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Gastroretentive delivery of rifampicin: *In vitro* mucoadhesion and *in vivo* gamma scintigraphy

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ABSTRACT

Rifampicin, a first line anti-tubercular drug, has maximum solubility and permeability in the stomach. An oral multi-particulate formulation with site specific sustained delivery of rifampicin was developed. This oral gastroretentive rifampicin formulation consisted of rifampicin pellets for immediate release as the loading dose and a bio/mucoadhesive rifampicin tablet for extended release.

Immediate release pellets of rifampicin were prepared by extrusion–spheronization process and were evaluated for physico-mechanical properties: usable yield, size, shape, abrasion resistance, mechanical crushing force, residual moisture and drug release. For the mucoadhesive rifampicin formulation, statistical experimental strategy was utilized to simultaneously optimize the effect of two independent variables namely amount of Carbopol and MCC. The two dependent responses selected were, work of adhesion; estimated using Texture Analyzer and $T_{50\%}$; determined from dissolution studies. Graphical and mathematical analysis of the results allowed the identification and quantification of the formulation variables influencing the selected responses.

To study the gastrointestinal transit of the optimized gastroretentive formulation, the *in vivo* gamma scintigraphy was carried out in six healthy human volunteers, after radiolabeling the formulation with ^{99m}Tc. The transit profiles demonstrated that the dosage form was retained in the stomach for more than 320 min. The human data validates the design concept and signifies the potential of the developed system for stomach targeted delivery of rifampicin for improved bioavailability.

1. Introduction

The gastrointestinal (GI) tract is the most common route of drug administration in the body. Ease of drug administration for compliant therapy, large surface area for systemic absorption, and flexibility of the GI tract to accommodate different formulations are some of the advantages of the GI tract in drug delivery. In addition to drug formulations that deliver the drug for a prolonged period of time, it is important to achieve spatial placement of the dosage form in the GI tract for efficient therapy. Site-specific drug delivery, using novel formulation designs, would improve local therapy in the GI tract, optimize systemic absorption, and would minimize premature drug degradation (Rubinstein and Friend, 1994).

In recent years, oral site-specific drug delivery systems have been devised to improve the absorption of drugs characterized by a poor peroral bioavailability. These strategies are of great interest for oral administration of molecules whose absorption is limited to a specific area of the gastrointestinal tract (Streubell et al., 2006). The drug localization at targeted sites can be achieved through the development of bio/mucoadhesive interactions between the drug carrier system and the intestinal mucosa. The concept of bioadhesion combines the advantages of extended the residence time at the absorption site and allows an intimate contact of the drug with the biological membrane has been applied to various formulations for targeting different parts in the GI tract.

Stomach-specific Rifampicin (RIF) delivery, for instance, would be highly beneficial in the treatment of tuberculosis (TB). RIF is the critical component in the therapeutic armamentarium for TB having potential sterilizing effect even on dormant TB bacilli. TB has made a comeback with vengeance, especially, in Asia, South America and Africa. TB, a potentially fatal lung disease, is a leading killer of young adults worldwide. The poor and impaired bioavailability of RIF from a number of dosage forms of RIF continues to be a subject of much concern (Shishoo et al., 2001a,b; du Toit et al., 2006). In addition, poor patient compliance is the most common reason for chemotherapy failure of TB (Shishoo et al., 2001a,b). Hence, there is a need to develop an anti-TB oral drug delivery system that is convenient for patients with improved and assured bioavailability.

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As of now, the only available treatment lies in the effective utilization of the available anti-TB drugs. Development of carrier/delivery systems that release drugs in a sustained manner at therapeutic concentration over a period of time can ensure patient compliance in terms of reducing dosing frequency, and may also minimize the risk of emergence of drug resistant mutants and potential toxicity. Various carrier systems such as liposomes and microspheres have been developed for the sustained delivery of anti-TB drugs with better chemotherapeutic efficacy (Dutt and Khuller, 2001; Vyas et al., 2004; Pandey et al., 2004). However, these formulations have to be injected either subcutaneously or intravenously, which, in general, is not acceptable (Prabakaran et al., 2004).

For improving the bioavailability of RIF, fabrication of an oral multi-particulate system which attains site specific sustained delivery of RIF in stomach could be a step in the right direction (Shishoo et al., 2001a; du Toit et al., 2006). Furthermore, RIF exhibit permeability in the stomach (Mariappan and Singh, 2003). However, multiparticulate dosage form of RIF which is easy to manufacture and directly addresses RIF bioavailability concerns and poor patient compliance is yet to be developed (du Toit et al., 2006).

The system which provides controlled delivery of RIF, in a near zero order rate is an ideal release profile for controlled drug delivery that in turn would improve safety profile of the drug and enhance activity duration for drug exhibiting short half-life. RIF has relatively short biological half-life, 2–5 h (Anonymous, 2008). The prolonged treatment of TB with conventional therapy of RIF and adverse side effects demanded and necessitated the development of controlled release formulation. Controlled release of anti-TB drugs hold promise for reducing the dosing frequency and improving patient compliance. Till more potent drugs become available, these systems could be cost-effective, feasible and save valuable life and resources in the management of TB (Khuller and Pandey, 2003). Also, such once daily formulations will improve patient compliance and may reduce duration of therapy (Prabakaran et al., 2004).

