Research Article

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Formulation and optimization of gastroretentive bilayer tablets of calcium carbonate using D-optimal mixture design

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Abstract: Gastroretentive bilayer tablets of calcium carbonate (CC) were developed using D-optimal mixture design. The effect of formulation factors such as levels of HPMC K100 M (X1), sodium bicarbonate (X2), and HPMC E15 LV (X3) on responses like floating lag time (R1) and release of CC at 1 h (R2) and 6 h (R3) was elucidated. The optimized formulations developed by numerical optimization technique were found to have short floating lag time (2.85 ± 0.98 min), minimum burst release ($27.02 \pm 1.18\%$), and controlled yet near complete release ($88.98 \pm 2.75\%$) at 6 h. *In vivo* radiographic studies in rabbits indicated that optimized batch displayed a mean gastric retention time (GRT) of 5.5 ± 1 h which was significantly prolonged ($P < 0.05$) compared to the conventional tablets that displayed a GRT of less than 1 h. The studies proved that the gastroretentive tablets can be a promising platform to improve bioavailability of nutrients having absorption window in upper gastrointestinal tract.

Keywords: calcium carbonate, floating tablets, gastroretentive tablets, mixture design, optimization

1 Introduction

Calcium is the major component of the skeletal system which accounts for about 1–2% of the adult body weight (1). The recommended dietary allowance (RDA) of calcium varies from 800 to 1,300 mg/day for adolescents, 1,000 mg/day for adults, and 1,200 mg/day for elderly. Globally, more than 800 million people are undernourished and about 3.5 billion people are at risk of calcium deficiency due to inadequate dietary supply (1). It has been estimated that more than 6% of global mortality and morbidity burdens are associated with undernourishment and micronutrient deficiencies. Approximately, 90% of those at risk of calcium deficiency were found to reside in Africa and Asia and nearly 75–100% of Indians are found to be calcium-deficient. Sensitive population include children, elderly pregnant patients, and postmenopausal syndrome (PMS) women. Calcium deficiency can retard growth and cognitive development, impair immunological functioning, and increase the risks of noncommunicable diseases including skeletal, cardiovascular, and metabolic disorders (2). Calcium deficiency may lead to brittle or weak bones, bone fractures, delays in children's growth and development, problems with proper blood clotting, weakness and fatigue, heart problems involving blood pressure and heart rhythms, osteoporosis (3), etc.

Oral calcium is considered to be the first line therapy for calcium deficiency (4,5). Calcium supplementation (6) is currently done by conventional tablets containing calcium salts such as CC or calcium citrate (7). Calcium carbonate is the least expensive and most widely used salt of calcium. Nearly 85% of all calcium supplements sold in the US contain CC. However, only about 30% of the available elemental calcium is actually absorbed and bioavailable following oral administration (8). The likely reason for the poor bioavailability is that calcium absorption is pH-dependent, site-specific, and limited by the carrier-mediated transport (7). The soluble calcium normally is

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well-absorbed from the duodenum due to the presence of carrier protein ‘calbindin’ at active absorption sites (9). However, conventional calcium tablets exhibit poor bioavailability as they may quickly cross the absorption sites allowing a fraction of the dose to be absorbed. Moreover, the conventional tablets are likely to saturate the carrier proteins located in the duodenum and therefore hamper the complete absorption of the whole dose of calcium resulting in poor oral bioavailability. In this context, there is a need to develop a gastroretentive drug delivery system (GRDDS) for CC that has the potential to overcome the above-mentioned limitation of the conventional tablets as no such product is available in the Indian as well as the global market. The GRDDS, by virtue of its buoyancy, is likely to be retained proximal to the absorption site and stays afloat in the gastric fluid in which CC is known to possess a good solubility. Various technologies have been developed for gastroretention of the drug delivery systems which include low density or floating systems ($<1\text{ g/cm}^3$) that remain buoyant above the gastric contents, high density systems ($>1\text{ g/cm}^3$) that are retained at the antrum of the stomach, bioadhesive systems that adhere to the gastric mucosa, and expandable systems that swell or unfold to a large size to prevent the passage of the dosage form through the pyloric sphincter, magnetic, and superporous systems (10,11).

The development of GRDDS tablet for CC seemed quite challenging considering its high dose (200 mg) and high density ($\sim 2.71\text{ g/cm}^3$). To meet the challenge, we aim to develop a gastroretentive system for CC using a D-optimal design. The GRDDS tablets are first of its kind that have both floating and bioadhesive properties for site-specific delivery of calcium in the upper part of the gastrointestinal tract. In this context, the objective of the work was to model the effect of the composition of the bilayer tablets, namely, the proportion of binder (Hydroxypropyl methyl cellulose E15 LV), matrix material (Hydroxypropyl methylcellulose K100 M), and effervescent agent (sodium bicarbonate) on dissolution and floating lag time. In addition, we plan to validate the polynomial models by preparing the optimized formulation with the most desirable attributes using regression analysis and analysis of variance (ANOVA). Finally, image analysis of the optimized bilayer tablet formulation in rabbits to assess the *in vivo* gastroretention would be the integral part of the investigation. The present work would be the ‘first of its kind’ as, to the best of our knowledge, no extensive work has been undertaken to develop a bilayer GRDDS for calcium.

