Preparation and Evaluation of Novel Expandable Drug Delivery System

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ABSTRACT

Aims: The purpose of this research is to develop a novel expandable gastroretentive dosage form (GRDF), based on unfolding mechanism. It consists of a drug loaded bilayer polymeric film, folded into a hard gelatin capsule. Gastric retention is achieved due to unfolding of the dosage form within 15-20 min. Furosemide is selected as the drug candidate for this work. Due to its narrow absorption window, Furosemide has to be administered to the upper parts of the intestine in order to maintain sustained therapeutic levels. This may be achieved by a GRDF.

Methodolgy: Films were prepared by solvent-casting technique using Ethyl cellulose, HPMC E15 and Eudragit RLPO as polymers and dibutyl phthalate as the plasticizer in both layers. The film with zigzag folding in the capsule was shown to unfold in the gastric juice and provide drug release up to 12 h in the acidic medium. The films were evaluated for weight & thickness variation, mechanical properties, *in vitro* drug release and unfolding behaviour based on the mechanical shape memory of polymers. Absence of drug polymer interaction and uniform drug dispersion in the polymeric layers was revealed by DSC, XRD studies and SEM. The GRDF location in the gastrointestinal tract was determined by X-ray studies.

Results: X-ray studies revealed that the GRDF is retained in the stomach up to 6± 0.5 h in fasting condition and 8 h in fed state.

Conclusion: The polymers used in the development of GRDFs were safe and proper combination of these polymers will yield a novel expandable GRDF with good *in vitro* drug release in acidic media, mechanical properties, unfolding behaviour. These outcomes demonstrate that the GRDF may be used to improve furosemide therapy and can be applied to extend the absorption of other narrow absorption window drugs that require continuous input.

Keywords: Furosemide, Expandable drug delivery systems, Gastric retention, Mechanical Properties, Hydroxy Propyl Methyl Cellulose, Ethyl cellulose

1. INTRODUCTION

Oral delivery of drugs is the most preferred route of drug delivery, due to ease of administration, patient compliance and flexibility in formulation. Conventional immediate oral dosage forms provide a specific drug concentration in the systemic circulation with limited control over drug delivery but limited in retention of the dosage form in the stomach [1]. Approaches to increase the gastric residence time of drug formulation include (a) High Density Systems (b) Floating

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Systems (c) Bio/Muco Adhesive Systems (d) Swelling and Expanding Systems (e) Incorporation of Passage Delaying Food Agents (f) Ion Exchange Resins (g) Raft Systems (h) Superporous Hydrogels (i) Magnetic Systems(j) Bioadhesive Liposomal Systems. However, it is recognized that there are many physiological constraints which may limit development of such delivery systems [2].

The purpose of this research was to develop a novel expandable GRDF, based on unfolding mechanism. It consists of a bilayered polymeric film in which the drug is loaded in one layer, folded into a hard gelatin capsule. Gastric retention is achieved due to unfolding of the dosage form in the stomach within 15 min of administration. The film with zigzag folding in the capsule was shown to unfold in the gastric juice and provide drug release up to 12 h in the acidic medium. The research on expandable GRDF was initiated by the team Klausner et al, as they worked on Riboflavin and Levodopa expandable GRDFs [3,4]. Recently Intec Pharma developed an expandable GRDF Accordion Pill Carbidopa/Levodopa for the treatment of Parkinson's disease. The dosage form works on unfolding mechanism but it is prepared with variable polymers and novel technology and got Success in Phase II clinical studies [5].

Furosemide (4-chloro-N-furfuryl-5-sulphamoylanthranilic acid or 5 (aminosulfonyl)-4-chloro-2[(2-furanylmethyl) amino] benzoic acid) is a loop diuretic that is used orally in the treatment of edematous states associated with cardiac, renal and hepatic failure and the treatment of hypertension [6]. The usual dosage is 40 to 120mg/day. Martindale reports that furosemide is practically insoluble in water, corresponding to <0.1 mg/mL [6,7]. It works by inhibiting the $Na^+/K^+/ 2Cl^-$ transporter in the ascending limb of the loop of henle. Furosemide is fairly rapidly absorbed from the gastrointestinal (GI) tract with half life of 30–120 min. Its bioavailability was reported to be about 60–70%, but the absorption is variable and erratic 7. Furosemide is most rapidly absorbed from the upper GI tract following dissolution in the stomach [8]. Based on these parameters expandable GRDFs were designed to overcome poor bioavailability and dosing intervals (usually 3-4 times/day). In vitro studies were carried out and compared with marketed dosage form LASIX ® 20 mg Tablets (Sanofi aventis, Canada).

