FORMULATION AND EVALUATION OF GASTRORETENTIVE DOSAGE FORM OF CALCIUM

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THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

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For the award of the degree of

Doctor of Philosophy

In the Faculty of

Pharmacy

by

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Under the Guidance of

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December 2021

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"A successful man is he who receives a great deal from his fellowmen, usually incomparably more than corresponds to his service to them. The value of a man, however, should be seen in what he gives and not in what he is able to receive" With these words of Albert Einstein, I would like to take the privilege to thank the selfless people from the core of my heart who with their constant support, affection, inspiration and encouragement made me feel comfortable to successfully complete this venture. I would like to express my gratitude & indebtedness to my parents and to my beloved **sister,** whose full-hearted co-operation, love and moral support made this day possible in my life.

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LIST OF ABBREVIATIONS

AIDS	Auto immune deficiency syndrome
ALP	Allopurinol
ANNOVA	Analysis of variance
API	Active pharmaceutical Ingredient
AUC	Area under curve
BD	Bulk density
CA	Cefuroxime Axetil
CC	Calcium carbonate
CDDS	Controlled drug delivery systems
CI	Compressibility index
CMC	Carboxy Methyl Cellulose
DoE	Design of experiment
DP	Dipyridamole
FRS	Floating raft system
FTIR	Fourier Transform Infra red
GBP's	Gabapentin's
GRDDS	Gastro retentive drug delivery system
GRFS	Gastro retentive floating system
GRT	Gastric residence time
HME	Hot melt extrusion
HPMC	Hydroxy propyl methylcellulose
HR	Hausner's ratio
ICH	International Conference of Harmonization
MMC	Migrating myoelectric cycle
PVA	Polyvinyl Alcohol
RDA	Recommended dietary intake
RH	Relative Humidity
RPM	Rotation per minute
S.D	Standard deviation
TD	Tapped density
USP	United states pharmacopoeia

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ABSTRACT

Background:

Calcium is mainly absorbed from the stomach and upper gastro intestinal tract and due to site specificity and p^H dependency leading to lower bioavailability and it quickly passes from the absorption site. In such scenario, gastro retentive drug delivery system could be potential alternative to overcome the limitations associated with currently available dosage forms.

Objective: To develop and evaluate gastroretentive systems as sigle unit bilayer tablets and insitu gelling raft forming solution of calcium carbonate (CC) with objective to prolong the gastric residence time (GRT) and thereby increasing the bioavailability.

Methodology: The experimental mixture designs were 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 and insitu gelling raft forming systems. The effect of formulation factors on the responses like floating lag times, percent release at 1^{st} hr and 6^{th} hr were evaluated. In vivo animal studies were performed in normal rabbits using X-ray imaging technique for evaluating the gastroretentive potential of the optimized formulations.

Results:

In case of optimised tablets, the floating lag time (R1), release of calcium carbonate at 1 hour (R2) and 6 hour (R3) were found to be 2.85±0.98min, 27.02±1.18% and 88.98±2.75% respectively. While, optimised insitu gelling raft forming solutions displayed short buoyancy lag time (10.90 ± 0.56 sec), minimum burst release (20.74 ± 1.08%) in 1h and controlled yet near complete release (87.25±1.81%) in 6h.

In vivo radiographic studies in rabbits indicated that the optimized batches displayed a mean gastric retention time of 5.5±1h and 5.64 ± 0.43h that was significantly higher ($P < 0.05$) compared to the available marketed formulations that exhibited a mean gastric retention time of less than one hour.

Conclusion - The experimental data for the optimized formulations was in agreement with that predicted by the mathematical models proving the validity of the models generated. The studies proved that the gastroretentive tablets and GRFS systems can be a promising platform to improve bioavailability of nutrients having an absorption window in the upper gastrointestinal tract.

Keywords: Calcium carbonate; gastro retention; bioavailability; floating lag time; raft forming in situ gelling system; tablet.

INTRODUCTION

INTRODUCTION

Oral route of drug administration is widly used to deliver the therapeutic agents as these are having minimum cost of therapy and can be administered easily as a result, patient compliance is quite high. Oral drug delivery systems account for more than half of all drug delivery systems on the market[1]. Controlled drug delivery systems (CDDS) are modified release systems that release the contents at a predetermined, predictable and controlled manner. There are various advantages of the CDDS like, maintains optimum concentration of drug in blood and releases drug in reproducible manner and over an extended time. It also increases the activity of short half life drugs, reduces side effects, frequency of doses , drug wastage and optimizing therapy and improving patient care[2]. However, CDDS are ineffective for medications which possess absorption window in the upper gastrointestinal system.Gastroretentive drug delivery systems (GRDDS) are known to address most of the limitations of the CDDS. These systems are said to prolong the gastric retention time and at the same time release the contents at a predetermined, predictable and controlled manner. Gastric emptying time is determined by several factors that must be considered when developing the dosage forms. Gastroretentive approaches are used for drugs that are administered for local action in the stomach and which are not stable in the environment of intestine drugs which have a narrow absorption window in the GIT and have low solubility at high pH. GRDDS is a site-specific technique that operates by increasing the gastric residency time (GRT), which will help to prolong the time between the drug contact and its absorption window for maximum site specific absorption. Hence improves drug solubility thereby enhancing oral bioavailability[4]. Floating drug delivery systems, Bioadhesive systems, raft forming systems, swelling and expandable systems, magnetic systems, and high-density systems are some of the

