Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/yjkkc824

DEVELOPEMENT AND IN VIVO EVALUATION OF GASTRO RETENTIVE FLOATING MICROBALLOONS OF PIRENTANIDE

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ABSTRACT
The present investigation deals with the development and evaluation of floating microballoons of Piretanide to extend the gastric residence time (GRT) and prolong the drug release. In the present work, floating microballoons of Piretanide were formulated using Eudragit RS 100, Eudragit S 100, and HPMC K4M and ethyl cellulose polymers by the solvent evaporation method. The prepared microballoons were evaluated for their physicochemical properties, *in-vitro* drug release, and *in-vitro* buoyancy. The *in-vivo* Radiographic study showed that the barium sulphate loaded optimized formulation remained buoyant up to 5.5 h in the stomach. The *in-vivo* pharmacokinetic study was conducted in healthy albino rabbits revealed that the oral bioavailability of optimized formulation was increased significantly when compared to the marketed formulations. The increased bioavailability may be due to the floating mechanism of the dosage form in the stomach for longer duration.

Key Words: Eudragit S 100, Eudragit RS 100, Ethyl Cellulose, Floating Microballons, Hydroxy Propyl Methyl Cellulose (HPMC), Piretanide.

INTRODUCTION:
Gastro retentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs (Gupta G et al, 2012). It improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment (Vyas SP et al, 2002).
Microballoons (Hollow microsphere) are in the strict sense, empty particles of spherical shape without core. These Microballoons are characteristically free-flowing powders comprising of proteins or synthetic polymers, ideally having a size less than 200 micrometer (Gattani YS et al, 2009). The slow release of drug at desired rate and better-floating properties of floating Microballoons mainly depends on the type of polymer, plasticizer, the solvents employed for the preparation and the release of the drug can be modulated by optimizing polymer concentration and the polymer - plasticizer ratio (Lenkalapally Matsyagiri et al, 2019).

Piretanide is absorbed mostly in the stomach and upper small intestine, possibly due to its weak acidic properties (pKa 3.93) and is characterized by a short half-life (1-2 h). The narrow absorption window of Piretanide leads to its low bioavailability (60-70%). The narrow absorption window of Piretanide in the upper part of the gastrointestinal tract provides a rationale for developing a gastro retentive dosage form (Pusp RN et al, 2007).

MATERIALS AND METHODS:

Materials: Piretanide was purchased from Yarrow chem. Products, Mumbai, India. Vitamin E TPGS, Eudragit RS 100, Eudragit S 100, HPMC K_4M, Ethyl cellulose, Ethanol, Dichloromethane chemicals of Laboratory-grade from SD Fine chemicals Pvt. Ltd., were used.

Methods:

Drug Excipient Compatibility Study:

Differential Scanning Calorimetry: The physicochemical compatibilities of the drug and the excipients were tested by differential scanning calorimetric (DSC) analysis. DSC thermograms of the drug alone and optimized formulation were derived from DSC (Perkin-Elmer, 4000). The instrument was calibrated with an indium standard. The samples (2-4 mg) were heated (20-30°C) at a constant scanning speed (10°C / min) in sealed aluminum pans, using nitrogen purged gas (Lenkalapally Matsyagiri et al, 2019).

FTIR Spectroscopy: Drug-polymer compatibility studies were carried out using the FTIR spectrophotometer by KBr pellet technique. Pure drug and optimized formulation were subjected to FTIR study. Compatibility studies were carried out to know the possible interactions between Piretanide and excipients used in the formulation (Awasthi R et al, 2013).

Formulation Development: As the drug Piretanide poorly water-soluble, before formulating it as floating microballoons, it was converted to freely soluble solid dispersion using Vitamin E TPGS as a solubility enhancing carrier. The composition of solid dispersions prepared is given in below Table1. Solid dispersions were prepared by solvent evaporation method. Drug and carrier were dissolved in a suitable quantity of methanol, and solvent was slowly evaporated. The obtained solid residue was collected and evaluated (Saniya Jawed et al, 2017).

