

Full Length Research Paper

Rifabutin loaded floating gellan gum beads: *In vitro* and *in vivo* evaluation

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Rifabutin loaded floating gellan gum beads were prepared by ionotropic gelation in acidic medium. Buoyancy to the beads was attributed to the use of gas-generating agent, which produced an extremely porous internal structure lighter than gastric juice. Beads exhibited excellent buoyancy and remained buoyant up to 18 h. Drug incorporation efficiency of the beads was found to be dependent on the concentration of Ca^{++} and gellan gum whereas drug release was dependent only on Ca^{++} concentration. When administered orally to albino rats, beads floated on the gastric juice and released the drug into the stomach. Furthermore, blood samples were withdrawn periodically and rifabutin concentrations in the blood were determined by high performance liquid chromatography (HPLC). Data obtained in this study demonstrated that floating beads bearing rifabutin were capable of stomach specific delivery of drug for longer period with increased bioavailability compared to pure drug as well as with non-floating drug containing beads. Overall, gellan gum loaded floating beads have a promising potential to treat multidrug resistant *Helicobacter pylori* infection.

Key words: Gellan gum, rifabutin, floating beads, *Helicobacter pylori*.

INTRODUCTION

Multiparticulate systems obtained by ionotropic crosslinking of carbohydrate polymers like alginates, carrageenan, xyloglucans, chitosan, gellan gum etc have been used to develop floating drug delivery systems. Various approaches (Whitehead et al., 2000; Iannuccelli et al., 1998; Sriamornsak et al., 2004) to induce buoyancy in crosslinked beads have been used. The use of sodium bicarbonate as buoyancy imparting agent to produce floating beads is simplest among the various approaches (Bussmer et al., 2003; Bulgarelli et al., 2000) and has been attempted successfully by many workers. Their floating property was based on the evolution of CO_2 when in contact with acidic environment followed by the ability of polymer gel to entrap it, which decreases their density below one. These beads have been used to achieve prolonged gastric residence time, For sustained

release / stomach specific drug delivery (Murata et al., 2000; Rajnikanth et al., 2007; Kedziericz et al., 1999; Ostberg et al.1994)) providing an opportunity for both local and systemic drugation.

Rifabutin, an antimycobacterial compound active against *Mycobacterium avium* and multidrug resistant *Mycobacterium tuberculosis* strains was selected as a model drug (Parente et al.,2003). It is incompletely absorbed (Martindale, 34th edition, 2005) after oral administration, leading to poor bioavailability. Recent literature has shown that it also possess very good activity against multidrug resistant *Helicobacter pylori* infection conferring up to 90% clinical Cure rates. Earlier literature showed that delivery systems of rifabutin viz. liposomal formulations, micro-spheres etc. were developed and were characterized for non- localized action (Gaspar et al., 2008), but for treatment of *H. pylori* and other localized infections, rifabutin formulations are not developed sofar. In view of the above facts, an attempt has been made to develop and characterize a floating gastro retentive drug delivery system, which not only

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Table 1. Formulation variables of floating beads of rifabutin.

Batch code	Gellan gum (%w/v)	Rifabutin (%w/w)	NaHCO ₃ (%w/v)	CaCl ₂ (%w/v)
A1	1	1	0.4	3
A2	1	1	0.4	4
A3	1	1	0.4	5
A4	2	1	0.4	3
A5	2	1	0.4	4
A6	2	1	0.4	5
A7	3	1	0.4	3
A8	3	1	0.4	4
A9	3	1	0.4	5

improves the bioavailability but is also suitable for stomach specific delivery of rifabutin.

MATERIALS AND METHODS

Rifabutin was a kind gift from Simpex Pharma Pvt Ltd, India. Deacetylated gellan gum (Kelcogel) was obtained as a gift sample from CP Kelco UK Ltd., Surrey, U.K. HPLC grade Water was purchased from Quilagens, India. All other chemicals purchased were of AR grade.

