FORMULATION AND EVALUATION OF GASTRO RETENTIVE FLOATING MICROBALLOONS OF ALENDRONATE SODIUM

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ABSTRACT
The aim of the research work was mainly focused on the formulation & evaluation of gastro retentive floating microballoons of Alendronate sodium using different polymers. It is used in the treatment of Osteoporosis and paget's disease, belongs to class II as per BBCS and hence it exhibits low aqueous solubility and high permeability and it undergo high first pass metabolism, which affects in low dissolution rate and in turn affect the bioavailability. Floating microballons were prepared by non-aqueous solvent evaporation method by using polymers like Eudragit S 100, Eudragit RS 100, HPMCK4M, Ethyl cellulose, solvents like Ethanol, Dichloromethane. Preliminary Solubility, acid stability studies were conducted for a drug. Drug-Excipient compatibility study was done by using DSC & FTIR and found that the drug was compatible with all the excipients. Floating microballons were evaluated for physicochemical properties by measuring the angle of repose, Carr's index, mean particle size and floating properties like drug entrapment efficiency, % yield, floating buoyancy, Drug content and *in vitro* drug release studies, conducted drug release kinetics, stability studies further conducted *in vivo* studies for optimized batch. Results shown that the *in vitro* evaluation studies demonstrated that microballoons prepared by using Eudragit S 100 and Eudragit RS 100 in 1:1 ratio (ASF10) shown maximum drug release. Hence it is selected as the optimized formulation and it followed zero order kinetics. The *in vivo* Radiographic images showed buoyant for up to 5.5 h. The mean area under plasma time curve AUC $_{0\text{-}t}$ and AUC $_{0\text{-}total}$ of reference formulation was 1023.01ng/ml×h and 1548.60 ng/ml×h and while AUC $_{0\text{-}t}$ and AUC $_{0\text{-}total}$ of test formulation was 1652.21ng/ml×h and 2939.76 ng/ml×h, This indicates that the overall absorption of Alendronate sodium 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 **(ASF10)** was increased significantly when compared to marketed formulation. Relative bioavailability with respect to marketed formulation was found to be 189.8 which is due to prolonged gastric residence time of Alendronate sodium floating microballoons. Microballoons were found to be stable at storage conditions for three months.

Keywords: Alendronate sodium, Floating microballoons, Gastro retentive drug delivery system, *in vitro* and *in vivo* Evaluation.

INTRODUCTION

Gastro retentive drug delivery system

Gastro retentive drug delivery systems can remain in the stomach for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged the gastric retention improves bioavailability, reduces the drug waste[1]. Their application can be advantageous in the case of drugs absorbed mainly from the upper part of GIT or unstable in the alkaline medium[2]. GRDDS can be used as carriers for drugs having narrow absorption windows. Microballoons are solid spherical particles having central hollow space. Floating microballons are non

effervescent multiple unit systems, floated on gastric fluid by low density[3]. Floating microballoons are gastro-retentive drug delivery systems based on non-effervescent approach. Microballoons are in strict sense, spherical empty particles without core. These are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 micrometer[4].

Alendronate sodium is a bisphosphonate drug that prevents osteoclastic bone resorption which is used for the prevention and treatment of osteoporosis and Paget's disease. Alendronate sodium is a second generation antiresorptive drugs, 10-100 times more potent than first generation antiresorptives. It exhibits low bioavailability.

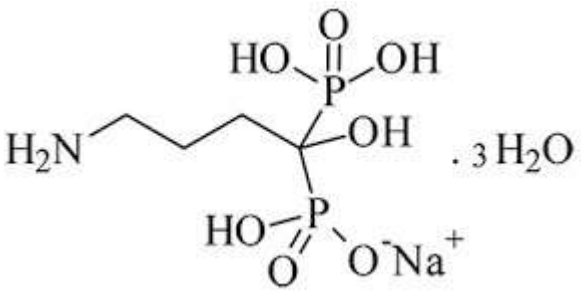

Figure 1: Structure of Alendronate sodium

MATERIALS AND METHODS

Materials

Alendronate sodium 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

Preliminary Solubility studies Alendronate sodium

The equilibrium solubility of Alendronate sodium was measured in 0.1M hydrochloric acid (pH of 1.2), phosphate buffer of pH 6.8, distilled water and phosphate buffer of pH 7.4 respectively in order to determine its solubility. Excess amount of the drug were added to 50 mL-stoppered conical flasks (n=3). The flasks were shaken mechanically at 37°C±0.5°C for 24 hrs, in a horizontal shaker. After 2 days of equilibrium, aliquots were withdrawn and filtered (0.22 µm pore syringe filter). Then, the filtered samples were assayed by UV-spectrophotometer at 264 nm for Alendronate sodium.

Determination of acid stability studies of a drug in 0.1N HCl

Stock solution of Alendronate sodium was prepared in 0.1 N HCl in order to determine its acid stability. At predetermined time points 1, 2, 4, 6, 8, 10, 12 and 24hrs, the samples were analyzed using UV-Visible spectrophotometer at 264 nm for Alendronate sodium to see whether there is any change in the absorbance and concentration in the prepared stock solutions.

Drug-excipient compatibility study

Differential scanning Calorimetry

The physicochemical compatibilities of the drug and the excipients were tested by differential scanning calorimetric (DSC) analysis.

FTIR spectroscopy

Compatibility studies were carried out to know the possible interactions between Alendronate sodium and excipients used in the formulation.

Preparation of Alendronate sodium floating microballoons

The floating microballoons were formulated by solvent evaporation method. The solution containing the drug is then introduced into an aqueous phase containing suitable additive (polymer/ surfactants) to form oil in water emulsion. Once the stable emulsion has formed, the organic solvent is evaporated either by continuous stirring or 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. The solvent removal leads to the precipitation of polymer at the oil/water interface of droplets, which forms cavity thus makes the microballoons. Those are collected and washed with excess amount of distilled water to remove any remnants. Collected microballons were dried at room temperature. (5-6).

Table 1: Composition of Alendronate sodium floating microballoons

Sl. No.	Materials	ASF 1	ASF 2	ASF 3	ASF 4	ASF 5	ASF 6	ASF 7	ASF 8	ASF 9	ASF 10	ASF 11	ASF 12	ASF 13	ASF 14	ASF 15
1	Drug (mg)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
2	Eudragit RS 100	35	35	35	70	70	70	35	35	70	70	NA	NA	NA	NA	NA
3	Eudragit S 100	35	70	105	35	70	105	105	105	70	70	NA	NA	NA	NA	NA
4	HPMC K4M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	35	35	35	70	70
5	Ethyl cellulose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	35	70	105	35	70
6	Ethanol	15	15	15	15	15	15	20	10	20	10	15	15	15	15	15
7	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

Evaluation of floating microballoons of Alendronate sodium

Physico chemical properties and floating properties of Alendronate sodium microballoons

Floating microballons were evaluated for physicochemical properties of all batches by measuring the angle of repose and Carr's index, mean particle size and floating properties like drug entrapment efficiency, percentage yield, floating buoyancy.