Gastroretentive drug delivery system by use of mucoadhesion technology provides spatial placement of the dosage form in the stomach and delivers the drug for a prolonged period of time (Streubell et al., 2006). Several other advantages of bio/mucoadhesive drug delivery systems, include, improved bioavailability of the drug; targeting a particular site and increased residence time. When combined with controlled release of a drug, gastroretention may lower the administration frequency (Woodley, 2001). Therefore, in order to target RIF at the site of maximum solubility and permeability, mucoadhesive property has been explored. The developed stomach targeted RIF delivery system comprised of loading and maintenance dose. The loading dose was formulated as pellets for immediate release and was evaluated for micromeritic and mechanical characteristics, whereas, maintenance dose was formulated as mucoadhesive tablet, was evaluated for its functionality; *in vitro* using texture analysis and *in vivo* for gastroretention using gamma scintigraphy.

2. Materials and methods

2.1. Materials

RIF was received as gift sample from Twilight Litaka Pharmaceuticals Pvt. Ltd., Pune, India. Microcrystalline cellulose (MCC, Avicel® PH 101, Signet Chemical Corporation, India), Lactose (Lactose (India) Ltd., India), Carbopol 71G (Arihant Trading Co., India), Hydroxypropylmethylcellulose (HPMC, Methocel E50, Colorcon Asia Pvt. Ltd., India), Polacrilin potassium U.S.P. (PP, Tulsion 339, Thermax Ltd., India), Aerosil® 200 (Evonik Ind., India) and magnesium stearate (Loba Chemie Pvt. Ltd., India) were used as excipients and obtained from the indicated sources. All other ingredients and reagents were of analytical grade and were used as received.

2.2. Preparation of RIF immediate release pellets

Powder components of the formulation (RIF—85%, w/w; MCC—5%, w/w; PP—5%, w/w and lactose—5%, w/w) were mixed in a small scale planetary mixer (Kalweka, Karnavati Eng. Ltd., India) for 10 min. Sufficient quantity of water was added as to form a damp coherent mass suitable for extrusion process. The wet mass was processed further for 10 min with occasional pauses to allow scraping of the bowl and blade of the planetary mixer. Extrudates were obtained using gravity fed cylinder extruder (R. R. enterprises, India), extruding at a constant speed of 125 rpm, through a roller die having holes 1 mm in diameter and 4 mm in length. A spheronizer (R. R. enterprises, India), equipped with a rotating plate of regular crosshatch geometry was used for the spheronization. The extrudates were spheronized for 10 min at 700 rpm and the pellets thus formed were dried in the Fluidized Bed dryer (Nero-Aeromatic, Switzerland) at 45 °C for 20 min.

2.3. Characterization of Immediate release RIF pellets

2.3.1. Usable yield (% theoretical)

The size distribution of pellets was determined by sieving using standard set of sieves (600–2360 μm) on a sieve shaker (Electromagnetic sieve shaker, EMS-8, Electrolab, India) for 5 min at a frequency of 50 Hz with amplitude of 1 mm. The fraction of pellets, 700–1190 μm, was considered as the usable yield (Howard et al., 2006).

2.3.2. Pellet size

Particle size for each batch was determined using Laser Light Scattering system (Malvern Mastersizer 2000, Malvern Instruments, Malvern, UK). All the measurements were carried out in triplicate and 50th percentile diameter of the cumulative particle size distribution was considered as mean pellet size (Koo and Heng, 2001).

2.3.3. Determination of the shape using Image analysis

For shape analysis, the images were captured using a stereomicroscope Leica S4E (Leica, Germany). The captured images were analyzed using Image analysis software (AnalySIS®, Soft Imaging system, v. 5.2, Münster, Germany). Analysis was carried out on 50 pellets from usable yield fraction. In this study, pellips was calculated for the characterization of the shape by using the following equation (Koo and Heng, 2001):

$$Pellips = \frac{P}{\pi \times d_{max}}$$

where P is the perimeter and d_{max} is maximum diameter of the pellet, calculated directly by using Image analysis software.

2.3.4. Mechanical crushing force

At least 20 pellets from the usable yield fraction of each formulation were evaluated for their diametral crushing force using a tablet strength tester (EH 01, Electrolab, India) (Pund et al., 2010).

2.3.5. Abrasion resistance

The resistance to abrasion was analyzed using Roche friabilator (Veego Instruments Corporation, India). A pre-weighed sample (approximately 6 g) taken from the usable yield fraction was placed in a friabilator along with 25 steel spheres, each 2 mm in diameter. After 100 revolutions at 25 rpm, the mass retained on the sieve (1190 μm) was weighed and the abrasion resistance was calculated as the percentage loss of mass between initial and final weights of

each pellet batch (Howard et al., 2006). Each batch was analyzed in triplicate.

2.3.6. Residual moisture

The residual water content present in the pellets after drying was determined by USP Method A using Karl Fischer titrator (Systronics Universal titrator 353, India). The equipment was pre-calibrated and standardised with disodium tartrate dihydrate. Pellets, approximately 250 mg, were accurately weighed and immediately placed in the moisture analyzer for titration with Karl Fischer reagent. Each batch was analyzed in triplicate (Pund et al., 2010).

2.3.7. Dissolution

Dissolution study was carried out in 0.1 N hydrochloric acid in USP dissolution apparatus I (Hanson Research Corporation, Chatsworth, CA) at 37 ± 0.2 °C, 100 rpm, for 45 min. A mean of six determinations was recorded. Samples withdrawn were analyzed by dual wavelength spectrophotometric method (Shishoo et al., 1999).