2. Materials and Methods

2.1. Materials

Furosemide was obtained as a gift sample from Dr. Reddys Laboratories, Hyd, A.P, India. Hydroxyl Propyl Methylcellulose (HPMC E 15), Ethyl Cellulose (EC) and Eudragit RLPO were procured from Loba chemicals Pvt Ltd., India. All other reagents used were of analytical grade.

2.2. Preparation of films

2.2.1. Preparation of primary layer

Expandable GRDFs were prepared by solvent casting method. Weighed quantity of EC, HPMC E15 and Eudragit RLPO were taken in a boiling tube. To this, 25 ml of solvent mixture of dichloromethane: methanol (1:1) was added and vortexed. Sufficient care was taken to prevent the formation of lumps. The boiling tube was set-aside for 6 hours to allow the polymers to swell. After swelling, measured quantity of di butyl phthalate was added to this mixture and vortexed. Finally weighed quantity of solid dispersion (1:3) of Furosemide with povidone was dissolved in 10 ml of solvent mixture, added to the polymer solution and mixed well. It was set-aside for some time to exclude any entrapped air and was then transferred into a previously cleaned anumbra petriplate. Drying of these patches for 8 hrs was carried out in oven (at 40^0C) placed over a flat surface. The patches formed were removed carefully, placed in a vacuum oven and vacuum was applied to remove traces of solvent if any.

2.2.2. Preparation of secondary layer

Weighed quantity (2 g) of EC was taken in a boiling tube. To this, 25 ml of solvent mixture of dichloromethane: methanol (1:1) was added and vortexed. Sufficient care was taken to prevent the formation of lumps. The boiling tube was set-aside for 1 hour to allow the polymer to dissolve. After that, measured quantity (1 ml) of di butyl phthalate was added to this mixture and vortexed. It was set-aside for some time to exclude any entrapped air and was then poured onto primary layer, which leads to formation of a bilayered film. For the preparation of GRDFs the composition of secondary layer is same for all formulations. Drying of these patches for 8 hrs was carried out in oven (at 40^0C) placed over a flat surface. The patches formed were removed carefully, placed in a vacuum oven and vacuum was applied to remove traces of solvent if any. On removal of the films they were checked for possible imperfections before being cut into 4cm×2cm rectangles and micro crystalline cellulose (MCC) was applied on to the film on both sides. These films are filled into hard

gelatin size 00 capsules by folding in a zigzag manner (Figure 1). The area of the petriplate used in the preparation of both layers is 64cm^2.

Figure 1. Folding pattern of expandable GRDFs (different views)

Table 1 Formulation Ingredients of Furosemide GRDFs.

	Primary layer					
Formulation	**Drug* (mg)**	**EC (mg)**	**HPMC E 15 (mg)**	**Eudragit RLPO (mg)**	**di butyl phthalate (µl)**	**DCM& Methanol (1:1) (ml)**
F1	160	500	300	200	500	35
F2	160	500	275	225	500	35
F3	**160**	**500**	**250**	**250**	**500**	**35**
F4	160	500	225	275	500	35
F5	160	500	200	300	500	35

*Solid dispersion equals to 160 mg of the drug

2.3. Optimization of GRDFs

The GRDFs were optimized for folding and unfolding patterns, drug release and integrity as described below.

2.3.1. Unfolding behaviour of GRDFs- in vitro

Films were folded by two methods. In both methods Avicel-101 was used as anti adherent agent. In the first method the film was rolled in a single direction, in the second method the film was folded in a zigzag manner and both films were inserted into individual capsule. In each case six capsules were taken for in vitro dissolution study in 900mL aqueous hydrochloric acid pH 1.2 at 37ºC ± 0.5ºC using the USPXXIII Apparatus1 (basket) at 100 rpm. Baskets were removed after 5, 10, 15, 20, 30, 60, 120, 240, 480 and 720 min and the films were examined for their unfolding behaviour.

2.3.2. Integrity of GRDFs

Initial trials were made with different grades of Eudragit and HPMC polymers with different ratios of solvent, plasticizer and anti adherent agents. Finally the films with EC (as secondary layer), HPMC E15, EC and Eudragit RLPO (as primary layer) got very good integrity for 12 hrs *in vitro.* Among the polymers used to prepare the film, EC plays an important role to maintain the integrity of the primary layer in combination with secondary layer.