Table 1: Compositions of solid dispersions of Piretanide

Sl. No.	Materials	PRT1	PRT2
1	Piretanide	10 mg	10 mg
2	Vitamin E TPGS	10 mg	20 mg
3	Methanol	10 mL	10 mL
Ratio of drug to polymer		1:1	1:2

Evaluation of Solid Dispersions:

Saturation Solubility:

Saturation solubility studies were conducted for prepared solid dispersions along with pure drug by adding an excess amount of drug in 2 mL of water and shaking it for 48-72 hours until equilibrium is attained (Peeyush Bhardwaj et al, 2010). Then the solution is centrifuged and the supernatant is analyzed for amount of drug dissolved by spectrophotometrically at 276 nm.

In-vitro Dissolution Study:

The drug release study was carried out using USP dissolution apparatus type XXIII basket type dissolution apparatus at 37 ± 0.5°C and at 50 rpm using 900 ml of 0.1N hydrochloric acid (pH 1.2) as a dissolution medium (Mali AD et al, 2015).

Formulation of Floating Microballoons:

Solid dispersion prepared with 1:2 ratio of drug to Vitamin E TPGS (PRT2) has shown improved solubility and dissolution and hence was chosen for preparing floating microballoons. The floating microballoons were formulated by solvent evaporation method. The polymer is dissolved in an organic solvent and the solid dispersion (10 mg) equivalent to 30 mg of drug is either dissolved or dispersed in the polymer solution. The solution containing the drug is then emulsified into an aqueous phase containing suitable additive (surfactants /polymer) to form oil in water emulsion. After the formation of a stable emulsion, the organic solvent is evaporated either by increasing the temperature under pressure. Stirring was continued for 6 h under 3 blade propellers at 500 rpm, 40°C until the smell disappears (Singh BN et al, 2000). The solvent removal leads to polymer precipitation at the oil/water interface of droplets, forming cavity and thus making them hollow to impart the floating properties. Then microballoons are collected and washed with excess amount of distilled water to remove any remnants. Collected microballons were dried at room temperature and subjected for further evaluation (Lenkalapally Matsyagiri et al, 2013).

Table 2: Composition of floating microballoons of Piretanide

Materials	PRT F1	PRT F2	PRT F3	PRT F4	PRT F5	PRT F6	PRTF 7	PRT F8	PRT F9	PRT F1 0	PRT F1 1	PRT F1 2	PRT F13	PRT F1 4	PRT F1 5
Piretanide SD Eqvt. to 10 mg	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Eudragit RS 100	10	10	10	20	20	20	10	10	20	20	NA	NA	NA	NA	NA
Eudragit S 100	10	20	30	10	20	30	30	30	20	20	NA	NA	NA	NA	NA
HPMC K4M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10	10	10	20	20
Ethyl cellulose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10	20	30	10	20
Ethanol	15	15	15	15	15	15	20	10	20	10	15	15	15	15	15
Dichloromethane	15	15	15	15	15	15	10	20	10	20	15	15	15	15	15
Ratio of drug to polymer	1:01:01	1:01:02	1:01:03	1:02:01	1:02:02	1:02:03	1:01:03	1:01:03	1:02:02	1:02:02	1:01:01	1:01:02	1:01:03	1:02:01	1:02:02
Ratio of solvent	1:01	1:01	1:01	1:01	1:01	1:01	2:01	1:02	2:01	1:02	1:01	1:01	1:01	1:01	1:01

***In-vivo* Evaluation of Microballoons:** The experimental protocol to carry out *in-vivo* studies were reviewed and approved by the IAEC. The *in-vivo* performance of the optimized formulations was evaluated on healthy albino rabbits (Lenkalapally Matsyagiri et al, 2019).

***In-vivo* Radiographic Studies:** *In-vivo* floating behavior of optimized floating microballoons formulation was studied in healthy albino rabbits, weighing 1.5 kg to 2 kg. The 3 healthy male albino rabbits were used for the study. Animals were maintained for one week in the animal house to acclimatize them and were fed a fixed standard diet, under standard laboratory conditions (Temperature 25 ± 2°C). To monitor the *in-vivo* transit behavior of the prepared floating microballoons. First X-ray was taken for all the rabbits to ensure the absence of radio-opaque material in the stomach. Radiopaque microballoons were prepared by incorporating 500 mg of barium sulfate into the polymeric solution, and a similar procedure by which optimized microballoons were prepared was followed. Optimized microballoons prepared with barium sulphate equivalent to rabbit dose (3.5 mg/kg) were administered to rabbits with a sufficient amount of water. Gastric radiography is done at intervals of 0.5, 2.5, and 4. 5, 5.5 h in both fed and unfed state (Mandal UK et al, 2016).

