

Bilayer Film Type of Unfolding Drug Delivery System for the Dual Release of Proton Pump Inhibitor and H₂ Receptor Antagonist

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Abstract

Aim: This study was aimed at formulating and evaluating a bilayer unfolding film type drug delivery system for the dual release of proton pump inhibitor, rabeprazole sodium (RS) and H₂ receptor antagonist, famotidine. **Materials and Methods:** Polymers such as polyvinyl alcohol hot, chitosan, and hydroxypropylmethyl cellulose E15 LV were used as film forming agents and glycerol as plasticizer. Enteric microspheres of RS were prepared by solvent evaporation and the films by solvent casting method. Microspheres were evaluated for production yield, entrapment efficiency, particle size, and *in vitro* drug release study. Optimized formulations of microspheres based on drug entrapment efficiency and *in vitro* drug release were incorporated into immediate release layer designed to disintegrate quickly. The individual layers, i.e., the immediate release layer containing RS microspheres and a gastro-retentive layer containing famotidine were subjected to various tests for uniformity of weight, thickness, folding endurance, uniformity of drug content, tensile strength, *in vitro* drug release, swelling index, and surface area. **Results and Discussion:** The immediate release layer disintegrated within 15 min while the gastro-retentive layer retained its integrity for more than 8 h. The evaluation of the assembled bilayer system of gastro-retentive layer and immediate release layer for *in vitro* drug release produced similar results as that for individual layers. *In vivo* X-ray radiography in rabbits confirmed the ability of the famotidine layer to be retained in the stomach for more than 8 h and the immediate release of the enteric RS microspheres for availability in the intestine. **Conclusion:** The bilayer unfolding film was successful in gastro retention and achieving the dual release of rabeprazole and famotidine and has the potential in the effective management of gastroesophageal reflux disease.

Key words: Bilayer film, chitosan, famotidine, gastro retention, solvent casting

INTRODUCTION

In recent years, scientific and technological advancements have been made in the research and development of rate-controlled oral drug delivery systems that overcome physiological adversities such as short gastric residence times and unpredictable gastric emptying times (GET).^[1]

Gastro-retentive drug delivery is an approach to prolonging gastric retention time (GRT), thereby targeting site-specific drug release in the upper gastrointestinal (GI) tract for local or systemic effects. Gastro-retentive dosage forms can remain in the gastric region for long periods and hence significantly prolong the GRT of drugs. Over the last few decades, several gastro-retentive drug delivery approaches were

designed and developed, and it including: High-density (sinking) systems that are retained in the bottom of the stomach; low-density (floating) systems that are buoyant in gastric fluid; mucoadhesive systems that causes bioadhesion to stomach mucosa; unfoldable, extendible, or swellable systems, which limit emptying of the dosage forms through the pyloric sphincter of stomach; super porous hydrogel systems; and magnetic systems.^[2] Drugs, which have narrow

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absorption window in GI tract, or primarily absorbed from the stomach and upper part of GI tract, act locally in the stomach, degrade in the colon and drugs that disturb normal colonic bacteria are benefited by formulating them as gastro-retentive dosage forms (GRDFs).^[3]

Expandable GRDFs are easily swallowed and reach a significantly larger size in the stomach due to swelling or unfolding processes that prolong their GRT. Hence, many of them consist of polymeric matrices which retain their integrity for several hours, and therefore, remain in the stomach even in the fed state. After drug release, their dimensions are minimized by erosion or break up with subsequent evacuation from the stomach. Gastro retention is enhanced by the combination of substantial dimensions with high rigidity of the dosage form to withstand the peristalsis and mechanical contractility of the stomach.^[4] Sustained and controlled drug release from these systems may be achieved by selecting a suitable hydrophilic polymer with the proper molecular weight, extent of cross-linking, and swelling properties. A simple and cost-effective approach for developing an unfolding system is a polymeric film made from such polymers that are folded in a characteristic manner into a hard gelatin capsule. On administration, after the dissolution of the capsule shell, the folded film on imbibition of stomach fluids, swell, and gradually unfolds to achieve dimensions that ensure retention of the film in the stomach.

It is known that proton pump inhibitors (PPI) are more efficient in inhibiting gastric acid secretion than the H₂ blockers but the latter are more effective in the suppression of 24 h gastric acid production including nocturnal gastric secretion.^[5] In fact, studies suggest that PPI may not control the gastric acidity effectively during the night, especially in gastroesophageal reflux disease (GERD) due to insufficient suppression of nocturnal gastric acidity. Patients with GERD benefitted more with the addition of H₂ receptor blockers to the existing PPI therapy.^[6] Therefore, in our investigation, we have taken a combination of rabeprazole sodium (RS), a PPI and famotidine, an H₂ receptor blocker. A dosage form that produces dual release of these two drugs would be in order such that there is prolonged delivery of famotidine which should take care of the nocturnal secretion of gastric acids and immediate release of RS that should bring about the instantaneous relief from the symptoms of GERD. The prescribed oral dose for RS in the treatment of GERD is 20 mg, once a day. Since RS is an acid labile drug, it is usually available as enteric-coated or delayed release tablets to protect it from stomach fluids.^[7] Famotidine has a low oral bioavailability of 40-45% due to incomplete absorption and an elimination half-life of 2.5 to 3.5 h. The recommended oral dosage of this drug in GERD is 20 mg, twice a day for 6 weeks.^[8] Thus, the GRDF that could provide sustained release of famotidine to the upper part of the intestine, where it is better absorbed by virtue of the lower pH that favors dissolution of famotidine could improve the bioavailability and enhance the therapeutic efficacy of the drug. We have

