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GASTRORETENTIVE FLOATING-BIOADHESIVE DRUG DELIVERY SYSTEM FOR REBAMIPIDE: DESIGN, *IN VITRO* AND *IN VIVO* EVALUATION

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ABSTRACT
Rebamipide is an amino acid analog of 2 (1H)-quinolinone used in the treatment of peptic ulcer. The objective of present investigation is to formulate and evaluate gastroretentive floating-bioadhesive tablets of rebamipide to increase the gastric residence time and further compare their pharmacokinetics with conventional immediate release tablets. Floating-bioadhesive tablets of rebamipide were prepared with combination of Polyox WSR 303 and CP 971P/ HPMC K4M and Sodium CMC by direct compression method. The prepared formulations were evaluated for hardness, thickness, weight variation, friability, drug content, *in vitro* buoyancy and drug release. The optimized formulation (RBF12) floated with a lag time of 28.3±3.2 sec, duration of floating 12 h and released about 99.91±1.84% of drug in 12 h, and then followed non-Fickian diffusion release mechanism with n value of 0.635. The RBF12 tablets with $BaSO_4$ remained in stomach for 5.13 ± 0.64 h (n=3) in radiological studies. The formulation, RBF12 exhibited maximum bioadhesive strength (1.346±0.110 N) than other formulations. The bioavailability studies were carried out for the optimized formulation (RBF12) and compared with that of reference IR tablets "Rebagen" in nine healthy human volunteers. Based on *in vivo* performance significant difference was observed between C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$, and MRT of RBF12 and IR tablets. The increase in relative bioavailability of RBF12 was 1.7 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: Floating-bioadhesive, rebamipide, *ex vivo* bioadhesion, *in vivo* radiological study, pharmacokinetics,

INTRODUCTION
Helicobacter pylori (H. pylori) is a bacterium that causes chronic inflammation in the stomach and is a common cause of ulcers worldwide. It is also responsible for chronic gastritis, peptic ulcer disease and gastric malignancy in majority of healthy populations (Everhart, 2000; Peterson et al., 2000; Megraud and Lamouliatte, 1992). The treatment of *H. pylori* remains a challenging proposition; although it is highly sensitive to most antibiotics, difficult to eradicate from human body even with the current best therapies. Conventional formulations such as immediate release tablets are used for eradication therapy, but they do not remain in the stomach for a longer period of time. Therefore, it is difficult to reach minimum inhibitory concentrations in the gastric mucus where *H. pylori* colonizes.

The therapeutic efficiency and bioavailability of drugs was improved by many novel techniques such as transdermal (Yang et al., 2015), iontophoretic, intranasal (Manda et al., 2012; Manda et al., 2014; Manda et al., 2011) and complexation technique (Popescu et al., 2015) for poorly water soluble drugs. Floating-bioadhesive drug delivery systems are also useful in this context. In order to extend the gastric residence time, gastro retentive drug delivery systems (GRDDS) have been developed, which is an approach to prolong gastric residence time and also to provide site-specific drug release in the upper gastrointestinal tract (GIT) for local or systemic effects (Vyas and Khar, 2006). These include high density (sinking) systems, low density (floating) systems, mucoadhesive systems, swelling and expanding systems, modified shape systems, (Doodipala et al., 2011), floating-bioadhesive systems (Ponchel and Irache, 1998) and other delayed gastric emptying devices which would improve the therapeutic effects of many drugs.

In floating drug delivery system (FDDS), drug remains buoyant in the stomach for a longer period of time without reducing the gastric emptying rate. This result in

retardation of drug release at the desired rate from the system, an increased gastric retention time (GRT) and helps in better control of fluctuations in plasma drug levels (Kawashima et al., 1991). But, the main drawback of FDDS is that it is effective only when the fluid level in the stomach is sufficiently high, which aids in the buoyancy of the dosage form (Chueh et al., 1995). This limitation can be overcome by using bioadhesive polymers which enable it to adhere to the mucous lining of the stomach wall (Chitnis et al., 1991). Floating-bioadhesive drug delivery systems offer the advantages of increased contact time with stomach mucosa resulting in more effective absorption, improving the bioavailability of drugs. with absorption window high in the stomach and proximal intestine and reduced dosing frequencies.

Previously, it was reported that levofloxacin single and mini floating tablets was found to be a potential candidate for targeted drug delivery and are anticipated to be useful in the treatment of *H. pylori* (El-Zahaby et al., 2014). In another study, lectin conjugated multi particulate floating systems of clarithromycin for eradication of *H. pylori* was reported based on the mucoadhesive property of gastric mucosa (Sunil and Manmohan, 2009).