2.3.8. Porosity

Pellet porosity was determined using Helium pycnometry (SmartPycno 30, Smart Instruments, India). All the values are mean of three replicates (Chopra et al., 2001; Pund et al., 2010).

2.3.9. Surface topography

Morphological examination of the surface of the pellets was carried out using a scanning electron microscope. Scanning electron microphotographs of pellets were obtained using JEOL (JEOL JSM-6100, Tokyo, Japan). The particles were vacuum dried, coated with thin gold–palladium layer by sputter coater unit (JEOL JFM-1100, Tokyo, Japan) and observed microscopically at an accelerating voltage of 5.0 kV.

2.4. Preparation and characterization of mucoadhesive RIF tablets

2.4.1. Experimental design

Method of dry granulation was employed, which involved precompression, size reduction of the slugs, screening, lubrication of granules and final compression to get tablets. The powder components of the formulation (RIF 150 mg/tablet, Carbopol 71G (4–19%, w/w), MCC (2.5–5%, w/w), HPMC (8 mg/tab), were mixed and compressed to form slugs with low hardness (3–4 kg). These slugs were then passed through sieve no. 24. The granules thus obtained were lubricated with a mixture of Aerosil 200 and magnesium stearate (1:1, 0.6%, w/w, previously passed through sieve no. 60). Granules were compressed on a single-station tablet compression machine (Cadmach, India). The combinatorial effect of amounts of MCC, and Carbopol on work of adhesion (W_{ad}) and $T_{50\%}$ (time required for 50% dissolution of the drug) were studied. The experiments were planned according to a duplicate four run, 2^2 full factorial design, using a software package NEMRODW (LPRAI SARL, Marseille, France). The compositions of all the formulations are listed in Table 1.

2.4.2. Work of adhesion, W_{ad}

Porcine gastric mucosa was used as the gastric mucosal surface and the mucoadhesive performance of the sample was tested using a texture analyzer (TA.XT2i plus, Stable Micro Systems, UK) equipped with 50 N load cell and a mucoadhesive holder. A sample was attached to the stainless steel cylindrical probe; 6 mm in diameter. The tissue (about 50 mm × 50 mm) was equilibrated for 15 min at 37 ± 0.2 °C before placing onto the holder stage of mucoadhesive holder. The texture analyzer was set to the 'adhesive test' mode with a test-speed of 0.5 mm/s. An acquisition rate of 200 points/s and a trigger force of 0.1 N were selected. The probe with the sample attached was then moved downward to contact with mucosal tissue at a specified force of 2 N and maintained 60 s. The probe was subsequently withdrawn at a test speed of 0.5 mm/s. The total amount of forces involved in the probe withdrawal from the tissue (W_{ad}) was then calculated from the area under the force versus distance curve using Texture Expert Exceed software (Ver. 2.64). Each sample was analyzed in triplicate.

Table 1
Experimental design and randomized run order and results of the combinatorial effects of formulation variables on work of adhesion (Y_1) and $T_{50\%}$ (time required for 50% dissolution of the drug) Y_2.

Run order	Carbopol 71G (%, w/w), X_1	Avicel PH 101 (%, w/w), X_2	W_{ad} (g s)[a], Y_1	$T_{50\%}$ (min)[b], Y_2
1	4	2.5	29.06	120
2	19	2.5	344	240
3	4	5	20.52	90
4	19	5	180	175
5	4	2.5	23.64	140
6	19	2.5	327.8	245
7	4	5	21.6	95
8	19	5	189.07	180

[a] Values shown are the means of 3 determinations.
[b] Values shown are the means of 6 determinations.

2.4.3. Determination of $T_{50\%}$ (time required for 50% dissolution of the drug)

Dissolution study was carried out in 0.1 N hydrochloric acid in USP dissolution apparatus I (Hanson Research Corporation, Chatsworth, CA) at 37 ± 0.2 °C with stirring speed of 100 rpm. A mean of six determinations was recorded. Samples withdrawn were analyzed by dual wavelength spectrophotometric method (Shishoo et al., 1999).

2.4.4. Assessment of in vivo gastroretention using gamma scintigraphy

Gamma scintigraphic studies were carried out to determine the location of RIF mucoadhesive formulation after oral administration and the extent of its transit through the gastrointestinal tract. Radio-labeling efficiency was evaluated using thin layer chromatography. Instant thin layer chromatography-silica gel strips (ITLC-SG) were used as stationary phase and 100% acetone as mobile phase (Rastogi et al., 2007).

$$\%\ \text{Radiolabeling} = \frac{\text{Radioactivity retained in the lower half of the strip}}{\text{Total count present with the strip}} \times 100$$

The RIF mucoadhesive formulation was radiolabeled with 500 μCi of $^{99m}TcO^{4-}$. After incorporating ^{99m}Tc onto the formulation, the radioactive dose was confirmed using dose calibrator.

Six healthy adult human volunteers were recruited from the B. V. Patel PERD Centre's volunteer data bank (inclusive of both; male and female, age: 24–38 years and weight: 55–75 kg). The study was carried out after getting approval from Institutional Ethics Committee. All the volunteers provided written consent to take part in the study. None of the volunteers had a history of gastrointestinal disorders. The volunteers who were smokers were abstained during the study.

