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Design and Evaluation of Floating-Bioadhesive Drug Delivery System for Risedronate Sodium to Improve the Oral Bioavailability

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Abstract

The objective of the present research work was to formulate and evaluate risedronate sodium floating-bioadhesive tablets (RSFBT) to increase the gastric residence time, and further compare their pharmacokinetics with conventional immediate release tablets. The RSFBT were prepared with combination of Gelucire 43/01 and Sodium CMC/ Gelucire 54/02 and CP 971P by direct compression method. The prepared RSFBT were evaluated for hardness, thickness, weight variation, friability, drug content, *in vitro* buoyancy and drug release. One of the optimized formulations (RSF10) floated with a lag time of 52.6±4.8 sec, duration of floating 12 h and released about 99.25±1.85 % of drug in 12 h, and then followed the non-Fickian diffusion release mechanism with n value of 0.635. The RSF10 of RSFBT loaded with $BaSO_4$ remained in stomach for 5.00 ± 0.86 h (n=3) in radiological studies. The formulation, RSF10 exhibited maximum bioadhesive strength (1.139±0.073 N) than other formulations. The bioavailability studies were carried out for RSF10 and compared with that of reference IR tablets, Actonel in eight healthy human volunteers. Based on *in vivo* pharmacokinetic study, significant difference was observed between C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$, and MRT of RSF10 and Actonel IR tablets. The increase in relative bioavailability of RSF10 was 1.59 fold when compared to reference IR tablets. The increased relative oral bioavailability may be due to the floating-bioadhesive mechanism of dosage form, which is desirable for drugs absorbed from the upper part of gastrointestinal tract.

Keywords

Risedronate sodium, floating-bioadhesive tablets, *in vivo* gastric residence time, *ex vivo* bioadhesion, bioavailability study.

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INTRODUCTION

The major challenge in the development of an oral sustained release drug delivery system is not just to sustain the release of drug but also to prolong the presence of the dosage form within the gastrointestinal tract (GIT) until all the drug is completely released at the desired period of time [1]. Gastro-retentive drug delivery systems have gained significant interest in the past few decades. Most of the conventional oral delivery systems have shown some limitations related to fast gastric-emptying time [2].

Garg and Gupta [3] classified the gastro-retentive dosage forms into four main classes: (A) floating systems [4], (B) expandable systems [5], (C) bioadhesive systems [6] and (D) high density systems [7]. Floating systems are of two types: (i) effervescent systems, depending on the generation of carbon dioxide gas upon contact with gastric fluids, and (ii) non-effervescent systems. The latter systems can be further divided into four sub-types, including hydrodynamically balanced systems [8], microporous compartment systems [9], alginate beads [10] and hollow microspheres or microballons [11]. In addition, super-porous hydrogels [12] and magnetic systems [13].

In floating dosage forms (FDs), the dosage form remains buoyant on the gastric fluid when the stomach is full. However, as the stomach empties and the tablet reaches the pylorus, the buoyancy of the dosage form may be reduced. It may be due to passage of the dosage form through the pylorus into the small intestine. Thus, the buoyancy of floating dosage form in the stomach may be limited to only 3–4 h. Furthermore, FDs do not always release the drug at the intended site. In a bioadhesive drug delivery system, the mucous secreted by the mucosa lining of stomach wall may detach the drug from stomach wall due to high mucous turnover. Then the detached tablet may emptyed from the stomach along with its contents [14]. A floating-bioadhesive drug delivery system (FBDDS) would overcome these drawbacks of floating and bioadhesive systems and would have a significant effect on improving the therapeutic effect of the drug involved [15].

Risedronate sodium (RS) is a potent pyridinyl bisphosphonate that binds to bone hydroxyapatite and inhibits osteoclast-mediated bone resorption. In preclinical studies, risedronate demonstrated potent anti-osteoclast and anti-resorptive activity, increasing bone mass and biomechanical strength. It is a third generation bisphosphonate and is relatively rapidly absorbed from the upper gastrointestinal (GI) tract with a short biological half-life of 1.5 h [16]. Due to these characters it is considered as a potential candidate for development of floating-bioadhesive drug delivery system.

The present investigation involved the preparation, *in vitro and in vivo* evaluation of risedronate sodium floating-bioadhesive tablets (RSFBT) by effervescent technique using a release retarding polymers, Sod CMC/Gelucire 43/01 and CP 971P/Gelucire 54/02, and calcium carbonate was used as gas former. The prepared tablets were evaluated for the physical characters such as *in vitro* buoyancy studies, drug release, swelling index, *ex vivo* bioadhesion study and *in vivo* radiological studies. Further, one of the optimized formulation was subjected to bioavailability study in healthy human volunteers.

MATERIALS AND METHODS

Materials

Risedronate sodium (RS) was received as generous gift sample from M/s Hetero Drugs Ltd., Hyderabad, India. Sodium carboxy methyl cellulose (SCMC) and Carbopol (CP 971P) was received as gift sample from M/s Aurobindo Pharma. Ltd., Hyderabad, India. Gelucire 43/01 and Gelucire 54/02 were purchased from Gattefose Pvt. Ltd., Mumbai, India. Sodium bicarbonate, microcrystalline cellulose (Avicel PH102), magnesium stearate (MS) and talc were purchased from S D Fine Chem Ltd., Mumbai, India. All other reagents used were of analytical grade.