2.3.3. Drug release

Initial trials were made without Eudragit RLPO, but there was no control over the drug release i.e., total drug was released in 4 hrs only. Drug release was prolonged by optimizing the EC concentration and inclusion of Eudragit RLPO in the primary layer. There was no drug in the secondary layer, but it gives good integrity and unfolding behaviour to the GRDF.

2.3.4. Solubility enhancement

To improve the solubility of the drug, solid dispersions were prepared by two methods i.e., physical mixing and solvent evaporation. In both methods the ratio of drug and polymer (povidone) varies from 1:1 to 1:3. Physical mixture was prepared by simply mixing the recrystallized drug and polymer in a motor with care to avoid any grinding action. In the solvent evaporation technique drug and polymer in different ratios were dissolved in methanol. The solvent was removed under reduced pressure in a rotary evaporator at 70^0 C. The dispersions were vacuum dried for 48 h in a desiccator at room temperature. The residue was ground and the particle size fraction was obtained by sieving. Good solubility enhancement was observed in case of 1:3 solid dispersion prepared by solvent evaporation technique. The solubility was increased from 24 µg/ml to 120 µg/ml in 0.1 N HCl (pH 1.2). In this work the term solid dispersion is the mixture of drug and polymer prepared by solvent evaporation technique

2.4. Characterization of GRDFs

2.4.1. Weight variation test

Each formulation was prepared in triplicate and ten patches each equivalent to 4cm×2cm was cut from each plate. Their weight was measured using Shimadzu digital balance. The mean ± SD values **(Table 2)** were calculated for all the formulations.

2.4.2. Thickness variation test

The thickness of the patches was measured by digital screw gauge (Digimatic outside micrometer, Mitutoyo, Japan). The mean ± SD values. **(Table 2)** were calculated for all the formulations.

2.4.3. In vitro drug release studies

Drug release from the formulations was studied by using USP dissolution tester XXIII Apparatus1 (basket) at 100 rpm in 900mL aqueous hydrochloric acid pH 1.2 at 37ºC ± 0.5ºC. The procedure is repeated for the marketed product LASIX ® 20 mg Tablets (Sanofi aventis, Canada), compared with optimized formulation. The *in vitro* drug release pattern was interpreted by using 'PCP Disso v2.08' soft ware and the data was fitted in various kinetic models and the values of the correlation coefficients were compared.

2.4.4. Measurement of Mechanical Properties

Mechanical properties of the GRDFs were evaluated using a microprocessor based advanced force gauze equipped with a motorized test stand (Ultra Test, Mecmesin, West Sussex, UK), equipped with a 25 kg load cell. Film strip with the dimensions 60 x 10 mm and free from air bubbles or physical imperfections, were held between two clamps positioned at a distance of 3 cm. A cardboard was attached on the surface of the clamp to prevent film from being cut by the grooves of the clamp. During measurement, the strips were pulled by the top clamp at a rate of 2.0 mm/s to a distance till the film broke.

The force and elongation were measured when the films were broken. Results from film samples, which were broken at end and not between the clamps were not included in observations. Measurements were run in six replicates for each formulation. The following equations were used to calculate the mechanical properties of the films.

$$\text{Tensile strength (kg.mm}^{-2}\text{)} = \frac{\text{Force at break (kg)}}{\text{Initial cross sectional area of the sample (mm}^2\text{)}}$$

And

$$\text{Elongation at break (\%mm}^{-2}\text{)} = \frac{\text{[Increase in length (mm)] 100}}{\text{[Original length] [Cross sectional area (mm}^2\text{)]}}$$

2.4.5. Scanning electron microscopy (SEM)

The morphology of the GRDFs was studied by scanning electron microscope (SEM). The film was examined in a JEM-1200 EX II electron microscope (Jeol, Tokyo, Japan) equipped with an EM-ASID 11 Scanning Image Observation Device using secondary electron imaging.

2.4.6. Differential scanning calorimetry (DSC)

Thermal analysis of drug-excipient compatibility was studied by Differential Scanning Calorimeter (METTLER). Pure drug, polymers and bilayer film were scanned in the temperature range of 50-250°C. Analysis was performed under a nitrogen purge at a rate of 10°C/min

2.4.7. X-ray diffraction (XRD)

XRD patterns were measured using a SIEMENSD-5000 X-ray diffractometer to characterize the crystallinity, amorphousness of furosemide, PVP and bilayer film of formulation F3.

2.4.8. In vivo (x-ray) studies

To make the GRDF X-ray opaque Barium Sulphate ($BaSO_4$) was incorporated. The films were prepared by replacing the drug with $BaSO_4$. In both layers 540 mg of $BaSO_4$ (15 % of film weight) was distributed equally (67.5 mg for each GRDF). These films were also evaluated for mechanical properties, unfolding behaviour *in vitro* and no difference was observed in their behaviour when compared with drug loaded GRDFs.