Preparation of floating beads

Floating beads, whose composition is shown in Table 1, were prepared by Ca⁺⁺ induced ionotropic gelation method (Srinatha and Pandit, 2008; Rajnikanth et al., 2007). Briefly, gellan gum solutions (1, 2 and 3% w/v) were prepared by dispersing the gum in hot (70°C) deionized water and stirred until solution was formed followed by addition of sodium bicarbonate as a gas generating agent (0.4% w/v) with continuous stirring. Rifabutin was dispersed into this solution at 46°C. Prepared bubble-free gellan-rifabutin dispersion was added drop wise with the help of a syringe fitted with a blunt-end needle (21G) to a solution containing 10% v/v acetic acid and different concentrations of calcium chloride. After curing, the separated beads were washed with cold deionized water until free from surface calcium and dried in an oven at 45°C for 6 h and then kept in a desiccator overnight.

Chromatographic system

The mobile phase consisted of 0.05 M potassium dihydrogen phosphate and 0.05 M sodium acetate (pH adjusted to 4.0 with acetic acid): acetonitrile (53:47, v/v with a flow rate of 1 ml/min at 25°C. The HPLC system consisted of a UV detector set to 275 nm. The analytical column was a Pursuit XRs C-8 column (USP-L7) with a flow rate of 1 ml/min at 25°C. Standard solution of rifabutin (100 µg/ml) was prepared by weighing the appropriate amount of bulk rifabutin and dissolving it in mobile phase. Further stock solutions were made by diluting the initial stock standard solutions with mobile phase. Seven-point calibration curve ranging from 1 to 7 µg/ml was used for the quantification of rifabutin. A stock solution of 1.0 µg/ml was stored at -30°C and a sample of this stock solution was always injected together with the analyzed samples to verify the precision of the obtained concentrations of rifabutin in samples and controls from their peak area concentration response.

Determination of drug incorporation efficiency

The drug incorporation efficiency of each formulation was determined by extracting first rifabutin from an accurately weighed quantity (100 mg) of beads using 0.01 M HCl (pH 2.0) and quantifying the amount of drug by HPLC method as earlier described.

Drug-polymer interactions

Fourier transform infrared spectroscopy (FTIR) was employed to study drug-polymer interactions. The spectra were recorded for pure drug, polymer and drug loaded beads using FTIR (Shimadzu, 8400S). Samples were prepared in KBr Disks (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm⁻¹ and the resolution was 2 cm⁻¹.

In vitro buoyancy of the beads

In-vitro study was performed using a USP dissolution apparatus (Electrolab, Mumbai) type II. Prepared beads were dispersed in 500 ml of 0.01 M HCl (pH 2.0) at 37±1°C with continuous agitation at 50 rpm with a paddle type apparatus. After that, floating beads were collected separately from the immersed beads.

The floating percentage was estimated as described by earlier workers (Srinatha and Pandit, 2008; Rajnikanth et al., 2007; Choi et al., 2002).

Morphology and bead size analysis

Size of prepared beads was determined using an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. Twenty dried beads were measured for calculating the mean diameter of beads. All readings are average of three trials ±S.D. The shape, surface morphology and internal structure of the dried beads were studied by scanning electron microscope (Leo 435VP, Variable pressure, Oxford, U.K.) at various magnifications.

In vitro drug release studies

In-vitro release of rifabutin from the beads was performed in USP dissolution apparatus (Electrolab, Mumbai) type II at 50 rpm in 500 ml 0.01 M HCl (pH 2.0) at 37±0.5°C. At predetermined intervals, 1 ml aliquot was withdrawn and replenished with an equal volume of fresh dissolution medium (USP31/NF26, Vol-3, Asian edition,