2 Materials and methods

2.1 Materials

Calcium carbonate (confirming to IP) and barium sulfate (X-ray grade) were purchased from Loba Chemie Pvt. Ltd, Mumbai. Sodium bicarbonate, potassium dihydrogen orthophosphate, sodium hydroxide pellets, hydrochloric acid, and talc were supplied from S.D. Fine Chemicals, Mumbai. Magnesium stearate was supplied by Central Drug House Pvt. Ltd, New Delhi. Hydroxypropyl methyl cellulose K100 M and hydroxypropyl methyl cellulose E15 LV were supplied by Colorcon Asia Pvt. Ltd, Goa.

2.2 Methodology

2.2.1 Fourier transform infrared spectrometry

Infrared spectrophotometry is a useful analytical technique utilized to check the chemical interaction between the formulations. The sample was powdered and intimately mixed with 10 mg of powdered potassium bromide (KBr). The powdered mixture was taken in a diffuse reflectance sampler and the spectrum was recorded by scanning in the wavelength region of $4,000\text{--}400\text{ cm}^{-1}$ in an FTIR spectrophotometer (Jasco 460 plus, Japan). The IR spectrum of the CC was compared with that of the physical mixture of check for any interaction of CC with any of the excipients used.

2.2.2 Preparation of gastroretentive floating bilayer tablets of CC: design of experiment (DoE)

A 3-factor 3-level D-optimal mixture design generated in Design Expert Software (version 10.0.6.0) was employed to study the effect of critical formulation on the product attributes of the floating bilayer tablets. The experimental design contained three factors or components, namely, the amounts of HPMC K100 M (X1), sodium bicarbonate (X2), and HPMC E 15 LV (X3). The sum of three components would be 100 where the proportions of X1, X2, and X3 were found to range from 50.00% to 79.00%, 20.00% to 49.00%, and 1.00% to 3.00%, respectively. The effect of formulation variables on responses like friability (R1), floatation time (R2), percent release at the end of 1 h (R3), and at the end of 6 h (R4) was systematically investigated. The compositions of formulations as per D-optimal

mixture and the constraints set on each component are shown in Table 1.

The bilayer tablets contained two layers, i.e., an effervescent floating layer and CC layer. All the ingredients were passed through a 250 μm sieve. The floating layer was prepared by direct compression of the blend of HPMC K100 M and sodium bicarbonate. Calcium carbonate layer was produced by wet granulation method. In brief, CC was blended with a solution of HPMC E15 LV in water. The quantity of HPMC E15 LV to be incorporated was predetermined by the experimental design. The wet mass was passed through a 12 mesh sieve of aperture size 1.67 mm and the wet granules produced were dried at 60°C for 30 min in a hot air oven. The dried granules were passed through the same sieve to break the lumps. The blend of the floating layer and dried granules of CC were separately lubricated with magnesium stearate (1.5% w/w) and talc (2.5% w/w) for 2–3 min. The lubricated blends were finally compressed to bilayer tablets weighing 420 mg on a bilayer tablet rotary press (Cronimach, Ahmedabad, Gujarat) using a 9 mm diameter die to a hardness of 5–7 kg/cm². The formulation variables employed to produce 16 batches of bilayer tablets as per the experimental design are portrayed in Table 2.

2.3 Evaluation of floating bilayer tablets:

2.3.1 Weight variation

Weight variation of the bilayer tablets from each batch was determined as per official method (12). Twenty tablets were selected at random and individual weight of the bilayer tablets was determined in an analytical balance (Model

220A XB, Precisa, Switzerland). The weights were recorded in mg; the mean and standard deviation values were computed. The average weight of the bilayer tablets and the acceptable limit were deduced.

2.3.2 Thickness and diameter

Tablet thickness and diameter of ten randomly selected bilayer from each batch were determined (13). The values were recorded in mm using a digital caliper (Mitutoyo digimatic caliper, Mitutoyo Corporation, Kawasaki, Japan). The mean and standard deviation of the thickness and diameter were calculated.

2.3.3 Hardness

The resistance of tablets to shipping or breakage under conditions of storage, transportation, and handling before usage depends on their hardness. Hardness of ten randomly selected bilayer tablets from each batch was measured using a Stokes Monsanto hardness tester (M/s Cambell Electronics, India) (14). The hardness was measured in terms of kg/cm². The mean and standard deviation values were computed.