2.4.8.1. Study protocol

The *in-vivo* study was carried out by administering GRDF to humans and monitoring them through a radiological method. Four healthy male subjects (mean age 27year: mean weight 60±10 kg) participated after giving informed consent. The study (approved by the Ethical Committee, UCPSc, Kakatiya University, Warangal) was conducted by administering one GRDF to each subject on two separate sessions.

a) Fasted state: The subjects fasted overnight then swallowed the film with 150 ml water. Afterwards the subjects were not allowed to eat.
b) Fed state: After a meal, the subjects swallowed the film immediately after ingestion of a standardized lunch composed of a bread and milk (150g solid, 200 ml liquid).
Afterwards the subjects were not allowed to eat.

In both cases 150 ml of water was given after every one hour. During the experiments the subjects remained in a sitting or upright posture. In each subject the position of the film was monitored by X-ray photographs (Konica Minolta, Siemens, Karlsruhe, Germany) of the gastric region at determined time intervals. All X-ray films were taken in anterior positions.

3. RESULTS AND DISCUSSION

3.1. Optimization of formulation

3.1.1. Unfolding behaviour

GRDFs prepared by both methods were evaluated for their *in vitro* unfolding behaviour. The GRDFs prepared by first method have not unfolded properly, but the GRDFs of second method unfolded within 15-20 min (Fig 2). Apart from folding pattern, for proper unfolding of a film, mechanical shape memory (resiliency to restore its original shape) is required. Such shape memory polymers may have the glass transition (*Tg*) at about room temperature [9]. The selection of plasticizer for GRDFs is very important because, only the plasticizers of similar solubility parameter ($MPa^{0.5}$) to that of EC (20 $MPa^{0.5}$) will have a greater effect on Tg suppression [10]. Initial trials were made with various plasticizers like Dibutyl phthalate (19 $MPa^{0.5}$), Diethyl phthalate (20.5 $MPa^{0.5}$), Triethyl citrate (20.4 $MPa^{0.5}$). But satisfactory results were obtained with only DBP.