approaches employed to enhace the gastric retention time[5]. Drugs with low acid solubility and those that meant for local action in colon are not good candidates for the GRDDS[6]. The GRDDS has recently gained a lot of attraction in the area of oral dosage forms. It is a commonly used method of retaining the medication type in the stomach for a long time and eventually releasing the drug, which may resolve many of the bioavailability issues[7]. GRDDS has greatly increased patient compliance by prolonging gastric retention time and controlling the drug release. The interest in these new delivery method has been sparked by some intrinsic shortcomings in traditional oral controlled drug delivery systems. Despite the numerous benefits, the difficulties of high subject variability in physiological condition in gastro intestinal tract, impact of food, and unpredictable rate of gastric emptying time restrict the amount of GRDDS available on the market[8].

BACKGROUND

Despite extensive studies into the development of oral controlled release systems, numerous physiological challenges such as pH variations in various segments of the gastrointestinal tract, trouble localizing the drug delivery mechanism in targeted area of GI tract, and highly variable sgastric emptying time have limited the develoment[7]. GRDDS can help resolve some of these issues, and they're especially helpful with medications that function locally in the stomach or have an absorption window in the uppermost gastro intestinal tract. For medications that are effective in the intestine, these mechanisms are very useful. GRDDS is a site-specific approach where control release of drug occur at specific absorption sites in the GIT staying in the stomach for a long time. Another benefit is that it aids in the improvement of bioavailability of therapeutic agents having absorption window in a specific GIT region.Gastric retention is done by preventing the doage form from passing the pyloric sphincter. Calcium is currently available in the form of immediate release capsules, liquid preparations such as syrups, and parenteral preparations. In primary level calcium is absorbed from the stomach and upper gastro intestinal tract[10-12].Due to the different properties of absorption, the gastric residence time of formulation containing calcium have to be changed and make it sustain release of calcium to go at the site of action in controlled manner. The orally administered calcium supplements suffer from common side effects such as constipation and stomach bloating. Patients who suffers hypohydrochloria syndrome, a regulated calcium delivery system can be advantageous because calcium will deliver in a controlled manner instead of burst phenomenon as in the conventional form[13].

Physiological Factors Consideration For Development Of GRDDS

1. Structure of stomach and its physiology:

The stomach is a digestive organ that is responsible for the accumulation and digestion of food. The stomach is the area of the gastrointestinal tract that lies between the cardiac and pyloric orifices. The stomach is comprised of three regions: upper part that is fundus, main part i.e. body, and the antrum, and it is located in upper left portion of the cavity of the abdomin, directly under the diaphragm as shown in figure 1. The stomach consists of goblet cells, parietal cells and chief cells. Goblet cells secrets mucus, parietal cells secrets hydrochloric acid and chief cells secrets pepsinogen, resulting in 2 to 3 liters of gastric juice each day[14].

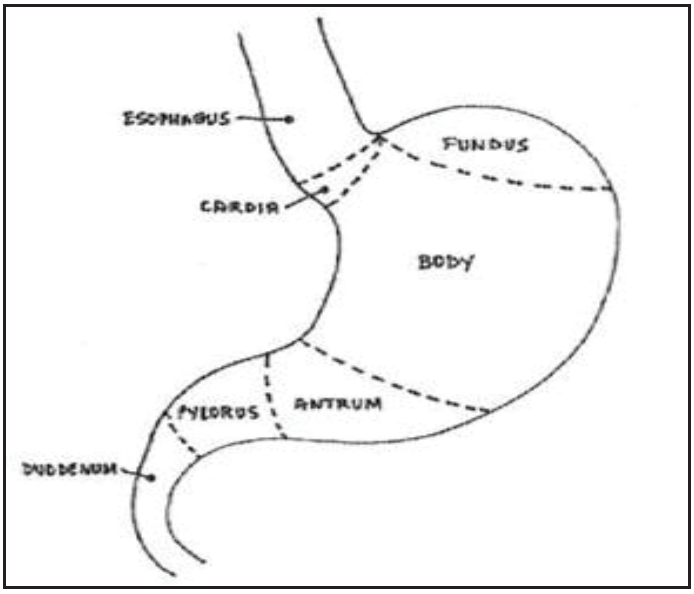


Figure 1: Structure of stomach

The stomach has an overall length of 0.2 m and consist of absorption surface area of 0.1 m^2. The fundus and body of the proximal portion of the stomach act as a reservoir for the material which is not digested and propel chyme to the antrum. It is the primary site for break down of food materials by mixing movements, as well as acting