2.3.4 Friability

The friability of bilayer tablets was determined by following the official procedure (15). Friability was determined by subjecting twenty randomly selected tablets of each batch to abrasion in automated USP friabilator (Electrolab, Mumbai, India) for 100 rotations. The de-dusted tablets were weighed and % friability was calculated using

Table 1: Independent variables showing experimental ranges of the D-optimal mixture design

Independent variables	Low value (%)	High value (%)
A: Fraction of HPMC K100 M (% w/w)	50	79
B: Fraction of sodium bicarbonate (% w/w)	20	49
C: Fraction of HPMC E15 LV (% w/v)	1	3
Dependent variables	Constraints	
Y1: Friability (%)	Minimize	
Y2: Floating lag time (min)	Minimize	
Y3: Drug release at 1 h (%)	Minimize	
Y4: Drug release at the end of 6 h (%)	Maximize	

Table 2: Composition of the model formulations (in mg) as per D-optimal mixture design

UN	Actual wt. of X1 ^a	Actual wt. of X2 ^a	Actual wt. of X3 ^a	CaCO ₃	Mg stearate	Talc	Total wt.
F1	77	73.41	3.59	250	6	10	420
F2	98.95	52.75	2.33	250	6	10	420
F3	119.61	30.8	3.61	250	6	10	420
F4	121.66	30.8	1.55	250	6	10	420
F5	97.79	51.59	4.65	250	6	10	420
F6	77	75.46	1.55	250	6	10	420
F7	97.79	51.59	4.65	250	6	10	420
F8	98.95	52.75	2.33	250	6	10	420
F9	91.89	60.57	1.55	250	6	10	420
F10	90.86	58.52	4.65	250	6	10	420
F11	109.34	41.58	3.1	250	6	10	420
F12	119.61	30.8	3.61	250	6	10	420
F13	98.95	52.75	2.33	250	6	10	420
F14	106.77	45.69	1.55	250	6	10	420
F15	87.78	63.14	3.1	250	6	10	420
F16	77	73.41	3.61	250	6	10	420

^a X₁, X₂, and X₃ represent the amounts of HPMC K100 M, sodium bicarbonate, and HPMC E15 LV, respectively. X₃ was used as 8% w/v and as binding solution in the bilayer tablets.

equation (1) for each batch of bilayer tablets and expressed as mean of 3 determinations. The tablets which tend to lose less than 1% of their weight are generally considered acceptable.

2.3.5 Content uniformity

Content uniformity test was performed as per USP procedure by random sampling ten tablets from each batch (16). The tablets were crushed and allowed to equilibrate with pH 1.2 buffer for 24 h. Subsequently, the solutions were filtered through (0.45 µm, Millipore) and suitably diluted to determine the content of CC using a flame photometer (Systronics, Flame photometer 128, Ahmedabad, Gujarat).

2.3.6 Floating lag time

The time required for the tablet to rise to the surface and remain afloat was considered as floating lag time (17). To record the floating lag time, the bilayer tablets were transferred to the dissolution medium taken in USP Type II dissolution apparatus in 900 mL of pH 1.2 buffer kept at 50 rpm and 37 ± 0.5°C. The floating lag time and floating lag time of bilayer tablets were recorded in triplicate for each batch of bilayer tablets produced.

2.3.7 In vitro release studies

The dissolution studies of the bilayer floating tablets were performed for a period of 6 h in USP dissolution apparatus-2 (Electrolab, Mumbai, India) at a paddle speed of 50 rpm in 900 mL of pH 1.2 buffer maintained at 37 ± 0.5°C (18). About 5 mL of samples were withdrawn at 1, 2, 3, 4, 5, and 6 h and immediately replaced with same amount of fresh dissolution medium maintained at the same temperature in order to maintain the sink condition. The aliquots sampled were filtered through 0.45 µ filters and analyzed using a flame photometer to determine the amount of CC released at different time points. The dissolution data recorded in triplicate was analyzed to calculate percentage cumulative calcium released at different time intervals.

2.3.8 In vitro release kinetics

In order to investigate the kinetics and mechanism of release of calcium from prepared tablets, the release data were examined using zero-order kinetic (19), first-order kinetic (20), and Higuchi kinetic (21).

For the zero-order kinetic, data obtained were plotted as cumulative amount of calcium released versus time whereas for the first-order kinetic, the obtained data were plotted as log cumulative calcium remaining versus time. For Higuchi kinetic, the obtained data were plotted as cumulative percentage calcium release versus square root of time.

2.3.9 Stability study

The optimized formulation was covered in aluminium foil and subjected to Real time stability condition for 6 months at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$. The samples were analyzed at 1, 3, and 6 months against the tablets on day “0” for physical characteristics, floating lag time, and Dissolution till 6 months.

2.3.10 *In vivo* X-ray imaging studies

In vivo animal studies were performed in normal rabbits using X-ray imaging technique for evaluating the gastro-retentive potential of the optimized tablet formulation as per the protocol approved by the Institutional Ethical Committee (IE-52, dated 12/10/2019) at *in vivo* Biosciences, Magadi Road, Bengaluru, India. Unisex rabbits of New Zealand white strain weighing 2–2.5 kg were housed under standard laboratory conditions at $25 \pm 2^\circ\text{C}$ and $55 \pm 5\% \text{ RH}$ with standard diet and tap water ad libitum (two groups of animals with four animals in each group were used for the studies). Prior to initiation of the studies, the animals were kept overnight under fasting condition in order to avoid difficulties during imaging. The first group of animals were orally administered with optimized batch of bilayer tablet formulation containing barium sulfate as a marker, while the control group of animals were orally treated with conventional tablets containing the same marker. The animals were held in the upright position for imaging to locate the position of both control and floating tablets in the GI tract under X-ray machine (Skanray Model: Microscan DR) at predetermined time intervals like 0, 2, 4, and 6 h, respectively.