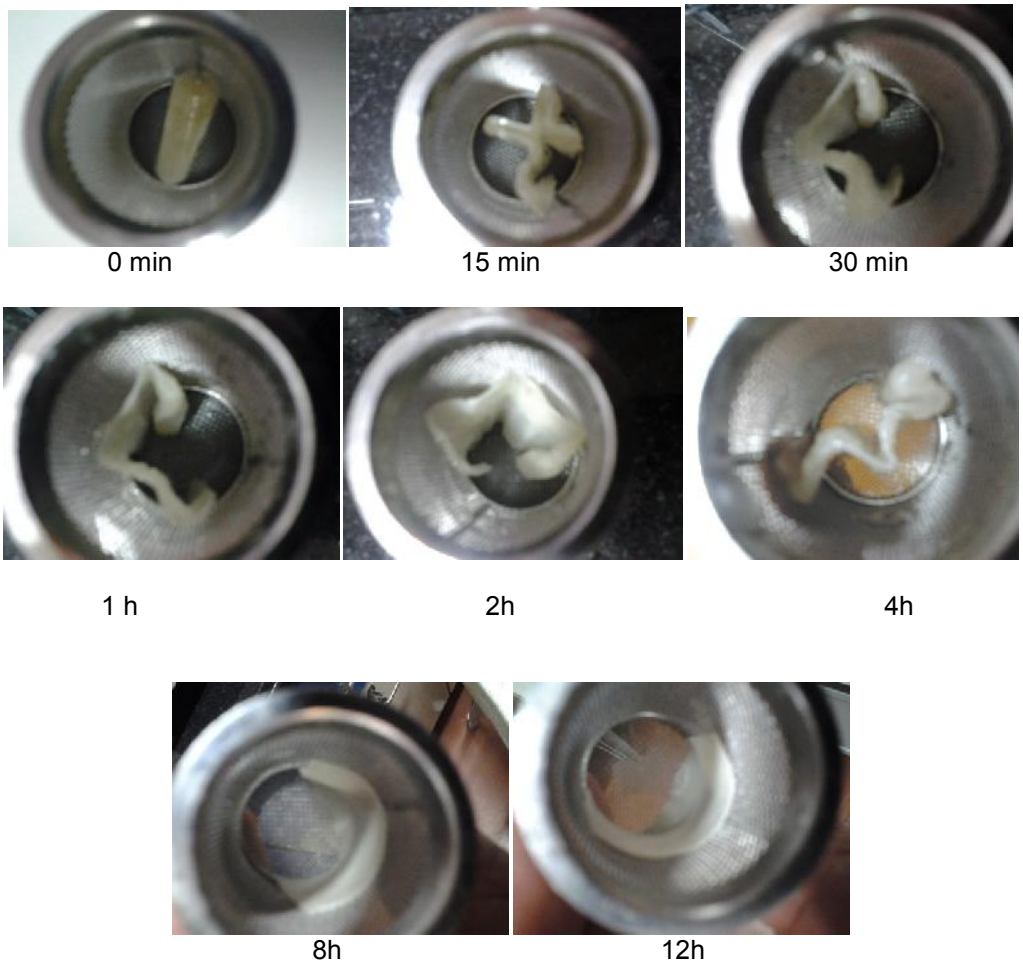


Figure 2 Unfolding behaviour of GRDF

3.1.2. Polymer content

In case of primary layer, EC content of less than 500 mg was insufficient to retard the drug release and retain the integrity. So formulations were prepared by keeping EC content constant and varying the contents of HPMC E 15 and Eudragit RLPO from 200 to 300 mg. In case of secondary layer, EC content of less than 2g was insufficient to retain the integrity and mechanical shape memory.

3.1.3. Plasticizer content

For secondary layer, plasticizer (DBP) concentration of less than 0.5mL was insufficient to form film. Plasticizer concentration of 1mL yielded more flexible films. Further increasing the concentration of plasticizer above 1mL resulted in enormous increase in the drying time. In case of primary layer 0.5mL of DBP yielded more flexible films.

3.1.4. Solvent volume

For secondary layer, solvent volume of 25mL was sufficient to cast the film. In case of primary layer , solvent volume of 14-20mL resulted in viscous solution; hence complete transfer of the solution could not be ensured. Solvent volume of 25-35 mL was sufficient to solubilize the drug and cast the films. Increasing the solvent volume above 35 mL resulted in the formation of patches with entrapped air bubbles.

3.2. Characterization of GRDFs

The results of weight variation test for various formulations were shown in Table 2. Results of weight variation test indicated uniformity in weight of the patches, as evidenced by SD values. In thickness variation test (Table 2), the thickness was found to be uniform.

Table 2 Evaluation of the GRDFs.

F.Code	Weight (mg)	Thickness (µm)	Tensile Strength (kg/mm^2)	Elongation at break ($\%mm^{-2}$)
F1	**450±3.66**	480±1.59	26.48±3.62	0.22±0.08
F2	462±3.98	489±2.64	29.62±2.27	0.46±0.09
F3	456±4.96	485±1.66	22.44±4.66	0.42±0.06
F4	470±3.64	483±2.42	24.62±4.62	0.38±0.08
F5	465±4.29	484±2.17	27.82±6.89	0.28±0.04

F.Code: Formulation Code; All values indicate mean±Standard Deviation

3.2.1. In vitro Drug Release Studies

Drug release was studied for all formulations from F1-F5. Based on the in vitro drug release, unfolding behaviour and mechanical properties, the formulation F3 was selected as the optimized formulation (Fig 3). Now the drug release from the marketed product (LASIX ® 20 mg Tablets) was studied and compared with formulation F3. The marketed product released 100% within 45 min, but formulation F3 showed that it was a controlled release formulation releasing the drug up to 12 hr and followed first order release (R^2=0.992) with diffusion control mechanism (Higuchi model, R^2=0.991).

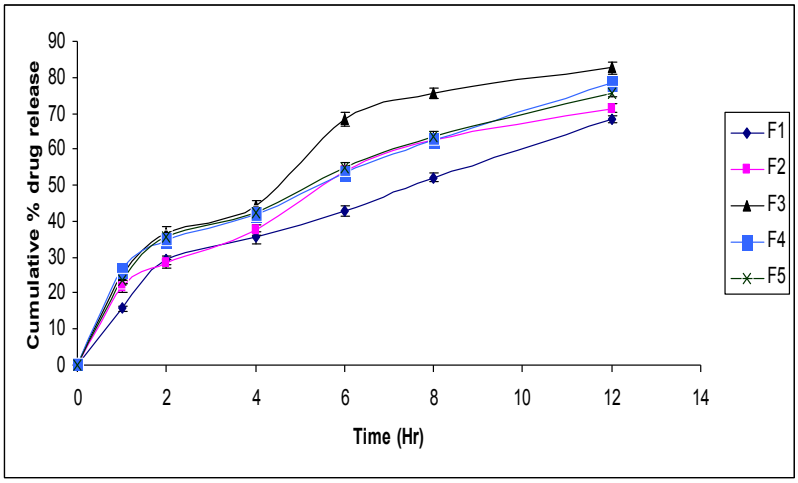


Fig 3 In vitro drug release from formulations F1-F5

3.2.2. Mechanical Properties of Films

The results of the mechanical properties i.e., tensile strength and elongation at break are presented in Table 2 and values indicated that no statistical difference was observed in tensile strength and elongation at break values between the formulations.