Radiolabeled mucoadhesive tablet was administered to volunteers in a sitting position, with 200 ml of water after overnight fasting. After administration, the subjects were imaged by laying down in a supine position under the gamma camera (Infinia, GE, USA). The gamma camera used for the study was low energy, high resolution scinticamera, with parallel hole collimator and interfaced to a dedicated computer. The images were recorded at 0, 1, 2, 3, 4, 5 and 6 h post-dose administration, anteriorly for 120 s/view

with a 10% window centred to include the 140 keV photopeak of ^{99m}Tc. The data was recorded using Xeleris Software and were stored as 256 × 256 pixel images. Scintigrams were used to determine formulation activities in the regions of interest (ROI). ROIs relating to the stomach were drawn on gamma images for each time point, and counts relating to ROIs were calculated using Xeleris software. All counts were corrected for background and isotope decay. Gastric emptying of the formulations was expressed in terms of remaining relative counts in ROI as a function of time. The time required for emptying of 50% of the formulation; T_{50}, from the stomach were calculated and used in evaluating gastric residence time.

3. Results and discussion

In spite of a rapid growth in the novel routes for drug delivery, a vast majority of therapeutic agents still require oral administration. There is a growing tendency to employ sophisticated systems that enable the controlled or timed release of a drug, thereby providing a better dosing pattern and greater convenience to the patient.

3.1. RIF immediate release pellets

The dose of the RIF was divided into two parts—loading dose of 300 mg in the form of pellets and maintenance dose of 150 mg in the form of mucoadhesive tablet. Immediate release dose was fabricated as pellets because pellets offer wide variety of advantages. Among the various pharmaceutical dosage forms, multiparticulate carriers (e.g., pellets, granules, etc.) are favoured due to their small size, which is responsible for their easy dispersibility within the GI tract with highly inter- and intra-reproducible gastrointestinal transit rate. These systems allow tailoring of the release profile of a drug, offer minimal risk of dose dumping, flexibility of blending units with different release patterns, show short and reproducible gastric residence time (Joshi et al., 2008). Pellets also reduce variations in gastric emptying rates and overall transit times. Thus inter- and intra-subject variability of plasma profiles, which is common with single unit regimens, is minimized. Therefore, it would be beneficial for the RIF which shows variable bioavailability.

In the present case, since the loading dose of RIF was formulated as pellets, their rapid disintegration was essential to get rapid dissolution. MCC, the extrusion–spheronization aid of choice, provides good binding properties and imparts cohesiveness required for the wet extrusion. MCC-based pellets produced via extrusion–spheronization are spherical with low friability, high density and smooth surface (Basit et al., 1999). However, MCC containing pellets prepared by extrusion–spheronization do not disintegrate which results in slow and incomplete dissolution of poorly water soluble drugs (Tho et al., 2003).

Our preliminary work revealed that the RIF pellets formulated only with MCC showed incomplete and slow dissolution. The slow dissolution of poorly water soluble drugs from microcrystalline cellulose pellets prepared by extrusion–spheronization has been widely documented. This slow dissolution rate derives from the pronounced contraction of the pellet during the drying phase, leading to reduced porosity which hinders entry of the dissolution medium into the pellet (Kleinebudde, 1994). Of the approaches that have been proposed for overcoming this limitation of MCC, the inclusion of superdisintegrants has received relatively little attention (Souto et al., 2005). Complete replacement of MCC with soluble spheronizing agent like lactose had improved the dissolution but pellets had low and insufficient mechanical strength. Hence, a combination of lactose and MCC was selected. Further, it was found to be essential to incorporate a superdisintegrant which remains unaffected by high pressure operations of extrusion and spheronization.

Table 2
Evaluation parameters for immediate release RIF pellets.

Sr. No.	Parameter	Result
1	Usable yield	90–92%
2	Pellet size	1116.06 μm
3	Pellips	0.960
4	Mechanical crushing force	5.2 N
5	Abrasion resistance	<1%
6	Residual moisture	3.12%
7	Dissolution	>75% at 45 min
8	Porosity	47.39%

PP, polymethacrylic acid, is a commercially available tablet superdisintegrant has remarkably high water uptake capacity (Borodkin, 1991). Use of PP in pellets is not yet reported. At 5% (w/w) concentration in RIF pellets, PP showed significant improvement in dissolution of RIF. This improvement in dissolution can be attributed to the high porosity of pellets 47.39% (Table 2) which can be observed in scanning electron microphotograph (Fig. 1).

Evaluation parameters for immediate release RIF pellets are listed in Table 2. For a successful extrusion–spheronization process and the formulation, a high percentage of pellets should be produced within a desired size range with sufficient mechanical robustness. Our results indicated that RIF pellets were produced with high usable yield (90–92%), good sphericity as indicated by pellips of 0.96, sufficient mechanical strength; as indicated from abrasion resistance <1% and mechanical crushing force 5.2 N, residual moisture 3.12% and dissolution >75% in 45 min (Table 2). This indicates that RIF pellets are produced with a good micromeritic, mechanical characteristics and dissolution even when the drug loading is high.