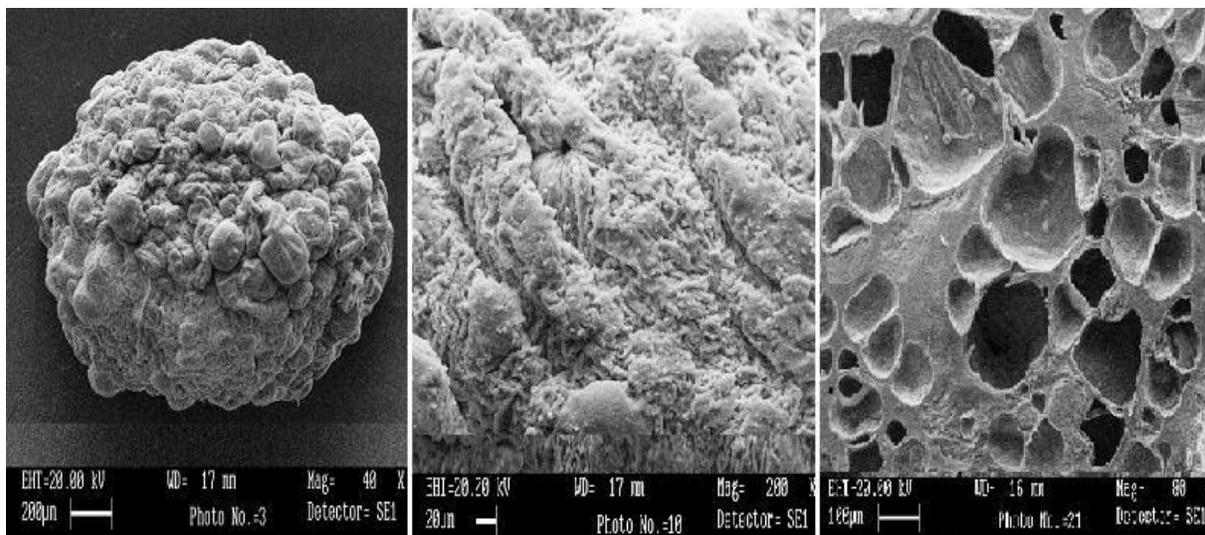


Figure 1. Scanning electron microscope images (a) shape of rifabutin loaded floating bead, (b) surface morphology, (c) transverse section.

2008). Withdrawn samples were analyzed by HPLC method as described earlier.

Animal studies

Albino rats (Male 6 to 8 weeks old, weighing approximately 200 g) were obtained from the animal house of the institute and divided into three groups (n=3). The animals were acclimated to the housing facilities for 5 days before initiation of the study. Free access to standard pellet chow was allowed throughout the experimental protocol. A single dose of rifabutin (10 mg) as 0.1%w/v dispersion in gellan gum was administered orally to albino rats (group I), whereas group II and III received floating and nonfloating beads bearing equivalent rifabutin.

The Animal Care and Use Committee of College of Pharmacy, IFTM, Moradabad India where the study was conducted approved all protocols.

Rifabutin levels in blood were determined by HPLC after an extraction procedure according to Battaglia et al. (1991) and Benedetti et al. (1995) with adaptations. Briefly, 500 µl of blood were mixed with 250 µl of mobile phase and extracted twice with 1 ml of a dichloromethane: isooctane mixture (2:3 v/v) under stirring (15 min), followed by a centrifugation step at 1200 x g for 10 min. The organic extracts were pooled and evaporated to dryness under nitrogen. The residue was dissolved in 1500 µl of mobile phase, filtered and then injected into the HPLC system. The pharmacokinetic parameters, namely the extent of absorption ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}) and time to reach maximum concentration (T_{max}) were calculated from the individual plasma-drug concentration data. C_{max} and T_{max} were obtained directly from the plasma values whereas ($AUC_{0-\infty}$) was obtained by adding the area under plasma drug concentration-time curve from time 0 to the last measurable concentration (AUC_{0-t}).

