IJPSR (2020), Volume 11, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 June 2019; received in revised form, 05 February 2020; accepted, 02 April 2020; published 01 May 2020

DEVELOPMENT OF FUROSEMIDE FLOATING MICROBALLOONS: *IN-VITRO* AND *IN-VIVO* EVALUATION

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Keywords:

Furosemide, Solvent evaporation, Gastric residence time, *In-vitro* evaluation, *In-vivo* evaluation, Floating microballons

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ABSTRACT: The present investigation deals with the development and evaluation of floating microballoons of furosemide to extend the gastric residence time (GRT) and prolong the drug release. In the present work, floating micro balloons of furosemide were formulated using eudragit RS 100, eudragit S 100, and HPMC K_4M and ethylcellulose polymers by the solvent evaporation method. The prepared micro balloons were evaluated for their physicochemical properties, *in-vitro* drug release, and *in-vitro* buoyancy. The *in-vitro* release studies demonstrated that micro balloons of furosemide prepared using eudragit RS 100 along with eudragit S 100 in 1:1 ratio (FSDF10) shown the maximum amount of drug release; hence it is considered as the optimized formulation. The *in-vitro* release kinetics revealed that the optimized formulation releases the drug in zero-order manner based on the regression values of kinetic models. The optimized formulation was used for *in-vivo* evaluation. The *in-vivo* Radiographic study showed that the $BaSo_4$ loaded optimized formulation remained buoyant up to 5.5 h in the stomach. The *in-vivo* pharmacokinetic study 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.

INTRODUCTION: Historically, oral drug administration has been the predominant route for drug delivery. During the past two decades, numerous oral delivery systems have been developed to act as drug reservoirs from which the active substance can be released over a defined period of time at a predetermined and controlled rate. However, this route has several physiological problems such as an unpredictable gastric emptying rate that varies from person to person a brief gastrointestinal transit time (8- 12 h).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(5).2248-61

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(5).2248-61

The existence of narrow absorption window in the upper GIT for several drugs [1] these difficulties have prompted researchers to design a drug delivery system that can stay in the stomach for prolonged and predictable period. Attempts are being made to develop a drug delivery system that can provide therapeutically effective plasma drug concentration for a longer period, thereby reducing the dosing frequency and minimizing fluctuation in plasma drug concentration at a steady state by delivering the drug in a controlled and reproducible manner.

Gastroretentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bio-availability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment.

It has applications also for local drug delivery to the stomach and proximal small intestines. Microballoons are gastro retentive drug-delivery systems with a non-effervescent approach and considered as one of the most favorable buoyant systems with the unique advantages of multiple unit systems as well as better floating properties. Microballoons (Hollow microsphere) are in the strict sense, empty particles of spherical shape without core. These microspheres are characteristically free-flowing powders comprising of proteins or synthetic polymers, ideally having a size less than 200 micrometer [2]. The slow release of drug at desired rate and better-floating properties of floating microspheres 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.

The drug, furosemide is a benzoic-sulfonamide-furan. It is a diuretic with fast onset and short duration that is used for edema and chronic renal insufficiency. Furosemide 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 furosemide leads to its low bioavailability (60-70%). The narrow absorption window of furosemide in the upper part of the gastrointestinal tract provides a rationale for developing a gastro retentive dosage form.

MATERIALS AND METHODS:

Materials: Furosemide was purchased from Yarrow chem. Products, Mumbai, India. Vitamin E TPGS, Eudragit RS 100, Eudragit S 100, HPMC K_4M, Ethylcellulose, 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 (20-300 °C) at a constant scanning speed (10 °C / min) in sealed aluminum pans, using nitrogen purged gas.

FTIR Spectroscopy: Drug-polymer compatibility studies were carried out using the FTIR spectrophotometer (Shimadzu) 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 furosemide and excipients used in the formulation. IR spectrum of pure drug and optimized formulation was seen in between 4000-400 cm^{-1}.

Formulation Development: As the drug furosemide 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.

TABLE 1: COMPOSITIONS OF SOLID DISPERSIONS OF FUROSEMIDE

S. no.	Materials	FSD1	FSD2
1	Furosemide	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. 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 HCl (pH 1.2) as a dissolution medium. 5 ml of sample solution was withdrawn at predetermined time intervals up to 12 h and the samples were filtered through Whatman filter paper, diluted suitably and

analyzed spectrophotometrically with UV-Visible spectrophotometer at a maximum absorbance wavelength of 276 nm. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. The dissolution studies were performed and the average percentage drug release was calculated.

Formulation of Floating Microballoons: Solid dispersion prepared with 1:2 ratio of drug to vitamin E TPGS (FSD2) 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 or by continuous stirring. Stirring was continued for 6 h under 3 blade propellers at 500 rpm, 40 °C until the smell disappears.

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.

TABLE 2: COMPOSITION OF FLOATING MICROBALLOONS OF FUROSEMIDE

S. no.	Materials	FSD F1	FSD F2	FSD F3	FSD F4	FSD F5	FSD F6	FSD F7	FSD F8	FSD F9	FSDF 10	FSD F11	FSD F12	FSD F13	FSD F14	FSD F15
1	Furosemide SD eqvt to 10mg	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
2	Eudragit RS 100	10	10	10	20	20	20	10	10	20	20	NA	NA	NA	NA	NA
3	Eudragit S 100	10	20	30	10	20	30	30	30	20	20	NA	NA	NA	NA	NA
4	HPMC K4M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10	10	10	20	20
5	Ethylcellulose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10	20	30	10	20
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

***In-vitro* Evaluation of Furosemide Micro-balloons:**

Micromeritic Properties: Microballoons are evaluated by their micromeritic properties such as particle shape and size, bulk density, tapped density, Hausner's ratio, and flow properties, which are determined by car's index and angle of repose [3].

Particle Size Measurement: Particle size of prepared microballoons was measured using an optical microscope, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer [4].

Scanning Electron Microscope (SEM): The surface morphology and surface characteristics of best formulation were carried out by scanning electron microscope (SEM). Microballoons were scanned and examined under Electron Microscope connected with a fine coat, Ion sputter. The sample was loaded on a copper sample holder and sputter-coated with carbon followed by gold [5].

Tapped Density: Tapped density and com-pressibility index are calculated by measuring the change in volume using a bulk density apparatus; angle of repose is determined by the fixed funnel method. The compressibility/carr's index was calculated using the following formula:

$$I = V_b - V_t / V_b \times 100$$

Where V_b is the bulk volume, and Vt is the tapped volume. The value given below 15% indicates a powder which usually gives rise to good flow characteristics, whereas above 25% indicate poor flowability. Angle of repose of the micro balloons was determined by the fixed funnel method.