***In vitro* release study:**

The *in vitro* drug release was carried out by using USP basket type dissolution apparatus containing 900 mL of 0.1N HCl (pH 1.2) as a dissolution medium at 37 ± 0.5 ºC at 50 rpm. At predetermined time intervals such as 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hrs 5 mL of sample was withdrawn and the samples were filtered through whatman filter paper, diluted suitably and analyzed spectrophotometrically at 299 nm. After withdrawal of the test sample, equal amount of fresh dissolution medium was added immediately to maintain 900 mL of dissolution media. The dissolution studies were performed and the average percentage drug release was calculated.

Drug Release Kinetics floating microballoons of Alendronate sodium

To describe the kinetics of the drug release from matrix tablet, mathematical models such as zero-order, first-order and Higuchi, models were used. The criterion for selecting the most appropriate model was chosen based on the goodness-or-fit test [7-8].

Stability Studies of floating microballoons of Alendronate sodium

Stability studies were conducted according to international conference on harmonization (ICH) guidelines (9-11). Optimized Microballoons **(ASF10)** were enclosed in polyethylene covers and placed in Dessicator containing saturated sodium chloride solution (75 % RH). The Dessicator was stored at 40°C for 3 months (12-16).

***In vivo* evaluation of floating microballoons of Alendronate sodium**

***In vivo* Evaluation of Gastric residence time in Rabbits**

In vivo floating behavior of optimized floating microballoons formulation was studied in healthy albino rabbits, weighing 1.5 kg to 2.5 kg. The study was based on the principle of monitoring radiological activity. Animals were maintained under standard laboratory conditions (Temperature 25±2^{0}C). The 3 healthy male albino rabbits were used to study the *in vivo* transit behavior of the formulated microballoons. Animals with any inflammation, dermatitis, infection or apparent abnormalities of the urinary tract were also excluded from the study. None of the animals should have symptoms or past history of gastro-intestinal disease. First X- ray was taken for all the rabbits to ensure absence of radio opaque material in the stomach(17-22). During the study food was not allowed to eat by animals but provided with water. Radio opaque microballoons were prepared by incorporating 500 mg of barium sulfate into polymeric solution and similar procedure by which optimized formulation was prepared was followed and administered to rabbits with sufficient amount of water. X-ray study was conducted both in fed and unfed state.

Fasting state : The $BaSO_4$ loaded microballoons were administered orally with sufficient amount of water through a mouth gag introduced in between the two jaws of rabbit.

Fed state: All the rabbits were fasted for 12 hrs before initiating the study and fed with a low calorie diet. Half an hour later, $BaSO_4$ loaded microballoons were administered orally with sufficient amount of water through a mouth and Gastric radiography is done at the time intervals of 0.5, 2.5, 4.5, 5.5 hrs and in between the radiographic imaging animals were not allowed to take any food but freed and permitted to move and carry out normal activities(23).

The *in vivo* performance of the optimized formulation **ASF10** was evaluated by pharmacokinetic study on

healthy albino rabbits obtained from Mahaveera Enterprises ,No. 203, Harsha Homes, 2-2-185/55/ E, Hyderabad-500013, Telangana, India and made comparison with the marketed formulation tablet **Fosamax 35 mg.**

Experimental design:

An open label, balanced, randomized, single-dose crossover study was designed in each treatment group with a wash out period of 7 days between successive treatments. Six healthy albino rabbits with body weight range of 1.5-2.5 Kg were selected after thorough physical examination. The test product was Ursodeoxycholic acid microballoons **(ASF10, 35 mg).** The reference product was tablet of Alendronate sodium **(Fosamax 35 mg).** Pharmacokinetic Parameters are assessed by administering the formulations in white albino rabbits weighing 1.5 to 2.5 kg. Healthy rabbits are divided into 2 groups (n=6 for each group). Group I animals are treated with optimized formulation **(ASF10)** and Group II animals are treated with marketed formulation **(Fosamax 35 mg).** At the predetermined time intervals 0.5 mL of blood samples were withdrawn from ear marginal vein and analyzed using HPLC.

Blood sample collection:

During each period, 0.5 ml venous blood samples were collected from the marginal ear veins of each rabbit in Accuvet tubes (Quantum Biologicals Pvt. Ltd., Chennai, India) containing K_3 EDTA. Blood Samples were collected at predetermined time intervals of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.00 and 24.00hrs and samples are stored in frozen conditions at - 20°C with appropriate labeling of subject code number, study date and collection time prior to analysis.

Sample preparation and extraction

Drug from the plasma is extracted using protein precipitation extraction technique. Blood samples are collected in Accuvet tubes (Quantum Biologicals Pvt. Ltd., Chennai, India) containing K_3 EDTA are immediately placed on ice and taken to the lab where they are centrifuged at 5000rpm for 5 min at room temperature. The resulting plasma samples are stored at deep freezer until analysis. Drug is extracted by using methanol as a precipitating solvent. Plasma sample is vortexed for one minute and the solution is centrifuged at 7000rpm for 10 min. The supernatant is taken and transferred to HPLC vials. The concentration of drug in plasma samples of each rabbit was analyzed by HPLC. (13).

Determination of pharmacokinetic parameters:

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 ($t_{½}$), were calculated in each case using the data by Kinetica^{TM} 2000 software (Inna Phase Corporation, U.S.A). Percent relative bioavailability of the optimized formulations with reference to the marketed preparation is studied.

Statistical analysis

All the data was statistically analyzed using a personal computer with Graph pad Prism and the significance of the difference between the pharmacokinetic parameters of IR tablet and Floating microballoons was evaluated by student paired t-test. A p-value less than 0.05 were termed significant.