Mechanism of drug release

In-vitro data were fitted to Higuchi's square root model $Q=K\sqrt{t}$ (Higuchi, 1963) to analyze the kinetics of drug release from the prepared beads, where Q is the amount of the drug released in time

"t", K is the release constant of respective equation. Further, data were fitted to Korsmeyer-Peppas's (Korsmeyer et al., 1983; Peppas, 1985) power law equation.

$$Mt/M_{\infty} = Kt^n \text{ ----- (1)}$$

Where Mt/M_{∞} is the fraction of drug released in time "t", K is the structural and geometric constant, and n is the release exponent.

Statistical analysis

All the data were analyzed by student's t test and one-way ANOVA to determine the statistical difference in the results. A probability value $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In vitro buoyancy of beads

There was no lag time as the beads floated immediately when placed in 0.01 M HCl for up to 18 h, which corroborated well with the density of beads. The propensity of beads to exhibit buoyancy depends on the true density of the fabricated beads. The density of beads was in the range of 0.7013 ± 0.068 to 0.8411 ± 0.056 g/cm³ (data not shown), that is less than the density of the gastric juice (approx. 1.004 g/cm³), which was in agreement with the previous work of Soppimath et al. (2001), that fabricated beads would exhibit excellent buoyancy *in vivo*.

Morphological properties and size of prepared beads

The scanning electron micrographs of the beads were recorded to study the shape, the surface and the cross-sectional morphologies of the floating beads as shown in

Table 2. Physiochemical characteristics of floating beads of rifabutin.

Batch code	Bead diameter (mm)	Incorporation efficiency (%)	Buoyancy (%)	n value
A1	1.26±0.042	40.32±1.37	98.77±1.79	0.53
A2	1.22±0.061	44.71±1.41	96.71±2.02	0.54
A3	1.20±0.046	48.89±1.34	94.67±1.97	0.41
A4	1.46±0.022	46.13±1.27	97.13±1.79	0.41
A5	1.42±0.054	48.69±1.34	95.34±2.13	0.41
A6	1.40±0.052	53.87±1.33	92.41±1.88	0.42
A7	1.52±0.031	48.62±1.28	95.77±1.76	0.45
A8	1.49±0.033	53.87±1.31	94.41±1.98	0.46
A9	1.48±0.021	60.68±1.24	90.24±1.71	0.46

Figure 1. All the rifabutin loaded floating beads were found discrete and spherical (except A1 and A2) in shape with rough outer surfaces and large internal pores (Figure 1). The large number of observed pores appears to be directly related to the presence of sodium bicarbonate, which was in agreement with that of Choi et al. (2002). The bead diameter varied from 1.20 to 1.52 mm (Table 2). As the amount of gellan gum in the beads was increased, the mean diameter also increased ($p < 0.05$) at low Ca^{++} concentration. However, upon increasing the Ca^{++} concentration, there was a reduction in the mean size of the beads when compared to the beads obtained at low Ca^{++} concentration. This could be attributed to more extended gel bead shrinkage during gelation by Ca^{++} , which is consistent with the work of Sriamornsak et al. (1999).

Incorporation efficiency of floating beads

The incorporation efficiency of fabricated beads was in the range of 40 to 60% (Table 2). Overall, the incorporation efficiency of fabricated beads was low. This could be due to the use of sodium bicarbonate as gas-generating agent which when reacted with acetic acid released CO_2 . The released CO_2 entrapped in the gel network in turn increasing the porosity, resulted in the decrease in the strength of the bead wall. This caused the drug diffuse out into the coagulation fluid without any hindrance causing lesser entrapment.