designed a novel bilayer unfolding film type drug delivery system that produces gastro retention of famotidine while simultaneously bringing about the immediate release of RS. Since RS needs to be protected from the gastric acids before reaching the intestine, we have prepared enteric microspheres for incorporation into the immediate release layer. After the disintegration of this layer, the microspheres whose size would be smaller than the pyloric sphincter diameter (2 mm) pass on to the small intestine where the drug is released immediately for absorption.^[9] The sustained release layer containing famotidine will be retained in the stomach due to the expansion and increase in size of the unfolding film that slowly releases the drug. This investigation is an attempt to determine the feasibility of developing and evaluating a Bilayer Unfolding Film type drug delivery system for dual release of famotidine and RS, which would be useful in the control of GERD or other gastric disorders related to hyperacidity.

MATERIALS AND METHODS

Famotidine was supplied by Zydus Roche, and RS was supplied by Dr. Reddy's Laboratories, Hyderabad. Polyvinyl alcohol (PVA) Hot was obtained from Central Drug House Ltd. (New Delhi), and chitosan was obtained from NICE Chemicals (Kochi). Hydroxypropylmethyl cellulose (HPMC) E15 LV and Eudragit L100 (EL) were obtained from Yarrow Chem Products (Mumbai). All other chemicals used were of laboratory grade.

Fabrication of famotidine layer (Layer A)

Famotidine containing layer/film was prepared by solvent casting method using chitosan or HPMC in combination with PVA and glycerol as plasticizer. Briefly, the drug was dissolved in 10 ml of 10% acetic acid solution. In case of the chitosan films, the polymer was dissolved in 1% w/v acetic acid solution while PVA hot was dissolved in water by heating on a water bath. Polymer solutions were mixed, and drug solution was added to it, followed by glycerol which was used as plasticizer. Then, the solutions were poured into Petri plates of 9.5 cm diameter and kept for drying under controlled temperature (40°C). The dried films were cut into rectangular size of 3 cm × 1.5 cm so that each film contained about 40 mg of drug. The films were packed in aluminum foil and stored in a desiccator until further use. The same procedure was used for the HPMC films, with the difference that HPMC E15 LV instead of chitosan was dissolved in distilled water followed by the addition of PVA and drug solutions. The compositions of Layer A are given in Table 1.

Fabrication of films containing RS (Layer B)

Steps involved:

1. Preparation of enteric microspheres of RS.
2. Preparation of microsphere loaded film.

Table 1: Composition of Layer A

Ingredients	FA	FB	FC	FD	FE	FF	FG	FH
Famotidine (mg)	620	620	620	620	620	620	620	620
PVA Hot (mg)	900	800	700	600	900	800	700	600
Chitosan (mg)	100	200	300	400	-	-	-	-
HPMC E15 LV (mg)	-	-	-	-	100	200	300	400
Glycerol (ml)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6

HPMC: Hydroxypropylmethyl cellulose, PVA: Polyvinyl alcohol

Preparation of enteric microspheres of RS

Microspheres were prepared by solvent evaporation technique using drug:polymer ratios of 1:1, 1:2, and 1:3. EL was dissolved in 10 ml ethanol using a magnetic stirrer. Pure RS was dissolved separately in 5 ml of ethanol. The drug solution was added to the polymer solution. Magnesium stearate was added to this solution and was mixed for 15 min with magnetic stirrer. The resulting dispersion was then poured into 250 ml beaker, containing the mixture of 50 ml liquid paraffin and 5 ml of 0.2% Span 60 (external phase) while stirring. A mechanical stirrer with a 3 blade paddle was used. Stirring (at 500 rpm) was continued for 4 h, until the alcohol was evaporated completely. Microspheres formed were filtered using vacuum assisted filtration. The residue was washed 4-5 times by 25 ml of n-hexane and petroleum ether. Microspheres were dried at room temperature for 24 h and kept in desiccator until further use.

Evaluation of RS microspheres

Prepared microspheres were evaluated for particle size, percentage yield, drug entrapment efficiency, and *in vitro* drug release studies.

Particle size analysis was carried out using digital imaging microscopy (Motic, DMBI-223) under $\times 45$ magnification.