Methods

Determination of acid stability of a drug in 0.1 N HCl

Stock solution of risedronate sodium was prepared in 0.1 N HCl in order to determine its acid stability. At predetermined time points like 1, 2, 3, 4, 6, 8, 10, 12 and 24 h, the samples were assayed using UV-Visible spectrophotometer at λ_{max} 261 nm to see whether there is any change in the absorbance and concentration in the prepared stock solutions.

Preparation of floating-bioadhesive tablets of risedronate sodium

Gelucire (43/01 or 54/02) was melted in a large china dish at 70°C and the required quantity of risedronate sodium was added to melted mass. Previously prepared geometric mixture of Sod. CMC/CP 971P and sodium bicarbonate was added to drug - gelucire mixture and stirred well to mix. This mass was removed from a hot plate and subjected to scrapping until it attained room temperature. The coherent mass was passed through # 22 and the resulting granules were passed through # 44 to separate fines. The granules were collected and mixed with magnesium stearate and talc and the composition is shown in table 1. The hardness is adjusted to 5

kg/cm^2. The lubricated blend was compressed using 16 station punching machine.

Evaluation of tablets

Physical characterization of prepared floating-bioadhesive tablets

The prepared floating-bioadhesive tablets of risedronate sodium were evaluated for uniformity of weight using 20 tablets [17], hardness (Monsanto tester) using 5 tablets, thickness (vernier caliperse) using 5 tablets, friability (Roche friabilator) using 10 tablets [18], drug content using 10 tablets, in vitro buoyancy using 3 tablets and in vitro dissolution studies using 3 tablets. The results were expressed as mean ± S.D in Table 2.

***In-vitro* buoyancy studies**

The *in vitro* buoyancy was characterized by floating lag time (FLT) and total floating time (TFT). The test was performed using United States Pharmacopeia (USP 24) type-2 apparatus using 900 mL of 0.1N HCl with a paddle rotation of 50 rpm at 37°C ± 0.5°C. The tablets of risedronate sodium floating-bioadhesive were placed in dissolution vessels and the time required for the tablet to rise to the surface of the dissolution medium and the duration of time the tablet constantly floated on the dissolution medium were noted as FLT and TFT, respectively [19].

***In-vitro* dissolution studies**

The *in vitro* drug release of risedronate sodium floating-bioadhesive tablets (RSFBT) were conducted using USP 24 type-2 apparatus (Electrolab, TDT-06T). The dissolution test was performed using 900 mL of 0.1N HCl (pH 1.2), at 37 ± 0.5°C and 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at pre-determined time intervals, and replaced with fresh dissolution medium. The samples were filtered through a 0.45-µm membrane filter and diluted to a suitable concentration with 0.1N HCl. Absorbance's of these samples were measured at 261 nm using UV/Visible spectrophotometer (Elico, SL 210, India).

Analysis of drug release kinetics

The drug release profiles all the formulations were fitted to different kinetic models to explain the release of risedronate from the floating-bioadhesive tablets. The model with the highest correlation coefficient (R^2) was considered to be the best fitting one. In the present study, the *in vitro* drug release profiles were subjected to zero-order [20], first-order [21], Higuchi [22] and Korsmeyer-Peppas [23] kinetic models.

Zero-order: $Q_t = Q_0 + k_0 t$ (1)

First order: $\log C = \log C_0 - k_1 t/2.303$ (2)

Higuchi: $Q_t = k_2 t^{1/2}$ (3)

$Q_t/Q_\infty = kt^n$ (4)

Where Q_0, Q_t and Q_∞ are the amounts of drug dissolved at zero time, at t time and at ∞ time. The C_0 and C are the concentrations of the drug at zero time and at time t, and k_0, k_1, k_2 and k refer to the rate constants obtained from the linear curves of the respective models, and n refers to the release exponent indicative of the mechanism of drug release. If the value of n is 0.5 or less, the release mechanism follows Fickian diffusion, while the higher values ($0.5 < n < 1$) indicates a non- Fickian model (anomalous transport). The non-Fickian model corresponds to coupled diffusion/polymer relaxation. If the n-value is 1, the drug release follows zero order and case II transport. The case II transport generally refers to the dissolution of polymeric matrix due to the relaxation of the polymer chain. However, the mechanism of drug release is regarded as super case-II transport if n values are higher than 1 [24]. This mechanism could result from increased plasticization at the relaxing boundary, i.e., gel layer.

Physical stability studies

Physical stability studies were conducted according to International Conference on Harmonization (ICH) guidelines [25]. One of the optimized formulations of risedronate sodium floating-bioadhesive tablet was enclosed in polyethylene bottle and placed in a desiccator containing saturated sodium chloride solution (75% RH). The desiccator was stored at 40°C for 3 months. At predetermined time intervals, the tablets were examined for hardness, FLT, TFT, drug content and drug release. Finally, the tablets were tested for any statistical difference using the Students paired t-test, the differences were considered to be significant at $p < 0.05$.