***In-vivo* Pharmacokinetic Evaluation of the Optimized Microballoons:** Six healthy albino rabbits with body weight range of 1.5-2.5 Kg were selected through physical examination. An open-label, balanced, randomized, single-dose complete crossover study design in which six healthy albino rabbits received one treatment (product) each with a washout period of 7 days was designed and pharmacokinetic parameters are assessed. Healthy rabbits are divided into 2 groups (n=6 for each group). Group I animals are treated with optimized formulation (PRTF10) and group II animals are treated with marketed formulation (Arelix). At the predetermined time intervals of 0, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, and 24.00 h, 0.5 ml of blood samples were withdrawn from marginal ear vein and analyzed using HPLC.

Pharmacokinetic parameters such as peak plasma concentration (C_{max}), time at which C_{max} occurred (t_{max}), area under the curve (AUC), biological half-life (t½), were calculated in each case using the data by kinetica TM 2000 software (Inna phase corporation, U. S. A) Using the non-compartmental approach. Percent relative bioavailability of the optimized formulations with reference to the marketed preparation is studied (Bagre A et al, 2017).

RESULTS AND DISCUSSION:

Solubility studies

Table 3: Solubility studies of Piretanide

Solvent	Solubility (mg/mL)				
	1	2	3	Average	SD
Double distilled water	0.01	0.02	0.015	0.015	0.05
0.1N Hydrochloric acid	0.025	0.035	0.042	0.034	0.008
pH 6.8 Phosphate buffer	0.034	0.056	0.057	0.049	0.01
pH 7.4 Phosphate buffer	0.045	0.058	0.052	0.052	0.06

Solubility of pure drug was determined in different solvents and the values obtained are given in the **Table 3**. From the results obtained it was observed that the drug was very freely soluble in distilled water. Solubility was found to be comparatively lesser in 0.1N Hydrochloric acid and the solubility was increased with increase in pH.

Acid stability of Piretanide

Table 4: Acid stability of Piretanide in 0.1N Hydrochloric acid (n=3)

Time (h)	Absorbance	Concentration (µg/ml)
0	0.362	10.00±0.07
1	0.366	10.04±0.19
2	0.365	10.07±0.08
4	0.367	10.05±0.05
6	0.365	10.03±0.06
8	0.366	10.02±0.21
12	0.367	10.18±0.34
24	0.366	10.14±0.07

Drug Excipient Compatibility Study:

Differential Scanning Calorimetry: DSC thermogram of the pure drug is shown in **Fig. 1** endothermic peak was observed at 194.1°C indicates the drug melting point for the pure drug.
The shift in the endothermic peak of the drug was very less (190.5°C), which indicates that the drug and polymers used were compatible with one another in the DSC of optimized formulation **Fig. 2**.