Rebamipide is an amino acid analog of 2 (1H)-quinolinone. It acts by the decrease in oxygen radicals, increase in blood flow and production of protective prostaglandins in ulcer mucosa, which accelerates the process of healing. It is better absorbed from the upper part of GIT following oral administration (Nebiki et al., 1998). It has half-life of 1.75 ± 0.63 h and bioavailability of approximately 10%. Due to the above mentioned conditions it's a good candidate for the development of gastroretentive drug delivery system.

The present investigation was focused on the development of floating-bioadhesive tablets of rebamipide by direct compression method using combination of Polyox WSR303 and Carbopol 971P, and HPMC K4M and Sod. CMC polymers. The prepared tablets were evaluation for the physical characters such as *in vitro* buoyancy studies, drug release, swelling index, *ex vivo* bioadhesion study and in vivo radiological studies. Further, the optimized formulation was subjected to bioavailability study in healthy human volunteers.

MATERIALS AND METHODS

Materials: Rebamipide was received as a generous gift samples from M/s Splendid Pharma. Ltd., Pune, India. Hydroxypropyl methylcellulose (HPMC K4M) and Polyox WSR 303 were received as gift samples from Orchid pharma Ltd., Chennai, India. Sod.CMC and Carbopol 971P were received from M/s Aurobindo Pharma. Ltd., Hyderabad, India. Sodium bicarbonate, magnesium stearate and talc were purchased from S.D. Fine-Chem. Ltd., Mumbai, India. Acetonitrile and methanol HPLC grade were purchased from Sigma Aldrich chemicals Dombivli, India. All other solvents and reagents used were of analytical grade.

Methods

Equilibrium solubility study of rebamipide

Excess amount of rebamipide was placed in 0.1 N HCl (pH 1.2), pH 4.5 acetate and pH 6.8 phosphate buffer and water in order to determine its solubility. The samples were shaken for 48 hrs at 37 °C in a horizontal shaker (Model 3015, GFL, Germany). The supernatant was filtered and the filtrate was diluted with the appropriate buffer and analysed by using UV/Visible spectrophotometer (Elico, SL210) at λ_{max} of 231 nm.

Determination of acid stability of rebamipide

Stock solution of rebamipide 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 (Elico, SL210) at λ_{max} of 231 nm to see whether there is any change in the absorbance and concentration in the prepared stock solutions.

Formulation of floating-bioadhesive tablets of rebamipide

Accurately weighed quantities of drug, Polyox WSR303/HPMC K4M/Carbopol 971P/Sod CMC, sodium bicarbonate and Avicel PH102 were passed through a sieve, no. 40, to get uniform sized particles, then they were taken in a mortar and triturated for 10 min with the help of a pestle. Then the mixture was transferred into a poly bag and further mixed for 5 min to ensure a homogeneous mass. To the mixture, magnesium stearate and talc were added and continued the mixing for another 2 min. Finally, each mixture was weighed and fed manually into the die of a 16 station punching machine (Cadmach, Ahmedabad, India) to produce the desired tablets using flat- faced round punches. The hardness is adjusted to 5 kg/cm^2

Evaluation of tablets

Physical characterization of prepared tablets

The prepared floating-bioadhesive tablets of rebamipide were evaluated for uniformity of weight using 20 tablets (Indian Pharmacopoeia, 1996), hardness (Monsanto tester) using 5 tablets, thickness (vernier caliperse) using 5 tablets, friability (Roche friabilator) using 10 tablets (Banker and Anderson, 1987), 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 will be 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 rebamipide floating-bioadhesive tablets were placed in dissolution vessels and 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 (Baumgartner et al., 2000).

***In-vitro* dissolution studies**

The *in vitro* drug release studies will be conducted using USP 24 type-2 apparatus (Electrolab, TDT-06T). The dissolution test is 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. Absorbances of these samples were measured using UV/Visible spectrophotometer (Elico, SL 210, India) at λ_{max} of 231 nm.

Analysis of drug release kinetics

The *in vitro drug* release profiles were fitted to different kinetic models to explain the release kinetics from the floating-bioadhesive tablets of rebamipide. 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 fitted to zero-order (Chen et al., 2000), first-order (Wagner, 1969), Higuchi (Higuchi, 1963) and Korsmeyer-Peppas kinetic models (Korsmeyer et al., 1983).

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 time t and at ∞ time. 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 the 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. This mechanism could result from increased plasticization at the relaxing boundary, i.e., gel layer (Ritger and Peppas, 1987).