2.3.11 Statistical analysis

The data generated during the *in vitro* and *in vivo* studies were statistically analyzed by ANOVA in GraphPad 5.0 Instat demo version software (GraphPad Inc. CA, USA). The probability value (*P*) of less than 0.05 was considered to be significant.

3 Results and discussion

The aim of the investigation was to produce bilayer tablets that were able to float at least for a period of 6 h

and at the same time ensure complete dose of the CC in the stipulated floating time of 6 h. Considering this, initially, we developed an effervescent floating matrix tablet of CC using HPMC K4M as a matrix material, sodium bicarbonate as effervescent agent, and HPMC E15LV as a binder. Even though the effervescent matrix tablets of CC were found to float for the period of 6 h, the release of calcium was substantially hampered even in sink conditions at pH 1.2, allowing a fraction of the calcium dose to be released in the stipulated time span of 6 h. Considering this, we planned to separate the floating layer from the CC layer in order to ensure a floating time of 6 h and at the same time ascertain a near complete release of calcium in a controlled fashion in pH 1.2 in the stipulated floating time. In order to systematically accomplish our goals, we planned to develop and optimize the formula or the composition of the bilayer floating effervescent floating tablet of CC using a D-optimal design. Mixture experimental designs are generally used to analyze the impact of formulation variables on the responses. D-optimal design is a mixture design that is used to evaluate the effect of changes in the composition on the responses and allows statistical optimization of the formulation with least number of experiments. The design comprises of a total of 16 points including 6 points for the modelling, 5 points to estimate lack of fit, and 5 points to estimate the pure experimental error (22). The buoyant layer was produced by direct compression of the blend of HPMC K100 M and sodium bicarbonate. On the contrary, CC layer was produced by wet granulation method using an aqueous solution of HPMC E15 LV as binder.

3.1 Drug-excipient compatibility studies

3.1.1 Fourier transform infrared spectroscopy

The FTIR spectrum of CC, the physical mixture of the CC with the other excipients used, and the bilayer tablet are portrayed in Figure 4a–c, respectively. The IR spectrum of CC displayed the characteristic absorption peak at $1,796 \text{ cm}^{-1}$ that can be assigned to $-\text{C}=\text{O}$ stretching. In addition, an intense band owing to the OH stretching was probably due to the moisture content in the compound. Similarly, the IR spectrum of the physical mixture depicted the broad band that can be assigned to OH stretching along with the characteristic absorption peak at the same position, though the peak intensity differed indicating the absence of any interaction between CC and

other excipients in the physical admixture. Likewise, the IR spectra of CC bilayer tablet did not reveal any significant shift in the peaks, though the intensity of the peak decreased indicating absence of any interaction between CC and other excipients during the tablet processing, thereby proving the integrity of CC in the bilayer tablet.

3.2 Characterization of tablets

All the batches of the bilayer tablets were found to comply with official tests for content uniformity and weight variation. The average diameter of different batches of tablets was found to range from 8.82 ± 0.09 mm to 8.89 ± 0.06 mm. The average thickness of different batches of tablets was found to range from 4.31 ± 0.12 mm to 4.42 ± 0.07 mm. The variation of the hardness and friability ranged from 4.27 ± 0.38 kg/cm² to 7.53 ± 0.19 kg/cm² and 0.02 ± 0.01 kg/cm² to 2.77 ± 0.64 kg/cm² for different batches of bilayer floating tablets. The hardness of the tablets was just sufficient to not hamper the complete release of CC as observed with some batches. The release of CC from most formulations was found to follow first-order kinetics. The mechanism of the release could be characterized to follow Higuchi diffusion model.

3.3 Data analysis of the D-optimal mixture design

The design expert[®] v-10 software was used to systematically analyze the experimental data obtained and generate mathematical models that define the relationship between the proportions of the three components (X1, X2, and X3) and the four responses, namely, friability, floating lag time (FLT), Rel_{1h}, and Rel_{6h}.

3.3.1 Model fitting and evaluation of the responses: Y1, Y2, Y3, and Y4

The experimental data were analyzed by fitting the data to the Scheffe polynomial equations (23). These equations are modified from the general polynomial equations to lack intercept and squared terms in order to fit the mixture designs. An attempt was made to fit the four responses, namely, Friability, FLT, Rel_{1h}, and Rel_{6h}, simultaneously quadratic, special cubic and cubic models, and statistically analyze the data by performing ANOVA. The statistical parameters used to analyze and select the best fit model

included *p* value of the model (must be <0.05), lack of fit (needs to be insignificant), coefficient of determination (*R*²), adjusted *R*², and predicted *R*² adequate precision and residual sum of squares (PRESS). The backward elimination procedure was employed to eliminate the insignificant terms from the models and include only the significant ones.