Drug-polymer interaction

Infrared spectra of drug, polymer and beads were recorded and compared. FTIR spectrum of pure rifabutin (Figure 2) exhibited bands appearing at 2931.60 and 2958.60 cm^{-1} due to asymmetric and symmetric $-\text{CH}$ stretching. The C-H deformation of CH_2 appeared at 1458.08, 1421.44 and 1373.22 cm^{-1} . Stretching vibration of $\text{C}=\text{C}$ appeared at 1508.23 and 1600.81 cm^{-1} . The band at 1560.3 and 1647.10 cm^{-1} are due to N-H deformation of 2° amine. The band at 1670.24 and 1730.03 cm^{-1} are due to the stretching vibrations of $\text{C}=\text{O}$ group, while those

appearing at 1247.86 and 1340.43 cm^{-1} are of phenolic C-O stretching. The C-O stretching of 2° alcohol appeared at 1118.64 cm^{-1} . The FTIR spectrum of pure gellan gum (Figure 2) exhibited bands at 1627.81 and 1409.87 cm^{-1} due to asymmetric and symmetric stretching of carboxylate group. The band at 2927.74 cm^{-1} is due to the stretching vibrations of $-\text{CH}_2$ group, while those appearing at 1153.35 and 1024.13 cm^{-1} are due to ethereal and hydroxylic C-O stretching. The band at 3427 cm^{-1} is due to the presence of OH^- group of glucopyranose ring. FTIR spectrum of rifabutin loaded gellan gum beads showed no evidence of interaction as all the major peaks of rifabutin found intact or exhibited very minor shift in frequencies.

In vitro drug release studies

Dissolution rates of the beads prepared with three calcium chloride concentrations (Figure 3) viz. 3, 4 and 5% studied in 0.01 M HCl (pH 2.0) showed that the drug release from all the batches of beads was very fast and 48 to 69% drug released within first hour. The gelation and aggregation of gellan gum occurs through a chemical bonding between calcium and carboxylic groups in the gellan chains (Kanesaka et al., 2004). Calcium being a hard electrophile interacts with carboxylate group of gellan gum through electrostatic attractive interactions.

Such interactions tend to readily hydrated and thus broken under aqueous conditions resulting in Rapid release of drug from the beads (Mutasem et al., 2008).

Effect of gellan gum concentration on drug release

It is imperative that, as the concentration of gellan gum was increased; more COO^- group side chains would be available for the formation of a stronger gellan- Ca^{++} network. Therefore, it was expected that with increasing gellan concentration, the drug release would be slower; however, this was not evident with fabricated beads. This could be because reaction between gellan gum and Ca^{++} carried out at acidic pH and the degree of ionization of