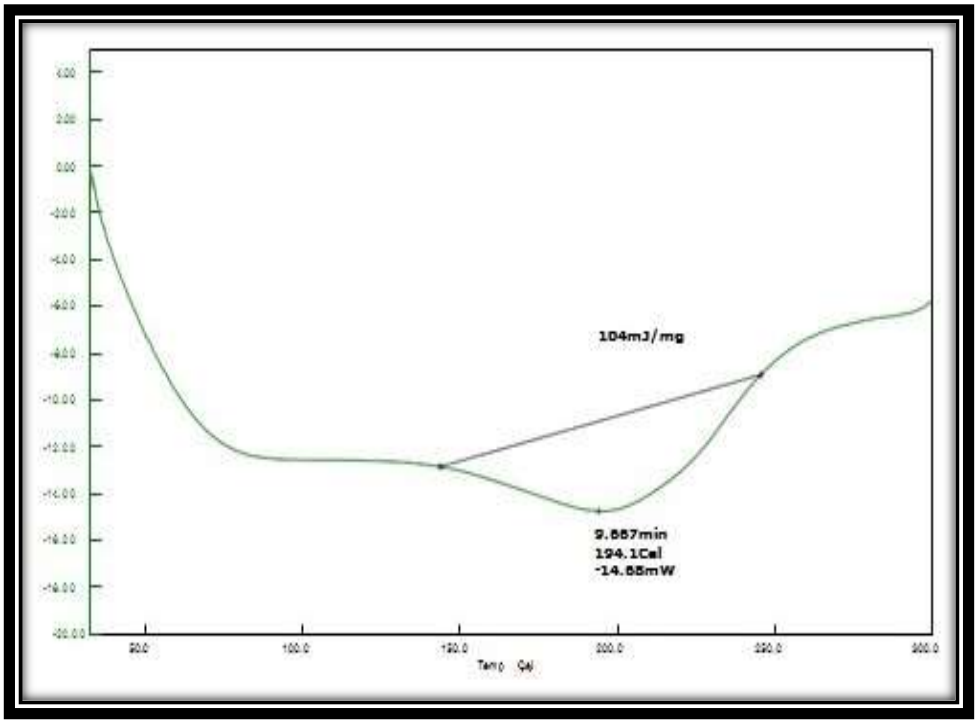

Figure 1: DSC thermogram of pure drug Piretanide

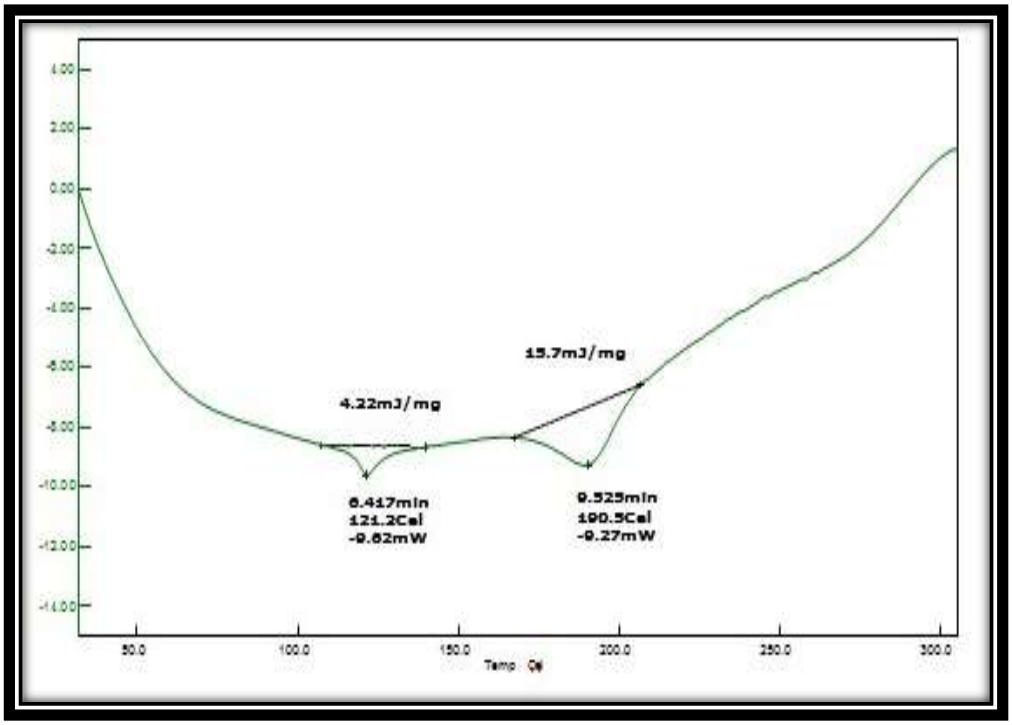

Figure 2: DSC thermogram of optimized formulation of Piretanide Microballoons

DSC thermogram of pure drug is shown in **(Figure 1)** Endothermic peak was observed at 194.1°C corresponding to the melting point of pure drug (206 ^{o}C). The shift in the endothermic peak of drug was very less in optimized formulation which indicates that the drug and polymers used were compatible with one another.