3.2. Mucoadhesive RIF tablet: combinatorial effects

Oral ingestion, the predominant and most preferred route for the delivery of a drug allows unassisted administration to the patient in absence of trained personnel. Controlled release oral drug delivery systems offer several advantages over immediate release dosage forms, including the minimization of fluctuations in drug concentrations in the plasma and at the site of action over

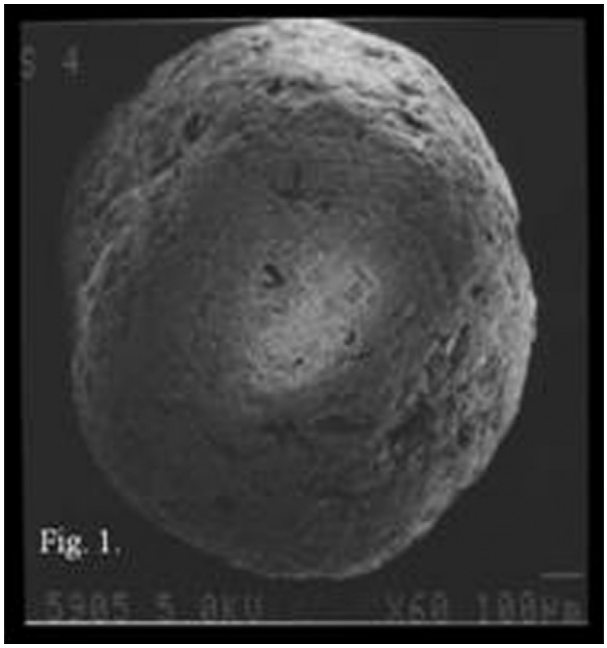

Fig. 1. Scanning electron microphotograph of immediate release RIF pellets.

prolonged period of time, resulting in optimized therapeutic efficiencies and reduced side effects; a reduction in the total dose administered (while providing similar therapeutic effects) and a reduction in the dosing frequency, leading to improved patient compliance (Streubell et al., 2006).

However, standard controlled release dosage forms offer only limited advantage for drugs that have an absorption window in the upper small intestine. Once emptied from the stomach, the passage of the dosage form through intestine is rapid, thus limiting the extent of absorption at stomach. In order to increase the bioavailability of this type of drugs, the residence time of the controlled release dosage forms in the upper gastrointestinal tract needs to be prolonged (Streubell et al., 2006; Murphy et al., 2009). It will also significantly increase the efficacy of therapy of chronic diseases and simplify the course of complex treatments involving several drugs (Hoichman et al., 2004).

The struggle to achieve improved bioavailability and/or prolonged residence time has led to an increased interest in bio/mucoadhesion (Thanos et al., 2003; Goto et al., 2006; Fransén et al., 2008). Bio/mucoadhesive drug delivery systems are designed to provide longer contact of the drug or delivery system with the crucial absorption region (Davis, 2005). There are several other advantages of bio/mucoadhesive drug delivery systems like, improved bioavailability, site-selective targeting and the increased residence time. When combined with controlled release of a drug, gastroretention may lower the administration frequency (Woodley, 2001). In addition, such system may provide the release of the drugs in a near zero order rate, which is an ideal release profile for controlled drug delivery that in turn would improve safety profile and enhance the activity duration for drug.

These modified release systems are more complicated than conventional tablets and capsules and require new methods for their evaluation. Texture analysis, a useful tool based on tensile strength measurement, has been extensively used as a valid means for mechanical characterization of pharmaceutical mucoadhesive dosage forms (Mortazavi and Smart, 1994; Park and Munday, 2002; Edsman and Hägerström, 2005). In the present study, the mucoadhesion using texture analysis has been evaluated through the W_{ad}.

Mucoadhesive polymers are used to immobilize a drug delivery system on a specific site for targeted release and optimal drug delivery due to intimacy and duration of contact. In the present study, Carbopol 74G, a directly compressible grade that provides controlled release along with bioadhesion was selected. The coexcipient MCC was selected as independent variable for studying the combinatorial effect since it can affect drug release due to its disintegrating nature. The experimental design methodology was exploited for systematically evaluating the effect of varying the amount of Carbopol (X_1) and MCC (X_2) as well as to highlight any interaction among the components on the *in vitro* mucoadhesion (Y_1) and $T_{50\%}$; time required for 50% dissolution of RIF (Y_2) (Table 1). This has facilitated the identification of the most significant factors influencing these properties and establishing their best levels for optimizing the considered experimental responses.

Mathematical relationship was generated between the factors and responses for determining the levels of factors, which yield optimum responses. A first order polynomial regression equation that fitted to the data is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 \quad (1)$$

where b represents the coefficient for the associated model term, b_0 is the intercept representing the arithmetic averages of all the quantitative outcomes of all experimental runs; b_1 and b_2 are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 are the levels of factors. The terms X_1X_2 represent the interaction term. The equation represents the quantitative effect of factors (X_1 and X_2) upon the each of the selected responses;