On eliminating the insignificant terms, the sequential *p* values for the four responses were found to be <0.0001, indicating the models generated were significant. Likewise, the lack-of-fit was insignificant (*p* > 0.05) for the three models analyzed as the *p* values for Y1, Y2, Y3, and Y4 were found to be <0.0001. Moreover, the selected models showed high *R*² values displaying a strong correlation between the adjusted *R*² and the predicted *R*². A high signal to noise ratio that exceeded 4 suggested an adequate signal.

The actual responses and polynomial equations for friability, FLT, Rel_{1h}, and Rel_{6h} in terms of the actual factors that are used as predictive models are represented in Tables 3 and 4.

The terms like *X*₁*X*₂ in the polynomial equation represent the nonlinear interaction between the factors on the response. A positive value signs of the coefficients in the interaction terms indicate a synergism where each factor potentiates the effect of the other. On the other hand, a negative sign indicates an antagonist effect where each factor counteracts the effect of the one factor. The curvilinear lines reveal nonlinearity, suggesting an interaction between the two factors on the response, whereas straight lines rule out interaction of the two factors on the response.

3.3.2 Friability

A friability limit of less than 1% is considered to be acceptable for compressed tablets as per the pharmacopoeia (15). However, effervescent tablets may have different limits for friability. The friability of the bilayer gastro-retentive tablets was found to range from $0.02 \pm 0.01\%$ for F12 to $2.77 \pm 0.64\%$ for F14 displayed in Table 3. Most of the batches of bilayer tablets produced were found to comply with the friability test except batches F4, F6, F9, and F14. The batches F4, F6, F9, and F14 exceeded the friability limit as they displayed friability values of $2.53 \pm 0.31\%$, $2.64 \pm 0.28\%$, $2.68 \pm 0.18\%$, and $2.77 \pm 0.64\%$, respectively. Coincidentally, the hardness of these batches failed to cross 4.5 kg/cm². The high friability values can be directly related to the low binder levels as it is observed that all the four batches were found to contain low binder levels (1% w/w).

Table 3: Response parameters of the model formulations of floating bilayer tablets of CC prepared as per D-optimal mixture design

RUN	X1 (% w/w) ^a	X2 (% w/w) ^a	X3 (% w/w) ^a	Friability	FLT (min)	% Rel _{1h}	% Rel _{6h}
F1	50	47.67	2.33	0.02 ± 0.01	38.47 ± 2.16	22.74 ± 0.57	81.85 ± 2.17
F2	64.25	34.25	1.5	0.25 ± 0.03	6.12 ± 0.40	38.75 ± 0.63	87.48 ± 2.41
F3	77.67	20	2.33	0.03 ± 0.01	12.47 ± 0.15	22.47 ± 0.35	82.41 ± 1.28
F4	79	20	1	2.53 ± 0.31	3.59 ± 0.21	53.85 ± 2.58	86.33 ± 2.57
F5	63.5	33.5	3	0.13 ± 0.02	37.88 ± 0.74	15.87 ± 2.54	54.20 ± 0.58
F6	50	49	1	2.64 ± 0.28	4.14 ± 0.08	54.82 ± 2.13	86.56 ± 1.55
F7	63.5	33.5	3	0.15 ± 0.02	37.54 ± 0.42	16.49 ± 0.31	54.08 ± 0.63
F8	64.25	34.25	1.5	0.25 ± 0.03	6.57 ± 0.26	36.07 ± 2.65	87.22 ± 1.19
F9	59.67	39.33	1	2.68 ± 0.18	2.85 ± 0.18	56.60 ± 1.59	82.29 ± 0.88
F10	59	38	3	0.10 ± 0.01	6.55 ± 0.57	15.01 ± 0.45	56.12 ± 2.03
F11	71	27	2	0.44 ± 0.10	17.31 ± 0.09	31.95 ± 2.89	86.31 ± 1.75
F12	77.67	20	2.33	0.02 ± 0.01	12.76 ± 0.60	21.70 ± 1.26	81.24 ± 0.27
F13	64.25	34.25	1.5	0.25 ± 0.03	4.74 ± 0.81	37.28 ± 1.87	88.72 ± 0.92
F14	69.33	29.67	1	2.77 ± 0.64	4.99 ± 0.96	55.61 ± 1.28	88.57 ± 2.80
F15	57	41	2	0.40 ± 0.10	36.55 ± 0.47	26.19 ± 1.54	85.19 ± 1.29
F16	50	47.67	2.33	0.03 ± 0.01	36.42 ± 3.59	24.85 ± 1.42	82.08 ± 1.45

Each data point represents mean ± S.D ($n = 3$).

^a X₁, X₂, and X₃ represent the amounts of HPMC K100 M, sodium bicarbonate, and HPMC E15 LV, respectively. X₃ was used as 8% w/v and as binding solution in the bilayer tablets.