as a pump to control emptying of gastric material by propelling activity. Particles or pellets easily sedimented and these particles can easily engulfed in the foldings of the antrum (density of 1.3 g/cm^3 or higher) may enable them to tolerate waves of peristalsis of the lining of stomach and provide sustained retention of gastric mattaer.The pylorus is a sphincter that connects the antrum and the duodenum. The diameter of human pylorus is about 12.8 ± 7.0 mm, and it serves as a strainer and a mechanical structure to prevent massive particles from passing through. Particles must be between 1 and 2 mm in size for passing through the pyloric valve and into the small intestine. To prevent premature gastric emptying and prolong gastric accumulation, and size-expandable GRDDS use dosage form which helps to increase in greater size than the pyloric diameter when administered[15, 16].

2. Gastric pH:

Because of variations in the condition of the stomach during measurement and other physiological as well as biological causes, physiological gastric pH in human being is not consistent and differs across various parts of the GI tract. It also experiences significant intra and inter-subject heterogeneity.The average pH gastric fluid in healthy subjects in fasting condition is 1.1±0.15. The pH falls below 5.0 in the fed condition, then steadily returns to fasting state levels for a few hours.In healthy males, the mean fed-state p is documented to be 3.6±0.415. Physiological modifications in the elderly impair secretion of gastric acid, resulting in decrease in chloride ions which is know as hypochlorhydria, which raises the pH frequency of their basal pH to greater than 5. The existence of medications like antagonists of H_2-receptor and inhibitors proton-pump, as well as pathological disorders like pernicious anemia and AIDS, greatly decrease secretion of gastric juice and boost the pH in gastric region. Because of the significant variations in gastric pH, clinical trials which involves

GRDDS musthave stringent screening procedures in place to detect certain causes and adapt adequate controls to eliminate bias and produce more accurate outcomes[17, 18].

3. Gastric emptying:

The method of moving the contents of the stomach into the duodenum is known as gastric emptying. This is achieved by three mechanisms: (1) peristaltic waves, (2) antrum systolic contractions, and (3) stomach shrinkage. Liquids usually drain faster than solids, and smaller structures usually empty faster than larger ones[19]. The time for which dosage form resides in the GI region is determined by gastric emptying time. As a result, this process is critical for drugs with a limited absorption window, which occurs mostly in the stomach and proximal small intestine. Any conditions which impact gastric emptying can also affect time the dosage form spends in connnection with the target site, and hence affects its oral bioavailability. Since gastric emptying is such a mechanism which is variable, being able to monitor and regulate it will expand the stomach's capacity which acts as a drug-absorbing organ, as well as the possibilities for developing new drug delivery system which is controlled release system[20] . Pattern of motility of stomach is contractile, and its tasks involves making food into small pieces which combines it with gastric fluid to provide "chyme," It causes emptying of matter in small intestine.The onset of migrating myoelectric cycle (MMC) is delayed in the fed state because of time require to empty gastric content which results in slow down of gastric emptying rate[16]. As a result, repeated feeding can delay emptying of gastric content and provide the benefit of longer dosage form retention. The primary factor for modulating GRT is condition or phase of the stomach, whether fed or fasted, concerning drug administration[21].In humans, different biological causes and pathological disorders have been shown to impair gastric motility. Gastric emptying is also said to be affected by different factors

like age, posture, mental fatigue, and states of disease involving the intestine, like gastroesophageal reflux, congenital heart disease, diabetes, and respiratory distress syndrome[22, 23].

Formulation approaches of GRDDS:

Various approaches to formulation development have been developed by researchers to enhance the GRT of the formulations with sustained and prolonged drug release. These approaches include sytems with high-density, mucoadhesive systems, raft forming systems and floating or low-density systems.

1. GRDDS based on high density:

The retention time of the formulation is enhanced in this method by increasing the density of the formulations above that of stomach fluid (1.004 g/cm^3). The formulation is usually developed with a density of 2.5 to 3.0 g/cm^3 such that it may tolerate peristaltic movement and remain steady during gastrointestinal disturbances [24]. High-density formulations (> 1.3 g/cm^3) get sedimented in the various folds of the stomach in the pyloric region, due to powerful gastric propulsions, this region helps to prevent premature emptying. depicted in figure 2.