Percentage Yield: The prepared microballoons of all batches were accurately weighed. The weight of prepared microballoons was divided by the total amount of all the excipients and drugs used in the preparation of the microballoons, which give the total percentage yield of floating microballoons [6, 7, 8]. It is calculated by using the following formula,

Percentage yield = Actual yield of product / Total weight of excipients and drug

Entrapment Efficiency: The amount of entrapped drug in the microballoons was calculated based on the total drug content and the unentrapped drug of the floating microballoons. The unentrapped drug was determined by taking one dose equivalent of floating microballoons and washed with 0.1N HCl to remove the free drug on the surface.

The drug content of microballoons was determined by dispersing 50 mg formulation (accurately weighed) in 10 ml 0.1 N HCl, followed by agitation with a magnetic stirrer for 12 h to dissolve the polymer and to extract the drug. Both the solutions of unentrapped drug and total drugs were filtered through a Whatman filter; the drug concentration was determined spectrophotometrically at 276 nm by making the desired dilution with 0.1N HCl9. Percentage entrapment efficiency was calculated as follows.

% Entrapment efficiency = Total drug content - unentrapped drug 100 / Total drug content

***In-vitro* Buoyancy:** Microballoons were spread over the surface of a USP dissolution apparatus type II filled with 900 ml of 0.1 N HCl. The medium was agitated with a paddle rotating at 50 rpm for 12 h. The floating and the settled portions of microballoons were recovered separately. The microballoons were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microballoons that remained floating and the total mass of the microballoons [10].

$$\text{Percentage buoyancy} = Q_f\ 100 / Q_f + Q_s$$

Where, Q_f and Q_s are the weight of the floating and the settled microballoons, respectively.

Drug Content: The drug content of each formulation equivalent to unit dose (30 mg) was determined by spectrophotometrically. Each formulation was taken and finely powdered in a glass mortar and dissolved in a solution of 0.1 N HCl for 6 h. The solution was then filtered, and absorbance was noted at 276 nm [11].

***In-vitro* Drug Release Study:** The drug release study was carried out using USP dissolution apparatus type XXIII basket type dissolution apparatus at 37 ± 0.5 ºC and at 50 rpm using 900 ml of 0.1 N HCl (pH 1.2) as a dissolution medium. 5 ml of sample solution was withdrawn at predetermined time intervals up to 12 h and the samples were filtered through whatman filter paper, diluted suitably and analyzed spectrphotometrically with UV-Visible spectrophotometer at a maximum absorbance wavelength of 276 nm. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample [12, 13, 14, 15]. The dissolution studies were performed and the average percentage drug release was calculated.

Drug Release Kinetic Studies: The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero-order, first-order, higuchi and Korsmeyer-Peppas and finding the R2 values of the release profile corresponding to each model [16, 17] using PCP Disso v 3 software.

Stability Studies: Microballoons were hermetically sealed in glass bottles and stored for 3 months at 4 ± 0.5 °C, room temperature and 40 ± 1 °C and 75% RH as per ICH guidelines [18,19] after every month, one bottle was used for evaluation. The microballoons were evaluated for physico chemical properties, drug content, percentage entrapment efficiency and percentage buoyancy and percentage of drug release.

***In-vivo* Evaluation Of Furosemide Microballoons:** The experimental protocol to carry out *in-vivo* studies were reviewed and approved by the Institutional Animal Ethical Committee of University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India (Registration No. IAEC/22/UCPSC/2018). The *in-vivo* performance of the optimized formulations was evaluated on healthy albino rabbits.

***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-rayy 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 $BaSO_4$ 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 [20].

Fasting State: The $BaSO_4$ loaded microballoons of furosemide were administered orally with a sufficient amount of water through a mouth gag introduced in between the two jaws of the rabbit. During the study, animals were not allowed to eat food, but the water was provided *ad libitum*, and in between radiographic imaging, animals are freed and allowed to move and carry out normal activities but not allowed to take any food.

Fed State: All the rabbits were fasted for 12 h before initiating the study and fed with a low-calorie diet. Half an hour later, $BaSO_4$ loaded microballoons of furosemide were administered orally with a sufficient amount of water through a mouth gag introduced in between the two jaws of rabbit and at different time intervals, rabbits were exposed to X-ray imaging, and floating behavior was studied.

***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 (FSDF10) and group II animals are treated with marketed formulation (Lasix). 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 (Tt_{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 [21].

RESULTS AND DISCUSSION:

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**.