Table 4: Summary of ANOVA for the response parameters of the model formulations of bilayer tablets prepared as per D-optimal mixture design

Response	F-value	p-value	R ²	Adj R ²	% C.V.
Y ₁	1544.43	<0.0001	0.9996	0.9989	2.81
Y ₂	160.02	<0.0001	0.9959	0.9896	4.36
Y ₃	527.56	<0.0001	0.9987	0.9968	2.68
Y ₄	125.72	<0.0001	0.9843	0.9765	2.41

Regression equations of the fitted model containing only the significant terms:

$$\sqrt{\text{Friability}} = + 0.18 \times X_1 + 0.32 \times X_2 - 17897.32 \times X_3$$

$$\ln \text{FLT} = + 0.24 \times X_1 + 1.19 \times X_2 - 25289.06 \times X_3$$

$$\text{Rel}_{1h} = + 0.40 \times X_1 + 4.38 \times X_2 - 64542.84 \times X_3$$

$$\text{Rel}_{6h} = + 0.74 \times X_1 + 0.88 \times X_2 - 1539.69 \times X_3$$

Y₁, Y₂, Y₃, and Y₄ represent friability, floating lag time (FLT), release at 1 h, and at 6 h, respectively.

The statistical analysis indicated that of the three factors, the influence of X₃ was greatest, followed by X₂, whereas the effect of X₁ was found to be the least. The amount of HPMC E15 LV (X₃) had a high negative coefficient (−17897.32) that implies the factor was found to have a substantial negative impact on friability. This decrease in friability can be explained on the basis of decrease in binder concentration produced tablets with low hardness. It was observed that the CC layer and not the buoyant layer was the major contributor to the tablet

friability. It is a common consensus that the hardness of the tablets was found to increase as the binder amounts increased (24). It could be concluded that the friability could be minimized merely using moderate to high levels of HPMC E15 LV. The negative impact of the binder on the friability was clearly visible in the 3D Plots captured in Figure 1a.

Of the three factors studied, HPMC K100 M and sodium bicarbonate were found to display a low positive coefficient values of 0.18 and 0.32, signifying a negligible influence on the friability. The possible reason for the poor effect noted with the two factors could be the fact that HPMC K100 M and sodium bicarbonate are the components of buoyant layer and not the CC layer.

3.3.3 Floating lag time

The FLT of the bilayer tablets was found to range from 2.85 ± 0.18 min for F₉ to 36.55 ± 0.47 min for F₁₅. The values are captured in Table 3 and representative pictures are portrayed in Figure 2. Leaving out batches F₄, F₆, F₉, and F₁₄ that failed to comply with the friability test, the batches F₁, F₅, F₇, F₁₅, and F₁₆ were associated with high FLT exceeding 30 min, whereas the batches F₃, F₁₁, and F₁₂ were more than 10 min. Rest of the batches, namely, F₂, F₆, F₈, F₁₀, and F₁₃, displayed acceptable lag time that was less than 10 min. A short lag time is preferable as

Figure 1: Response surface plots depicting the interaction effects of dependent factors on a Y: friability (a), FLT (b), % release at 1 h (c), and % release at 6 h (d).

prolonged lag time could eventually lead to system failure due to unanticipated or accidental rapid gastric clearance by the peristaltic action of the stomach and forcible gastric housekeeping waves. Generally, batches with high FLT contained higher levels ($\geq 2\%$) of binder HPMC E15 LV. Mathematical modelling of the experimental data suggested that the three factors investigated were found to have a substantial influence on FLT. Among the three factors explored, the effect of X3 was the most, followed by X2, while the effect of X1 was found to be the least. The amounts of HPMC E15 LV had a highest negative coefficient value (25289.06), which implies that the factor has most significant influence on floatation lag times. This can be related to the fact that higher binder amounts could result in more compact tablets with reduced porosity. The decreased tablet porosity is likely to substantially hinder the penetration of dissolution medium into the tablet matrix, which in turn would delay the generation of carbon dioxide that

may be required to initiate floatation (25). It could be summarized that the FLT could be minimized merely using moderate levels of HPMC E15 LV. The negative impact of the binder on the friability is clearly visible in the 3D Plots captured in Figure 1b. In contrast, the amounts of sodium bicarbonate with a positive coefficient value of 1.19 were found to display a mild impact on the FLT. The positive effect of bicarbonate can likely be attributed to the ability to generate carbon dioxide by a reaction of sodium bicarbonate and gastric fluid that would be efficiently entrapped in the polymeric gel layers, thereby decreasing FLT (26). Of the three factors investigated, the amount of HPMC K100 M was found to have minor effect with a positive coefficient of 0.24. The positive effect can be attributed to HPMC K100 M, a high viscosity hydrophilic material that could form a layer of strong gel matrix in the gastric fluids (27). The strong gel barrier in turn effectively entraps the carbon dioxide liberated *in situ*, thereby reducing the tablet density below

Figure 2: Photographs depicting the side view of bilayer tablets of CC in pH 1.2 buffer at 1 h (a), 2 h (b), 4 h (c), and 6 h (d).

unity to confer the tablet buoyant (28). However, the FLT in the present study was invariably affected by composition of the carbonate layer rather than the floating layer.