3.2.3. Scanning electron microscopy (SEM)

The cross sectional view of the GRDF shows that the presence of a secondary layer. (Fig 4). The secondary layer did not show any crystals on the surface indicated homogenous dispersion of the drug in the polymer matrices.

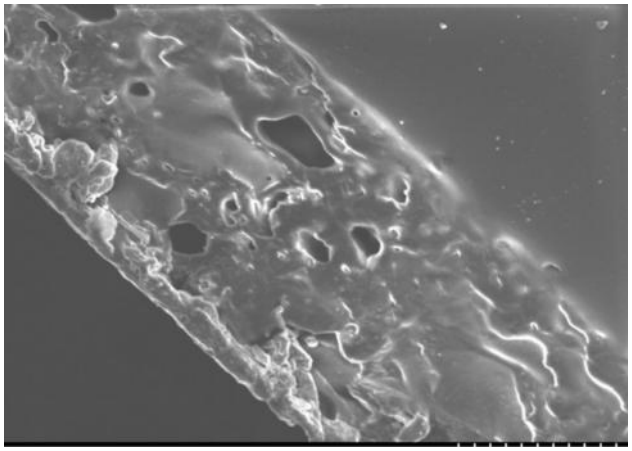


Fig 4 Scanning electron microscopy of the GRDF

3.2.4. Differential scanning calorimetry (DSC)

DSC studies revealed that furosemide exhibits a sharp endothermic peak at 220.8 ^{0}C corresponding to its melting point which is usually associated with decomposition of the drug. This could also be seen in the solid dispersion also. The peak did not appear in the thermogram of the polymeric film (F3) (Fig. 5) which indicated that the drug was uniformly entrapped in the polymeric matrices.

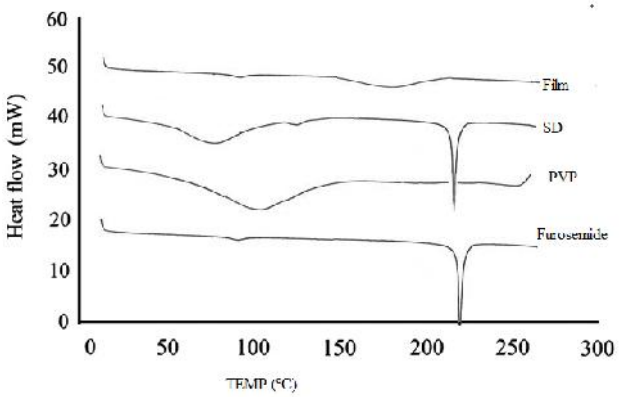


Fig 5 DSC thermograms of furosemide, PVP, Solid dispersion and GRDF

3.2.5. X-ray diffraction (XRD)

X-ray diffraction studies were carried out to reveal the crystalline modifications during the preparation of films (Fig. **6**). Results of the x-ray diffractograms showed that furosemide showed crystallinity where as PVP showed amorphous form. In case of the solid dispersion and film, the intensity of the peaks was decreased when compared with the pure drug, which indicated uniform molecular dispersion of furosemide in the polymeric layers.