Percentage drug entrapment was determined from drug content after extracting a suitable weight of the microspheres with phosphate buffer of pH 7.2 and measurement of absorbance at 284 nm using a UV spectrophotometer. The following formulas were used to determine percentage yield of microspheres and percentage drug entrapment.

$$\text{Percentage yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

$$\% \text{ Drug Entrapment} = \frac{\text{Actual Content}}{\text{Theoretical Content}} \times 100$$

***In vitro* drug release study of microspheres**

The dissolution rate of RS from the microspheres was studied at pH 1.2 for 2 h followed by pH 7.2 for 3 h using the USP dissolution apparatus with basket attachment. Accurately weighed microspheres equivalent to 20 mg of RS were taken for dissolution studies. The dissolution medium was kept at $37 \pm 0.5^\circ\text{C}$. Aliquots of sample were withdrawn at

predetermined intervals of time and analyzed for the drug by measuring the absorbance at 284 nm. The volume withdrawn at each time intervals was replaced with the same amount of fresh dissolution medium.^[10]

Preparation of microsphere loaded film (Layer B)

Films were prepared by solvent casting method. The weighed quantity of HPMC 15 LV was dissolved in distilled water. Optimized microspheres equivalent to 20 mg of RS were added to the above polymer solution. Glycerol was used as plasticizer. The above solution was poured into glass mold of 4.5 cm² area (3 cm \times 1.5 cm rectangular chamber) and kept for drying at 40°C for 2 h. The composition of Layer B is given in Table 2. The film was packed in aluminum foil and stored in desiccator until further use.

Evaluation of Layer A and Layer B

Uniformity of weight

The individual weights of 3 samples of each formulation of Layer A and Layer B were determined, and the average weight was calculated.

Film thickness

Thickness of 3 films of each formulation was determined using micrometer screw gauge and average was determined.

Folding endurance

Folding endurance of the films was determined by repeatedly folding a 3 \times 1.5 cm film at the same place till it broke. The number of times, the film could be folded at the same place without breaking gave the value of folding endurance of film. Folding endurance more than 300 was found to be an adequate indication of flexibility of the films. This study was performed in triplicate, and the average of three readings was calculated.^[11]

Tensile strength measurement

This mechanical property was evaluated using Linus bursting/tensile strength apparatus. The pressure gauge was selected depending on the sample to be tested by turning the gauge selector switch. Films of 9 cm diameter, free from air bubbles, or physical imperfections were placed on the

diaphragm plate, and the wheel on top of the diaphragm plate was rotated till it fits securely on the sample and does not rotate any further. The “Push” button was pressed till the sample bursts. The pressure gauge directly gives readings in kg/cm². Measurements were run in triplicate for each film. Tensile strength is the maximum stress applied to a point at which the film specimen bursts.

Measurement of surface area

This measurement was done for Layer A, the gastro-retentive layer which is expected to swell in gastric fluid and produce an increase in surface area. Layer B was expected to disintegrate immediately, and therefore, not subjected to this test. The swelling study was conducted in simulated gastric fluid of pH 1.2. The initial dimensions of the film samples were measured before the test. Then, the films were submerged in 50 ml of simulated gastric medium contained in a porcelain dish of 200 ml capacity. At definite time interval (5 min), the film was removed, excess moisture was blotted out with tissue carefully and increase in the dimensions of the film was determined at each time interval until a constant dimension was observed.

Swelling index

The film samples (4.5 cm²) were weighed and placed in a pre-weighed stainless steel wire sieve of approximately 800 μm mesh. The sieve containing film sample was submerged into 50 ml of simulated gastric fluid in glass mortar. At definite time intervals, the stainless steel mesh was removed, excess moisture blotted out by carefully wiping with absorbent tissue and reweighed. Increase in weight of the film as a result of moisture absorption was determined and this procedure was continued until constant weight was observed. The degree of swelling was calculated using the formula:

$$S.I = (w_t - w_0) / w_0$$

Where, S.I is the Swelling Index, w_t is the weight of film at time t , and w_0 is the weight of the film at time 0.^[12] Figure 1 shows the swelling index of all formulations in simulated gastric fluid.

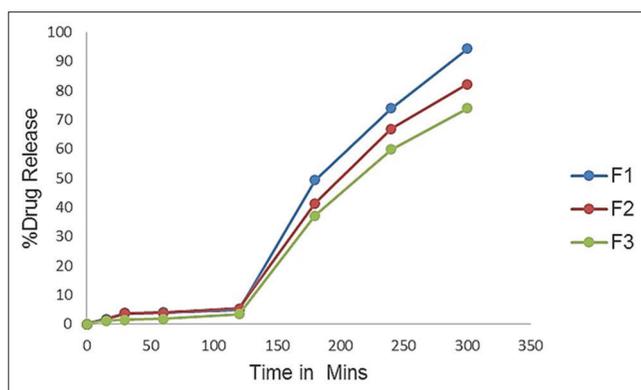


Figure 1: *In vitro* drug release profile of rabeprazole sodium from microsphere formulations

Uniformity of drug content

Drug content was determined for each of the formulations of Layer A containing famotidine by dissolving a film (4.5 cm² area) by homogenization in 100 ml of 10% acetic acid for 30 min with continuous shaking, followed by measurement of absorbance at 263 nm using the UV spectrometer.

In case of film formulations of Layer B containing RS, drug content was determined by extracting each film sample (4.5 cm² area) with 50 ml of ethanol by shaking on a bottle shaker for 2 h. The solution was filtered and diluted with sufficient phosphate buffer of pH 7.4 and its UV absorbance was measured at 284 nm.

Disintegration test

The film under test was placed in 900 ml simulated gastric pH of 1.2 and stirred at 100 rpm. Temperature was maintained at $37 \pm 0.5^\circ\text{C}$. Time taken for the films to start eroding was noted as the disintegration time. The test was carried out in triplicate and average time was determined.