RESULTS AND DISCUSSION

Evaluation of floating microballoons of Alendronate sodium

Solubility studies

Table 2. Solubility studies of Alendronate sodium

Solvent	Solubility (mg/mL)				
	1	2	3	Average	SD
Double distilled water	45.2	45.6	46.1	45.6	0.05
0.1N HCl (pH 1.2)	100.14	100.17	100.18	100.16	0.10
pH 6.8 Phosphate buffer	43.5	47.9	52.4	47.9	0.09
pH 7.4 Phosphate buffer	75.6	85.4	75.9	79.0	1.03

Acid stability of Alendronate sodium:

Table 3: Acid stability of Alendronate sodium (n=3):

Time (hr)	Absorbance	Concentration (µg/ml) (Mean±SD)
0	0.352	10.09±0.05
1	0.346	10.05±0.10
2	0.356	10.06±0.04
4	0.353	10.13±0.14
6	0.352	10.37±0.01
8	0.352	10.28±0.13
12	0.353	10.04±0.06
24	0.351	10.27±0.09

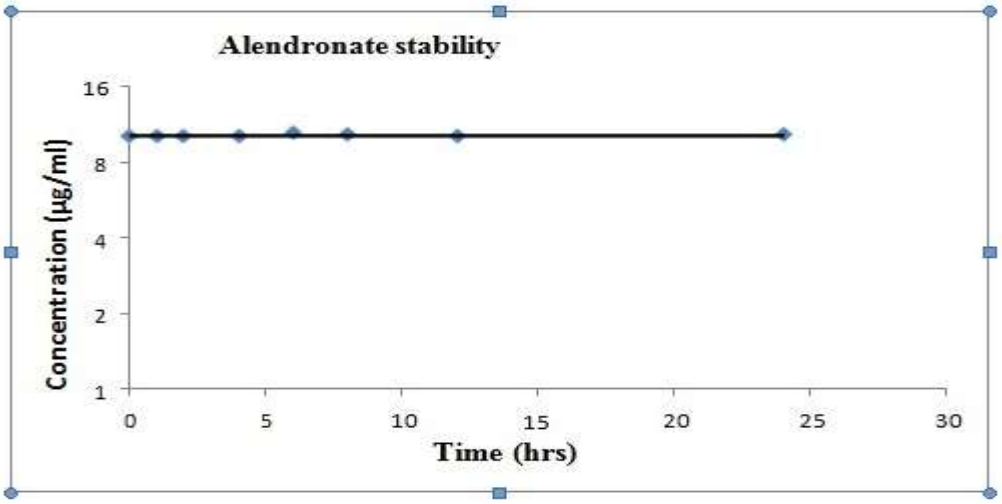

Figure 2: Acid stability profile of Alendronate sodium

Drug-Excipient Compatibility study

Differential Scanning Colorimetry (DSC)

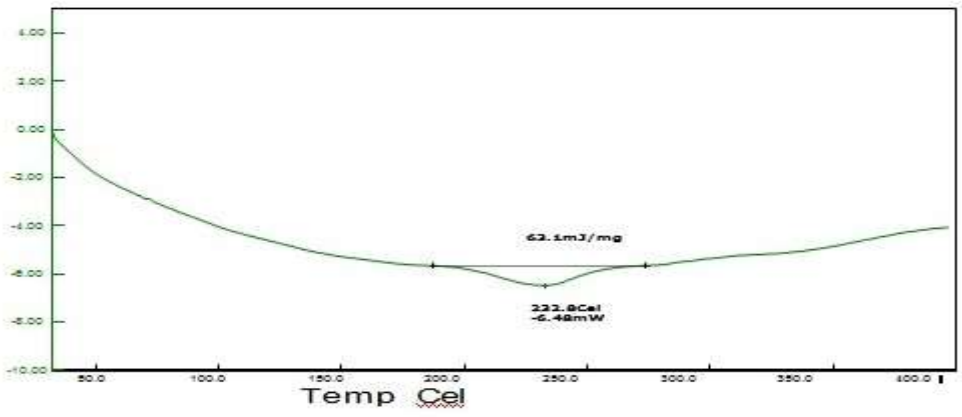

Figure 3: DSC thermogram of pure drug Alendronate sodium

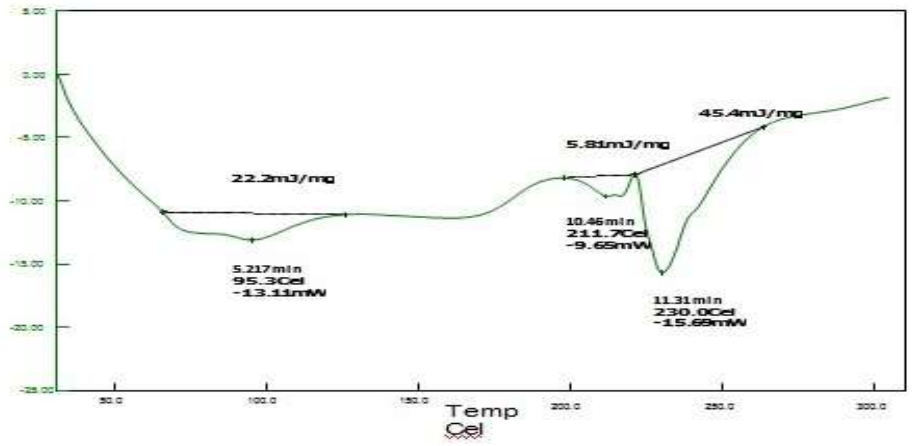

Figure 4: DSC thermogram of optimized formulation of Alendronate sodium

Fourier transform infrared (FTIR)

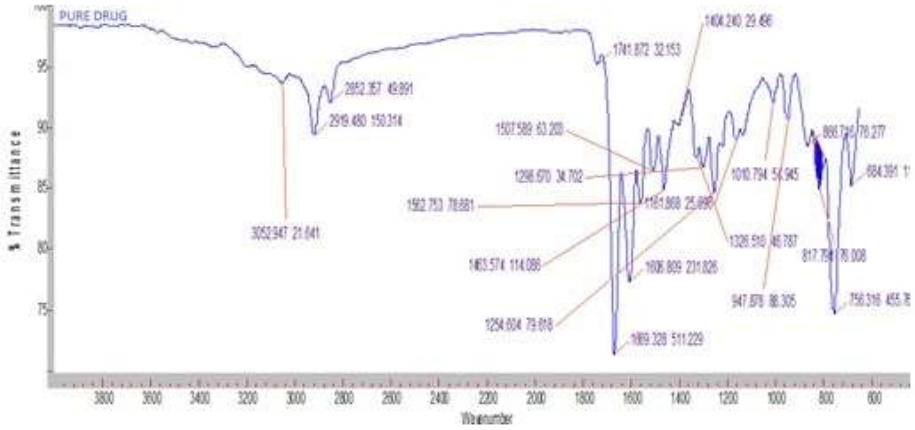

Figure 5: FTIR of pure drug Alendronate sodium

Table 4: FTIR data of pure drug Alendronate sodium

Sl. No	Frequency (cm^{-1})	Functional group
1	3052.94	OH
2	1328.50	P=O
3	2919.40	C-H
4	1404.24	P-C