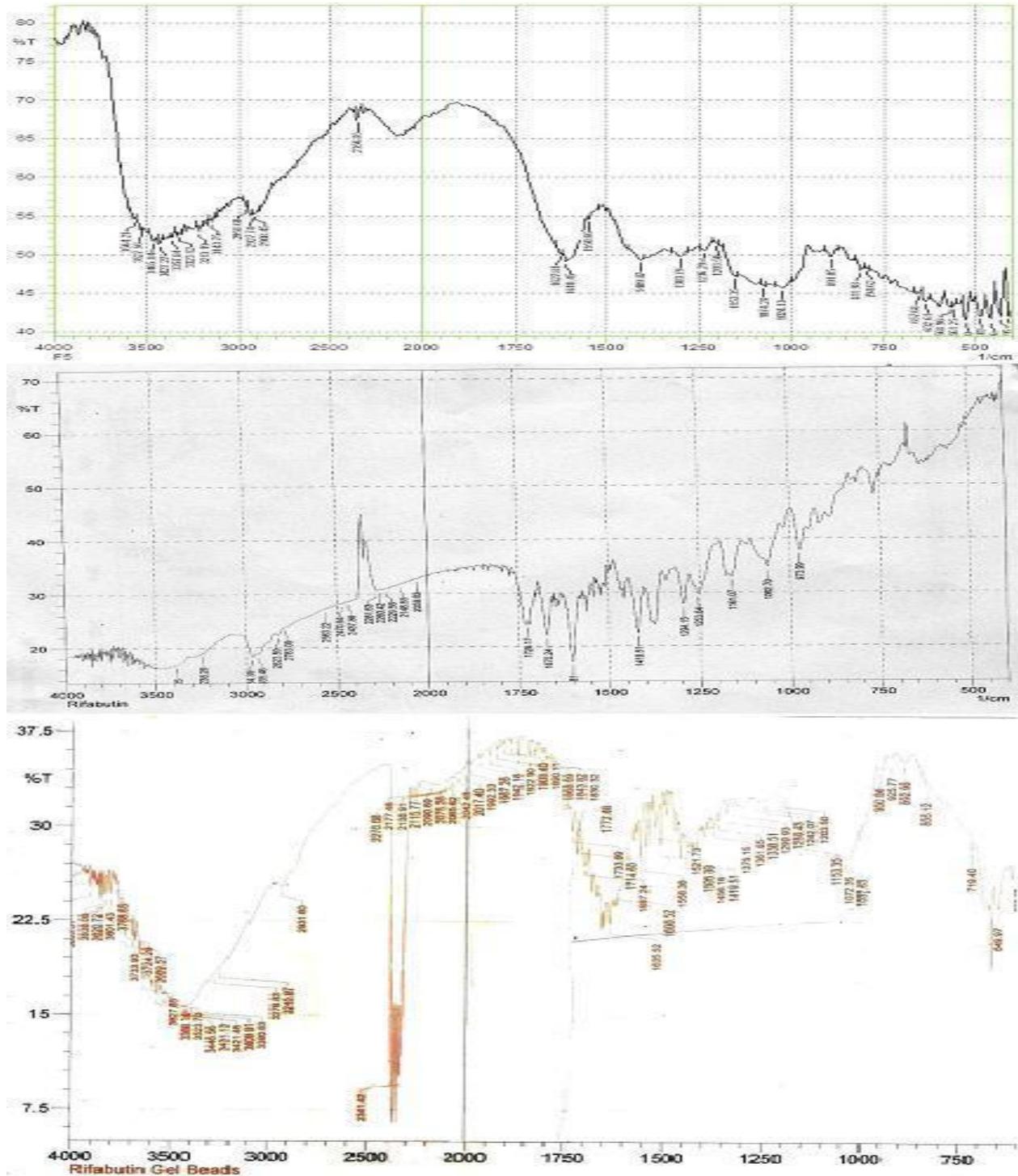


Figure 2. FTIR spectra of pure gellan gum, rifabutin and rifabutin loaded gellan beads.

COO⁻ group on gellan gum droplets depends on the pH of the coagulation media. At low pH, Ca⁺⁺ are less likely to crosslink and form a dense matrix due to less availability of COO⁻ groups since gellan droplets have lower anionic character at acidic pH that could not retard the drug release from the beads.

Drug release mechanism

The correlation coefficient (r^2) values for various equations viz., Q vs. t, log cumulative percentage drug release vs. t, and Q vs. $t^{1/2}$ (data not shown) suggest that the drug release from fabricated beads predominantly followed

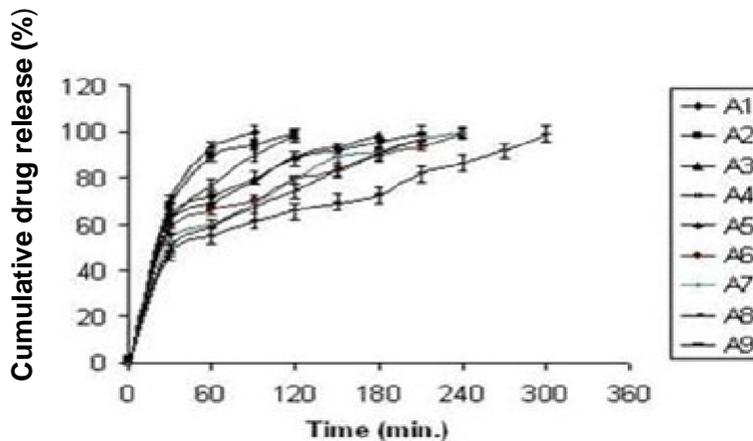


Figure 3. *In vitro* rifabutin release from floating beads in 0.01M HCl (pH 2.0).