In vitro drug release study

The USP Type II dissolution apparatus was used for this study. For Layer A, the dissolution medium used was 900 ml of simulated gastric fluid of pH 1.2 at 100 rpm. Temperature of dissolution medium was maintained at $37 \pm 0.5^\circ\text{C}$. For the purpose of the study, the film formulation was folded in a zig-zag manner and placed within a hard gelatin capsule (size 1) before positioning in the release medium. Samples of 5 ml were withdrawn at predetermined time intervals and replaced with fresh medium. Samples were filtered and necessary dilutions were made. Absorbance was read at 263 nm using UV spectrophotometer.^[13,14]

A similar dissolution study was conducted for Layer B, with the difference that after 2 h in simulated gastric fluid of pH 1.2, the medium was changed to phosphate buffer, pH 7.2, and the study was continued for a further 3 h. Absorbance was measured at 284 nm using UV spectrophotometer.

Assembling of bilayer unfolding film system

Optimized formulations for Layer A (separately for chitosan and HPMC E15) and Layer B were chosen. Surfaces of the two Layers were moisturized with 10% HPMC solution. Layer A was then placed on Layer B so that the moisturized surfaces were in contact with each other. A weight of 1 Kg was placed over the conjoined film and dried in a hot air oven at 40°C for 10 min. The films were allowed to cool, and the formed bilayer film was then stored in a desiccator until further use. Thus, two formulations of bilayer systems: BL1 and BL2 were identified and subjected to evaluation. Table 3 gives the composition of the bilayer systems.

Evaluation of bilayer unfolding film system

The assembled bilayer system was evaluated for *in vitro* disintegration, dissolution, and *in vivo* X-ray radiography studies.

Disintegration test

The same method described under evaluation of individual layers was used to determine the disintegration behavior of the bilayer system.

In vitro drug release study

The dissolution behavior of the bilayer film was studied at pH 1.2 for 9 h followed by pH 7.2 for 3 h using USP Type II dissolution apparatus. The dissolution medium was kept at 37 ± 0.5°C. Aliquots of the medium were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 263 nm for famotidine for first 9 h and 284 nm for RS for the first 2 h and the final 4 h. The volume withdrawn at each time intervals was replaced with the same amount of fresh dissolution medium. Simultaneous UV estimation by the Vierordt's simultaneous equation method previously standardized was used in the determination of both drugs.

In vivo X-ray radiography studies

To visualize the course of movement and behavior of the bilayer system after *in vivo* administration, an X-ray procedure was conducted using New Zealand White rabbits. For animal testing, approval was obtained from the Institutional Animal Ethics Committee (Ref. KSHEMA/IAEC/10/2014) of K.S. Hegde Medical Academy, Mangalore, Karnataka. The bilayer film was modified by replacing the drug with 80 mg of a radioopaque agent, i.e., barium sulfate in each layer, and remaining ingredients were used in the same quantities

as mentioned previously. Microspheres of barium sulfate were prepared and incorporated in Layer B. The films were folded in zig-zag fashion and placed in size 1 hard gelatin capsules. Three rabbits of either sex or body weight of 2.5-3.5 kg were fasted overnight. One rabbit served as the control and was administered a capsule containing 80 mg of barium sulfate only. The second and third rabbits were administered formulations BL1 and BL2, respectively. X-ray photographs were taken for the duration of 8 h at 0, 0.5, 2, 4, and 8 h periods.^[15] Light food was given to the rabbits 2 h after administration of the capsules.

RESULTS AND DISCUSSION

Evaluation of enteric microspheres of RS

The particle size analysis done by optical microscopy gave reproducible results. The particle size of the microspheres was found to be more for F3. It is observed that the particle size for all formulations increased with increased polymer concentration and found to be within the range of 25-70 µm. Production yield was found to be less for F3, which contained 3 parts of the polymer. This may be due to increasing in free polymer concentration. Digital microscopic images of the microspheres showed spherical shape of the particles. Drug entrapment efficiency for all the ratios of microspheres was found to be in the range of 95-100%.

The *in vitro* release profiles of microspheres from different formulations are represented in Figure 1. For the first 2 h in simulated gastric fluid of pH 1.2, drug release was considerably less, indicating good resistance to the stomach pH. There was a sharp increase in the release as soon as the pH of the dissolution media was changed to 7.2. This is due to the dissolution of enteric polymer used in the intestinal pH of 7.2. Drug release was found to be the greatest for F1 and was found to decrease in the formulations with increasing concentration of EL polymer.

The best formulation was found to be F1, which was selected based on the parameters such as particle size (smallest), drug content (maximum), and *in vitro* drug release (maximum at the end of 5 h) as shown in Table 4. This formulation was, therefore, selected for incorporation into the immediate release layer of bilayer film.