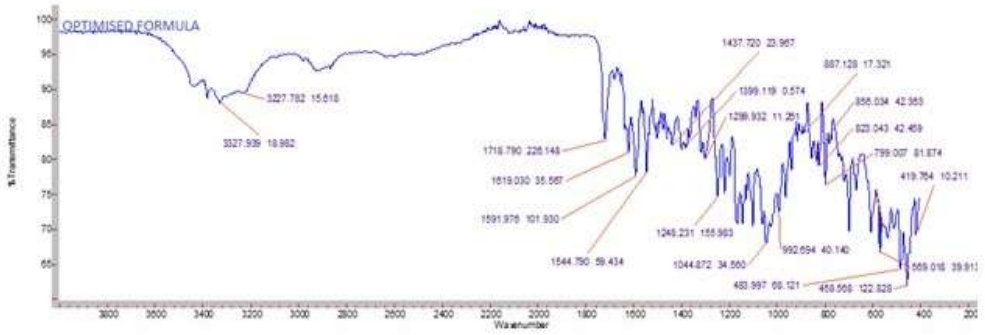

Figure 6: FTIR of optimized formulation of Alendronate sodium

Table 5 : FTIR data of optimized formulation of Alendronate sodium

Sl. No	Frequency (cm^{-1})	Functional group
1	3327.63	OH
2	1248.23	P=O
3	3227.78	C-H
4	1356.98	P-C

Table 6: Physico chemical properties of Alendronate sodium microballoons

Formulation	Bulk Density*	Tapped Density*	Compressibility Index*	Angle of Repose*	Mean particle Size (µm)**	Drug content
ASF1	0.71±0.06	0.64±0.28	9.86±0.18	15.3±0.02	124.36±1.36	98.74±0.19
ASF2	0.73±0.16	0.63±0.73	13.70±0.21	16.5±0.18	144.35±3.44	99.03±0.16
ASF3	0.77±0.34	0.68±0.22	11.69±0.07	16.4±0.22	154.45±2.25	97.69±0.37
ASF4	0.81±0.28	0.71±0.19	12.35±0.02	15.5±0.32	144.24±0.54	98.74±0.19
ASF5	0.77±0.55	0.66±0.25	14.29±0.16	15.7±0.18	155.33±2.56	99.04±0.11
ASF6	0.76±0.18	0.65±0.33	14.47±0. 21	15.8±01.1	133.55±2.56	99.03±0.11
ASF7	0.78±0.54	0.65±0.72	16.67±0.29	16.2±0.27	147.38±3.68	98.75±0.28
ASF8	0.73±0.19	0.64±0.19	12.33±0.06	15.4±0.32	136.26±4.45	98.75±0.31
ASF9	0.74±0.38	0.64±0.04	13.51±0.87	15.9±0.33	127.37±3.56	99.31±0.41
ASF10	0.77±0.32	0.69±0.07	10.39±0.09	13.4±0.28	129.31±2.46	98.06±0.52
ASF11	0.78±0.28	0.68±0.11	12.82±0.91	15.4±0.42	115.36±2.48	98.75±0.32
ASF12	0.75±0.15	0.67±0.32	10.67±0.09	16.2±0.33	125.35±2.48	99.06±0.02
ASF13	0.77±0.04	0.68±0.19	11.69±0.23	15.8±0.29	155.34±3.56	97.86±0.28
ASF14	0.77±0.07	0.68±0.26	11.69±0.34	15.8±0.19	165.34±2.34	98.59±0.03
ASF15	0.81±0.12	0.68±0.92	16.05±0.65	16.1±0.23	127.36±2.35	99.09±0.02

*****All values represent Mean±SD: n=3 ****** All values represent Mean±SD: n=100

Table 7: *In vitro* evaluation of Alendronate sodium microballoons

Formulation Code	% Yield	% Entrapment Efficiency	% Buoyancy
ASF1	86.4	83.4	77.8
ASF2	83.4	93.2	85.4
ASF3	82.6	94.6	85.1
ASF4	76.8	90.6	81.6
ASF5	81.2	90.2	85.5
ASF6	83.4	93.5	86.5
ASF7	81.4	90.7	82.9
ASF8	82.4	88.5	84.5
ASF9	81.6	90.5	81.2
ASF10	86.5	93.6	88.5
ASF11	71.5	69.8	66.5
ASF12	66.8	66.5	66.9
ASF13	65.4	63.5	65.3
ASF14	65.4	63.5	64.9
ASF15	60.2	62.3	63.8