FTIR

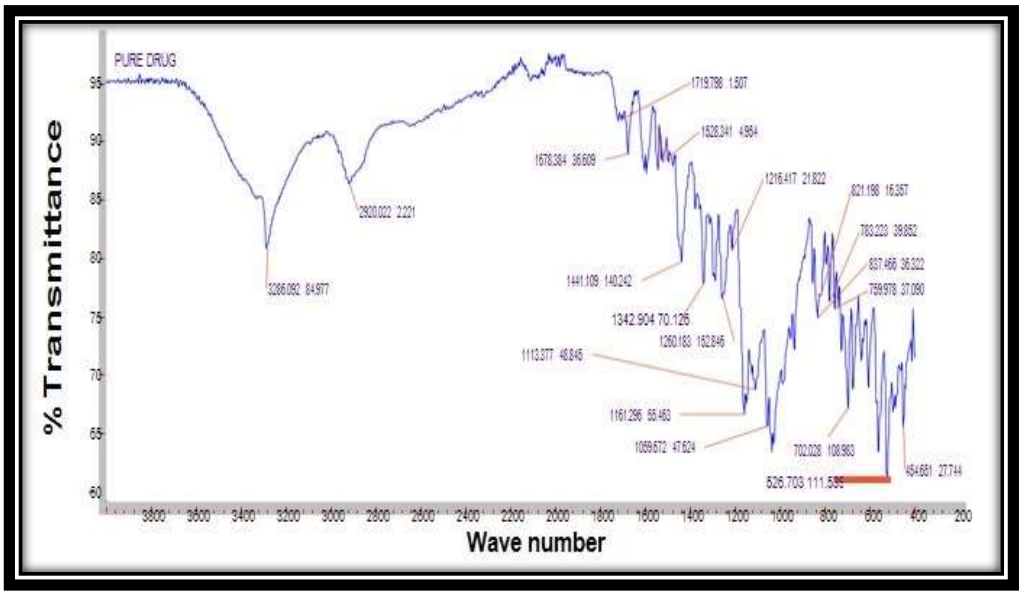

Figure 3: FTIR of pure drug Piretanide

Table 5: FTIR data of pure drug Piretanide

Sl. No	Frequency(cm^{-1})	Functional group
1	3286.09	OH
2	1678.38	C=O
3	1440.2	-NH
4	1047.62	C-O

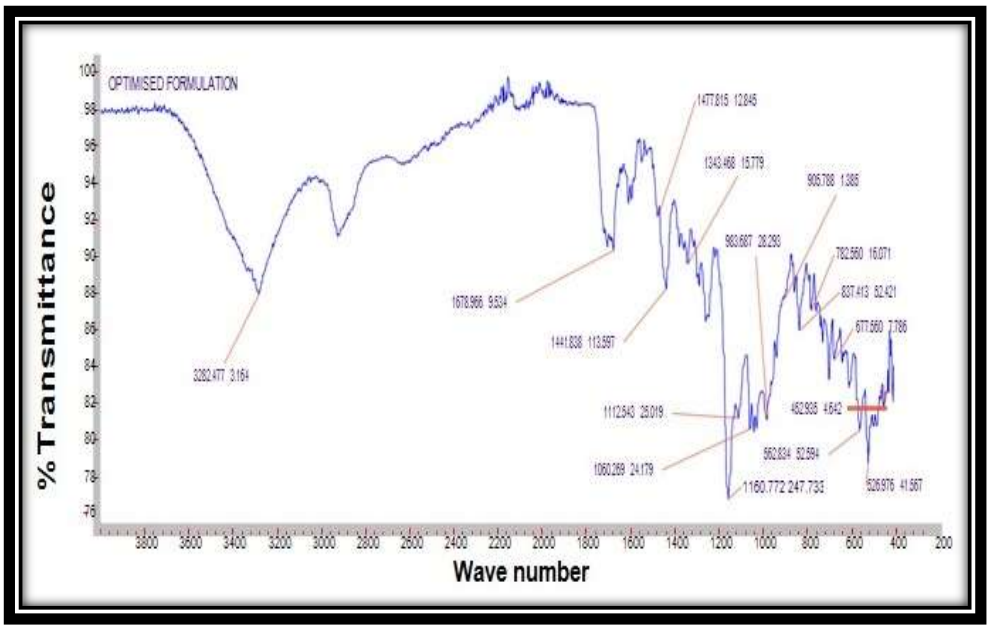

Figure 4: FTIR of optimized formulation of Piretanide Microballoons

Table 6: FTIR data of optimized formulation of Piretanide

Sl. No	Frequency(cm^{-1})	Functional group
1	3262.47	OH
2	1678.96	C=O
3	1441.83	-NH
4	1160.77	C-O

The drug-excipient compatibility study was done by Fourier transform infrared (FT- IR) spectroscopy study. The prominent peaks of Piretanide pure drug were shown at 3286.09cm^{-1} (due to –O-H), 1678.38cm^{-1} (due to C=O), 1440cm^{-1} (due to –N-H) and1047.62 cm^{-1}(due to –C-O) **(Figure 3 & Table 5)**. These prominent peaks of drug were also observed in the IR spectrum of optimized formulation **(Figure 4 & Table 6)** which indicates that the drug was not interacted with the polymers used in the study which confirms the stability of the drug.

