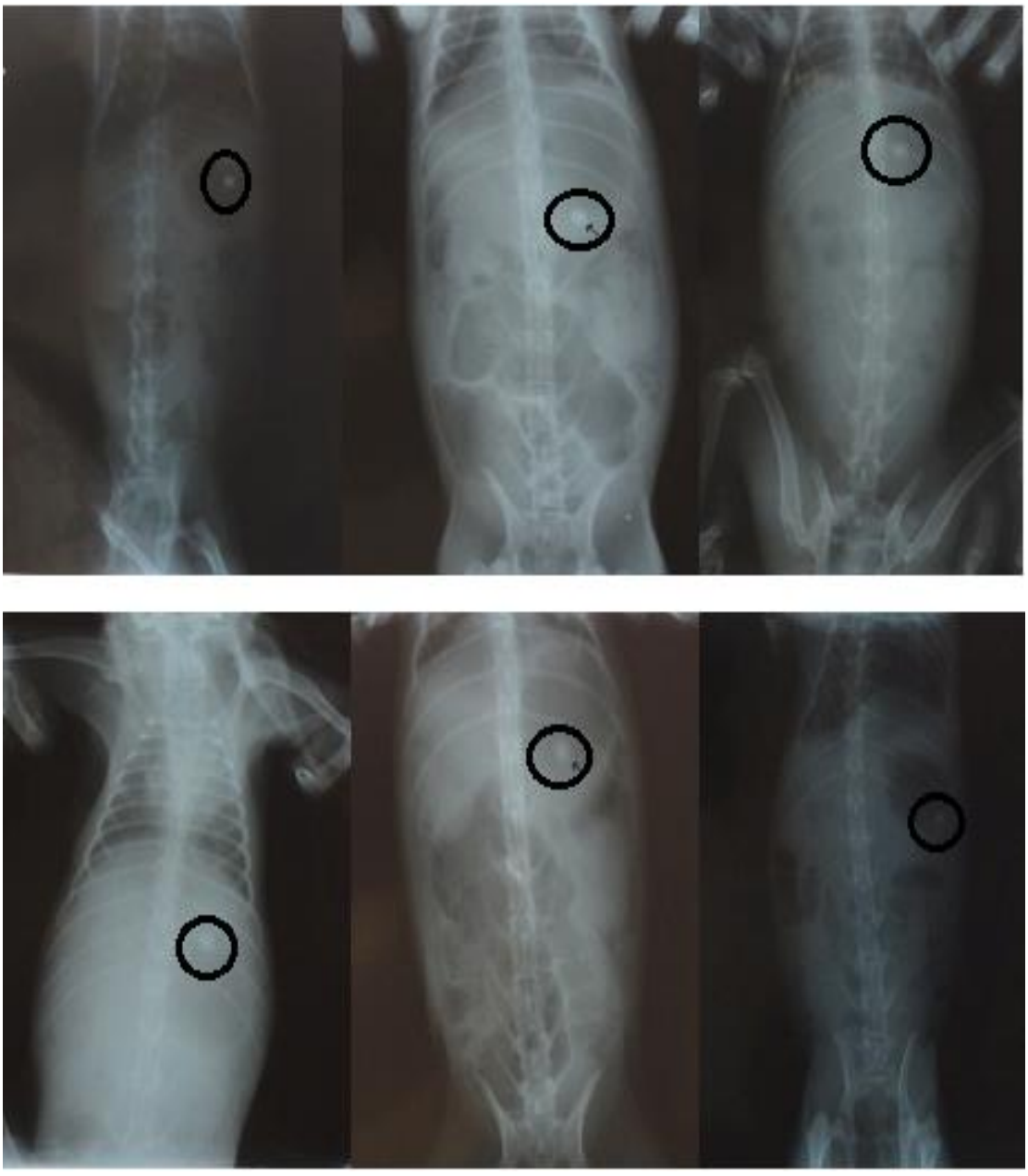


Fig. 11.14. X-ray images of all 6 rabbits showing the presence of floating tablet in rabbit stomach at 5th hour

11.6.5. Stability studies

The results of the stability studies are shown in the Tables 11.5, 11.6 and Fig. 11.15. These results clearly indicate that these gastroretentive pellets were stable up to 6 months under accelerated stability conditions of 40±2 °C and 75±5% RH.

Table 11.5. Results of stability studies of optimized GRPs

Test	0 days	3 months	6 months
Colour of pellets	red	red	red
Friability (%)	0.62	0.60	0.56
Assay (%)	99.56±1.34	97.97±1.48	96.61±1.25
Floating time (h)	5.75	5.83	6.0
Drug release at 6 h	99.12±0.84	97.11±1.03	95.36±0.93

Table 11.6. Drug release of optimized pellets stored at accelerated stability conditions

Time (h)	% Cumulative release of rifampicin at 6 h		
	0 months	3 months	6 months
0	0	0	0
0.5	8.82±0.67	7.86±0.71	7.14±0.68
1	17.41±0.51	15.94±0.85	14.18±0.59
2	37.2±0.74	34.12±0.76	32.38±0.87
3	55.6±0.82	50.34±0.82	48.19±0.79
4	75.34±0.61	73.32±0.91	71.21±1.08
6	99.12±0.84	97.11±1.03	95.36±0.93

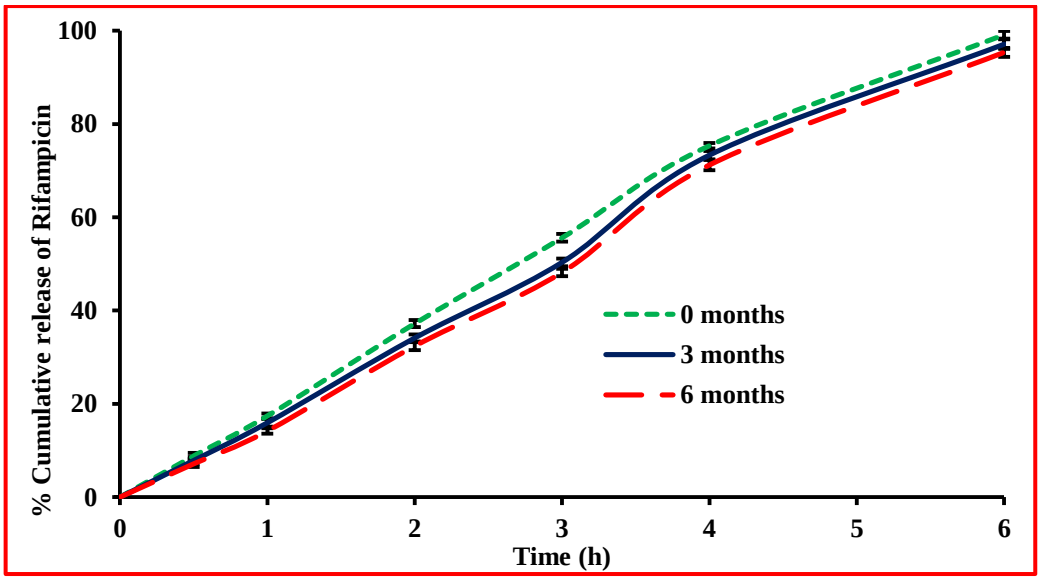


Fig. 11.15. Release profile of optimized formulation stored at accelerated conditions