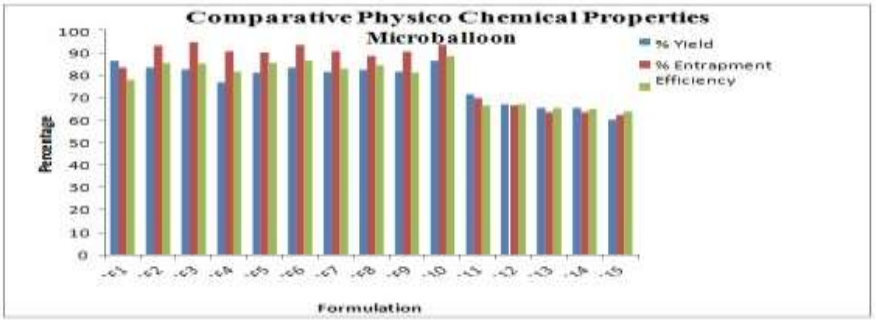

Figure 7: Comparative physico chemical properties of microballoons

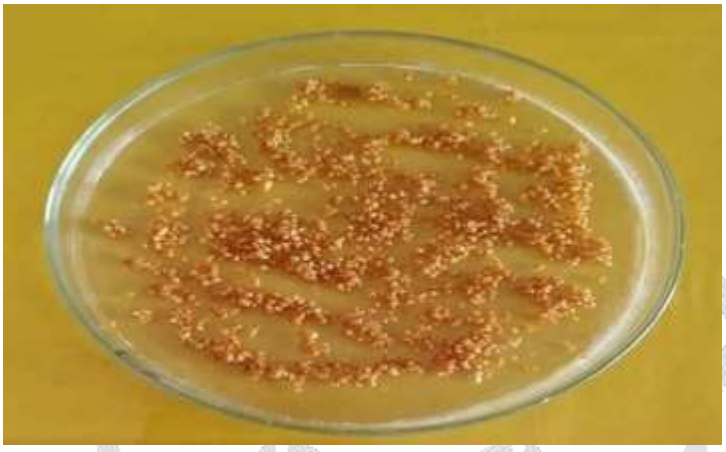

Figure 8: Prepared microballoons

***In vitro* buoyancy of Alendronate sodium floating microballoons**

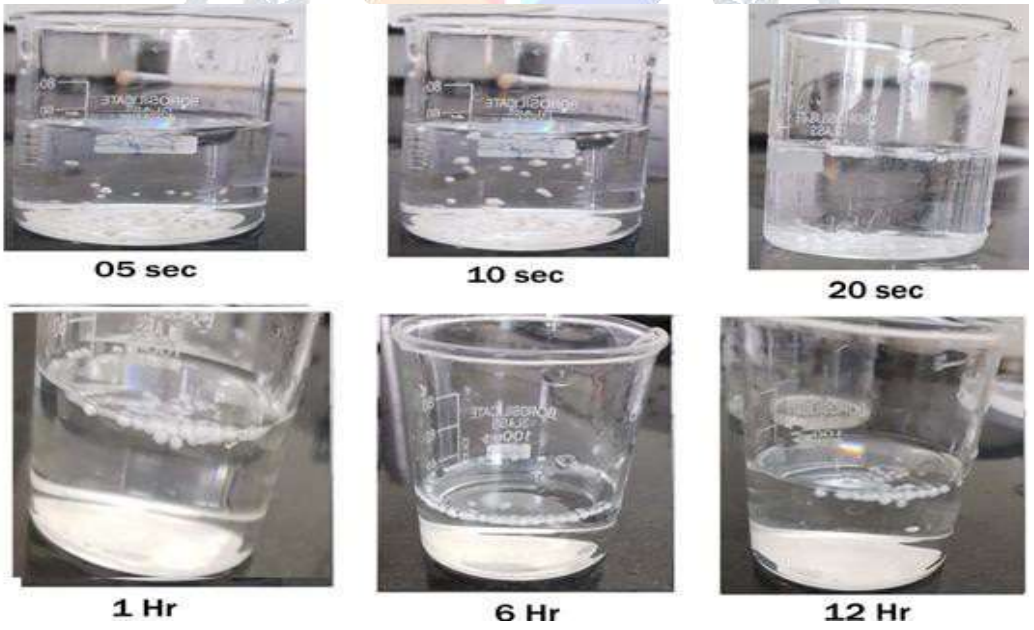

Figure 9: *In vitro* buoyancy of Alendronate sodium floating microballoons in 0.1N HCl

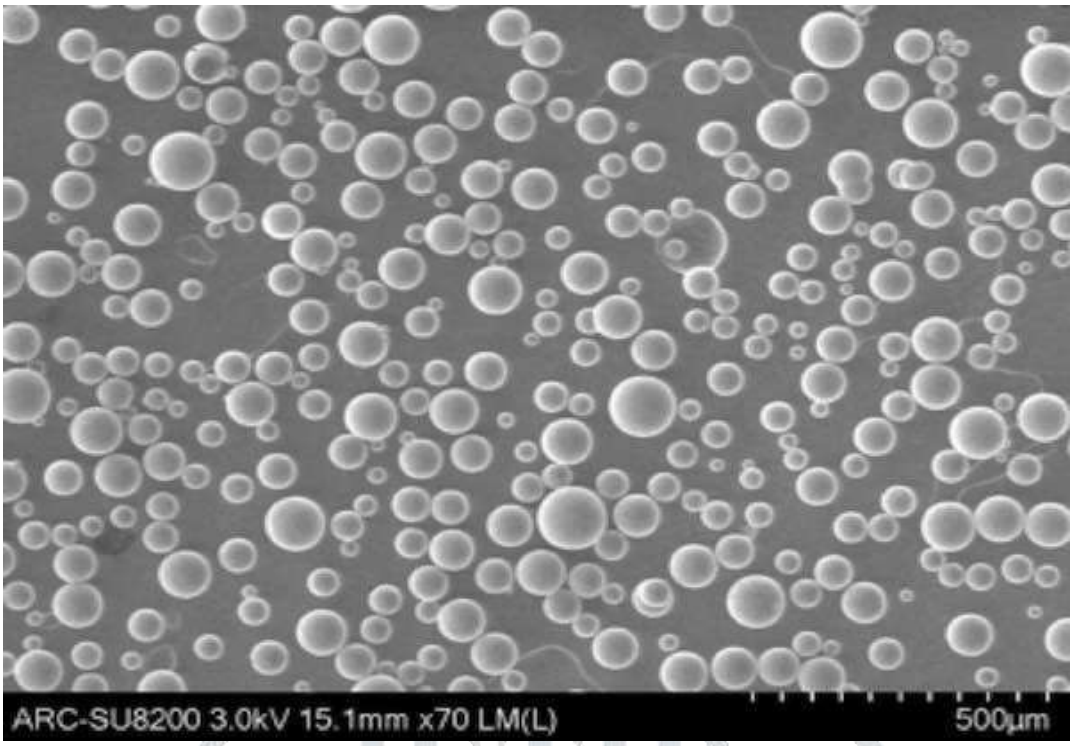

Figure 10: Scanning electron micrographs of optimized floating microballoons of Alendronate Sodium

Scanning electron microscope (SEM)

Scanning electron microscope was used to study the surface morphology of the floating microballoons. The surface morphology of optimized formulation (ASF10) was shown in the **Figure 10.** From the SEM micrographs it is apparent that the Alendronate sodium loaded microballoons were predominately spherical in appearance. The surface was observed to be smooth, dense and less porous, where as the internal core was highly porous and irregular with numerous depressions that are expression of evaporation of water, ethanol and dichloromethane The less porous outer surface and highly porous internal surface supported controlled release of drug from the microballoons and good buoyancy.

Table 8: Percentage drug release data of Alendronate sodium Microballoons

Time (Hr)	Percentage drug release							
	ASF1	ASF2	ASF3	ASF4	ASF5	ASF6	ASF7	ASF8
0	0	0	0	0	0	0	0	0
0.5	24.6±0.12	18.5±0.36	14.7±0.71	19.6±0.78	5.7±0.65	5.1±0.57	15.8±0.45	15.6±0.25
1	43.8±0.13	33.5±0.75	24.8±0.15	36.2±0.29	10.2±0.36	7.8±0.69	25.6±0.20	23.4±0.43
2	63.8±0.19	50.3±0.54	43.5±0.26	56.4±0.56	18.6±0.24	11.4±54	41.5±0.06	43.6±0.85
3	86.9±0.23	62.8±0.25	57.6±0.38	64.5±0.51	24.6±0.59	19.5±32	56.7±0.12	8.6±0.29
4	97.6±0.56	73.6±0.85	65.7±0.79	74.5±0.29	33.5±0.16	28.5±0.29	65.3±0.56	8.5±0.75
6	100.2±0.89	83.7±0.68	73.6±0.54	86.5±0.67	51.2±0.19	44.5±0.17	71.5±0.28	71.3±0.64
8		100.1±0.75	85.6±0.47	98.5±0.82	63.7±0.21	56.4±0.25	83.5±0.26	85.6±0.45
10			100.1±0.26	100.2±0.38	81.5±0.31	66.7±0.36	97.8±0.87	100.2±0.75
12					100.1±0.88	73.6±0.89	100.2±0.56	