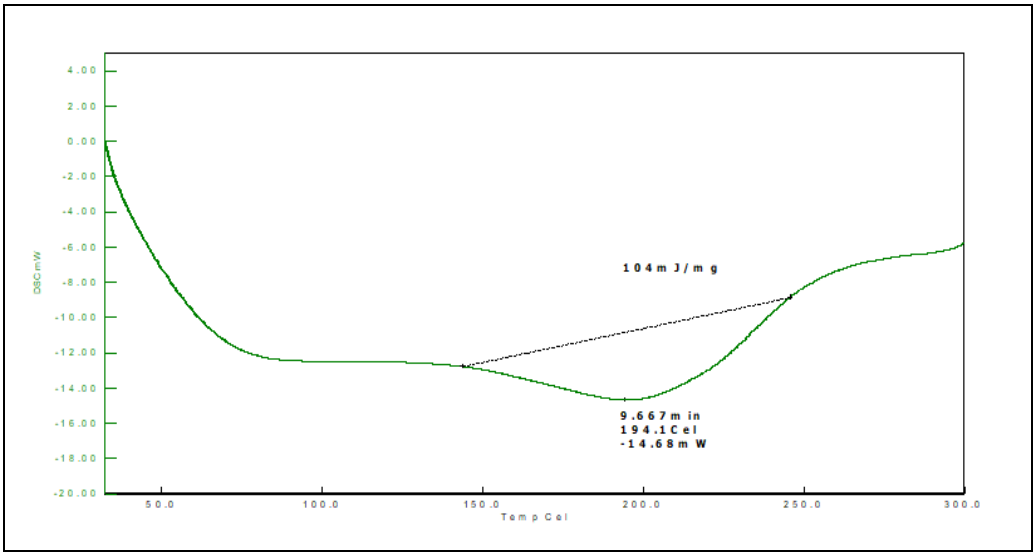

FIG. 1: DSC THERMOGRAM OF PURE DRUG

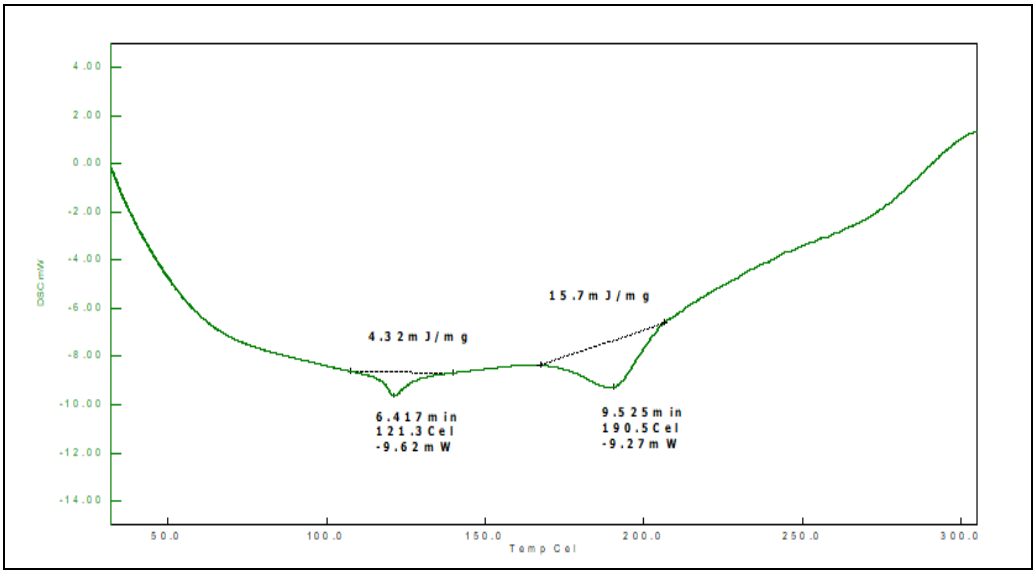

FIG. 2: DSC THERMOGRAM OF OPTIMIZED FORMULATION

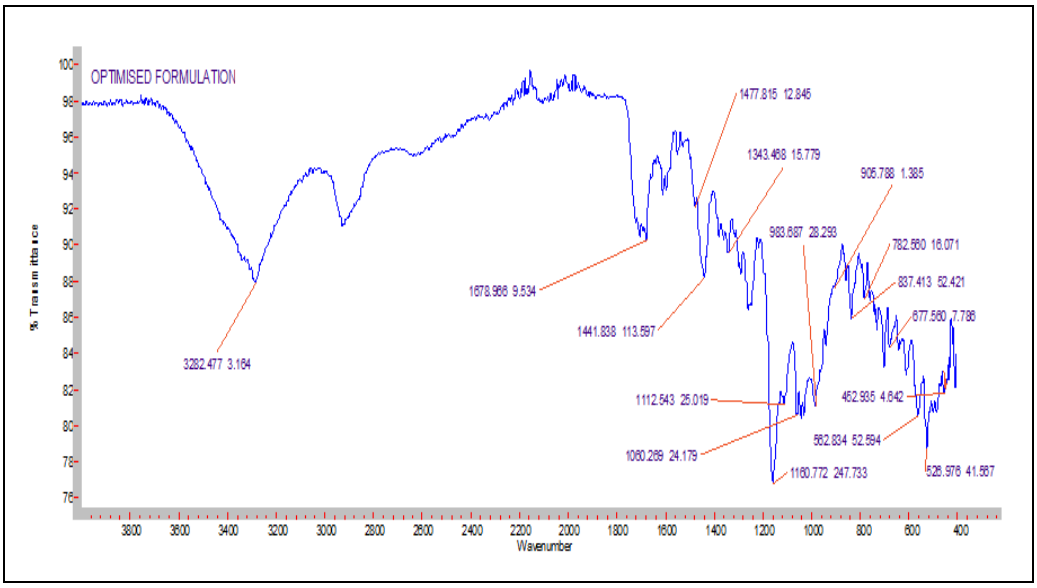

FIG. 3: FTIR OF PURE DRUG

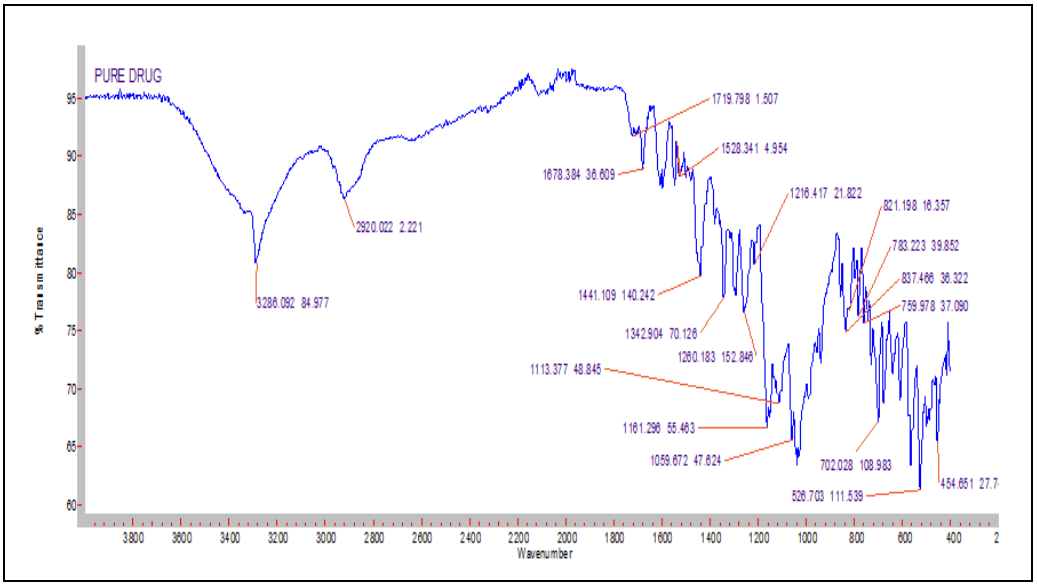

FIG. 4: FTIR OF OPTIMIZED FORMULATION

FTIR Spectroscopy: The drug-excipient compatibility study was done by Fourier transform infrared spectroscopy study. The prominent peaks of furosemide pure drug **Fig. 3** were shown at 3286.09 cm^{-1} (due to –N-H), 1678.38 cm^{-1} (due to C=O), 2920.02 cm^{-1} (due to $–CH_2$) and 1047 cm^{-1} (due to -C-O). These prominent peaks of the drug were also observed in the IR spectrum of optimized formulation of drug **Fig. 4** with various recipients, which indicates that the drug was not interacted with the polymers used in the study, which confirms the stability of the drug.

Evaluation of Furosemide Solid Dispersion: Solubility and dissolution study of solid furosemide dispersion reveals that solid dispersion prepared with a 1:2 ratio of drug to vitamin E TPGS (FSD2) has shown more improved solubility and dissolution and hence chosen for preparing floating microballoons **Table 3** and **4**, **Fig. 5**.