***In vivo* radiographic studies**

$BaSO_4$ was used to make the tablet X-ray opaque. For *this* study, $BaSO_4$ was loaded in optimized formulation of risedronate sodium floating-bioadhesive tablets (RSF10) with following composition: risedronate sodium 35 mg, Gelucire 54/02 35 mg, CP 971P 50 mg, $BaSO_4$ 45 mg, $NaHCO_3$ 30 mg, Avicel PH102 99 mg, magnesium stearate 3 mg and talc 3 mg. The tablets were prepared by direct compression method.

In this study, x-ray technique will be used to determine the gastric residence time of gastroretentive tablets. To make the tablet X-ray opaque, $BaSO_4$ was used. Three healthy male volunteers will participate after giving an informed written consent. The subjects weighed in between 62-74 kg, in height from 167-172 cm, and in the age group of 23-27 years. The study was conducted under the guidance of an expert radiologist. After

overnight fasting, the volunteers were fed with low calorie food (100 g of bread). Half an hour later, a $BaSO_4$-loaded optimized formulation (RSF10) was given to every volunteer with a glass of water. During the study, the subjects were not allowed to eat but water was made available *ad libitum*. At different time intervals like, 0.5, 2.5, 4.5 and 5.5 h, the volunteers were exposed to abdominal x-ray imaging in a standing position [26]. The distance between source of x-rays and the subject was kept constant for all images. Thus, the observation of the tablet movements could be easily noticed. The mean gastric residence time was calculated.

***Ex-vivo* bioadhesion study**

The bioadhesive strength of risedronate sodium floating-bioadhesive tablets were determined using an ultra-test (Mecmesin, West Sussex, UK) equipped with a 5-kg load cell. For this study, porcine gastric mucosa will be obtained from slaughter house. The mucosal membrane was excised by removing the underlying connective tissue and was secured tightly to a circular stainless steel adapter of a diameter 2.2 cm provided with the equipment. The tablets of risedronate sodium floating-bioadhesive was placed over another cylindrical stainless steel adaptor of similar diameter. The tablet with a backing membrane was adhered on to it using a solution [27] of cyanoacrylate adhesive. During the study, 100 µl of 1% w/v mucin solution was used to moisten the porcine gastric mucosal membrane. The upper support was lowered at a speed of 0.5 mm/s until contact was made with the tissue at the predetermined force of 0.5 N for a contact time of 180 sec. At the end of the contact time upper support was withdrawn at a speed of 0.5 mm/s to detach the membrane from the tablet. The peak detachment force was expressed as mean ± SD in triplicate for all the formulation.

Comparative bioavailability study

Subjects: The mean age of volunteers was 23±2 years, mean height was 167.5 ± 8.5 cm, and mean body weight was 65.4±8.6 kg. Eight healthy volunteers for risedronate sodium floating-bioadhesive tablets were selected for the study. Before starting the study, each candidate signed an informed consent form. They were judged to be healthy based on medical history, physical examination, haematological and biochemical laboratory tests. The bioavailability protocol was approved by an Institutional Ethics Committee, TPCP, Warangal, India.

Study design: A single dose, randomized, two-way cross-over study was designed with eight subjects in each treatment group. A one-week washout period existed between treatments of the study. After overnight fasting, in three study periods for each subject the assigned formulation (35 mg of Floating-bioadhesive RSF10/ Actonel immediate tablets) was administered orally with 240 ml of water.

One week before and during the study, they were not allowed to take alcohol or any other medication. The subjects fasted overnight and 5 hrs after tablet administration, but water was made available *ad libitum*. Study medication was administered according to randomization schedule. Subjects received standard meals after 5 hrs of tablet administration. Blood samples were collected at predetermined time intervals such as 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h. Blood samples (5 ml) were obtained from forearm vein using sterile disposable needle and collected into 10 ml sterile test tubes. The samples were centrifuged immediately at 4000 rpm for 15 min. and the separated plasma was transferred into 2.5 ml of Eppendorf tubes and stored at −80° C till the time of analysis.

Chromatographic conditions: The serum concentrations were determined by reverse phase HPLC equipped with a pump model, Shimadzu, LC-10AT and an SPD-10A detector. Mobile phase was prepared with Buffer: Methanol (85:15 v/v), pH 6.75 adjusted with sodium phosphate, etidronate disodium hydrate and disodium EDTA and pumped isocratically at 1 mL/min through a Hibar, Lichrosphere (5 µm, 250 × 4.6 mm) column. The UV-Visible detector was adjusted to 262 nm.

Extraction procedure: About 5 ml of blood samples were collected from the volunteers at different time points, which were centrifuged at 4000 rpm for 15 min and serum was collected. About 150 µl of serum was diluted with 1.4 ml of double distilled water and it was deproteinized by the addition of 450 µl of trichloro acetic acid (TCA, 10%). This mixture was centrifuged at 1500 rpm for 15 min. The supernatant was collected 50 µl of 1.25 M calcium chloride and 57 µl of 30% w/v NaOH were incorporated to increase the precipitate formation [28]. Then the samples were mixed and centrifuged at4500 rpm for 10 min. The precipitate was collected and dissolved in 400 µl of 1M Hydrochloric acid, 25 µl of 1.25 $CaCl_2$ and 90 µl of 30% NaOH. The samples were centrifuged for 10 min at 4500 rpm and then the precipitate formed was dissolved in 80 µl of 0.025M disodium EDTA and 920 µl of mobile phase, mixed and about 20 µl sample was injected into the HPLC column.