3.3.4 Release at 1 h

The percentage calcium release at the end of first hour was found to range from $15.87 \pm 2.54\%$ for F5 to $55.61 \pm 1.28\%$ for F14 as per Table 3. The dissolution profiles of the model formulation were presented in Figure 3. The three formulation factors investigated were found to have a significant influence on the release of calcium at the

end of 1 h. Among the three factors, the effect of X3 was most significant, followed by X2, while the effect of X1 was found to be the least. The batches F4, F6, F9, and F14 were deemed to be unsuitable as they were found to exhibit a burst release of $53.85 \pm 2.58\%$, $54.82 \pm 2.13\%$, $56.60 \pm 1.59\%$, and $55.61 \pm 1.28\%$, which coincidentally corresponded well with the high friability values of 2.53 ± 0.31 , 2.64 ± 0.28 , 2.68 ± 0.18 , and 2.77 ± 0.64 , respectively. The binder concentration displayed high negative coefficient (64542.84) indicating it had most significant effect on the release of calcium at 1 h. On the contrary, higher binder levels produced more compact tablets that effectively prevented the initial burst release.

Figure 3: Comparative cumulative amount of calcium release from bilayer tablets of batches F1–F8 (a) and F9–F16 (b).

Literature citations in the past have indicated that increase in binder concentrations in the matrix tablets substantially reduced the burst release during the first hour (29). In contrast, sodium bicarbonate with a coefficient of 4.38 was found to display a mild positive effect on the release at 1 h. The tendency of bicarbonate to produce effervescence that renders the tablet porous could be the likely reason for the better calcium release at 1 h. Previous reports have indicated that increase in the bicarbonate amounts would increase the drug release from matrix tablets (30). In summary, the burst release could be minimized by using moderate to high levels of HPMC E15 LV. The negative effect of the binder on the burst release is clearly evident in the 3D Plots captured in Figure 1c.

Of the three factors investigated, HPMC K100 M was found to have a negligible influence on the release at 1 h. The likely reason for the same would be that HPMC K100M was not a part of the matrix in the CC layer. The rest of the batches that were devoid of initial burst release can be considered to be more suitable as they displayed a controlled pattern of calcium release.

3.3.5 Release at 6 h

The percentage calcium release by 6 h was found to range from $54.08 \pm 0.63\%$ for F7 to $88.72 \pm 0.92\%$ for F13 as is presented in Table 3. The total of 12 batches including F1–F3, F5, F7, F8, F10–F13, F15, and F16 that were devoid of initial burst release were found to display a controlled pattern of calcium release. The batches F4, F6, F9, and F14 were found to exhibit a burst release exceeding 50% as they displayed low hardness and high friability. However, the subsequent release of calcium from these tablets appeared to be retarded. The likely reason for the retarded release could be that the dissolution media that has been already saturated with the dissolved CC (>50% CC in dissolved state) is less likely to create a sink condition to generate the concentration differential for further dissolution of CC from the matrix tablet.

The three factors investigated were found to significantly influence the release at 6 h. Of the three factors investigated, the influence of X3 was the most, followed by X2, whereas the effect of X1 was found to be the least. The amount of binder HPMC E15 LV (X3) had a high negative coefficient (-1539.68) that implies the factor was found to have the considerable influence on the release at 6 h. An increase in the concentration of binder effectively controlled the release of calcium from the matrix tablets. The negative influence of the binder could be explained by the fact that higher binder amounts produced compact and denser tablets that displayed controlled release of calcium during 6 h. The results also imply that the release rate could be modulated by merely varying the concentration of the binder HPMC E15 LV. HPMC E15 LV alone is reported to effectively control the drug release from the matrix tablets (31). To conclude, the complete release of calcium could be ensured by just using moderate levels of HPMC E15 LV. The negative influence of the binder on the release at 6 h is clearly observed in the 3D Plots captured in Figure 1d. On the contrary, sodium bicarbonate with a coefficient of 0.88 was found to exert a mild positive influence on the release at 6 h. The ability of bicarbonate to render the tablet porous, especially in those with lower binder levels, might be the probable reason for the higher release observed (32). Of the three factors studied, HPMC K100 M was found to have a negligible influence on the calcium release at 6 h. As described earlier, the poor impact of HPMC K100 M could be due the fact that the high molecular weight polymer did not constitute the matrix material in CC layer.

Figure 4: FTIR Spectrum of CC (a), physical mixture (b), and bilayer tablet (c).

At the end of the studies, it could be concluded that the batches F2, F8, and F13 that contained moderate amount of binder were found to be most suitable formulations as they were found to comply with the official friability limits, devoid of the initial burst effect, displayed a short FLT, and resulted in a controlled yet complete release of calcium by 6 h.