TABLE 3: SOLUBILITY OF FUROSEMIDE SOLID DISPERSIONS

Formulation	Solubility (mg/mL)			
	1	2	3	Average
Pure drug	0.01	0.02	0.015	0.015
FSD1	8.75	8.35	8.15	8.417
FSD2	12.57	15.54	13.45	13.853

TABLE 4: PERCENTAGE DRUG RELEASE OF FUROSEMIDE SOLID DISPERSIONS

Time (min)	Percentage of drug release		
	Pure drug	FSD1	FSD2
0	0	0	
5	2.3	11.2	15.6
10	5.4	18.9	25.6
20	7.8	27.8	35.9
30	12.5	35.9	45.9
40	13.9	45.6	55.6
50	15.9	52.6	62.5
60	19.8	55.9	71.5

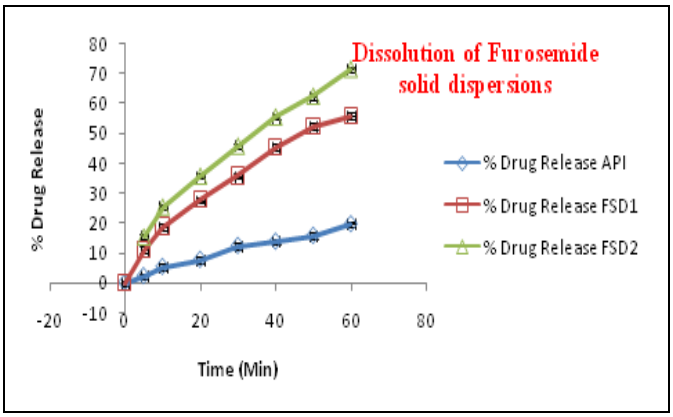

FIG. 5: DISSOLUTION PROFILES OF FUROSEMIDE SOLID DISPERSIONS

***In-vitro* Evaluation of Furosemide Micro-balloons:** The particle size was measured using a calibrated optical microscope, and the average particle size of floating microballoons was found to be in the range of 120-180 µm **Table 5** and **6**. The measured tapped density bulk density, compressibility index, and angle of repose are within limits, which indicates good flow properties of microballoons **Table 5** and **6**. The floating microballoons were prepared and the percentage yield was calculated for all the formulations. The results of % yield are shown in **Table 7**. The percentage yield was in the range of 60-90 % for all the formulations. It was found to be less than 70% yield with ethyl cellulose and HPMC K_4M, and for the optimized formulation, the yield was 84.5%. The entrapment efficiency of floating micro-balloons of furosemide was calculated, and the results are depicted in **Table 7**. The entrapment efficiency was in the range of 60-90% for all the formulations and was found to be 94.6% for optimized formulation.

The entrapment efficiency was low, with formulations prepared with ethyl cellulose and HPMC K_4M. There was no effect of the solvent ratio observed in the % Entrapment Efficiency. The percentage buoyancy was calculated for all the formulations, and it was found that all the formulations were able to float on the dissolution medium (0.1 N HCl) over a period of 12 h. Even after 12 h of agitation of the dissolution medium, the microballoons continued to float without any apparent gelation. The high buoyancy of the microballoons is mainly due to the presence of pores and cavities which were formed during solvent evaporation. The percentage buoyancy was slightly less with formulations prepared with ethyl cellulose and HPMC K_4M and decreased as the concentration of the polymers increased. This is because of the high viscosity of the polymer solution, which in turn is the reason for the less formation of pores and cavities in microballoons during solvent evaporation. The results of *in-vitro* buoyancy studies are shown in **Table 7**. The percentage buoyancy was in the range of 60-90 % for all the formulations and was found to be 91.2% for optimized formulation. The drug content of all the prepared formulations was found to be within the acceptable range of 90.0 -110.0%. Values obtained are given in **Table 5** and **6**.

TABLE 5: OBSERVATIONS OF *IN-VITRO* EVALUATION PARAMETERS OF FLOATING MICROBALLOONS OF FUROSEMIDE

Parameter	FSDF1	FSDF2	FSDF3	FSDF4	FSDF5	FSDF6	FSDF7	FSDF8
Mean particle size (µm)**	135.24±1.34	143.34±3.45	156.32±1.56	144.24±3.25	156.54±2.35	131.23±3.26	145.39±2.34	135.26±2.54
Bulk density*	0.7±0.19	0.72±0.97	0.78±0.65	0.82±0.06	0.78±0.88	0.77±0.54	0.77±0.43	0.72±0.32
Tapped density*	0.65±0.05	0.64±0.18	0.69±0.92	0.72±0.22	0.66±0.18	0.66±0.19	0.64±0.28	0.65±0.17
Compressibility index*	7.14±0.18	11.11±0.26	11.54±0.19	12.20±0.92	15.38±0.94	14.29±0.22	16.88±0.91	9.72±0.84
Angle of repose*	14.6±0.49	16.6±0.28	16.5±0.29	15.6±0.34	15.8±0.18	15.9±0.28	16.3±0.91	15.5±0.18
Drug content	98.78±0.19	99.02±0.02	96.68±0.19	07.89±0.11	98.02±0.76	98.54±0.54	98.72±0.63	98.29±0.82

TABLE 6: OBSERVATIONS OF *IN-VITRO* EVALUATION PARAMETERS OF FLOATING MICROBALLOONS OF FUROSEMIDE

Parameter	FSDF9	FSDF10	FSDF11	FSDF12	FSDF13	FSDF14	FSDF15
Mean particle size (µm) **	125.35±2.38	127.35±3.24	121.35±3.36	128.35±3.36	145.32±3.69	161.23±3.38	125.64±3.39
Bulk density*	0.77±0.76	0.78±0.82	0.81±0.95	0.76±0.33	0.78±0.28	0.75±0.18	0.82±0.19
Tapped density*	0.66±0.21	0.69±0.28	0.71±0.84	0.68±0.93	0.68±0.84	0.65±0.83	0.71±0.89
Compressibility index*	14.29±0.72	11.54±0.92	12.35±0.99	10.53±0.17	12.82±0.25	13.33±0.39	13.41±0.43
Angle of repose*	16.2±0.38	14.2±0.32	15.5±0.48	16.3±0.11	15.8±0.17	16.1±0.81	16.5±0.11
Drug content	99.04±0.07	99.38±0.06	98.06±0.17	99.08±0.04	97.67±0.11	98.03±0.18	94.58±0.17

*All values represent mean ± SD; n = 3 ** all values represent mean ± SD; n = 100