Pharmacokinetic analysis: The pharmacokinetic parameters of test formulation (RSF10) and reference formulation (Actonel) were estimated for each volunteer by using a computer programme,

Kinetica 2000 (Version 3.0, Innaphase Corporation, Philadelphia, USA). Non-compartmental analysis was used to calculate pharmacokinetic parameters, C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$ and MRT values. C_{max} and t_{max} were read directly from the observed mean plasma drug concentration against time profile. AUC_{0-t} was calculated by the trapezoidal rule and the total $AUC_{0-\infty}$ was calculated according to the equation.

$$AUC_{0-\infty} = AUC_{0-t} + C_t/K_E$$

Where, Ct is the last measurable concentration and K_E is the elimination rate constant obtained from terminal log-linear portion of the plasma concentration-time profile [29]. The mean residence time (MRT) was calculated using following equation.

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$$

Where, AUMC is the area under the first moment of the concentration time curves.

The extent of absorption $AUC_{0-\infty}$ from the test formulation relative to the marketed one was calculated as the relative bioavailability.

RESULTS AND DISCUSSION

Stability of drug in 0.1 N HCl

The concentration of the drug was noted in acidic medium (0.1N HCl) for 24h and found to have no degradation (Figure 1). Therefore, the drug was found to be stable in gastric environment.

Physical characterization of RSFBT

The tablets were prepared by direct compression method and evaluated for their physical characters. The tablet weights ranged between 298.40±6.88 and 303.45±7.23 mg, the hardness varied between 5.92±0.23 and 6.21±0.44 kg/cm^2, thickness between 5.42±0.07 and 5.62±0.07 mm and friability ranged between 0.28 and 0.42 %. The drug content of all the formulations varied between 98.55±1.65 and 101.30±1.70 %. Thus, the physical parameters of the prepared tablets were within pharmacopoeial limits.

In vitro buoyancy

All the formulations were prepared by melt granulation technique and sodium bicarbonate was used as a gas generating agent. Formulations RSF1-RSF6 prepared with combination of Gelucire 43/01 and Sodium CMC floated with a lag time of 46.6±3.5 (RSF1) to 70.2±6.2 (RSF6) sec. Formulations RSF7-RSF12 prepared with combination of Gelucire 54/02 and CP 971P showed a floating lag time of 38.5±2.5 (RSF7) to 64.0±5.5 (RSF12) sec. Tablets of all formulations showed good in vitro buoyancy with maximum floating lag time of 70.2±6.2 sec (Table 2), The floating lag time slightly changed by increasing either of the components Gelucire and Polymer. All formulations remained buoyant for more than 12 h in dissolution medium (0.1 N HCl, pH 1.2) except RSF1, RSF2, RSF7 and RSF8.

In vitro drug release studies

All the formulations were subjected to in vitro drug release studies in 0.1 N HCl. The drug release profiles of formulations RSF1-RSF6 prepared with combination of Gelucire 43/01 and Sodium CMC were shown in Figure 2.

Formulations RSF1 and RSF2 prepared with same amount of Gelucire 43/01 (17.5 mg/tablet) and different amount of Sodium CMC i.e., 25 mg and 50 mg respectively in order to study the effect of sodium CMC concentration on drug release. Formulation RSF1 and RSF2 released about 99.87±1.45% and 99.21±1.44 % drug in 8 and 10 h respectively and couldn't sustain the drug release for 12 h, indicating less concentration of lipid polymer. Moreover, the drug release was decreased as increasing the concentration of Sodium CMC. The formulation RSF3 released about 92.65±1.65% of drug in 10 h, which couldn't satisfactory sustained drug release. Similarly, Formulation RSF4 sustained the drug release for 12 h and released 99.65±1.40% of drug in 12 h. The formulations RSF5 and RSF6 contain same amount (52.5 mg/tablet) of Gelucire 43/01 and 25 mg and 50 mg of sodium CMC respectively. Due to the more concentration of lipid polymer the drug release from these formulations was found to be 95.65±1.56 % and 89.44±1.29 % respectively. From the results, it was also observed that as increasing concentration of lipid polymer, the floating lag time was decreased and duration of floating was increased.

The drug release profiles of formulations RSF7-RSF12 prepared with combination of Gelucire 54/02 and CP 971P were shown in Figure 3.

Formulations RSF7 and RSF8 prepared with same amount of Gelucire 54/02 (17.5 mg/tablet) and different concentration 25 mg and 50 mg of CP 971P respectively in order to study the effect of CP 971P concentration on drug release. Formulation RSF7 and RSF8 released about 98.69±2.42 % and 99.66±1.25 % drug in 8 and 10 h respectively and couldn't sustain the drug release for 12 h, indicating less concentration of lipid polymer. Moreover, the drug release was decreased with increase in the concentration of CP 971P. The formulation RSF9 released about 93.89±1.56 % of drug in 10 h, which couldn't satisfactory sustained drug release. Similarly, the formulation RSF10 released 99.25±1.85 % of drug and satisfactorily sustained the drug release for 12 h. The formulations RSF11 and RSF12 contain same amount (52.5 mg/tablet) of Gelucire 54/02 and 25 mg and 50 mg of CP 971P respectively.