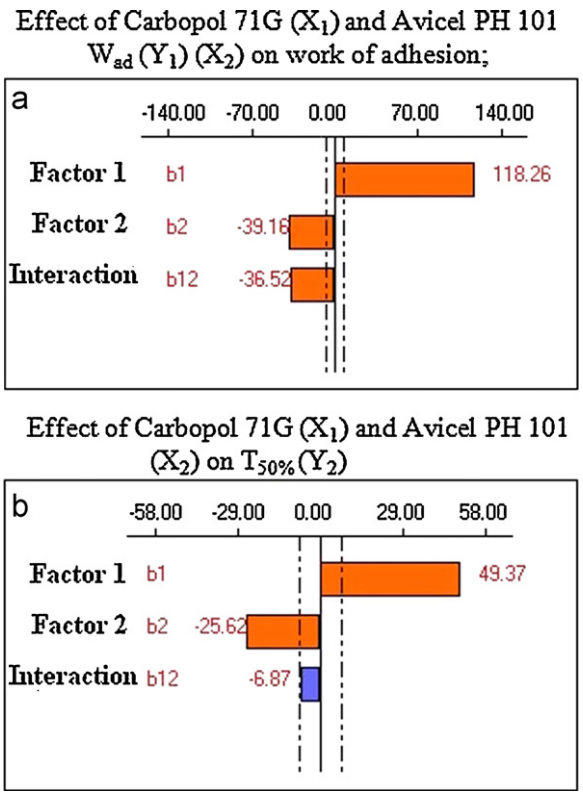

Fig. 2. . Graphical representation of effect of factors on selected responses. (a) Effect of Carbopol 71G (X_1) and Avicel PH 101 (X_2) on work of adhesion; W_{ad} (Y_1). (b) Effect of Carbopol 71G (X_1) and Avicel PH 101 (X_2) on time required for 50% dissolution of RIF; $T_{50\%}$ (Y_2).

Y_1 and Y_2. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors. A positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors. Analysis of variance (ANOVA) was applied for estimating the significance of the model, at 5% significance level. The coefficients for each model and their tests of significance are summarized in Table 3. The equations of the responses are given below:

$$Y_1 = 141.96 + 118.26b_1 - 39.16b_2 - 36.52b_{12} \quad (2)$$

$$Y_2 = 160.63 + 49.38b_1 - 25.62b_2 - 6.87b_{12} \quad (3)$$

The mathematical model is considered significant if the P-value is less than 0.05. In addition, graphical analysis of responses was carried out. This allowed the identification of important factors for the considered responses to be pointed out and the optimum factor level to be selected. The bar graphs were constructed in which the bars that exceed the two reference lines, calculated according to the experimental variance, correspond to the factors that are active on the response. In particular, the active factors are those where a level change determines a response variation which is statistically different from the variation due to the experimental error (Furlanetto et al., 2003; Pund et al., 2010).

The graphs showing the influence of selected formulation variables on the selected responses are presented in Fig. 2. These plots demonstrate that the *in vitro* mucoadhesion and $T_{50\%}$ are significantly influenced by the levels of Carbopol and MCC in the formulation. With increasing levels of Carbopol, the work of adhesion and $T_{50\%}$ are increased significantly, as evident from the positive sign of coefficients of term X_1 in Eqs. (2) and (3). There appears to be a negative influence of amount of MCC on both the selected responses; as observed from the graph (Fig. 2) and equa-

Table 3
Parameter estimates for coefficients (b) in Eqs. (2) and (3).

Component	Work of adhesion (W_{ad}, Y_1)		Time required for 50% dissolution of RIF ($T_{50\%}$, Y_2)	
	Coefficient estimate	P value	Coefficient estimate	P value
Model	141.96	**<0.01**	160.625	**<0.01**
X_1	118.26	**<0.01**	49.38	**<0.01**
X_2	−39.16	**<0.01**	−25.62	**0.0412**
X_1 X_2	−36.52	**0.0113**	−6.87	6.5

Significant values are written in bold type.

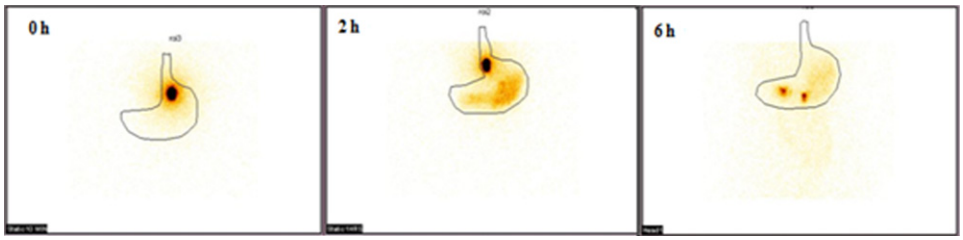

Fig. 3. . Representative gamma scintigraphic images of mucoadhesive RIF formulation in human volunteer (volunteer no. 2, at 0, 2 and 6 h).

tions (Eqs. (2) and (3)). There seems to be an optimal ratio between Carbopol and MCC for each response. With the help of polynomial equation, the formulation was optimized for both the responses. The final optimal formulation parameters were calculated by satisfying the requirements for each response. Thus, to maximize the gastroretention and to sustain the release of mucoadhesive tablets of RIF, it was desirable to maximize X_1 and minimize X_2, without affecting the tabletting performance. The optimal calculated parameters were 19% (w/w) of Carbopol and 2.5% (w/w) of MCC corresponding to formulation run nos. 2 and 6 (Table 1) having W_{ad} 327.8–344 g s and $T_{50\%}$ 240–245 min.

3.3. Gamma scintigraphy study

In recent years, there has been a tendency to employ sophisticated systems that enable controlled or timed release of a drug, thereby providing a better dosing pattern and greater convenience to the patient. Although much about the performance of such systems can be learned from *in vitro* release studies using conventional and modified dissolution methods, evaluation *in vivo* is essential in product development. These gastroretentive systems are more complicated than conventional dosage forms and require new methods for their evaluation. The technique of gamma scintigraphy has become the most popular method to investigate the gastrointestinal performance of the product (Wilding et al., 2001; Davis et al., 1992). It is commonly used non-invasive method to study the gastric emptying process of solids (Podczeck et al., 1999).