***In vivo* floating behavior:**

The optimized floating microballoons formulation prepared were tested for *in vivo* floating behavior in healthy albino rabbits. Radiographic images obtained at 0.5hrs, 2.5 hrs, 4.5 hrs & 5.5 hrs are shown in Figure 8 & 9. It was observed from the images that the formulation was remained buoyant for up to 5.5 hrs in the stomach indicating the uniform distribution of formulation in the stomach. But in unfed state the formulation remained buoyant in the stomach only up to 2.5 hrs this is because in fasting condition myoelectric migrating contractions forces the contents to duodenum from stomach. The forceful waves will remove all the contents of stomach including dosage form. This will not take place in fed state. Therefore from these studies it was clearly observed that the floating microballoons should be given to patients after a standard diet.

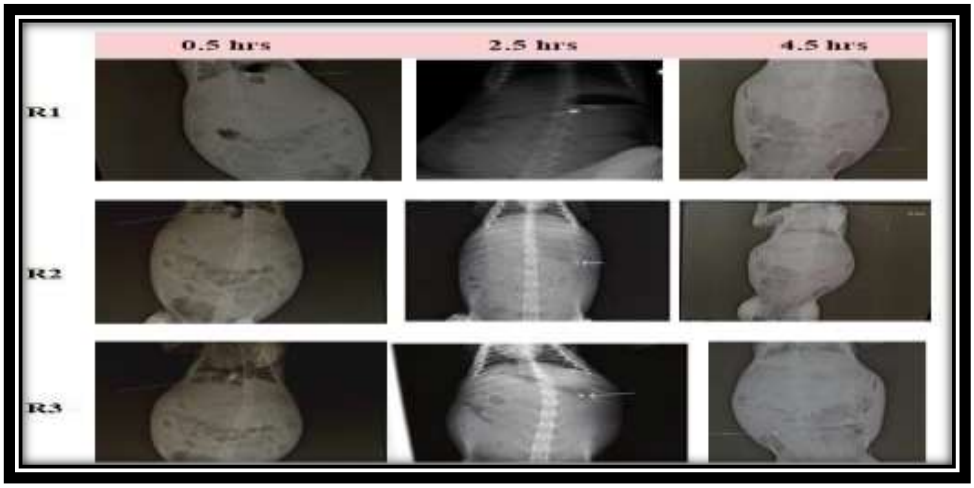

Figure 5: X-ray images of optimized microballoons of Piretanide in the gastric region of rabbit during unfed state at 0.5 hrs, 2.5 hrs, and 4.5hrs.

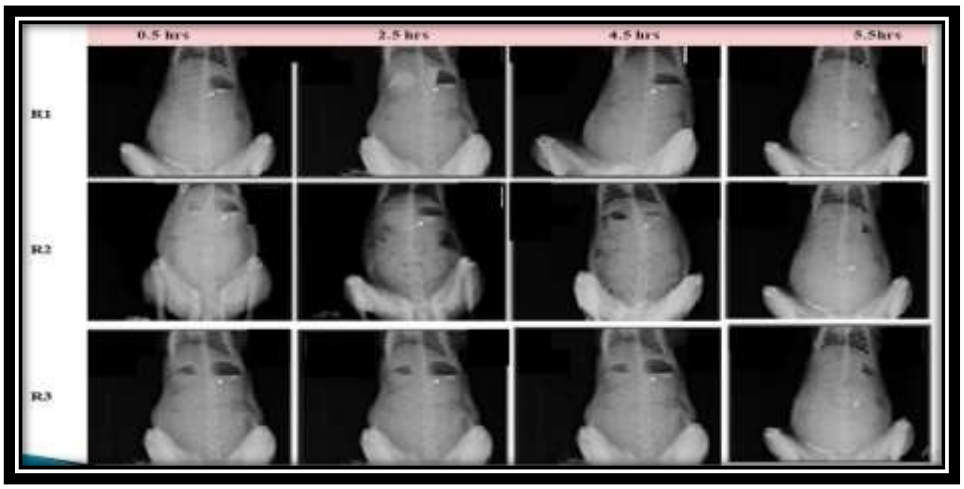

Figure 6: X-ray images of optimized microballoons of Piretanide in the gastric region of rabbit during fed state at 0.5 hrs, 2.5 hrs, 4.5hrs, and 5.5hrs.