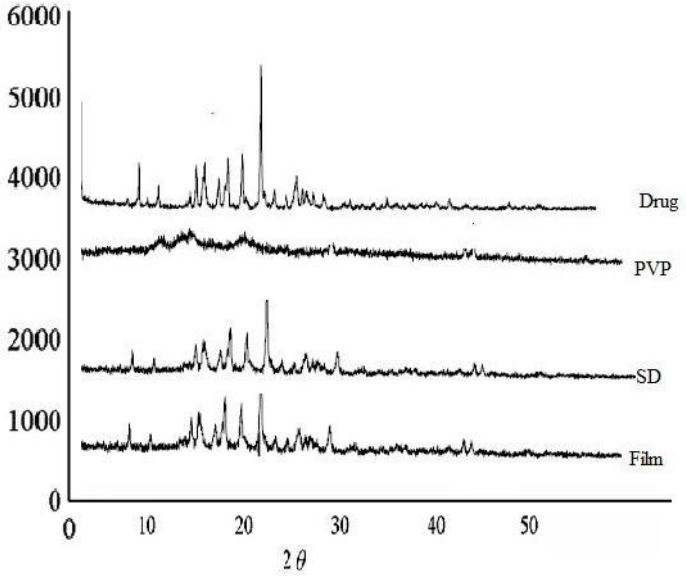


Fig 6 X-ray diffraction patterns of furosemide, PVP, Solid dispersion and GRDF

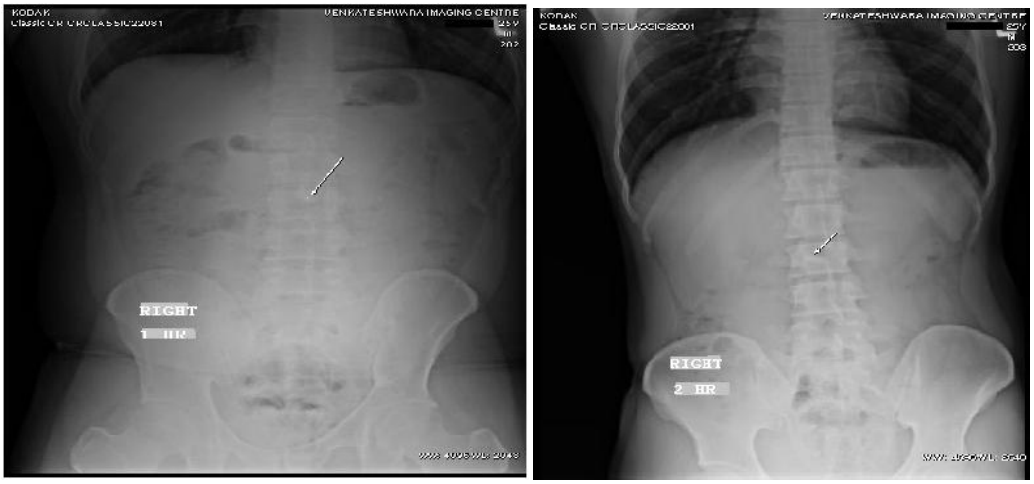
3.2.6. In vivo (x-ray) studies

The behaviour of the GRDFs in the human stomach was observed in real time using a radiographic imaging technique. In radiographic images made 1 hr after the administration, the films were observed in the stomach. In the next pictures taken at 2, 4, 6 hrs the film had altered its position in the stomach. This provided evidence that the films did not adhere to the gastric mucosa. The gastric residence time of optimized GRDFs were evaluated by conducting in-vivo X-ray studies in healthy human volunteers both in fasting and fed conditions. From the radiographic images following results were obtained.

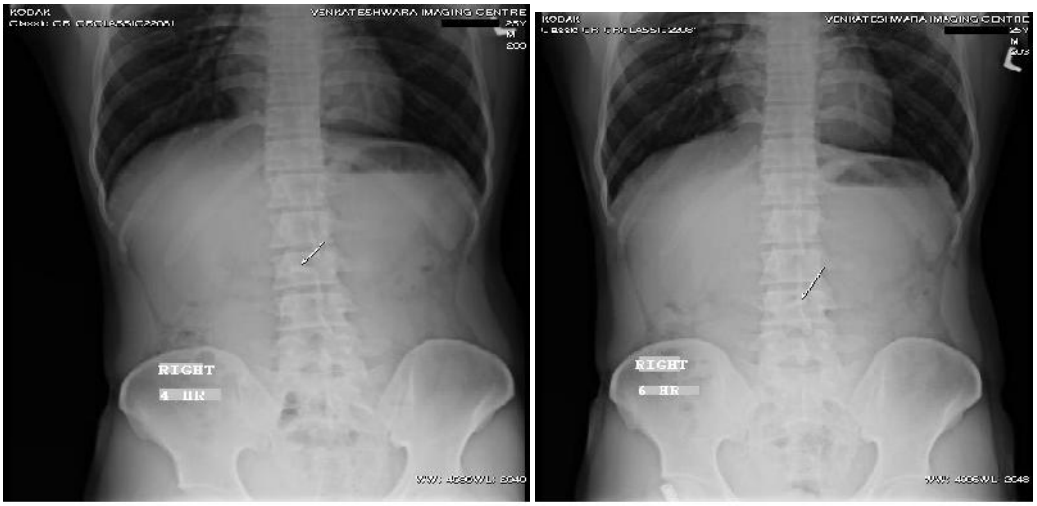
Table 3: Results of in-vivo x-ray studies

Condition	Gastric residence time (h)
Over night fasting state	Up to 6± 0.5
Fed state	Up to 8

From above results it was observed that the mean gastric residence time for the developed GRDFs was 6± 0.5 hr in overnight fasting state. But in fed state the gastric residence time was observed for 8 hrs.