The objective of the present *in vivo* gamma-scintigraphy study was to provide the proof of concept that the mucoadhesive capability of the RIF gastroretentive tablet was useful for increasing the gastric residence time of the dosage form. ^{99m}Tc is the most popular of radionuclide because of its versatile chemistry, near-ideal energy (140 keV), low radiation dose and short half-life (6 h). In addition, it is readily available through the use of portable generators (Wilding et al., 2001). We assessed that the labeling method for visualizing the system over time was adequate to the objective of the study as the radiolabeling efficiency was found to be >90%. Therefore, ^{99m}Tc acts as the marker for locating the transit of the mucoadhesive RIF tablet in the gastrointestinal tract.

A representative gamma scintigraphy image of one of the volunteer at 0, 2 and 6 h is shown in Fig. 3. It is evident from the images that the formulation is retained in the stomach for more than 6 h. Mean gastric emptying of the formulations, expressed in terms of

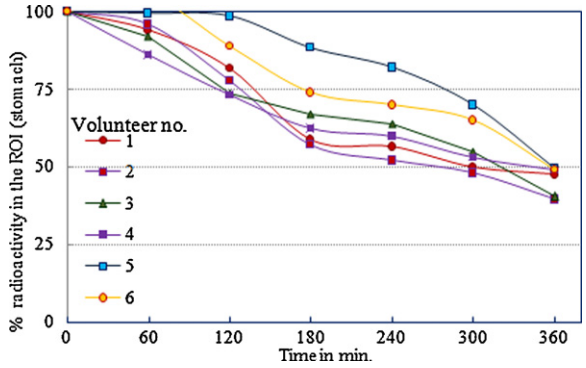

Fig. 4. . Gastric emptying curves for the radiolabeled mucoadhesive RIF formulation for 6 volunteers.

remaining relative counts in the ROI as a function of time shows $T\gamma_{50}$ (time required for 50% of the formulation to empty from the stomach) is 320 min (Fig. 4).

4. Conclusion

A novel gastroretentive delivery system of RIF was developed for targeting its sustained release in the stomach, a region where RIF has maximum solubility and permeability. This novel delivery system is a revitalized formulation which holds promise to give a new lease of life to an old but potential molecule.

The human gamma scintigraphy imaging data validated the design concept and signifies the potential of the developed system for stomach targeted delivery of RIF. From our *in vitro* characterization and *in vivo* imaging studies, the formulation is found to be promising and could further be considered for human *in vivo* bioavailability studies. Till more potent drugs become available, these systems could be cost-effective, feasible and save valuable life and resources in the management of TB.

The manufacturing technology used for the preparation of novel gastroretentive RIF formulation is relatively simple, which can be easily adopted in industrial units on a commercial scale.