Table 7: Plasma concentration of Piretanide conventional tablets (Arelix) in rabbits (n=6) at different time intervals (Reference formulation)

Time (hrs)	Plasma Concentration (ng/mL)							
	Animal 1	**Animal 2**	**Animal 3**	**Animal 4**	**Animal 5**	**Animal 6**	**Average**	**SD**
0	0	0	0	0	0	0	**0**	0
0.5	4.5	4.6	4.8	5.2	4.9	4.7	**4.78**	0.25
1	5.5	5.7	6.2	5.8	5.9	6.2	**5.88**	0.28
1.5	7.2	7.4	7.5	7.1	6.9	7.5	**7.27**	0.24
2	10.5	10.6	11.2	10.9	11.6	10.8	**10.93**	0.41
2.5	12.5	13.2	13.9	14.2	12.8	13.2	**13.30**	0.64
3	15.2	15.5	16.2	15.5	16.3	15.8	**15.75**	0.43
4	12.5	13.2	13.8	14.5	15.2	15.6	**14.13**	1.19
6	10.5	11.2	10.8	10.9	11.3	12.5	**11.20**	0.70
8	8.5	8.8	8.9	9.2	9.5	8.8	**8.95**	0.35
12	6.5	6.6	6.8	7.1	6.2	6.4	**6.60**	0.32
24	4.2	4.5	4.4	4.8	5.2	5.3	**4.73**	0.45

Table 8: Plasma concentration of Piretanide floating microballoons (PRTF10) in rabbits (n=6) at different time intervals (Test Formulation)

Time (hrs)	Plasma Concentration (ng/mL)							
	Animal 1	**Animal 2**	**Animal 3**	**Animal 4**	**Animal 5**	**Animal 6**	**Average**	**SD**
0	0	0	0	0	0	0	**0**	0
0.5	3.2	3.5	3.8	3.9	3.3	3.3	**3.50**	0.29
1	4.5	4.9	5.1	4.2	4.8	5.1	**4.77**	0.36
1.5	6.8	7.2	6.9	6.5	6.6	7.5	**6.92**	0.38
2	8.9	9.1	8.8	9.3	9.4	9.6	**9.18**	0.31
2.5	11.5	12.5	13.5	10.2	11.5	11.9	**11.85**	1.11
3	14.8	15.2	16.5	14.5	13.5	13.8	**14.72**	1.08
4	16.9	17.5	18.2	16.2	14.9	15.5	**16.53**	1.24
6	18.5	21.2	23.2	21.2	19.2	21.2	**20.75**	1.68
8	14.5	15.5	14.3	12.9	13.5	16.8	**13.92**	1.04
12	12.5	13.2	13.5	10.5	12.6	15.5	**13.80**	1.05
24	3.5	3.5	3.5	3.8	3.8	3.5	**3.60**	0.15