Table 2: Composition of Layer B

Formulation code	Microsphere (mg)	HPMC E15 LV (mg)	Glycerol (ml)
FX	40	0.15	0.2
FY	40	0.20	0.2
FZ	40	0.25	0.2

HPMC: Hydroxypropylmethyl cellulose

Table 3: Composition of Bilayer unfolding film formulations

Formulation	Layer A		Layer B	
	Drug	Polymer	Drug	Polymer
BL1	Famotidine	PVA Chitosan	Enteric microspheres of rabeprazole	HPMC E15 LV
BL2	Famotidine	PVA HPMC E15	Enteric microspheres of rabeprazole	HPMC E15 LV

HPMC: Hydroxypropylmethyl cellulose, PVA: Polyvinyl alcohol

Evaluation of “Layer A” and “Layer B”

All film formulations were smooth, non-tacky, homogeneous, and translucent in nature except for Layer B which was slightly opaque since it contained uniformly distributed enteric microspheres of RS.

Thickness and uniformity of weight

It was observed that weight and thickness of the entire film in each of formulations of Layer A was uniform. In case of formulations of Layer B, increase in the weight and thickness was due to increase in the concentration of HPMC E15 polymer.

Folding endurance

Folding endurance testing was done to check the ability of the films to withstand mechanical handling and folding while placing them inside the capsule. Folding endurance of all the formulations of both Layer A and Layer B was found to be more than 300 which was considered to be a satisfactory indication of sufficient mechanical strength and flexibility to resist breaking as a result of folding.

Results for uniformity of weight, thickness uniformity, and folding endurance of both the layers are given in Table 5.

Tensile strength

Tensile strength gives an indication of strength and elasticity of the films. For Layer A formulations, tensile strength increased as the chitosan content increased. In fact, PVA itself is a polymer with superior tensile strength and the combination with chitosan produced good films with sufficient flexibility to enable folding and at the same time resisted breaking or disintegration for a substantial period during the drug release studies. On the other hand, the use of HPMC in the films had the opposite effect and appeared to decrease the tensile strength.

Surface area

Increase in the surface area for Layer A is an important criterion for gastro retention. The surface area of all the formulations was found to double within 30 min. Chitosan and HPMC are polymers which take up water rapidly and swell, contributing to the expansion of the films. Thus, the surface area measured was the greatest for the formulations FD and FH, which had larger amounts of these water-swallowable polymers. Hence, it is clear that the surface area increased with increasing concentration of chitosan and HPMC.

Swelling index

As it was earlier explained, swelling is a contributing factor in the expansion of the film and hence influences gastro

Table 4: Characterization of microsphere formulations

Formulation	Average particle size (µm)*	Percentage yield (%)	Drug entrapment efficiency*	Maximum percentage drug released at 5 h*
F1	25.16±2.54	89.28	98.47±0.65	94.35±0.39
F2	43.35±0.22	90.85	97.30±0.21	82.2±0.28
F3	68.80±1.46	66.25	96.35±0.42	73.95±0.52

*Mean of 3 replications±SD, SD: Standard deviation

Table 5: Results for uniformity of weight, thickness, folding endurance, and tensile strength

Formulation	Weight (mg)*	Thickness (mm)*	Folding endurance	Tensile strength (kg/cm ²)*
Layer A				
FA	0.150±0.004	0.303±0.028	>300	1.9±0.05
FB	0.149±0.012	0.262±0.057	>300	2.5±0.24
FC	0.151±0.004	0.273±0.023	>300	3.2±0.19
FD	0.156±0.002	0.263±0.024	>300	6.0±0.32
FE	0.148±0.006	0.253±0.030	>300	0.8±0.16
FF	0.143±0.004	0.236±0.012	>300	1.2±0.21
FG	0.145±0.012	0.24±0.017	>300	1.6±0.095
FH	0.149±0.013	0.256±0.009	>300	2.0±0.08
Layer B				
FX	0.175±0.010	0.216±0.020	>300	1.0±0.11
FY	0.221±0.010	0.26±0.008	>300	1.85±0.28
FZ	0.265±0.010	0.28±0.014	>300	2.0±0.31

*Mean of 3 replications±SD, SD: Standard deviation

retention.^[16] Polymer swelling depends on the hydrophilic properties of the polymers which are used in the formulation. For Layer A, swelling index was found to increase with increasing amounts of chitosan and HPMC, and therefore, greatest for FD and FH, which had the largest amount of these polymers. These formulations also showed rapid rate of swelling since they achieved maximum swelling in 1 h and started disintegrating soon after while the other formulations took double the time. Thus, swelling index and surface area are directly proportional to each other. Similar was the case for Layer B, the greatest swelling was observed for FZ, however, extent of swelling for these formulations was comparatively less since they eroded simultaneously.

Drug content

Drug content for all the formulations of both the layers was found to be in the range of 90-100%.

Disintegration test

Disintegration test was carried out for both the layers. When designing the formulations, the objective was to make Layer A last longer in the stomach for the sustained release of famotidine and Layer B to disintegrate quickly for the immediate release of enteric microspheres of RS. For Layer A, the formulations FA, FB, FE, and FF showed gastro retention for more than 9 h without any sign of disintegration. The formulations FC, FD, FG, and FH disintegrated before 8 h, so they were not considered for the optimization. In FA and FB, gastro retention was found to increase with increasing concentration of PVA due to its tough and slow swelling properties; moreover, chitosan presents in the same layer contributes for faster swelling and slow erosion properties. In FE and FF, gastro retention was achieved due to the slow swelling property of PVA hot. Layer B being immediate release layer, all the formulations showed disintegration

within 15 min. This may be due to the rapid swelling and faster erosion properties of HPMC E15 polymer. Formulation FX disintegrated the most rapidly, the film being thinnest due to its lower content of this polymer.