Table 9: Percentage drug release data of Alendronate sodium Microballoons

Time (Hr)	Percentage drug release						
	ASF9	ASF10	ASF11	ASF12	ASF13	ASF14	ASF15
0	0	0	0	0	0	0	0
0.5	6.5±0.69	5.3±0.10	6.4±0.98	5.4±0.54	4.2±0.59	5.9±0.15	8.2±0.24
1	10.2±0.54	9.8±0.21	10.2±0.35	9.5±0.11	6.5±0.11	11.6±0.19	15.3±0.32
2	15.8±0.35	18.5±0.32	16.4±0.49	11.6±0.18	11.3±0.16	16.8±0.21	19.5±0.57

3	24.5±0.78	24.6±0.52	21.2±0.12	16.7±0.25	13.5±0.23	23.8±0.26	21.5±0.65
4	36.8±0.42	35.6±0.75	27.5±0.91	21.3±0.36	16.5±0.29	29.6±0.41	26.9±0.26
6	54.6±0.76	55.9±0.69	41.2±0.87	36.5±0.14	26.5±0.35	43.5±0.48	41.5±0.49
8	65.4±0.25	66.5±0.79	46.8±0.74	41.3±0.65	36.9±0.45	51.2±0.31.2	49.8±0.61
10	84.6±0.78	87.9±0.26	66.8±0.69	56.5±0.56	46.8±0.54	72.5±0.87	61.5±0.71
12	100.1±0.54	100.3±0.71	73.5±0.32	66.5±0.65	57.9±0.72	81.5±0.95	71.5±0.15

Dissolution studies of all the formulations were carried out using USP basket type dissolution apparatus. The dissolution profiles were compared among different formulations. The cumulative percentage drug release was decreased with increase in the polymer concentration. Based on the results of *in vitro* drug release studies it was found that ASF10 has shown sustained drug release for 12h with zero order drug release. The results of the *in vitro* drug release studies are shown in the **Table 8 and 9.**

Release Kinetics of Floating Microballoons

Table 10: Release kinetic parameters of Alendronate sodium Microballoons

Formulation code	Release Kinetics Parameters				
	Zero order	First order	Higuchi model	Korse- meyer peppas	Hixon - crowell
ASF1	0.100	0.991	0.804	0.827	0.952
ASF2	0.587	0.992	0.966	0.959	0.985
ASF3	0.744	0.989	0.985	0.978	0.979
ASF4	0.507	0.992	0.954	0.956	0.979
ASF5	0.998	0.938	0.868	0.998	0.964
ASF6	0.988	0.979	0.879	0.990	0.990
ASF7	0.747	0.986	0.988	0.982	0.974
ASF8	0.733	0.984	0.979	0.969	0.973
ASF9	0.996	0.939	0.870	0.986	0.967
ASF10	0.996	0.938	0.871	0.987	0.966
ASF11	0.984	0.976	0.896	0.989	0.985
ASF12	0.990	0.973	0.870	0.990	0.983
ASF13	0.994	0.972	0.849	0.993	0.981
ASF14	0.988	0.966	0.889	0.991	0.981
ASF15	0.961	0.977	0.930	0.986	0.979

Data of the *in vitro* release of optimized formulation (ASF10) was fit into kinetic models to explain the release kinetics of Alendronate Sodium from microballoons. The kinetic models used were zero order, first order, and Higuchi and Korsmeyer-peppas models. The *in vitro* drug release kinetics based on the regression values reveals that the optimized formulation (ASF10) releases the drug in zero order manners. **(Table 10).**

Stability data of optimized microballoons formulation (ASF10)

Table 11: Stability data of optimized microballoons formulation (ASF10)

Optimized formulation ASF10	Bulk density	Tapped density	Compressibility index	Angle of repose	% Buoyancy	Drug content	Mean particle Size (µm)
1st Month	0.77±0.12	0.68±0.01	10.31±0.10	13.1±0.21	81.2±0.30	98.01±0.51	129.01±2.39
2nd Month	0.76±0.11	0.67±0.03	10.28±0.11	12.8±0.10	80.1±2.10	97.06±0.48	129.01±1.99
3rd Month	0.75±0.08	0.66±0.04	10.19±0.13	12.5±0.09	80.1±1.10	97.02±0.47	128.02±1.56

The stability studies were conducted only on optimized formulation (ASF10). The stability study was conducted for 3 months and the results were analyzed. No significant change was observed in particle size, flow properties,

drug content, percentage buoyancy and percentage drug release of microballoons. Microballoons were found to be stable at storage conditions for three months **(Table 11 and 12).**

Table 12: Percentage drug release of optimized microballoons formulation (ASF10) of Alendronate Sodium during stability studies.

SF10	1st Month	2nd Month	3rd Month
0	0	0	0
0.5	5.1±0.9	4.9±0.1	4.6±0.2
1	9.7±0.19	9.6±0.13	9.1±0.09
2	18.4±0.31	17.9±0.29	16.9±0.21
3	24.4±0.50	24.1±0.47	23.8±0.42
4	35.2±0.71	34.8±0.69	33.3±0.61
6	55.8±0.67	54.7±0.61	54.1±0.58
8	66.1±0.76	65.4±0.62	65.9±0.59
10	87.7±0.22	87.1±0.19	87.5±0.12
12	100.2±0.69	100.0±0.59	100.8±0.51

***In vivo* floating behavior:**

The optimized floating microballoons formulation prepared was 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 11 & 12.** It was observed from the images that the formulation was remained buoyant for up to 6 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 3.5 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.