Due to the more concentration of lipid polymer the drug release from these formulations was found to be 96.41. ±1.50 % and 90.25 ±1.59 % respectively. From the results, it was also observed that as increasing concentration of lipid polymer, Gelucire 54/02 the floating lag time was decreased and duration of floating was increased.

Kinetic models of drug release

The drug release profiles of all the formulations of risedronate sodium floating-bioadhesive were fitted to different kinetic equations. The r^2 values for Zero-order model ranged from 0.928 (RSF3) to 0.979 (RSF12). Similarly, r^2 values for Higuchi model ranged from 0.935 (RSF2) to 0.975 (RSF10). All the formulations followed the Peppas model and r^2 values were ranged from 0.955 (RSF1) to 0.998 (RSF11) due to high coefficient of determination (r^2). The optimized formulation RSF10 followed Peppas model (r^2=0.990) with non-Fickian diffusion drug release mechanism (n=0.635). The value of release exponent n for all the formulations ranged from 0.539 (RSF8) to 0.0.652 (RSF5). All the formulations have n values between 0.5 and 1, indicating anomalous transport (non-Fickian). The release rate constants (k) of all the formulations were significantly different. The value of k for formulations RSF1-RSF6 prepared with combination of Gelucire 43/01 and Sodium CMC was ranged from 16.36 (RSF12) to 24.25 (RSF7), and that of formulations RSF7-RSF12, prepared with combination of Gelucire 54/02 and CP 971P was ranged from 17.29 (RSF6) to 23.65 (RSF1). Higher the value of k, greater the amount of drug released.

Physical stability studies

The optimized (RSF10) RSFBT were selected for stability studies. The stability studies were conducted for three 3 months and Student's paired t-test was used for analysing the data. No significant difference ($p > 0.05$) was observed in all the tested parameters i.e., tablet hardness, drug content, *in vitro* buoyancy or *in vitro* dissolution (Table 4). Therefore, the formulation RSF10 was stable for at least 3 months.

***In vivo* buoyancy study in human volunteers**

The study aimed to examine whether the RSFBT could be retained buoyant in the stomach or not. The *in vivo* buoyancy of tablet was confirmed by X-ray imaging at different time intervals like 1, 2.5, 4 and 5.5 h post-administration of the $BaSO_4$-loaded RSF10. Figure 4 showed the gastric retention of $BaSO_4$-loaded RSF10 in one volunteer (RSA). The first radiographic image was taken at 1 h post-administration of tablet and the tablet was observed in the stomach. In the next pictures (2.5 h and 4 h), it was observed that the tablet appeared more or less at the same position in the stomach. Later on, the tablet slightly changed its position and still remained within the stomach for 5.5 h. The mean gastric retention time was found to be 5 ± 0.86 h (n=3).

Bioadhesive study of RSFBT

The bioadhesive strengths of the developed formulations of RSFBT are shown in Figure 5. The formulations RSF1-RSF6 prepared with combination of Gelucire 43/01and Sodium CMC, in which Sodium CMC has bioadhesive property. The Gelucire 43/01 and other excipients such as $NaHCO_3$, Avicel PH101, Magnesium stearate and talk were not reported to have any bioadhesive property. From the results it was observed that BS was increased by increasing the amount of Sodium CMC. The results of bioadhesion study showed that the maximum bioadhesion strength was observed for the formulation RSF6 (0.883±0.104 N) than other formulations in this series. Similarly, formulations RSF7-RSF12 were prepared with combination of Gelucire 54/02 and CP 971P. From the results it was observed that bioadhesive strength was increased by increasing the amount of CP 971P, in this series formulation RBF12 exhibited maximum bioadhesive strength (1.141±0.109 N) than other formulations.

Comparative bioavailability study RSFBT

The bioavailability study of RSFT (test) and reference formulation (Actonel) was conducted according to the protocol. The drug was well tolerated with no other symptoms or disturbances during the two treatments. The serum samples were analyzed by HPLC method [28]. The pharmacokinetic parameters used to assess the bioavailability of test versus reference were $AUC_{0-\infty}$ for the extent of absorption and C_{max}, t_{max} for the rate of absorption. The mean serum concentration-time curves for test (RSF10) and reference (Actonel) conventional formulations are shown in Figure 6. The C_{max} value for reference formulation was found to be 14.82±1.92 ng/ml, whereas C_{max} value for test was found to be 12.70±1.01 ng/ml. The t_{max} values for both reference and test formulation was found to be 1.06±0.18 h and 2.88±0.35 h respectively. Half-life value for reference was found to be 1.57±0.33, and for test 4.29±0.32 h. $AUC_{0-\infty}$ values for reference and test were 51.05±9.04 µg×h/ml and 81.02±11.00 µg×h/ml, respectively. Mean residence time (MRT) values for reference and test formulations were 4.55±0.33 and 8.35±0.39 h respectively. In the present study student's paired t- test showed that there was significant difference ($P < 0.05$) between two formulations in their tested pharmacokinetic parameters, $AUC_{0-\infty}$, C_{max}, t_{max} and MRT. The

increased relative bioavailability of test formulation was 1.59 fold when compared to reference formulation.