Results for tensile strength, percentage drug content, disintegration time test, surface area, and swelling index are given in Table 6.

In vitro drug release studies

The *in vitro* release profiles of different formulations are shown in Figures 2-4.

Release of famotidine was observed to be sustained from all formulations of Layer A and was found maximum for formulations FD and FH, which had larger amounts of readily erodible polymers, chitosan, and HPMC. Release of drug was found to increase with decrease in the concentration of PVA Hot and increase in the concentration of chitosan and HPMC.

After the disintegration of Layer B, the release of RS from the microspheres was almost negligible for all 3 formulations in the first 2 h of the study in simulated gastric fluid. This is in conformance to the protection offered by enteric polymer EL to the gastric labile drug, RS as a result of the former's insolubility in this medium. However, there was a sharp increase in drug release when the medium was changed to phosphate buffer in the next 3 h, and this release was the greatest for formulation FX. This observation could be attributed to the low concentration of swellable polymer, HPMC in that formulation resulting in a much thinner film that disintegrated faster than the others.

The best formulation for Layer A and Layer B was selected based on the parameters, disintegration time, and *in vitro* cumulative percentage drug release. FB and FF formulations

Table 6: Results for percentage drug content, disintegration time, surface area, and swelling index

Formulation	% Drug content	Disintegration time (min)	Surface area*	Swelling index*
Layer A				
FA	98.90±0.70	644.6±0.56	10.58±0.18	2.5739±0.15
FB	96.64±0.10	579.0±0.68	16.52±0.125	3.862±0.26
FC	97.89±0.29	465.0±0.80	20.46±0.48	4.062±0.05
FD	99.94±0.45	383.3±0.45	25.92±0.25	5.042±0.12
FE	94.60±0.36	606.6±0.85	10.35±0.252	3.818±0.5
FF	92.32±0.48	550.0±0.49	10.80±0.21	3.85±0.25
FG	98.16±0.58	403.3±0.55	11.50±0.42	4.10±0.42
FH	97.73±0.73	251.6±0.75	13.00±0.20	4.25±0.42
Layer B				
FX	99.38±0.23	4.90±0.311	-	1.10±0.14
FY	96.80±0.45	7.90±0.117	-	1.25±0.15
FZ	94.88±0.59	12.50±0.48	-	1.38±0.131

*Mean of 3 replications±SD, SD: Standard deviation

were considered optimal for Layer A since they showed greatest drug release during the period they remained intact without disintegration. FX was considered the best for Layer B since it took the least time to disintegrate during which maximum drug was released based on the disintegration time and *in vitro* drug release studies as shown in Table 6 and Figures 2-4, respectively, the optimized formulation was found to be FB and FF for Layer A which, i.e., the gastro-retentive layer and FX for Layer B or immediate release layer of the bilayer system.

Kinetic analysis of *in vitro* drug release data

The *in vitro* release data of optimized formulations were fitted to various models such as zero order, first order, Higuchi matrix, and Korsmeyer-Peppas model. Based on the regression values, drug release kinetics is best described by the first order model for FB and FF and by the zero order model in case of FX. The release exponent (*n*) of the Peppas model described the mechanism of drug release from the matrices and was calculated by regression analysis using the following equation.^[17]