TABLE 7: PHYSICOCHEMICAL PROPERTIES OF PREPARED MICROBALLOONS OF FUROSEMIDE

Formulation Code	% Yield	% EE	% B
FSDF1	85.4	85.4	81.5
FSDF2	84.3	94.5	86.5
FSDF3	86.2	96.5	86.9
FSDF4	77.8	96.5	91.2
FSDF5	82.5	92.5	82.2
FSDF6	94.3	95.8	88.9
FSDF7	82.5	91.5	87.5
FSDF8	93.6	91.2	85.6
FSDF9	82.7	91.5	82.5
FSDF10	84.5	94.6	91.2
FSDF11	72.6	71.2	67.5
FSDF12	67.5	66.5	67.8
FSDF13	68.9	64.5	66.9
FSDF14	66.5	66.3	65.2
FSDF15	62.5	63.5	65.3

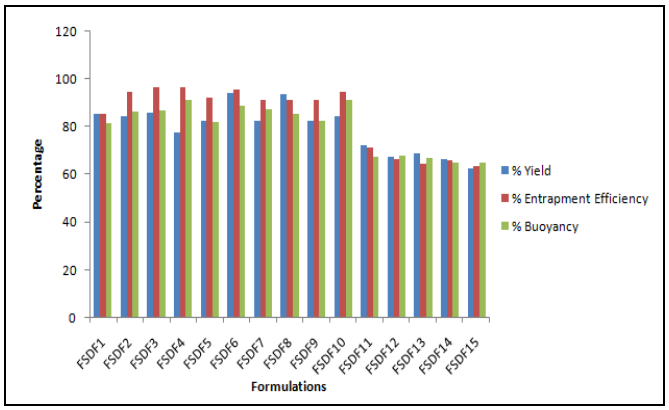

FIG. 6: COMPARATIVE PHYSICOCHEMICAL PROPERTIES OF MICROBALLOONS OF FUROSEMIDE

Scanning Electron Microscope (SEM): The surface morphology of the floating microballoons was studied using a scanning electron microscope (SEM). The surface morphology of optimized formulation FSDF10 was shown in **Fig. 7** from the SEM micrographs it is apparent that the furosemide loaded microballoons were predominantly spherical in appearance. The surface was observed to be smooth, dense, and less porous, whereas the internal core was highly porous and irregular with numerous depressions that are an expression of evaporation of water, ethanol, and dichloromethane. The less porous outer surface and highly porous internal surface supported the controlled release of drug from the microballoons and good buoyancy.

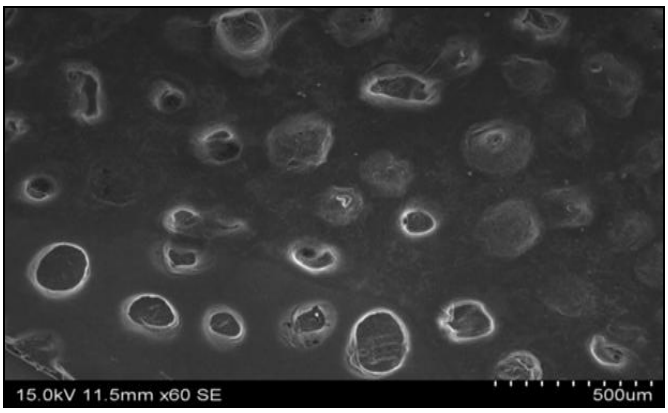

FIG. 7: SEM IMAGE OF FUROSEMIDE LOADED OPTIMIZED MICROBALLOONS

***In-vitro* Drug Release Study:** Dissolution studies of all the formulations were carried out using USP dissolution apparatus XXIII basket type dissolution apparatus. The dissolution profiles were compared among different formulations.

The cumulative percentage drug release was decreased with an increase in the polymer concentration. Based on the results of *in-vitro* drug release studies, it was found that FSDF10 has shown sustained drug release for 12 h with zero-order drug release. The results of the in vitro drug release profile were shown in **Table 8, 9,** and **Fig. 8-10**.

TABLE 8: PERCENTAGE DRUG RELEASE DATA OF FUROSEMIDE MICROBALLOONS

Time (H)	Percentage drug release							
	FSDF1	FSDF2	FSDF3	FSDF4	FSDF5	FSDF6	FSDF7	FSDF8
0	0	0	0	0	0	0	0	0
0.5	25.6±0.02	20.6±0.02	15.6±0.12	20.4±0.93	6.5±0.32	7.2±0.98	17.8±0.32	16.5±0.54
1	44.2±0.23	34.5±0.32	25.9±0.24	37.2±0.26	11.2±0.42	10.2±0.45	26.5±0.18	25.6±0.18
2	64.5±0.42	51.3±0.47	44.2±0.43	57.6±0.47	21.2±0.09	13.5±0.57	43.5±0.38	44.5±0.17
3	87.5±0.21	63.8±0.98	58.6±0.66	65.5±0.32	26.5±0.58	21.3±0.17	58.7±0.97	61.2±0.23
4	98.5±0.22	75.4±0.26	66.9±0.85	75.6±0.82	35.4±0.41	31.2±0.83	66.9±0.11	71.2±0.24
6	100.1±0.31	82.5±0.32	74.5±0.41	87.9±0.17	53.2±0.95	46.5±0.37	73.5±0.81	73.5±0.38
8		100.2±0.04	86.4±0.33	99.7±0.02	65.4±0.73	58.4±0.88	85.6±0.92	87.6±0.52
10			100.1±0.58	100.1±0.04	83.5±0.29	68.5±0.21	98.6±0.37	100.2±0.54
12					100.2±0.11	75.4±0.65	100.2±0.45	

TABLE 9: PERCENTAGE DRUG RELEASE DATA OF FUROSEMIDE MICROBALLOONS

Time	Percentage drug release						
(H)	FSDF9	FSDF10	FSDF11	FSDF12	FSDF13	FSDF14	FSDF15
0	0	0	0	0	0	0	0
0.5	7.6±0.11	5.5±0.94	6.4±0.65	5.6±0.34	5.6±0.10	7.5±0.54	10.2±0.89
1	11.2±0.09	10.2±0.03	10.2±0.88	10.2±0.98	8.5±0.16	13.6±0.36	16.5±0.78
2	16.8±0.29	21.2±0.46	16.4±0.74	14.5±0.28	13.5±0.21	18.9±0.46	22.5±0.38
3	25.6±0.28	26.5±0.87	21.2±0.44	18.5±0.88	15.6±0.27	25.9±0.03	23.6±0.27
4	37.8±0.32	36.9±0.45	27.5±0.57	23.6±0.67	18.9±0.37	32.5±0.19	29.5±0.51
6	55.6±0.46	61.2±0.04	42.3±0.32	38.9±0.03	28.6±0.55	46.5±0.28	43.5±0.46
8	66.5±0.76	68.5±0.26	48.9±0.56	43.5±0.05	38.9±0.46	53.6±0.97	52.6±0.47
10	85.9±0.74	88.9±0.18	68.5±0.33	58.6±0.24	48.9±0.73	75.4±0.56	63.9±0.65
12	100.1±0.85	100.3±0.67	75.4±0.63	68.9±0.46	59.6±0.89	83.6±0.58	73.5±0.99