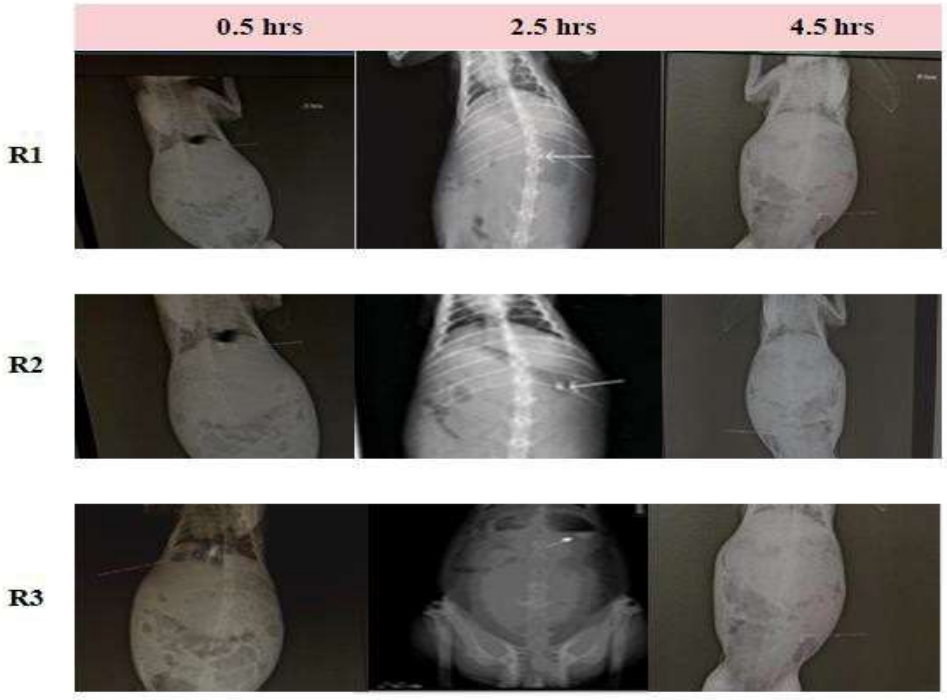

Figure 11: X-ray images of Alendronate sodium optimized formulation in the gastric region of rabbit during unfed state at 0.5 hrs, 2.5 hrs, 4.5hrs.

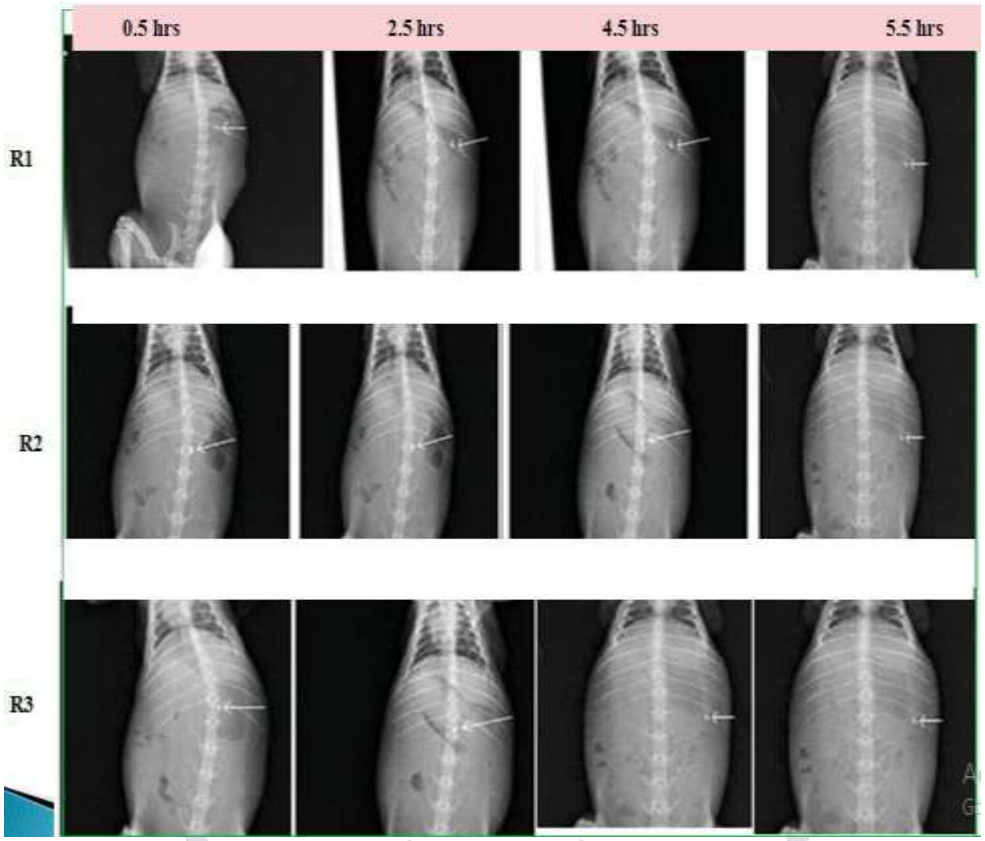

Figure 12: X-ray images of optimized formulation in the gastric region of rabbit during fed state at 0.5 hrs, 2.5 hrs, 4.5hrs, 5.5 hrs.

***In vivo* pharmacokinetic results**

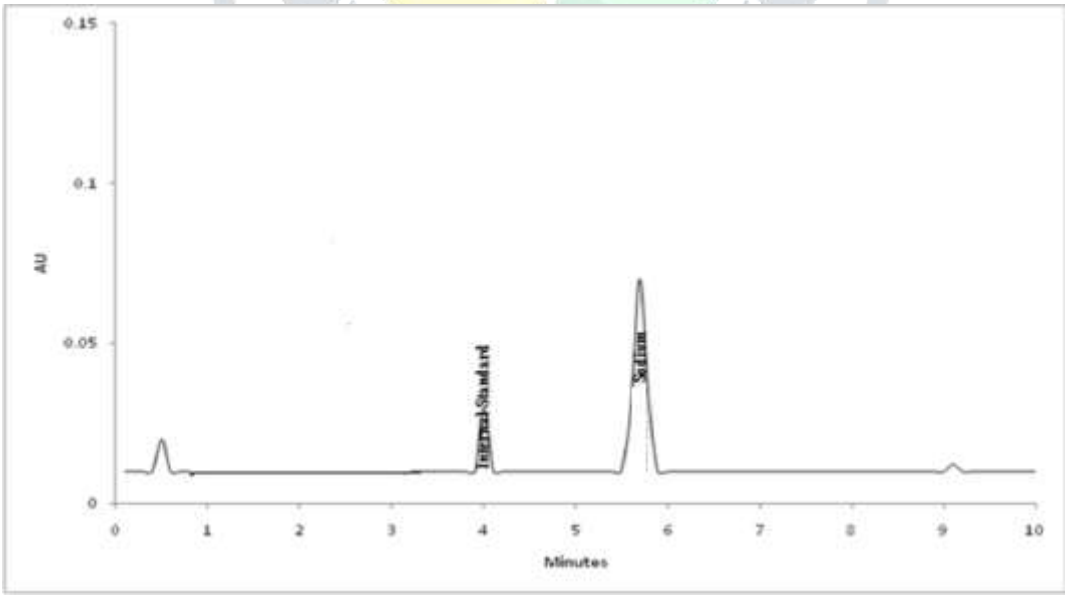

Figure 12: HPLC chromatogram of Alendronate sodium (RT 5.68 min) and internal standard (RT 4min)