Tablet swelling and erosion studies

The swelling behaviour of the tablets will be determined in triplicate. The rebamipide floating-bioadhesive tablets were weighed (W_0) and placed in a glass beaker containing 200 mL of 0.1 N HCl, maintained at 37 ± 0.5 °C. At regular time intervals, the tablets were removed and the excess surface liquid was carefully removed by a filter paper (Patel et al., 2009). The swollen individual tablet was then reweighed (W_1). The wet tablets were then dried in an oven at 40° C for 24-h and finally weighed until constant weight was achieved (final dry weight, W_2). The percentage swelling and erosion at different times was estimated from the following equations:

$$\% \text{ Swelling} = \frac{(W1 - W0)}{W0} \times 100$$

$$\% \text{ Erosion} = \frac{(W0 - W2)}{W0} \times 100$$

Physical stability studies

Physical stability studies were conducted according to ICH guidelines. One of the optimized formulations of rebamipide floating-bioadhesive (RBF12) tablets were 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 (Tadros, 2010). 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 rebapimide floating-bioadhesive tablets (RBF12) with following composition: 125 mg drug, 75 mg $BaSO_4$, 60 mg Sod. CMC, 60 mg $NaHCO_3$, 130 mg Avicel PH102, 5 mg magnesium stearate and 5 mg talc. The tablets were prepared by direct compression method.

Three healthy male volunteers will participate after giving an informed written consent. The subjects weighed in between 64-75 kg, in height from 165-173 cm, and in the age group of 22-26 years. The radiological study protocol was approved by an Institutional Ethics Committee, Talla Padmavathi College of Pharmacy, Kakatiya University, Warangal, India.

In this study, x-ray technique was used to determine the gastric residence time of tablets. To make the tablet X-ray opaque, $BaSO_4$ was used. 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 of rebamipide floating-bioadhesive (RBF12) 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. 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 (El Gamal et al., 2011). The mean gastric residence time was calculated.

***Ex-vivo* bioadhesion study**

The bioadhesive strength was determined using an ultratest (Mecmesin, West Sussex, UK) equipped with a 5-kg load cell. For this study, porcine gastric mucosa was obtained from slaughterhouse. The mucosal membrane was excised by removing the underlying connective tissue (Fig. 1) and was secured tightly to a circular stainless steel adapter of a diameter 2.2 cm provided with the equipment. The tablets of rebamipide floating-bioadhesive were placed over another cylindrical stainless steel adaptor of similar diameter. The tablet with a backing membrane was adhered on to it using a solution of cyanoacrylate adhesive. During the study, 100 µl of 1% w/v mucin solution was used to moisten the porcine gastric mucosal membrane (Yamsani et al., 2007). 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 in human volunteers

Subjects: The mean age of volunteers was 22.5 ± 3.2 years, mean height was 167.5 ± 8.5 cm, and mean body weight was 63.5 ± 6.4 kg.

Nine healthy male volunteers for rebamipide floating-bioadhesive and floating 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, Talla Padmavathi College of Pharmacy, Kakatiya University, Warangal, India.

Study design

Rebamipide floating-bioadhesive tablets: A single dose, randomized, three-way cross-over study was designed with nine 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 (Floating-bioadhesive RBF12/Rebagen 200 mg) 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^{o} C till the time of analysis.

Chromatographic conditions

Rebamipide serum concentrations were determined by reversed phase HPLC equipped with a pump model, Shimadzu, LC-10AT and an SPD-10A detector. Mobile phase was prepared with acetonitrile: water: methanol: acetic acid (38:60:1:1 v/v) 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 280 nm.

Sample preparation for analysis

The serum samples were extracted by liquid-liquid extraction method. To 1 ml of serum, 0.4 ml of phosphate buffer PH 7 and 100 µl of internal standard (carbamazepine, 2 µg/ml) was added and vortexed for 5 min in a test tube. To this mixture 7 ml of chloroform: isopropyl alcohol was added as extracting agent in 95: 5 v/v proportion and vortexed for 3 min then centrifuged at 2500 rpm on cooling centrifuge for 15 min at 4 ^{o}C (Manglani et al., 2006). The organic phase was separated into another test tube and evaporated to dryness in a vacuum oven. To the dried residue 0.4 ml of diethyl ether was added and shaken for 10 sec and the ether layer was discarded. The dried residue was reconstituted with100 µl of mobile phase from which 20 µl was injected into the HPLC column.

Pharmacokinetic analysis

The pharmacokinetic parameters of test formulation and reference formulation 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. The mean residence time (MRT) was calculated using following equation (Shargel et al., 2005).

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

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

Equilibrium solubility study of rebamipide

The solubility studies were conducted in different media and values are shown in table 2. The solubility of the drug was determined in different media like, 0.1 N HCl (pH 1.2), pH 4.5, pH 6.8 and water. The drug showed greater solubility in 0.1 N HCl (1.85 ± 0.32 mg/ml) and lesser solubility in water (1.32 ± 0.26 mg/ml).

Determination of acid stability of rebamipide

From the acid stability study results, it was observed that there was no change in drug concentration until 24 h indicating stability of drug in 0.1 N HCl.

Physical characteristics of prepared tablets of rebamipide

All the prepared formulations were subjected to the hardness, thickness, weight variation, friability, drug content FLT and TF.