3.4 Optimization

A numerical optimization technique using the desirability approach was employed to develop two new floating bilayer tablet formulations with the desired responses. The compositions of the optimized batches of floating bilayer tablet along with predicted and experimental

Table 5: Composition of the optimized formulations and comparison of experimental values of the response parameters with the predicted values

Composition (X1:X2:X3) ^a	Responses ^b	Predicted value	Experimental value ^c	Prediction error %
77.96:20:2.04	Y1	0.08	0.07 ± 0.81	−14.29
	Y2	2.73	2.91 ± 0.57	+6.19
	Y3	27.30	28.73 ± 0.85	+4.98
	Y4	87.58	87.10 ± 1.69	−0.55
55.43:43.07:1.49	Y1	0.07	0.08 ± 0.49	+12.50
	Y2	8.50	9.13 ± 0.24	+6.90
	Y3	34.80	36.41 ± 1.12	+4.42
	Y4	88.70	88.05 ± 1.96	−0.74

^a X₁, X₂, and X₃ represent the amounts of HPMC K100 M, sodium bicarbonate, and HPMC E15 LV, respectively. X₃ was used as 8% w/v and as binding solution in the bilayer tablets. ^b Y1, Y2, Y3, and Y4 represent friability, floating lag time (FLT), release at 1 h, and at 6 h, respectively.

^c Each data point represents mean ± S.D (*n* = 3).

values for the response parameters are portrayed in Table 5. The prediction error for the response parameters was found to range from −14.29 to +12.50. The low values of prediction errors prove the validity of the mathematical models generated by ANOVA and regression analysis. The *in vitro* calcium release from the optimized formulation of bilayer tablets was found to follow first-order kinetics.

3.5 Stability study

The results of real time stability studies for optimized formulation batch carried out as per ICH guidelines did not show any physical change in the tablets during the study period. The characteristic peaks of CC were clearly evident in the spectra of the tablets too, proving the integrity of CC and at the same time ruling out the possibility of any chemical interaction between CC and other excipients used in the formulation. The representative spectra of CC and tablet mixture are captured in Figure 4. No significant difference was noted in the

content uniformity, FLT, burst release, and the amount released at 6 h proving the stability of the formulation (Table 6).

3.6 *In vivo* radiographic studies

The representative images of the *in vivo* radiographic studies (33) with the bilayer floating tablets are captured in Figure 5. The *in vivo* studies revealed that the mean gastric retention time for the tablets from the optimized batch correlated well with the *in vitro* floating time. The studies indicated that the bilayer floating tablets from the optimized batch remained in the stomach for a mean period of 5.5 ± 1.0 h in rabbits which was significantly higher (*p* < 0.05) than the conventional tablets that displayed a mean gastric retention time of less than 2 h. The bilayer tablets by virtue of the floating properties were found to be well-retained in the stomach, despite the action of peristalsis and forcible housekeeping waves compared to the conventional tablets. As the tablets are well-retained in the stomach proximal to the absorption

Table 6: Responses of the optimized formulation on real time stability studies (25°C, 60% RH)

Condition	Time point	Content uniformity ^a (%)	Floating lag time (min) ^a	Release at 1 h (%) ^a	Release at 6 h (%) ^a
25°C/60% RH	Initial	94.56 ± 0.81	2.89 ± 1.87	27.28 ± 0.73	88.81 ± 1.54
	1 M	96.01 ± 0.93	3.02 ± 0.98	25.81 ± 1.85	86.37 ± 2.03
	3 M	95.41 ± 0.57	2.96 ± 0.31	26.09 ± 1.54	88.41 ± 1.45
	6 M	94.85 ± 0.62	2.99 ± 2.53	27.44 ± 1.29	88.56 ± 1.30

^a Each data point represents mean ± S.D (*n* = 3).

Figure 5: Radiographic images portraying the bilayer floating tablet containing barium sulfate as a radio opaque marker in the stomach of rabbits at 0 h (a), 2 h (b), 4 h (c), and 6 h (d); the tablet is pointed by a circle.

window and probably release the contents in a controlled manner, they are less likely to saturate the calcium transporters situated in the duodenal region of the gastrointestinal tract and therefore may exhibit a superior bioavailability compared to the conventional tablets.

4 Conclusion

Floating bilayer tablets of CC were successfully developed employing D-optimal design. Of the three formulation factors investigated, the levels of HPMC E15 LV used as a binder in the CC layer significantly affected the friability, FLT, and release of calcium. The batches F2, F8, and F13 that contained moderate to high amount of binder were found to be most suitable as they were

complied with the official friability limits, devoid of the initial burst effect, displayed a short FLT, and resulted in yet a controlled and complete release of calcium by 6 h. Numerical optimization technique was successfully employed to develop optimized formulations by setting constraints on the responses. The experimental data for the optimized formulations were found to agree well with those predicted by the polynomial models proving the validity of the models generated. *In vivo* radiographic studies of the optimized bilayer tablet formulations in rabbits revealed that floating tablets were found to be retained in the stomach for 5.5 ± 1 h. The studies collectively proved that bilayer gastroretentive tablets possessing floating properties would be highly promising drug delivery platform for nutrients and therapeutic agents with absorption window in the upper part of the gastrointestinal tract.

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