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References

Anonymous, 2008. Rifampin. Tuberculosis 88, 151–154.
Basit, A.W., Newton, J.M., Lacey, L.F., 1999. Formulation of ranitidine pellets by extrusion–spheronization with little or no microcrystalline cellulose. Pharm. Dev. Technol. 4, 499–505.
Borodkin, S., 1991. Ion exchange resin delivery system. In: Tarcha, P.J. (Ed.), Polymers for Controlled Drug Delivery. CRC Press, Florida, pp. 215–229.
Chopra, R., Newton, J.M., Alderborn, G., Podczeck, F., 2001. Preparation of pellets of different shape and their characterization. Pharm. Dev. Technol. 6, 495–503.
Davis, S.S., Hardy, J.G., Newman, S.P., Wilding, I.R., 1992. Gamma scintigraphy in the evaluation of pharmaceutical dosage forms. Eur. J. Nucl. Med. 19, 971–986.
Davis, S.S., 2005. Formulation strategies for absorption windows. Drug Discov. Today 10, 249–257.
du Toit, L.C., Pillay, V., Danckwerts, M.P., 2006. Tuberculosis chemotherapy—current drug delivery approaches. Respir. Res. 7, 1–18.
Dutt, M., Khuller, G.K., 2001. Sustained release of isoniazid from a single injectable dose of poly (DL-lactide-co-glycolide) microparticles as a therapeutic approach towards tuberculosis. Int. J. Antimicrob. Agents 17, 115–122.
Edsman, K., Hägerström, H., 2005. Pharmaceutical applications of mucoadhesion for the non-oral routes. J. Pharm. Pharmacol. 57, 3–22.
Fransén, N., Björk, E., Edsman, K., 2008. Changes in the mucoadhesion of powder formulations after drug application investigated with a simplified method. J. Pharm. Sci. 97, 3855–3864.
Furlanetto, S., Maestrelli, F., Orlandini, S., Mura, P., 2003. Optimization of dissolution test precision for a ketoprofen oral extended-release product. J. Pharm. Biomed. Anal. 32, 159–165.
Goto, T., Morishita, M., Kavimandan, N.J., Takayama, K., Peppas, N.A., 2006. Gastrointestinal transit and mucoadhesive characteristics of complexation hydrogels in rats. J. Pharm. Sci. 95, 462–469.
Hoichman, D., Gromova, L.I., Sela, J., 2004. Gastroretentive controlled-release drugs. Pharm. Chem. J. 38, 621–624.
Howard, M.A., Neau, S.H., Marvin, J.S., 2006. PEO and MPEG in high drug load extruded and spheronized beads that are devoid of MCC. Int. J. Pharm. 307, 66–76.
Joshi, A., Pund, S., Nivsarkar, M., Vasu, K., Shishoo, C., 2008. Dissolution test for site-specific release isoniazid pellets in USP apparatus 3 (reciprocating cylinder): optimization using response surface methodology. Eur. J. Pharm. Biopharm. 69, 769–775.
Khuller, G.K., Pandey, R., 2003. Sustained release drug delivery systems in management of tuberculosis. Indian J. Chest Dis. Allied Sci. 45, 229–230.
Kleinebudde, P., 1994. Shrinking and swelling properties of pellets containing microcrystalline cellulose and low substituted hydroxypropylcellulose. I. Shrinking properties. Int. J. Pharm. 109, 209–219.
Koo, O.M.Y.K., Heng, P.W.S., 2001. The influence of microcrystalline cellulose grade on shape and shape distributions of pellets produced by extrusion–spheronization. Chem. Pharm. Bull. 49, 1383–1387.
Mariappan, T.T., Singh, S., 2003. Regional gastrointestinal permeability of rifampicin and isoniazid (alone and their combination) in the rat. Int. J. Tuberc. Lung Dis. 7, 797–803.
Mortazavi, S.A., Smart, J.D., 1994. An in-vitro method for assessing the duration of mucoadhesion. J. Control. Release 31, 207–212.
Murphy, C.S., Pillay, V., Choonara, Y.E., du Toit, L.C., 2009. Gastroretentive drug delivery systems: current developments in novel system design and evaluation. Curr. Drug Deliv. 6, 451–460.
Pandey, R., Sharma, S., Khuller, G.K., 2004. Nebulization of liposome encapsulated antitubercular drugs in guinea pigs. Int. J. Antimicrob. Agents 24, 93–94.
Park, C.R., Munday, D.L., 2002. Development and evaluation of a biphasic buccal adhesive tablet for nicotine replacement therapy. Int. J. Pharm. 237, 215–226.
Prabakaran, D., Singh, P., Jaganathan, K.S., Vyas, S.P., 2004. Osmotically regulated asymmetric capsular systems for simultaneous sustained delivery of antitubercular drugs. J. Control. Release 95, 239–248.
Podczeck, F., Course, N.J., Newton, J.M., 1999. Determination of the gastric emptying of solid dosage forms using gamma-scintigraphy: a problem of image timing and mathematical analysis. Eur. J. Nucl. Med. 26, 373–378.
Pund, S., Joshi, A., Vasu, K., Nivsarkar, M., Shishoo, C., 2010. Multivariate optimization of formulation and process variables influencing physico-mechanical characteristics of site-specific release isoniazid pellets. Int. J. Pharm. 388, 64–72.
Rastogi, R., Sultana, Y., Aqil, M., Ali, A., Kumar, S., Chuttani, K., Mishra, A.K., 2007. Alginate microspheres of isoniazid for oral sustained drug delivery. Int. J. Pharm. 334, 71–77.
Rubinstein, A., Friend, D.R., 1994. Specific delivery to the gastrointestinal tract. In: Domb, A.J. (Ed.), Polymeric Site-Specific Pharmacotherapy. John Wiley & Sons, Chichester, U.K., pp. 282–283.
Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Kotecha, J.S., Shah, P.B., 1999. Stability of rifampicin in dissolution medium in presence of isoniazid. Int. J. Pharm. 190, 109–123.
Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Vora, M.J., 2001a. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int. J. Pharm. 228, 53–67.
Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., 2001b. Impaired bioavailability of rifampicin from fixed dose combination formulations with isoniazid. Indian J. Pharm. Sci. 63, 443–449.
Souto, C., Rodríguez, A., Parajes, S., Martínez-Pacheco, R., 2005. A comparative study of the utility of two superdisintegrants in microcrystalline cellulose pellets prepared by extrusion–spheronization. Eur. J. Pharm. Biopharm. 61, 94–99.
Streubell, A., Siepmann, J., Bodmeie, R., 2006. Drug delivery to the upper small intestine window using gastroretentive technologies. Curr. Opin. Pharmacol. 6, 501–508.
Thanos, C.G., Liu, Z., Goddard, M., Reineke, J., Bailey, N., Cross, M., Burrill, R., Mathiowitz, E., 2003. Enhancing the oral bioavailability of the poorly soluble drug dicumarol with a bioadhesive polymer. J. Pharm. Sci. 92, 1677–1689.
Tho, I., Sande, S.A., Kleinebudde, P., 2003. Disintegrating pellets from a water-insoluble pectin derivative produced by extrusion/spheronization. Eur. J. Pharm. Biopharm. 56, 371–380.
Vyas, S.P., Kannan, M.E., Jain, S., Mishra, V., Singh, P., 2004. Design of liposomal aerosols for improved delivery of rifampicin to alveolar macrophages. Int. J. Pharm. 269, 37–49.
Wilding, I.R., Coupe, A.J., Davis, S.S., 2001. The role of γ-scintigraphy in oral drug delivery. Adv. Drug Deliv. Rev. 46, 103–124.
Woodley, J., 2001. Bioadhesion new possibilities for drug administration? Clin. Pharmacokinet. 40, 77–84.