The physicochemical characteristics of the tablets are summarized in Table 3. The hardness of all tablets ranged from 5.18 ± 0.23 to 5.31 ± 0.45 kg/cm^2 and that of thickness from 5.28 ± 0.13 to 5.58 ± 0.16 mm. All the tablet formulations showed acceptable physicochemical properties and complied with the pharmacopoeial specifications for weight variation, drug content and friability (Banker and Anderson, 1987). The weight of the tablets ranged from 499.20 ± 5.90 to 503.80 ± 7.07 mg. Drug content results were found to be good among different batches; the percentage of drug content ranged from 98.55 ± 1.46 to 101.30 ± 1.72. The percentage friability for all formulations was less than 1%, indicating good mechanical resistance.

In vitro buoyancy studies

All the formulations were prepared by effervescent technique. Sodium bicarbonate was used as a gas generating agent. Formulations RBF1-RBF6, prepared with combination of Polyox WSR 303 and CP 971P floated with a lag time of 15.3±2.5 (RBF1) to 35.7±3.1(RBF6) sec. Formulations RBF7-F12, prepared with combination of HPMC K4M and Sod.CMC showed a floating lag of 17.3±3.1 (RBF7) to 27.6±2.5 (RBF12) sec. Tablets of all formulations showed good in vitro buoyancy with maximum floating lag time of 35.7±3.1 sec (Table 4), regardless of viscosity of polymer used. This was mainly due to evolution of carbon dioxide entrapped inside the hydrated polymeric matrices, resulting from the interaction between gas generating agent ($NaHCO_3$) and dissolution medium (0.1N HCl, pH 1.2), and this lead to the lowering of density of matrices to float. The floating lag time could not change with different viscosity grades of polymers and the type of filler used. All formulations remained buoyant for more than 12 h.

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 RBF1-RBF6 prepared with combination of Polyox WSR 303 and CP 971P shown in Figure 3. Formulation RBF1 and RBF2 released about 87.87±1.56% and 94.43±1.83 % drug in 8 and 10 h respectively and couldn't sustain the drug release for 12 h, indicating less concentration polymer. The formulation RBF3 released about 99.04±1.67% of drug in 12 h. Similarly Formulation RBF4 sustained the drug release for 12 h and released 98.26±1.86% of drug in 12 h. Formulations RBF5 and RBF6 released about 96.35±1.33% and 90.70±1.45% respectively in 12 h. From the results, it was also observed that as increasing concentration of CP 971P, the drug release was decreased.

All the formulations were subjected to in vitro drug release studies in 0.1 N HCl. The formulations RBF7-RBF12 prepared with combination of HPMC K4M and Sodium CMC were shown in Figure 4. Amount of drug release from these formulations ranged from 84.48±1.85% (RBF7) to 99.91±1.84 (RBF12). All the formulations formulation sustained the drug release for 12 h. The optimized formulation released about 99.91±1.84% of drug in 12 h.

Analysis of drug release kinetics

The drug release profiles of all the formulations of rebamipide floating-bioadhesive were fitted to

different kinetic equations and are shown in Table 4. The r^2 values for Zero-order model ranged from 0.921 (RBF1) to 0.983 (RBF12). Similarly, r^2 values for Higuchi model ranged from 0.949 (RBF9) to 0.986 (RBF12). All the formulations followed the Peppas model and r^2 values were ranged from 0.963-0.998 due to high coefficient of determination (r^2). The optimized formulation RBF12 followed Peppas model (r^2=0.998) with non-Fickian diffusion drug release mechanism (n=0.635). The value of release exponent n for all the formulations ranged from 0.652 (RBF1) to 0.0.679 (RBF7) and that of optimized formulation was 0.635. 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 RBF1-RBF6 prepared with combination of Polyox WSR303 and CP 971P was ranged from 4.32 (RBF6) to 7.71 (RBF1), and that of formulations RBF7-RBF12, prepared with combination of HPMC K4M and Sod. CMC was ranged from 5.89 (RBF12) to 6.60 (RBF7). Higher k values meant higher quantities of drug released.

Studies on tablet swelling and erosion

The swelling and erosion studies were conducted on the optimized formulations RBF4 and RBF12. The percentage of swelling is shown in the Fig.5. The percentage swelling of optimized rebamipide floating-bioadhesive tablets RBF4 and RBF12 were determined at different time intervals. The maximum swelling was observed at 4 h and was found to be 140.21±5.11%, and 158.01±5.90% for RBF4 and RBF12 respectively. The erosion was increased with increase in time (Fig.6).