Table 1: Formulation of floating-bioadhesive tablets of risedronate sodium (weights in mg/tablet)

Ingredients	RSF1	RSF2	RSF3	RSF4	RSF5	RSF6	RSF7	RSF8	RSF9	RSF 10*	RSF11	RSF12
Risedronate sodium	35	35	35	35	35	35	35	35	35	35	35	35
Gelucire 43/01	17.5	17.5	35	35	52.5	52.5	-	-	-	-	-	-
Sod CMC	25	50	25	50	25	50	-	-	-	-	-	-
Gelucire 54/02	-	-	-	-	-	-	17.5	17.5	35	35	52.5	52.5
CP 971P	-	-	-	-	-	-	25	50	25	50	25	50
Sodium bicarbonate	30	30	30	30	30	30	30	30	30	30	30	30
Avicel PH102	186.5	161.5	169	144	151.5	126.5	186.5	161.5	169	144	151.5	126.5
Magnesium Stearate	3	3	3	3	3	3	3	3	3	3	3	3
Talc	3	3	3	3	3	3	3	3	3	3	3	3
Total tablet weight	300	300	300	300	300	300	300	300	300	300	300	300

***Lead formulation is used in pharmacokinetic study**

Table 2: Physical characters and in vitro buoyancy of RSFBT.

Formulation code	Hardness (Kg/cm^2), (n=6)	Thickness (mm), (n=6)	Weights (mg), (n=20)	Friability (%), (n=10)	Drug content (%), (n=3)	Floating lag time (s) (n=3)	Duration of floating (h) (n=3)
RSF1	6.16±0.45	5.45±0.06	300.65±5.82	0.33	99.65±1.75	46.6±3.5	8
RSF2	6.07±0.40	5.49±0.05	301.34±6.55	0.37	100.23±1.45	52.7±4.4	10
RSF3	5.98±0.33	5.53±0.03	303.45±7.23	0.40	100.66±1.35	55.5±5.3	>12
RSF4	6.14±0.38	5.55±0.06	298.40±6.88	0.33	99.54±1.26	60.4±4.5	>12
RSF5	6.19±0.28	5.49±0.04	303.45±5.65	0.35	100.22±1.18	65.0±5.2	>12
RSF6	6.12±0.24	5.44±0.06	300.10±5.54	0.31	99.23±1.43	70.2±6.2	>12
RSF7	5.92±0.32	5.52±0.08	298.60±5.38	0.42	98.55±1.65	38.5±2.5	8
RSF8	6.21±0.44	5.43±0.09	300.22±5.45	0.28	101.30±1.70	43.4±3.2	10
RSF9	5.98±0.48	5.58±0.06	301.20±6.25	0.36	100.54±1.30	49.5±4.3	>12
RSF10	6.17±0.51	5.42±0.07	303.70±6.75	0.33	99.35±1.55	52.6±4.8	>12
RSF11	6.11±0.32	5.62±0.07	304.45±7.65	0.35	99.22±1.56	59.4±4.2	>12
RSF12	6.15±0.39	5.59±0.04	300.60±7.56	0.34	99.25±1.78	64.0±5.5	>12

Table 3: Mathematical models and drug release kinetics of risedronate sodium floating-bioadhesive tablets.

Formulation Code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	r^2	k_0	r^2	k_1	r^2	k_2	r^2	k	N
RSF1	0.931	10.81	0.624	0.484	0.953	38.26	0.955	23.65	0.645
RSF2	0.942	10.32	0.537	0.435	0.935	34.29	0.968	21.54	0.633
RSF3	0.928	9.69	0.518	0.419	0.948	31.38	0.974	20.33	0.649
RSF4	0.944	8.66	0.622	0.339	0.944	31.38	0.988	19.75	0.578
RSF5	0.957	7.54	0.466	0.316	0.972	29.33	0.989	17.55	0.652
RSF6	0.950	7.11	0.543	0.304	0.965	28.21	0.985	17.29	0.638
RSF7	0.962	9.26	0.613	0.398	0.966	38.31	0.989	24.25	0.643
RSF8	0.971	9.34	0.529	0.328	0.971	32.56	0.987	21.56	0.539
RSF9	0.945	8.54	0.617	0.304	0.959	31.26	0.997	20.54	0.632
RSF10	0.978	7.65	0.551	0.248	0.975	28.75	0.990	19.40	0.635
RSF11	0.965	7.16	0.227	0.227	0.969	28.16	0.998	17.66	0.644
RSF12	0.979	6.26	0.225	0.225	0.972	27.45	0.996	16.36	0.629

k_0, k_1, k_2 and k refer to the rate constants of the respective models, and n refers to the release exponent.

Table 4: Stability studies of optimized (RSF10) RSFBT

Characteristic	0 day *	15th day *	30th day *	60th day *	90th day *
Hardness (kg/cm^2)	6.15±0.54	6.12±0.38	6.11±0.50	6.09±0.45	6.07±0.51
Drug content (%)	99.45±1.65	99.46±1.57	99.24±1.55	99.30±1.85	99.15±1.58
Floating lag time (s)	51.85±5.32	51.92±6.12	51.56±5.25	51.41±5.22	51.38±4.95
Duration of floating (h)	>12	>12	>12	>12	>12
Drug released at 12 h (%)	99.44±1.85	99.32±1.55	99.24±1.62	99.20±1.74	99.15±1.50

***The difference was not statistically significant ($p > 0.05$)**

Table 5: Pharmacokinetic parameters of risedronate test (RSF10) and reference (Actonel) formulation, n=8.