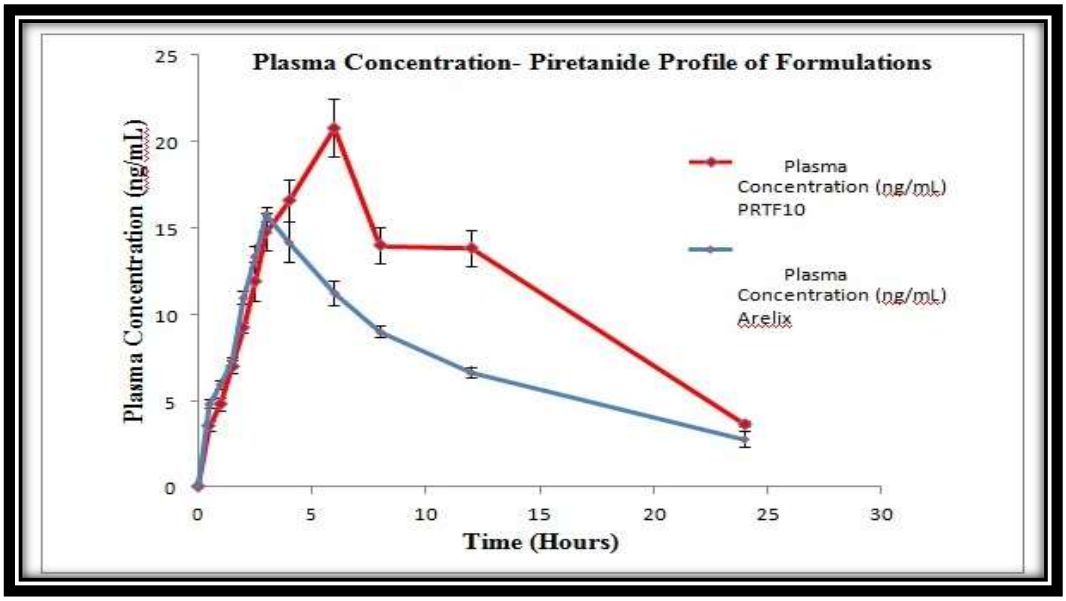

Figure 7: Mean plasma concentration time profile of Piretanide test (PRTF10) and Reference (Arelix) formulations

***In vivo* pharmacokinetic study**

Table 9: Mean Pharmacokinetic Parameters of Piretanide as Reference and Test Tablets in Rabbits (N=6)

Pharmacokinetics parameters	Unit	Reference	Test
C_{max}	ng/ml	15.75	20.75
t_{max}	h	3	6
AUC_{0-t}	ng/mlXh	184.54	269.19
$AUC_{0-\alpha}$	ng/mlXh	302.4	327.9
$t_{1/2}$	h	7.45	18.72

The *in vivo* pharmacokinetic study was conducted in healthy albino rabbits. In this study, the pharmacokinetics parameters of Piretanide floating microballoons were compared with IR Tablets (Arilex).The mean plasma concentration – time profile obtained from the study is shown in **Figure 7**. Various pharmacokinetic parameters were estimated such as C_{max}, t_{max}, AUC and relative bioavailability are given in **Table 9**. The significance of the difference between the treatments was evaluated by using Graph pad Prism by student paired t- test. The results showed that the difference between all pharmacokinetic parameters of IR and Floating microballoons were statistically significant ($p<0.050$).

The mean t_{max} of reference formulation was 3 hrs. This indicates that the drug release from the reference formulation was rapid while in the test formulation the mean t_{max} was 6 hrs. This indicates that the test formulation was effective in delaying the peak plasma concentration, thus showing prolonged plasma concentration of Piretanide from the floating microballoons. The mean biological half-life ($t_{1/2}$) of Piretanide from test and reference formulations was 18.72h and 7.45h respectively. The difference observed here is due to prolonged absorption of test formulation there is prolonged continuous release of drug into blood stream. Therefore, the test formulation shows to have longer half-life i.e., the drug stays in the plasma for a longer time than the reference formulation. The lower half-life of reference preparation indicates rapid removal of drug from plasma where as higher half – life of test formulation indicates prolonged release.The mean area under plasma time curve AUC_{0-t} and $AUC_{0-total}$ of reference formulation was 184.5458 ng/ml×h and 302.4ng/ml×h and while AUC_{0-t} and $AUC_{0-total}$ of test formulation was 269.19583 ng/ml×h and 327.9 ng/ml×h,

This indicates that the overall absorption of Piretanide was more in the test formulation with respect to the reference product at the same dose. It was observed from the results that the oral bioavailability of optimized formulation **(PRTF10)** was increased significantly when compared to marketed formulation. Relative bioavailability with respect to marketed formulation was found to be 108.4 which are due to prolonged gastric residence time of Piretanide floating microballoons.

CONCLUSION:

Gastro-retentive drug delivery system for Piretanide was successfully prepared and evaluated by the solvent evaporation technique using Eudragit RS 100, Eudragit S 100, HPMC K4M, ethyl cellulose polymers. From the drug-excipient compatibility studies, it was observed that, there was no interaction between drug and excipients used in the formulations. Prepared floating microballoons showed significant floating ability, good buoyancy, and sustained drug release. *In vitro* drug release of microballoons was influenced by polymers concentration. *In-vivo* bioavailability study was conducted in rabbits optimized formulation (PRTF10) showed increased bioavailability when compared to the reference marketed tablets (Arelix) due to controlled floating technology. Microballoons prepared in this study provide a promising gastro retentive drug delivery system to deliver Piretanide with sustained-release in order to improve oral drug bioavailability.

CONFLICT OF INTEREST: No conflict of interest.

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