Physical stability studies

The optimized rebamipide floating- tablets (RBF12) was selected for stability study based on physical characters and *in vitro* drug release. Before and after conducting the stability studies for 3 months, the results were analysed statistically by using Student's paired t-test. No significant difference ($p > 0.05$) was observed in the tablet hardness, FLT, TFT, drug content or *in vitro* dissolution (Table 5). The drug content was slightly decreased from 101.26 ± 1.59% to 100.85 ± 1.69% after storage at 40 ^{o}C under 75% RH. But the difference was not statistically significant ($p > 0.05$). Thus, the RBF11 rebamipide floating-bioadhesive tablets were found to be stable.

***In vivo* evaluation of gastric residence time**

The floating-bioadhesive tablets (RBF12) prepared for radiological studies were characterized for hardness (5.16 ± 0.33 kg/cm^2), thickness (5.34 ± 0.11 mm), weight variation (501.45 ± 5.65 mg), friability (0.37%), FLT (68.65 ± 5.42 sec) and TFT was greater than 12 h. The increased lag time of $BaSO_4$-loaded rebamipide floating-bioadhesive tablets, compared to the original formulation RBF12 (27.3 ± 3.1 s) was expected because of high density of $BaSO_4$ (4.5 g/cm^3). Figure 7 shows the radiographic images taken at different periods after administration of $BaSO_4$-loaded rebamipide floating-bioadhesive tablets in one volunteer (A). The first radiographic image was taken at 0.5 h post-administration of tablet and the tablet was observed in the stomach. The next pictures were taken at 2.5, 4.5 and 5.5 h; the tablet had altered its position, yet remained within the stomach till the end of 5.5 h. The mean gastric residence time was found to be 5.13 ± 0.64 h (n=3).

***Ex vivo* bioadhesion study**

The bioadhesive strength is an important property for gastroretentive drug delivery systems. The developed formulations RBF1-RBF6 contained a combination of Polyox WSR303 and CP 971P, in which CP 971P has potential bioadhesive property. Polyox WSR303 polymers were also reported to have the bioadhesive property. Other materials used in the study such as $NaHCO_3$, Avicel pH 102, magnesium stearate and talc were not reported to have bioadhesive properties. The bioadhesive strengths (BS) of the developed formulations are shown in Figure 8. From the results it was observed that BS was increased by increasing the concentration of CP 971P. The results of bioadhesion study showed that the bioadhesion was significantly higher for the formulation RBF6 (1.436±0.114 N) than other formulations. Similarly, formulations RBF7-RBF12 were prepared with combination of HPMC K4M and Sod. CMC, in this series formulation RBF12 exhibited maximum bioadhesive strength (1.346±0.110 N) than other formulations.

Comparative bioavailability study

The bioavailability study was successfully conducted according to the study protocol. The serum samples were analyzed by RP-HPLC method. The retention time of drug rebamipide is 4.81 min and the internal standard carbamazepine is 7.42 min. These retention times were nearly closer to the reported values (29). 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 rebamipide serum concentration-time curves for test (RBF12) and reference (Rebagen Tab 300 mg) immediate formulations are shown in Figure 9. The C_{max} value for Rebagen formulation (reference) was found to be 0.295±0.047 µg/ml and that of test formulation (RBF12) was found to be 0.252±0.011 µg/ml. The t_{max} values for both reference and test formulation was found to be 2.167±0.500 h and 3.444±0.500 h respectively. Half-life value for reference was found to be

2.029±0.200, and that of test is 4.116±0.407 h (Table 6). The AUC_{0-24} values for reference and test were 9.461±0.760 µg×h/ml and 16.110±1.580 µg×h/ml, respectively. $AUC_{0-\infty}$ value reference formulation was 9.564±0.772 µg×h/ml and that of test formulation was 16.289±1.667 µg×h/ml. Similarly, mean residence time (MRT) value reference formulations were 4.731±0.211 and that of test formulation was 8.791±0.409 h. In the present study student's paired t- test was used to compare pharmacokinetic data of reference and test formulation. The data showed that there was significant difference ($P < 0.05$) between two formulations in their tested pharmacokinetic parameters, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max} and MRT. The increase in relative bioavailability of test formulation was found to be 1.7 times when compared to reference formulation.

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

Ingredients	RBF1	RBF2	RBF3	RBF4	RBF5	RBF6	RBF7	RBF8	RBF9	RBF10	RBF11	RBF12*
Rebamipide	200	200	200	200	200	200	200	200	200	200	200	200
Polyox WSR 303	100	80	60	40	20	0	-	-	-	-	-	-
Cabopol 971P	0	20	40	60	80	100	-	-	-	-	-	-
HPMC K4M	-	-	-	-	-	-	100	80	60	40	20	0
Sod.CMC	-	-	-	-	-	-	0	20	40	60	80	100
Sodium bicarbonate	60	60	60	60	60	60	60	60	60	60	60	60
Avicel PH102	130	130	130	130	130	130	130	130	130	130	130	130
Magnesium Stearate	5	5	5	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5	5	5	5
Total tablet weight	500	500	500	500	500	500	500	500	500	500	500	500

*Lead formulation is used in pharmacokinetic study

Table. 2: Solubility of rebamipide in different media (Mean±SD).