Pharmacokinetic parameter	Risedronate sodium reference formulation (Actonel) Mean ± SD	RSFBT test formulation (RSF10) Mean ± SD
C_{max} (ng/ml)	14.82±1.92	12.70±1.01
t_{max} (h)	1.06±0.18	2.88±0.35
$t_{1/2}$ (h)	1.57±0.33	4.29±0.32
AUC_{0-24} (ng.h/ml)	50.60±8.78	79.91±10.86
$AUC_{0-\infty}$ (ng.h/ml)	51.05±9.04	81.02±11.00
MRT (h)	4.55±0.33	8.35±0.39

By student paired *t*-test, $p < 0.05$ is considered statically significant in all the parameters.

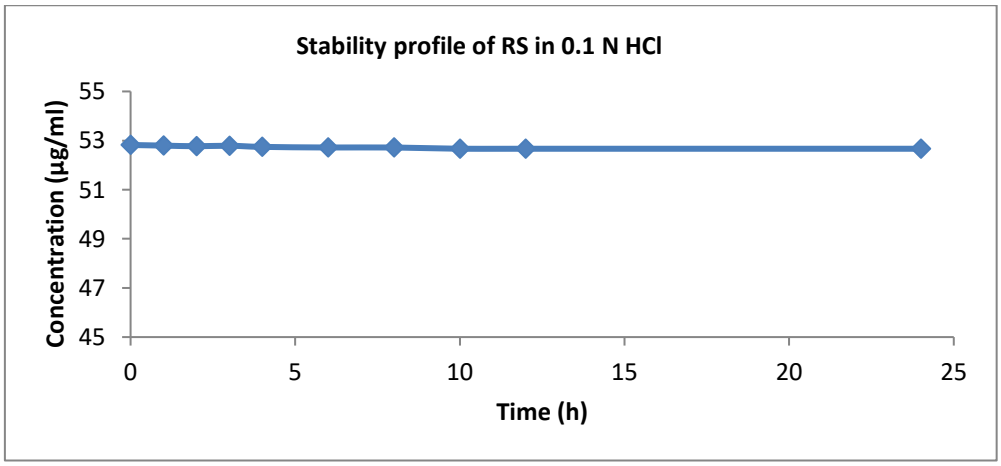

Figure 1: Stability profile of RS in 0.1 N HCl.

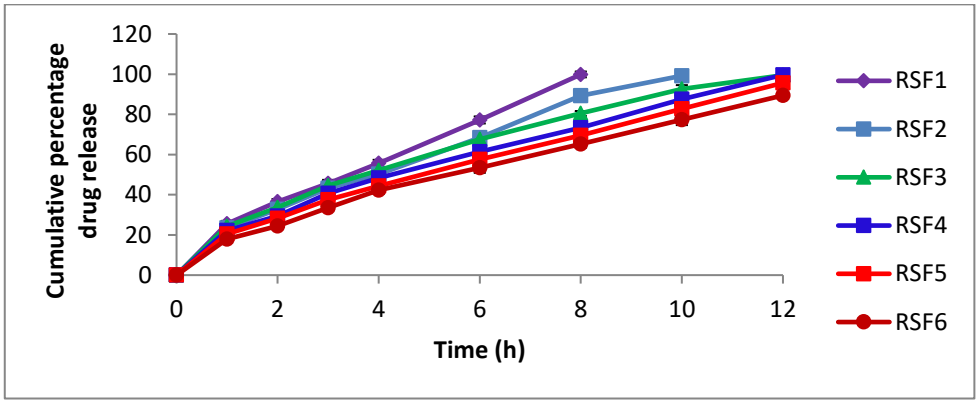

Figure 2: Cumulative release of floating bioadhesive tablets contain RS prepared with combination of Gelucire 43/01 and Sodium CMC in 0.1 N HCl (n=3, Mean±SD).

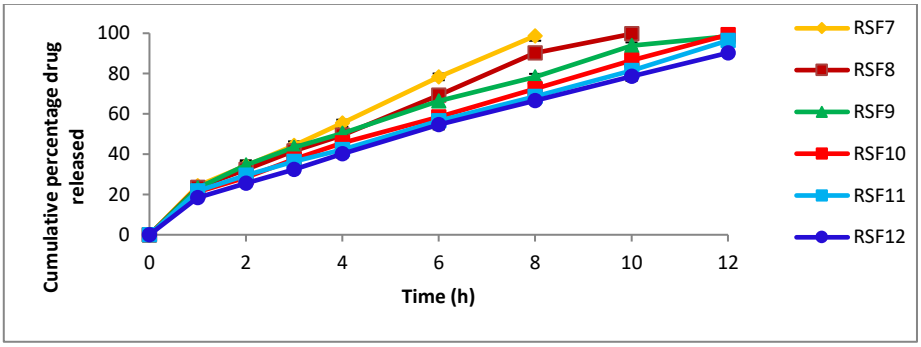

Figure 3: In vitro release profiles of floating bioadhesive tablets of RS prepared with combination of Gelucire 54/02 and CP 971P (n=3, Mean±SD).