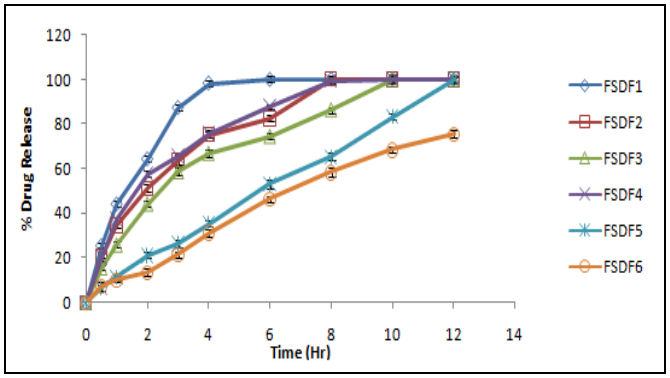

FIG. 8: *IN-VITRO* DISSOLUTION PROFILES OF FORMULATIONS FSDF1-FSDF6

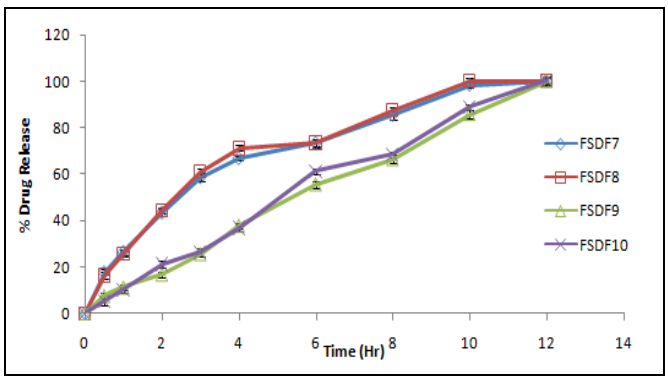

FIG. 9: *IN-VITRO* DISSOLUTION PROFILES OF FORMULATIONS FSDF7-FSDF10

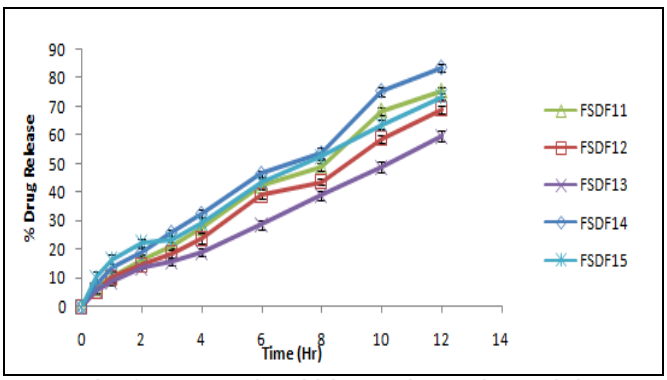

FIG. 10: *IN-VITRO* DISSOLUTION PROFILES OF FORMULATIONS FSDF11-FSDF15

Release Kinetics of Floating Microballoons: Data of the *in-vitro* release of optimized formulation (FSDF10) was fit into kinetic models to explain the release kinetics of furosemide from microballoons. The kinetic models used were zero order, first order, Higuchi and Korsmeyer-peppas models. The *in-vitro* release kinetics revealed that the optimized formulation (FSDF10) releases the drug in a zero order manner based on the regression values **Table 10**.

TABLE 10: RELEASE KINETICS OF FUROSEMIDE FLOATING MICROBALLOONS

Formulation	Release Kinetics Parameters				
	Zero-order	First-order	Higuchi model	Korse-meyer peppas	Hixon -crowell
RSF1	0.073	0.991	0.795	0.823	0.947
RSF2	0.551	0.989	0.962	0.960	0.979
RSF3	0.723	0.989	0.985	0.977	0.978
RSF4	0.476	0.992	0.947	0.952	0.979
RSF5	0.995	0.946	0.886	0.998	0.971
RSF6	0.981	0.984	0.901	0.990	0.993
RSF7	0.711	0.987	0.987	0.981	0.972
RSF8	0.690	0.986	0.976	0.965	0.973
RSF9	0.995	0.943	0.881	0.996	0.970
RSF10	0.999	0.947	0.887	0.983	0.973
RSF11	0.987	0.974	0.892	0.991	0.985
RSF12	0.987	0.978	0.890	0.991	0.986
RSF13	0.989	0.977	0.880	0.989	0.984
RSF14	0.979	0.969	0.908	0.989	0.981
RSF15	0.941	0.974	0.947	0.985	0.972

Stability Studies: The stability studies were conducted on the optimized formulation (FSDF10), the stability study was conducted for 3 months and the results were analyzed. No significant change was observed in microballons and was found to be stable at storage conditions for three months **Table 11, 12**.

TABLE 11: STABILITY DATA OF OPTIMIZED MICROBALLOONS OF FUROSEMIDE (FSDF10)

Optimized formulation FSDF10	Bulk density	Tapped density	Compressibility index	Angle of repose	Mean particle size (µm)	Percentage buoyancy	Drug content
1st Month	0.77±0.81	0.68±0.27	11.52±0.91	13.9±0.31	126.31±3.02	81.2±0.87	99.35±0.01
2nd Month	0.76±0.79	0.67±0.26	11.06±0.87	13.8±0.29	125.29±2.09	81.1±2.1	99.21±0.09
3rd Month	0.75±0.76	0.66±0.24	11.05±0.85	13.5±0.24	124.25±1.89	80.09±1.9	99.05±0.11

TABLE 12: PERCENTAGE DRUG RELEASE OF OPTIMIZED MICROBALLOONS OF FUROSEMIDE (FSDF10) DURING STABILITY STUDIES

FSDF10	1st Month	2nd Month	3rd Month
0	0	0	0
0.5	5.4±0.04	4.9±0.01	4.7±0.19
1	10.1±0.11	9.1±0.9	9.1±0.8
2	21.1±0.31	20.9±0.18	19.9±0.28
3	26.1±0.71	25.9±0.65	25.1±0.61
4	35.1±0.31	34.8±0.28	34.1±0.25
6	60.1±0.01	59.1±0.21	58.1±0.19
8	67.1±0.21	66.11±0.19	65.8±0.15
10	87.1±0.11	86.9±0.10	85.8±0.12
12	100.1±0.61	100±0.58	100±0.53

***In-vivo* Evaluation of Furosemide Micro-balloons:**

***In-vivo* Floating Behaviour:** The optimized floating microballoons formulation prepared was tested for *in-vivo* floating behavior in healthy albino rabbits. Radiographic images obtained at 0.5 h, 2.5 h, 4.5 h, and 5.5 h at unfed state and fed state are shown in **Fig. 11** and **12,** respectively. It was observed from the images that the formulation remained buoyant for up to 5.5 h in the stomach, indicating the uniform distribution of formulation in the stomach. But in the unfed state, the formulation remained buoyant in the stomach only up to 4.5 h; this is because in fasting condition, myoelectric migrating contraction forces the

contents to duodenum from stomach. The forceful waves will remove all the contents of the stomach, including the dosage form. This will not take place in a fed state. Therefore from these studies, it was clearly observed that the floating microballoons should be given to patients after a standard diet.