Medium	Solubility (mg/ml)
pH 1.2	1.85 ± 0.32
pH 4.5	1.66 ± 0.22
pH 6.8	1.46 ± 0.43
Water	1.32 ± 0.26

Table 3: Physical characters of rebamipide floating-bioadhesive tablets.

Formulation code	Hardness (kg/cm^2) (n=6)	Thickness (mm) (n=6)	Tablet weight (mg) (n=20)	Friability (%) (n=10)	Drug content (%) (n=3)	Floating lag time (s) (n=3)
RBF1	5.30±0.28	5.40±0.12	501.80±6.16	0.43	99.03±1.96	15.3±2.5
RBF2	5.18±0.23	5.34±0.11	502.20±7.16	0.36	101.30±1.72	17.7±2.6
RBF3	5.18±0.29	5.38±0.16	501.90±7.75	0.41	99.40±1.49	20.0±2.0
RBF4	5.30±0.42	5.28±0.13	499.30±6.62	0.49	98.55±1.46	24.7±3.5
RBF5	5.26±0.26	5.58±0.16	499.20±5.90	0.44	99.67±1.44	29.3±5.5
RBF6	5.22±0.29	5.34±0.11	501.20±6.97	0.38	99.88±1.33	35.7±3.1
RBF7	5.34±0.36	5.52±0.17	500.10±6.10	0.45	98.91±1.89	17.3±3.1
RBF8	5.30±0.36	5.52±0.13	502.10±6.16	0.37	99.28±1.39	19.0±3.0
RBF9	5.26±0.47	5.42±0.14	503.80±7.07	0.42	99.38±1.24	21.3±3.1
RBF10	5.30±0.51	5.50±0.15	501.60±7.35	0.48	99.40±1.79	25.0±3.0
RBF11	5.24±0.50	5.48±0.13	501.70±6.95	0.42	100.48±1.88	27.3±3.1
RBF12	5.31±0.45	5.52±0.14	502.65±6.56	0.39	101.26±1.59	28.3±3.2

Table. 4: Mathematical models and drug release kinetics of rebamipide 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
RBF1	0.921	26.82	0.436	0.395	0.952	87.68	0.963	7.71	0.652
RBF2	0.933	22.35	0.455	0.396	0.963	79.85	0.972	7.67	0.654
RBF3	0.939	21.29	0.429	0.308	0.969	76.79	0.978	7.54	0.656
RBF4	0.941	18.73	0.436	0.484	0.951	72.62	0.986	5.87	0.659
RBF5	0.953	16.66	0.419	0.429	0.961	70.64	0.989	4.59	0.662
RBF6	0.959	12.31	0.379	0.491	0.972	65.26	0.985	4.32	0.665
RBF7	0.971	25.33	0.388	0.356	0.972	55.32	0.996	6.60	0.679
RBF8	0.984	21.64	0.408	0.393	0.963	57.63	0.997	6.75	0.656
RBF9	0.966	18.71	0.454	0.413	0.949	59.39	0.998	6.57	0.654
RBF10	0.965	15.32	0.436	0.365	0.956	62.27	0.996	6.34	0.645
RBF11	0.977	11.43	0.427	0.345	0.953	63.54	0.997	6.33	0.642
RBF12	0.983	10.56	0.421	0.329	0.986	65.65	0.998	5.89	0.635

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 5: Physical characters during storage - stability study of rebamipide floating-bioadhesive tablet (RBF12).

Characteristic parameter	0 day *	15th day *	30th day *	60th day *	90th day *
Hardness (kg/cm^2)	5.31±0.50	5.32±0.45	5.29±0.53	5.26±0.44	5.30±0.38
Floating lag time (s)	28.30±3.20	28.25±3.05	28.21±2.51	28.22±2.31	28.20±2.64
Duration of floating (h)	>12	>12	>12	>12	>12
Drug content (%)	101.26±1.59	101.31±1.63	101.32±1.74	101.26±1.75	100.85±1.69
Drug released at 12 h	99.91±1.84	99.69±1.44	99.45±1.32	99.31±1.31	99.26±1.43

* Statistically not significant ($p > 0.05$).

Table. 6: Pharmacokinetic parameters of rebamipide test (RBF12) and reference (RebagenTab) formulation, n=8.