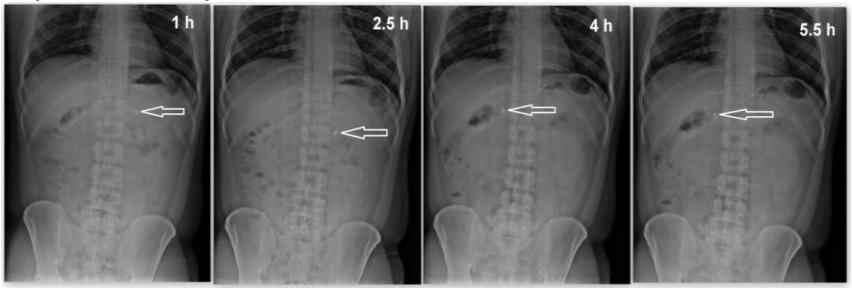

Figure 4: Radiographic images showing the presence of optimized $BaSO_4$-loaded RSFBT (RSF10) in the stomach of volunteers-1 at different time intervals (the tablet location is pointed out with an arrow).

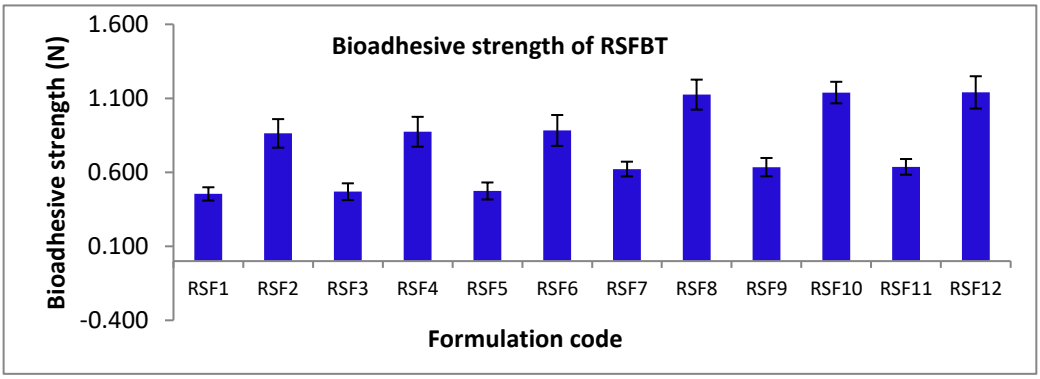

Figure 5: Bioadhesive strength of RSFBT (n=3, Mean±SD).

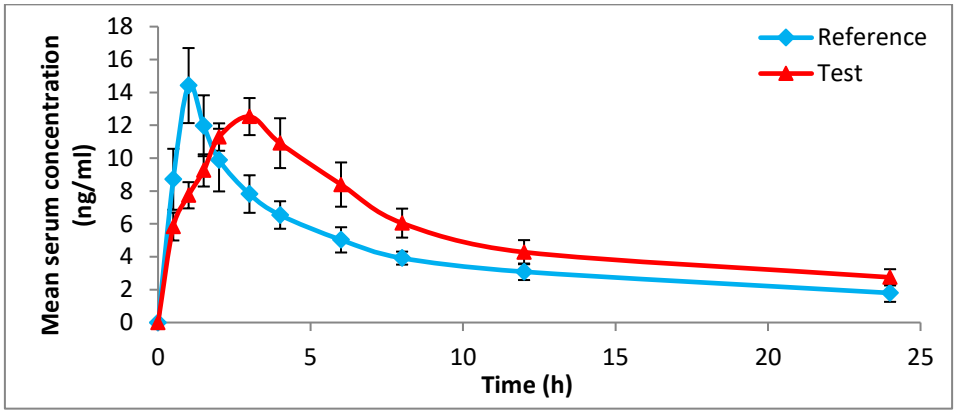

Figure 6: Mean serum concentrations (ng/ml) of RSFBT test (RSF10) and reference formulation in healthy human volunteers (n=8, Mean ± SD).

CONCLUSION

The RSFBT were prepared with combination of Gelucire 43/01 and Sodium CMC/ Gelucire 54/02 and CP 971P by direct compression method. The prepared RSFBT were evaluated for physical characters, *in vitro* buoyancy and drug release. One of the optimized formulations (RSF10) floated with a lag time of 52.6±4.8 sec and duration of floating 12 h. The RSF10 released about 99.25±1.85 % of drug in 12 h and then followed the non-Fickian diffusion release mechanism with n value of 0.635. The RSF10 of RSFBT loaded with $BaSO_4$ remained in stomach of volunteers for 5.00 ± 0.86 h (n=3) in radiological studies. The formulation, RSF10 exhibited maximum bioadhesive strength (1.139±0.073 N) than other formulations. The bioavailability studies were carried out for RSF10 and compared with that of reference IR tablets, Actonel in eight healthy human volunteers. Based on *in vivo* pharmacokinetic study, significant difference was observed between C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$, and MRT of RSF10 and Actonel IR tablets. The increase in relative bioavailability of RSF10 was 1.59 fold when compared to reference IR tablets. The increased relative oral bioavailability may be due to the floating-bioadhesive mechanism of dosage form, which is desirable for drugs absorbed from the upper part of gastrointestinal tract.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests

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