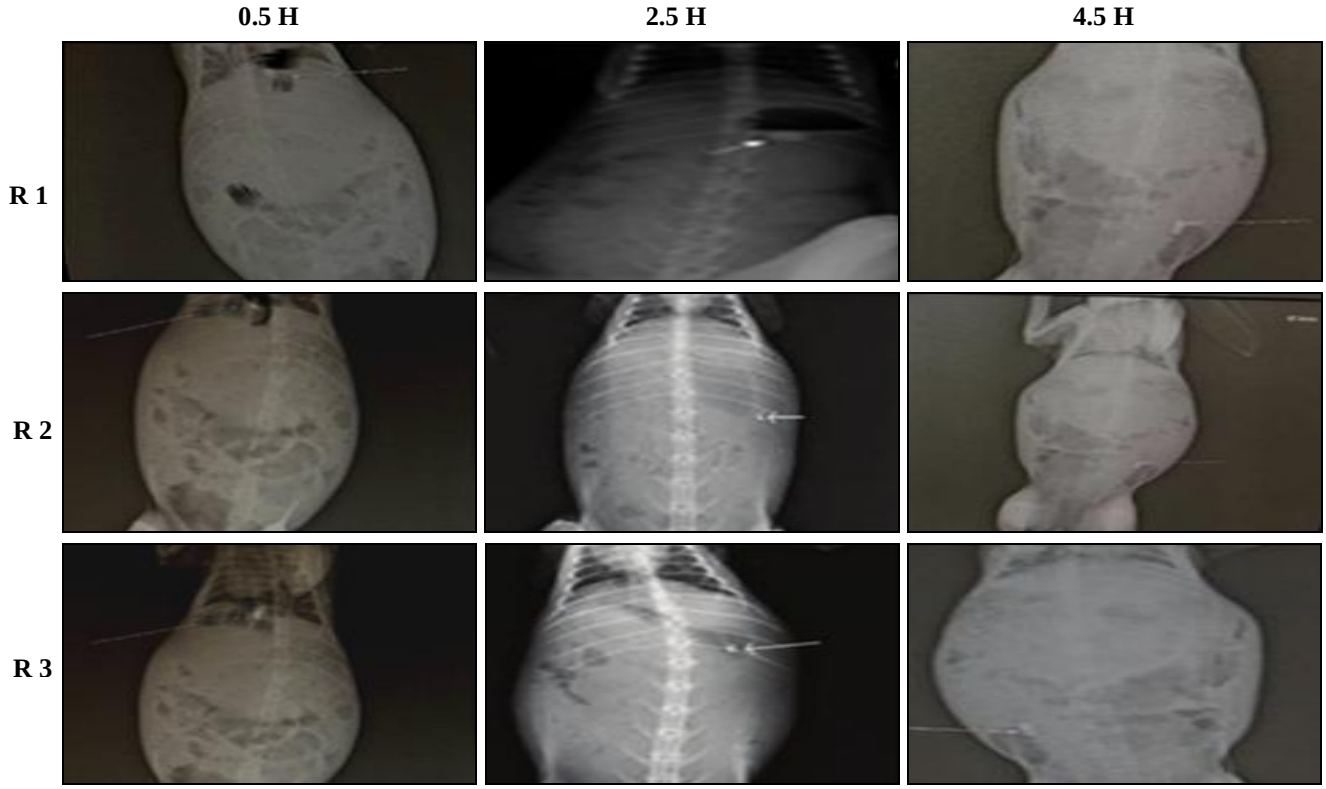

FIG. 11: X-RAY IMAGES OF OPTIMIZED MICROBALLOONS OF FUROSEMIDE IN THE GASTRIC REGION OF RABBIT DURING UNFED STATE AT 0.5 H, 2.5 H, 4.5