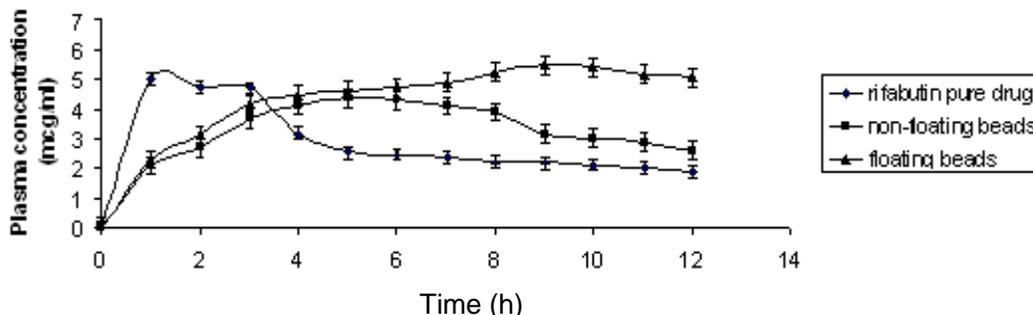


Figure 4. Mean plasma drug concentration ($\mu\text{g/ml}$) against time (h) profile after oral administration of rifabutin bearing floating, non-floating beads and pure drug dispersion.

Higuchi's square root of time kinetics as the values for Q vs. $t^{1/2}$ were always higher than other equations. The *in vitro* drug release data were fitted in exponential equation $M_t/M_\infty = Kt^n$. The drug release from the fabricated beads followed anomalous non-Fickian mechanism as indicated by the release exponent values (n).

Animal studies

After administered orally to albino rats ($n=3$), rifabutin loaded floating beads released the drug in the stomach by floating on the gastric juice. Their capacity to float in the stomach of the albino rats was confirmed visually upon excision. Figure 4 shows the mean plasma concentration-time profile after oral administration of 10 mg rifabutin as drug dispersion in gellan gum (0.1%w/v) in demineralized water, equivalent rifabutin encapsulated in non-floating and floating gellan gum beads. Statically significant differences ($p < 0.05$) were observed among the among the T_{max} values of floating, non-floating and pure drug dispersion, that were 9 ± 1.1 , 5 ± 1.2 and 1 ± 0.12 h for the formulations in the same order. The C_{max} values

for floating beads, non-floating beads and pure drug dispersion were 5.47 ± 0.8 , 4.38 ± 1.1 and 5.01 ± 1.3 $\mu\text{g/ml}$ respectively. The area under the drug plasma concentration-time curve (AUC_{0-t}) for drug encapsulated in floating, non-floating beads and pure drug dispersion estimated to be 49.45, 39.65 and 34.43, which were not only statistically different with each other but also clearly showed the superiority of rifabutin loaded floating beads in improving the bioavailability of rifabutin.

Conclusion

Floating beads bearing rifabutin were prepared by using gellan gum as a matrix. To assess the usefulness of intragastric floating properties of floating beads in sustained pharmacological action of the drug and improved bioavailability, non-floating beads bearing equivalent amount of rifabutin were also prepared. *In vitro* drug release studies carried out in 0.01 M HCl (pH 2.0) showed rapid drug release from floating beads; however, animal studies showed prolonged drug release from the floating beads. Data obtained in this study demonstrated that

that floating beads bearing rifabutin were capable of sustained delivery of drug for longer period with increased bioavailability. The floating gellan gum beads appear to be a promising vehicle for delivering rifabutin specifically to the gastric region in therapy of diseases in which a gastric-mucosa-specific drug delivery regimen is required, such as *H. pylori* infection.

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