Pharmacokinetic parameter	Rebamipide reference formulation (RebagenTab) Mean ± SD	Rebamipide test formulation (RBF12) Mean ± SD
C_{max} (µg/ml)	0.295±0.047	0.252±0.011
t_{max} (h)	2.167±0.500	3.444±0.500
$t_{1/2}$ (h)	2.029±0.200	4.116±0.407
AUC_{0-24} (µg.h/ml)	9.461±0.760	16.110±1.580
$AUC_{0-\infty}$ (µg.h/ml)	9.564±0.772	16.289±1.667
MRT (h)	4.731±0.211	8.791±0.409

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

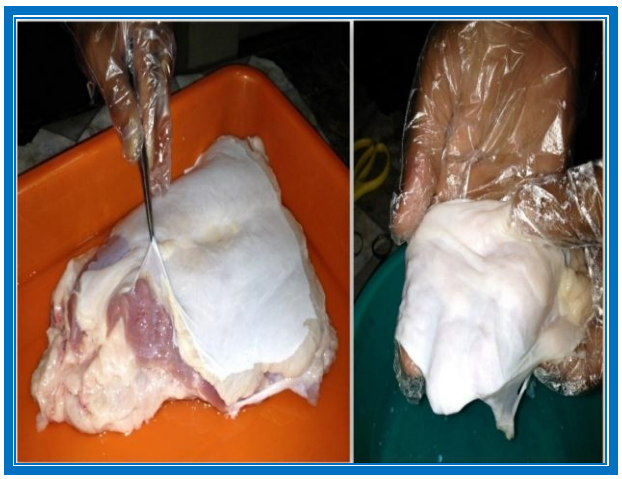

Figure. 1: Photographs showing the separation of porcine gastric mucosa from underlying connective tissue by forceps.

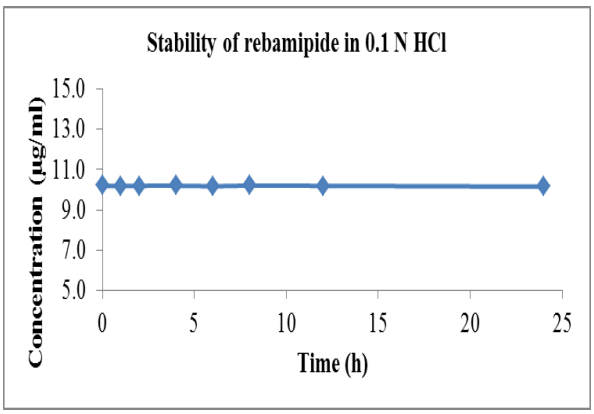

Figure. 2: Stability of rebamipide in 0.1 N HCl.

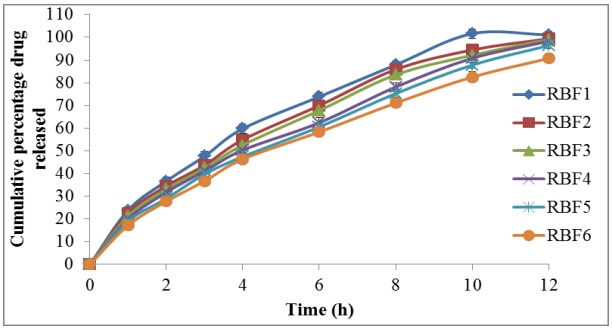

Figure 3: In vitro release profiles of rebamipide from floating-bioadhesive tablets prepared with combination of Polyox WSR 303 and CP 971P (n=3, Mean±SD)

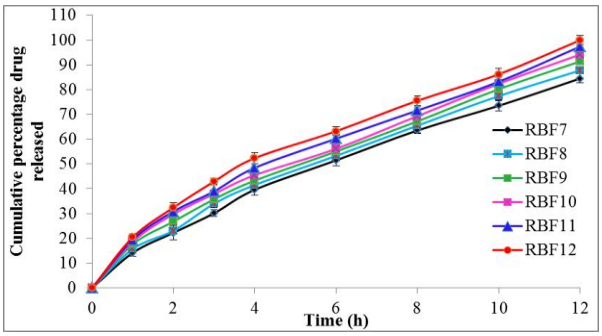

Figure 4: In vitro release profiles of rebamipide from floating-bioadhesive tablets prepared with combination of HPMC K4M and Sodium CMC (n=3, Mean±SD).

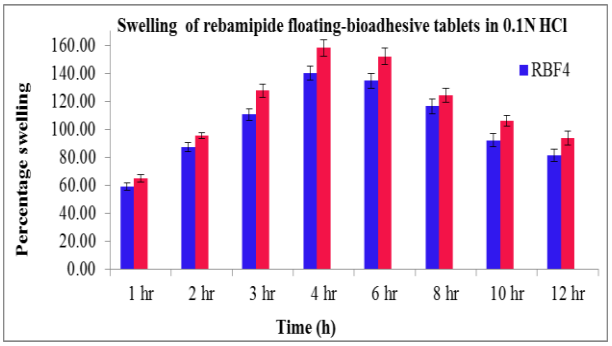

Figure 5: Extent of swelling of different formulations of rebamipide floating-bioadhesive tablets in 0.1 N HCl ((Mean ± SD, n=3).