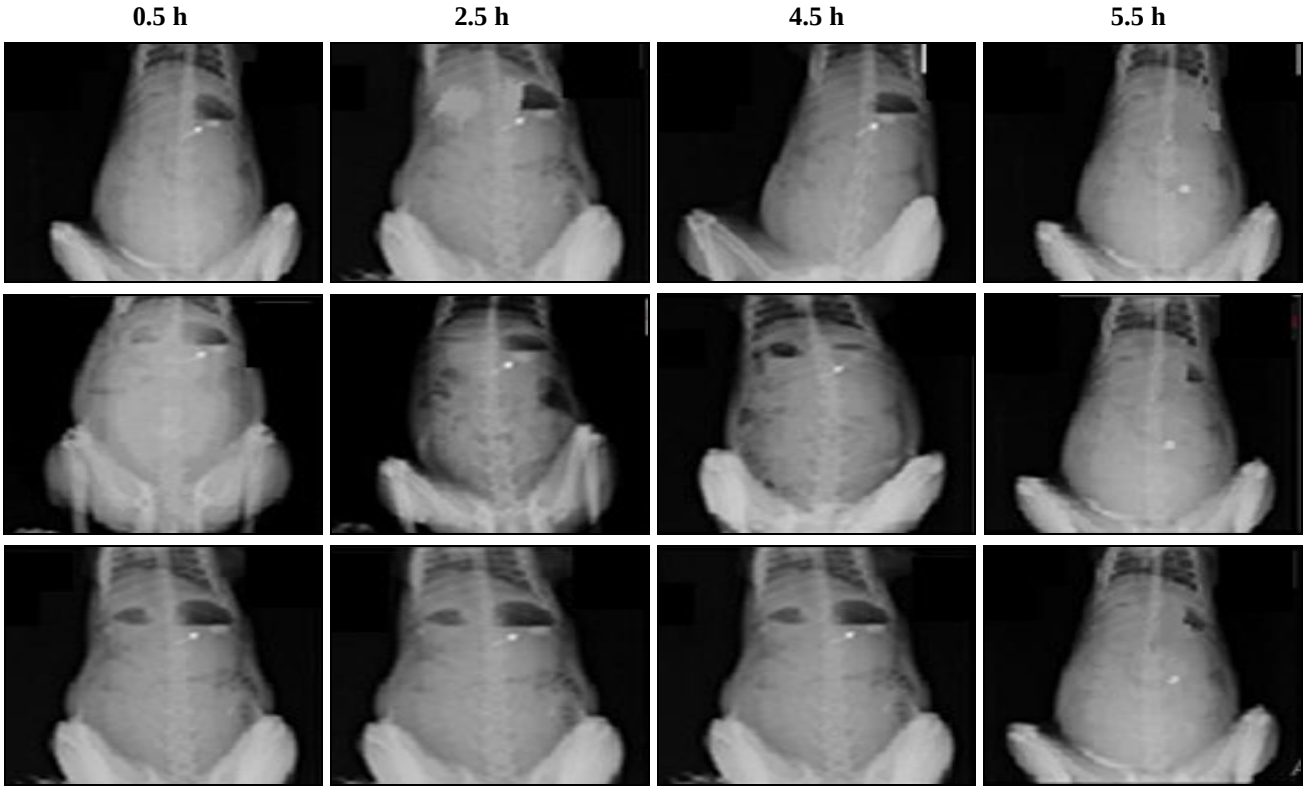

FIG. 12: X-RAY IMAGES OF OPTIMIZED MICROBALLOONS OF FUROSEMIDE IN THE GASTRIC REGION OF RABBIT DURING FED STATE AT 0.5 h, 2.5 h, 4.5 h, 5.5 h

***In-vivo* Pharmacokinetic Study:** The *in-vivo* pharmacokinetic study was conducted in healthy albino rabbits. In this study, the pharmacokinetic of optimized furosemide floating microballoons (FSDF10) **were** compared with IR tablets (Lithostat). The mean plasma concentration-time profile obtained from the study is shown in **Fig**. **13;** various pharmacokinetic parameters were estimated such as C_{max}, T_{max}, AUC are given in **Table 15**. The mean t_{max} of reference formulation was 3 h. This indicates that the drug release from the reference formulation was rapid, while in the test formulation, the mean t_{max} was 5.5 h. This indicates that the test formulation was effective in delaying the peak plasma concentration, thus showing prolonged plasma concentration of furosemide from the floating microballoons.

The mean biological half-life ($t_{1/2}$ of furosemide from test and reference formulations was 18.72h and 7.45 h respectively. The difference observed here is due to prolonged absorption of test formulation; there is a prolonged continuous release of the drug into bloodstream. Therefore, the test formulation shows to have a 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 the rapid removal of drugs from plasma, whereas the higher half-life of test formulation indicates prolonged release.

The mean area under plasma time curve AUC $_{0\text{-}t}$ and AUC $_{0\text{-}\alpha}$ of reference formulation was 184.5458 ng/ml × h and 302.4 ng/ml × h and while AUC $_{0\text{-}t}$ and AUC $_{0\text{-}\alpha}$ of the test formulation was 269.19583 ng/ml × h and 327.9 ng/ml × h, This indicates that the overall absorption of furosemide 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 (FSDF10) was increased significantly when compared to marketed formulation. Relative bioavailability with respective to marketed formulation was found to be 108.4, which is due to the prolonged gastric residence time of furosemide floating microballoons.

TABLE 13: PLASMA CONCENTRATION OF FUROSEMIDE CONVENTIONAL TABLETS (LASIX) IN RABBITS (N = 6) AT DIFFERENT TIME INTERVALS (REFERENCE FORMULATION)

Time (h)	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 14: PLASMA CONCENTRATION OF FUROSEMIDE FLOATING MICROBALLOONS (FSDF10) IN RABBITS (N = 6) AT DIFFERENT TIME INTERVALS (TEST FORMULATION)

Time (h)	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

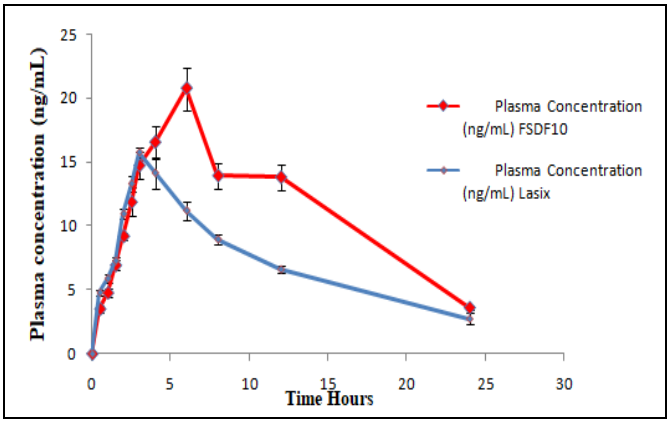

FIG. 13: MEAN PLASMA CONCENTRATION TIME PROFILE OF FUROSEMIDE TEST (FSDF10) AND REFERENCE (LASIX) FORMULATIONS

TABLE 15: MEAN PHARMACOKINETIC PARAMETERS OF FUROSEMIDE AS REFERENCE AND TEST TABLETS IN RABBITS (N=6)

Pharmacokinetic parameter	Unit	Reference	Test
C_{max}	ng/mL	15.75	20.75
T_{max}	h	3	6
AUC $_{0-t}$	ng/mL× h	184.54	269.19
AUC $_{0-\alpha}$	ng/mL × h	302.4	327.9
$T_{1/2}$	h	7.45	18.72

CONCLUSION: Gastro-retentive drug delivery system for furosemide was successfully prepared and evaluated by the solvent evaporation technique using eudragit RS 100, eudragit S 100, HPMC K_4M, ethylcellulose 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. From the percentage loading efficiency and *in-vitro* drug release studies, it was observed that FSDF10 formulation exhibits greater drug loading efficiency and sustained release behavior. On fixing the *in-vitro* drug release data of optimized formulation to various kinetic models, it was found that it exhibits the zero-order kinetics. $BaSO_4$ loaded optimized formulation FSDF10 selected for radiological study reveals that gastric retention time of floating microballoon in unfed state was 4.5 h, and in the fed state it was 5.5 h. *In-vivo* bioavailability study was conducted in rabbits optimized formulation (FSDF10) showed increased bioavailability when compared to the reference marketed tablets (Lasix) due to controlled floating technology.

Microballoons prepared in this study provide a promising gastro retentive drug delivery system to deliver furosemide with sustained-release in order to improve oral drug bioavailability. Thus, the prepared floating microspheres may prove to be potential candidates for multiple-unit delivery devices adaptable to any intragastric condition.

ACKNOWLEDGEMENT: Authors are thankful to the Principal, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, for providing necessary facilities.

CONFLICTS OF INTEREST: There are no conflicts of interest.

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How to cite this article:
Pancheddula M and Shayeda: Development of furosemide floating microballoons: *in-vitro* and *in-vivo* evaluation. Int J Pharm Sci & Res 2020; 11(5): 2248-61. doi: 10.13040/IJPSR.0975-8232.11(5).2248-61.