$$M_t/M_\infty = Kt^n$$

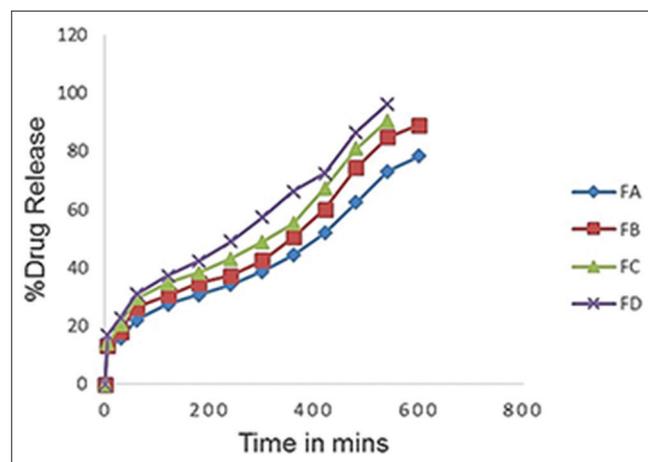


Figure 2: *In vitro* release profile of famotidine from film formulations, FA-FD

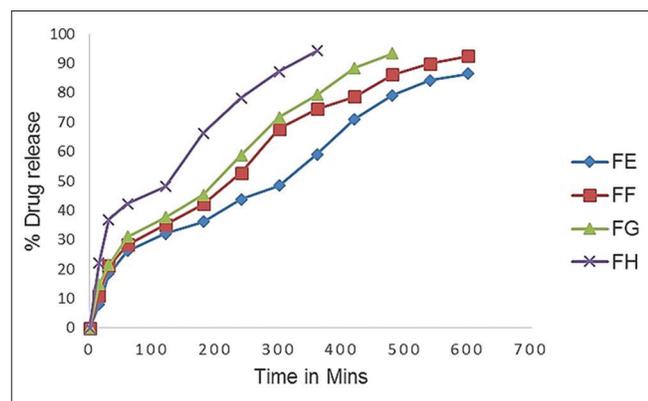


Figure 3: *In vitro* release profile of famotidine from film formulations, FE-FH

Where, M_t/M_∞ is the fraction of drug released (using values of M_t/M_∞ within the range 0.10-0.60) attimet and Kisa constant incorporating the structural and geometric characteristics of the release device. A value of $n = 0.5$ indicates case I (Fickian) diffusion, $0.45 < n < 0.89$ indicates anomalous (non-Fickian) diffusion, $n = 1$ indicates case II transport (Zero order release), and $n > 1$ indicates Super case II transport. The results of the kinetic analysis of *in vitro* drug release data from the formulations are given in Table 7. Accordingly, drug release follows non-Fickian diffusion in case of FA and FF and case II transport or zero order release in case of FX.

The values of the regression coefficient, R for the Higuchi Matrix equation, indicate that the drug release from the films could also be described by the matrix diffusion process since these films are basically hydrophilic polymer matrices.

Evaluation of bilayer unfolding film systems

Two bilayer systems: BL1, and BL2 were formulated after optimization of the two layers. In BL1, Layer A was FB and for BL2, FF was used. Layer B was FX for both systems. Both systems were found to be smooth and the layers intact with no signs of separation during handling. Figure 5 represents an image of the bilayer film folded in the zig-zag manner and placed in the gelatin capsule.

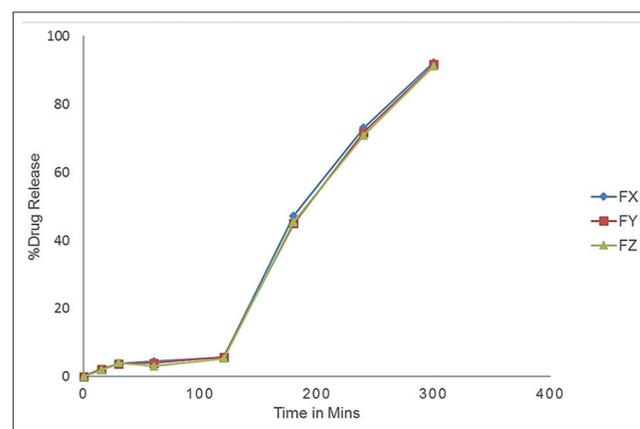


Figure 4: *In vitro* release profile of rabeprazole sodium from film formulations, FX-FZ

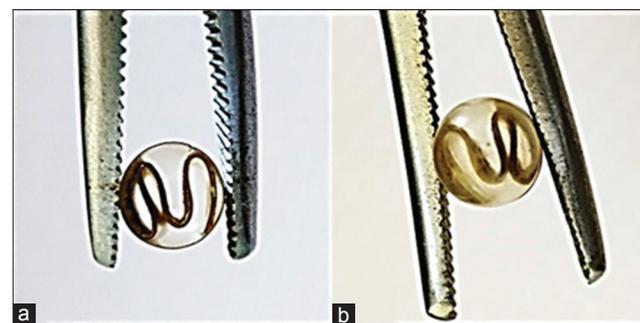


Figure 5: Images of bilayer film system folded and placed into body of transparent hard gelatin capsule: (a) Without the cap (b) After replacing the cap

Disintegration time

After the dissolution of the capsule, Layer B disintegrated to release the microspheres within 15 min for formulations BL1 and BL2 while the film unfolded. Layer A remained intact for more than 8 h with no sign of disintegration.

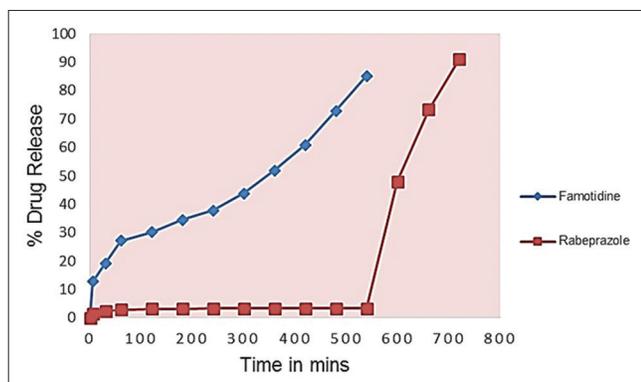


Figure 6: *In vitro* drug release profile of famotidine and rabeprazole sodium from bilayer film system, BL1

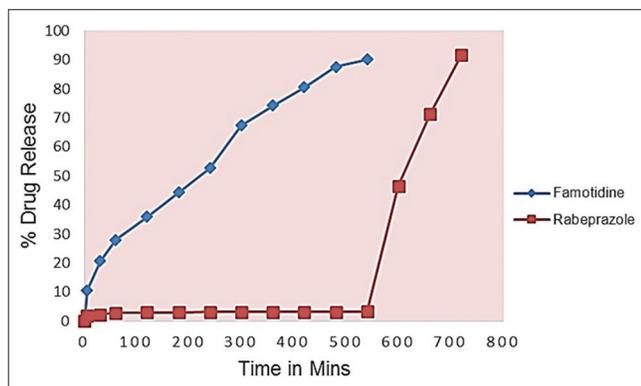


Figure 7: *In vitro* drug release profile of famotidine and rabeprazole sodium from bilayer film system, BL2

In vitro dissolution studies

Drug release from the bilayer film was no different from the individual layers already discussed. Sustained release was observed for famotidine and immediate release of RS from the microspheres. Adhesion of the two layers in no way affected the drug release behavior of the bilayer systems for both formulations as can be seen in the drug release profiles shown in Figures 6 and 7. Drug release was more than 90% for both formulations.