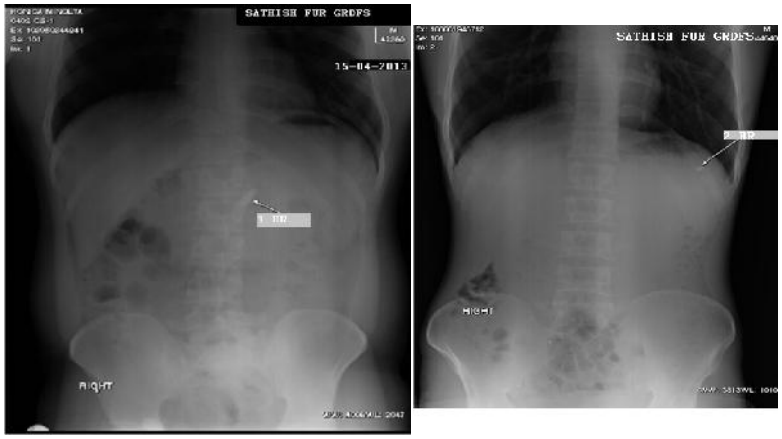


1 h **2h**

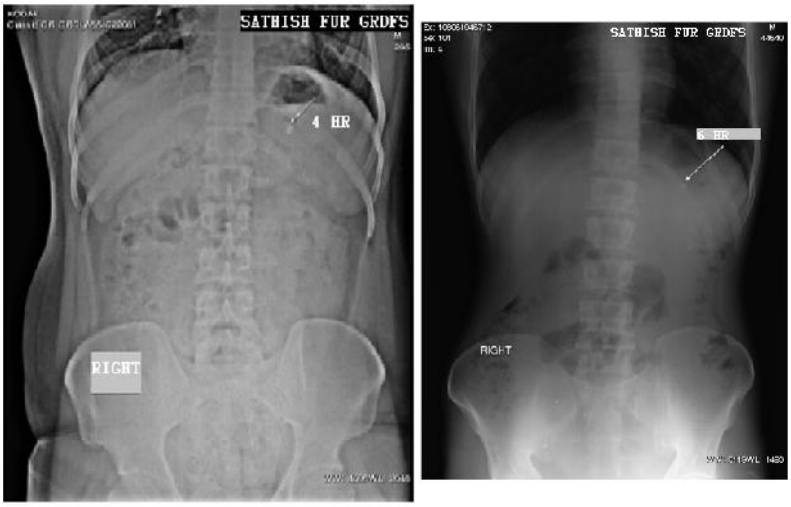


4h **6h**

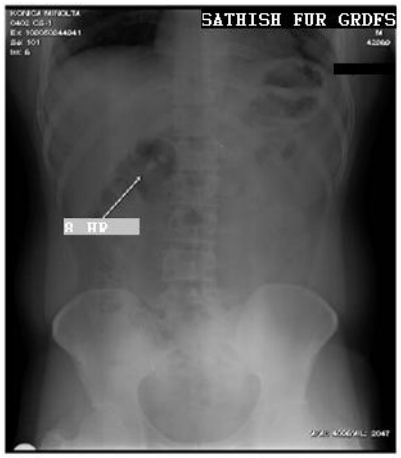
Fig. 7: In *vivo* x-ray studies in fasting state



1 h **2 h**



4 h **6 h**



8 h

Fig. 8: In *vivo* x-ray studies in fed state

4. CONCLUSION

The current research work demonstrates the successful development of a GRDF for a drug (Furosemide) with a narrow absorption window. It consists of a drug loaded bilayer polymeric film, folded into a hard gelatin capsule. Gastric retention is achieved due to unfolding of the dosage form in the stomach within 15-20 min of administration. The polymers used in the development of GRDFs were safe and proper combination of these polymers will yield a novel expandable GRDF with good *in vitro* drug release in acidic media, mechanical properties, unfolding behaviour. In fasting condition the myoelectric migrating contractions force the cssontents to duodenum from stomach. The forceful house keeping wave will remove all the contents including dosage form to leave stomach. But X-ray studies revealed that the GRDF is retained in the stomach up to 6± 0.5 h in fasting condition and 8 h in fed state. Further pharmacokinetic and pharmacodynamic studies have to be carried out in human volunteers.

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