Table 13: Plasma concentration of Alendronate sodium marketed tablets (Fosamax) 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	15.6	20.1	10.2	15.8	15.3	16.3	**15.55**	3.16
1	32.4	40.2	25.4	32.5	33.6	34.5	**33.10**	4.75
1.5	55.4	65.4	45.7	55.6	56.8	57.2	**56.02**	6.28
2	65.4	75.5	55.8	63.8	65.4	66.9	**65.47**	6.31
2.5	79.5	80.2	70.5	90.2	81.2	80.2	**80.30**	6.25
3	82.5	83.5	84.6	95.6	75.6	83.5	**84.22**	6.45
4	75.4	86.8	65.4	76.8	77.9	81.2	**80.50**	7.09
6	60.2	75.2	45.5	62.5	63.5	66.5	**62.23**	9.72
8	49.5	48.5	52.5	62.5	39.5	51.2	**50.62**	7.41
12	35.2	45.5	25.6	36.5	37.5	41.3	**36.93**	6.70
24	21.2	11.5	32.6	25.4	26.8	24.6	**23.68**	7.04

Table 14: Plasma concentration of Alendronate sodium floating microballoons (ASF10) 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	12.5	15.2	25.6	14.5	13.5	12.6	**15.65**	4.99
1	23.5	25.4	45.6	18.5	31.2	20.5	**27.45**	9.92
1.5	36.8	38.6	58.6	25.6	40.2	35.6	**39.23**	10.78
2	49.8	51.2	72.5	35.7	51.5	41.5	**50.37**	12.54
2.5	61.5	65.4	82.5	51.5	65.4	51.4	**62.95**	11.50
3	75.5	75.7	100.2	72.5	72.5	61.4	**76.30**	12.83
4	87.6	86.7	112.5	81.8	86.5	79.8	**89.15**	11.85
6	82.7	87.5	113.5	85.6	88.7	85.6	**90.60**	11.40
8	90.5	95.4	115.4	87.6	87.8	86.5	**93.87**	11.02
12	67.5	65.7	71.5	82.5	90.2	74.5	**75.32**	9.40
24	46.5	42.5	40.2	56.7	62.5	49.7	**49.68**	8.55

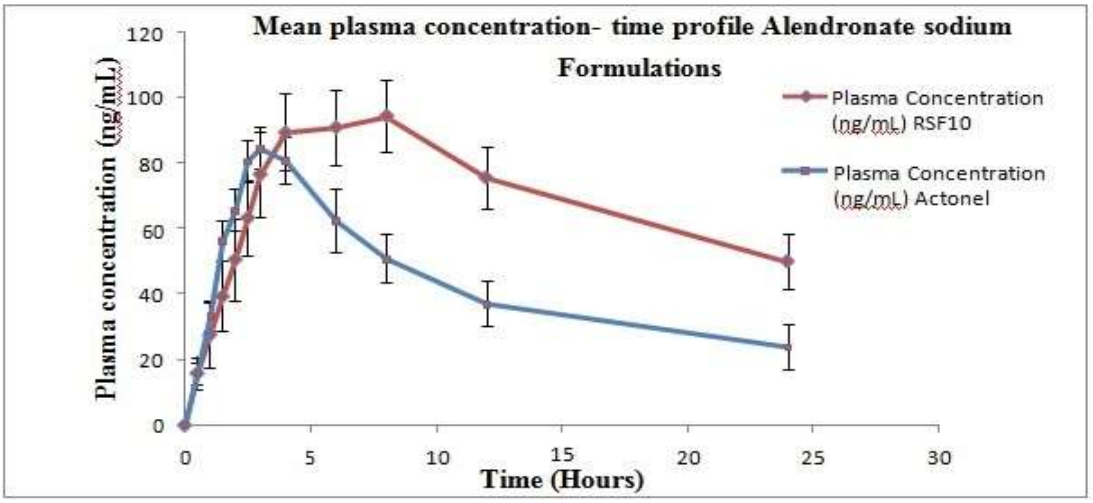

Figure 13: Mean plasma concentration time profile of Alendronate sodium test (ASF10) and reference (Fosamax) Formulations

Table 15: Mean pharmacokinetic parameters of Alendronate Sodium as reference and test tablets in Rabbits (n=6)

Pharmacokinetic parameter	Unit	Reference	Test
Cmax	ng/mL	84.21	93.86
tmax	h	3	8
AUC 0-t	ng/mL×h	1023.01	1652.21
AUC 0-α	ng/mL×h	1548.60	2939.76
t1/2	h	15.38	17.96

The mean area under plasma time curve AUC $_{0\text{-}t}$ and AUC $_{0\text{-}total}$ of reference formulation was 1023.01ng/ml×h and 1548.60 ng/ml×h and while AUC $_{0\text{-}t}$ and AUC $_{0\text{-}total}$ of test formulation was 1652.21ng/ml×h and 2939.76 ng/ml×h, This indicates that the overall absorption of Alendronate sodium 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 **(ASF10)** was increased significantly when compared to marketed formulation. Relative bioavailability with respect to marketed formulation was found to be 189.8 which is due to prolonged gastric residence time of Alendronate sodium floating microballoons.

CONCLUSION

In the present work, floating microballoons of Alendronate sodium were prepared using Eudragit RS 100, Eudragit S 100, HPMC K4M and Ethyl cellulose polymers. From the results of this study it indicates that solvent evaporation method can be employed successfully for preparing Alendronate sodium microballoons. Drug-excipient compatibility study was done by using DSC & FTIR and found that the drug was compatible with all the excipients used in the study. The *in vitro* studies demonstrated that microballoons of Alendronate sodium prepared using Eudragit S 100 along with Eudragit RS 100 in 1:1 ratio **(ASF10)** shown maximum amount of drug release. Hence, it is considered as the optimized formulation. The *in vitro drug* release kinetics states that the optimized formulation **(ASF10)** release the drug in zero order fashion on the basis of regression values of first order, Higuchi and Korsmeyer- peppas model respectively. The *in vivo* radiographic images showed that the $BaSO_4$ loaded optimized formulation **(ASF10)** was remained buoyant up to 5.5 h in the stomach .The *in vivo* pharmacokinetic study was conducted in healthy albino rabbits. It was observed from the results that the oral bioavailability of optimized formulation (**ASF10)** was increased significantly when compared to the marketed formulations. Relative bioavailability with respect to marketed formulation (Fosamax**)** was found to be 189.8. The increased bioavailability may be due to the floating mechanism of dosage form in stomach for longer duration.

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