In vivo X-ray radiography studies

X-ray radiographic studies in rabbits clearly indicate the gastro-retentive behavior of Layer A in BL1 and BL2. Figure 8a-d represent X-ray images of the control and test groups taken at 0, 0.5, 2, 4, and 8 h. In the control group who were given capsules of barium sulfate alone, evacuation of the radioopaque agent could be observed from the stomach within 30 min [Figure 8b]. In Test 1 and Test 2 groups, Layer A of the formulation was retained in the stomach for more than 8 h as seen in Figure 8c and 8d. There was a clear indication of the increase in the size of this layer although it retained its structural integrity and shape in the stomach. This confirms the ability for gastro retention of the gastro-retentive layer when exposed to stomach conditions. The images taken at 2 h for the two test groups show a slightly opaque, diffusive appearance (encircled areas) moving toward the region of the small intestine, probably produced by the dispersion of the microspheres after release from Layer B in the gastric fluid. Thus, this study proves the gastro-retentive ability of the two formulations of bilayered unfolding film system.

CONCLUSION

This investigation proves that it is possible to formulate a bilayer unfolding film type of drug delivery system for the

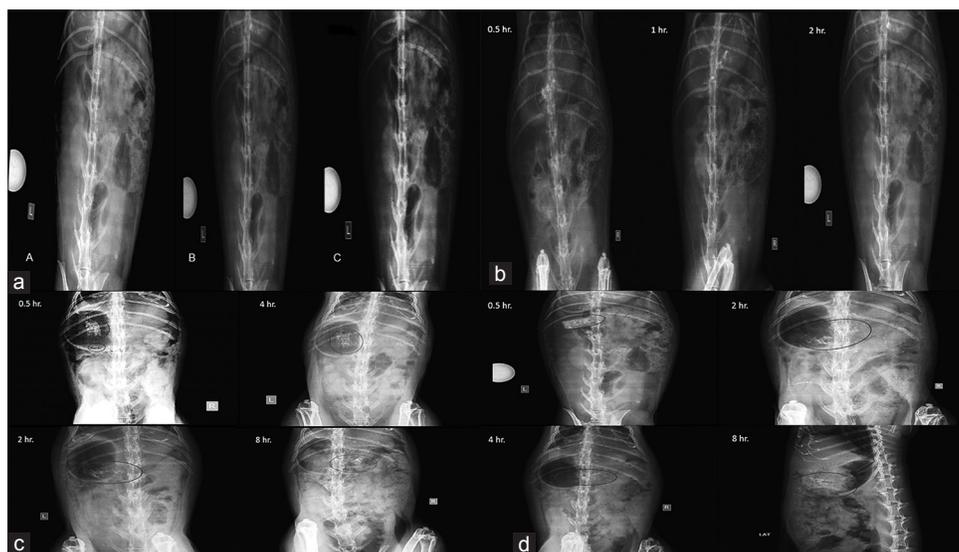


Figure 8: (a) X-ray images of (1) control, (2) test 1, and (3) test 2 at 0 h, (b) X-ray images of control administered with BaSO₄ capsule, (c) X-ray images of test 1 after administration of BL 1 at various time intervals, (d) X-ray images of test 2 after administration of BL 2 at various time intervals

Table 7: Results for *in-vitro* drug release kinetics of formulations

Model	FB	FF	FX
Zero order	$R=0.9517$ $k=0.0001$	$R=0.9030$ $k=0.1714$	$R=0.9428$ $k=0.2752$
First order	$R=0.9713$ $k=0.0000$	$R=0.9470$ $k=0.0037$	$R=0.8797$ $k=-0.0014$
Higuchi matrix	$R=0.9633$ $k=3.1575$	$R=0.9320$ $k=3.5186$	$R=0.8124$ $k=3.6916$
Korsmeyer-Peppas	$R=0.9378$ $k=3.3388$ $n=0.4833$	$R=0.9144$ $k=2.9811$ $n=0.5196$	$R=0.9121$ $k=0.0396$ $n=1.0096$
Best fit model	First order	First order	Zero order

dual release of rabeprazole and famotidine. The evaluation of this dosage form for gastro retention of famotidine and the immediate availability of rabeprazole has shown promising results indicating its potential in the effective management of GERD and similar disorders. To obtain the best possible benefits of this dosage form, it would be required to be administered at bedtime, preferably before the evening meal. Although the practice of prescribing two drugs in the treatment of GERD is not very common, this formulation could prove useful for the delivery of two drugs of which one would require gastro retention and the another would require rapid absorption. Simple, reproducible methods with inexpensive materials have been used in the fabrication of this system, and therefore, could be commercially viable.

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