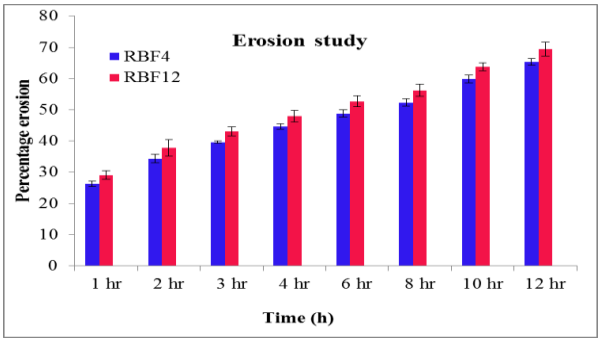

Figure 6: Extent of erosion of different formulations of rebamipide floating-bioadhesive tablets in 0.1 N HCl ((Mean ± SD, n=3).

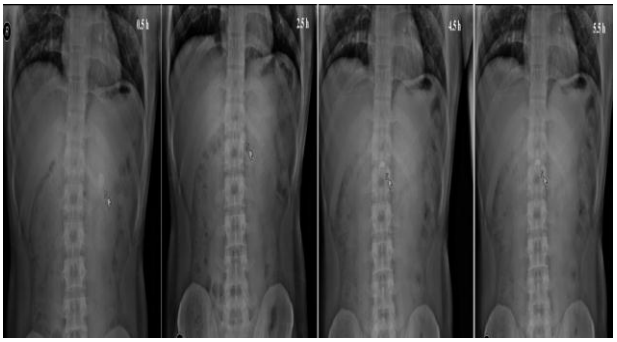

Figure 7: Radiographic images showing the presence of $BaSO_4$-loaded floating-bioadhesive tablets of rebamipide in the stomach of volunteer-A at different time points (the location of the tablet is shown with an arrow).

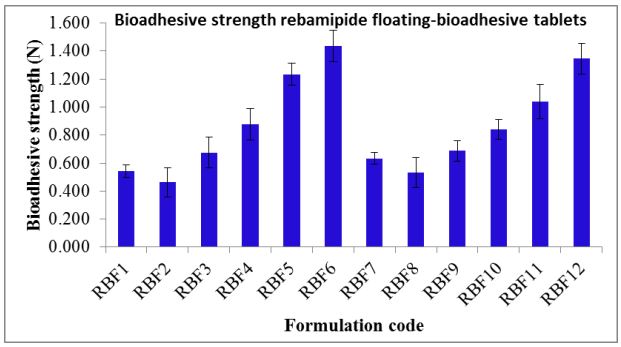

Figure 8: Bioadhesive strength of rebamipide floating-bioadhesive formulations (n=3, Mean±SD).

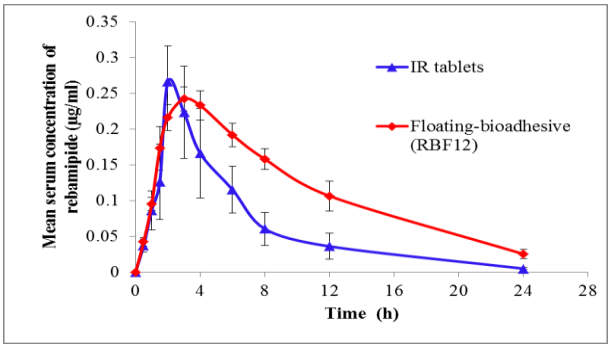

Figure. 9: Mean serum concentration (µg/ml) of rebamipide test (RBF12) and reference (Rebagen Tab) formulation in healthy human volunteers (n=9, Mean±SD).

CONCLUSION

Floating-bioadhesive tablets of rebamipide were prepared with combination of Polyox WSR 303 and CP 971P/ HPMC K4M and Sodium CMC. The optimized formulation (RBF12) floated with a lag time of 28.3±3.2 sec, duration of floating 12 h and released about 99.91±1.84% of drug in 12 h, and then followed the non-Fickian diffusion release mechanism with n value of 0.635. The RBF12 tablets with $BaSO_4$ remained in stomach for 5.13 ± 0.64 h (n=3) in radiological studies. The formulation, RBF12 exhibited maximum

bioadhesive strength (1.346±0.110 N) than other formulations. The bioavailability studies were carried out for the RBF12 and compared with that of reference IR tablets (Rebagen) in nine healthy human volunteers. Based on *in vivo* performance, significant difference was observed between C_{max}, t_{max}, $t_{1/2}$, $AUC_{0-\infty}$, and MRT of RBF12 and IR tablets. The increase in relative bioavailability of RBF12 was 1.7 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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