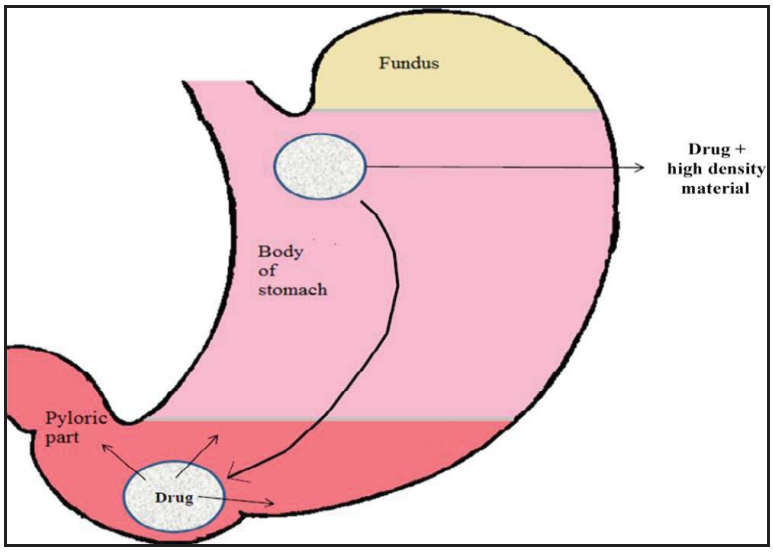


Figure 2: GRDDS based on high-density formulations

Titanium dioxide, zinc oxide, iron powder, and barium sulfates are used in the formulations to increase the density. Such high-density formulations are quite difficult to manufacture with increased dose size and this is one of the drawbacks of such formulations[25]. This system is widely utilized in veterinary medicines and has shown promising results while it has not shown that much effectiveness in human patients due to the differences in retention caused by mobility and posture[26-28].

2. GRDDS based on mucoadhesion:

The GRDDS can also be formed by using mucoadhesive and bioadhesive polymeric systems that get adhere to the gastric mucosal lining or mucus gel layering leading to the prolongation of retention time in GIT is shown in figure 3. Such dosage forms can easily survive under gastric motility for a longer duration of time[29].

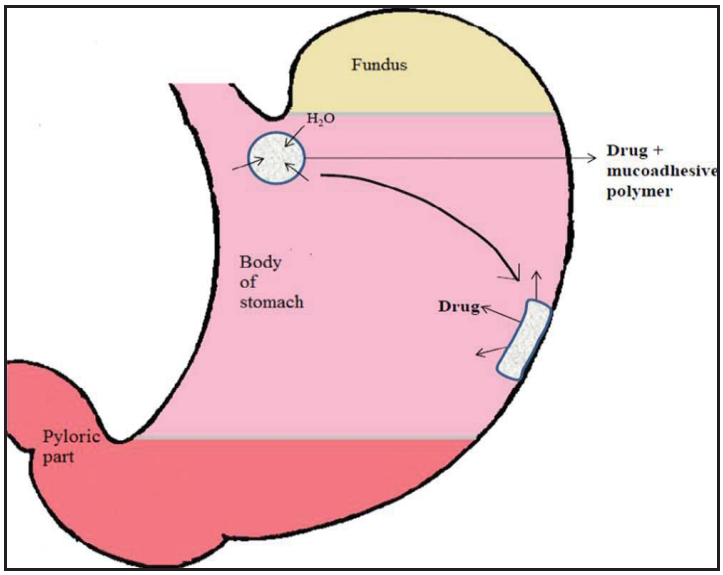


Figure 3: GRDDS based on mucoadhesion

These formulations are also beneficial for drug delivery which is site specific in the infected region of GI region to enhance the absorption of the active moiety[30]. The polymers used in this system are natural, semisynthetic, or synthetic. The mucoadhesion may be imparted by hydration, swelling, mechanical or chemical

bonding, or receptor activation in gastric cell lines[31]. Various mucoadhesive polymers used in GRDDS are presented in table 1.

Table 1: Application of polymers (mucoadhesive) in GRDDS

Anionic	Sodium alginate, Carboxymethyl cellulose, Carrageenan, Carbopol, Pectins, Polyacrylic acids, Hyaluronic acid, Polyvinyl sulphate, Dextran sodium
Cationic	Chitosan, Polyvinyl methyl imidazole, Polylysine, Polybrene
Non ionic	Polyvinyl pyrrolidone

Non-specific adhesion and binding of the dosage forms to the GI tract is the major drawback of these polymers. This non-specific adhesion may lead to the formation of drug-induced injuries and mucosal irritation. Apart from this, the efficiency of these formulations is limited by the amount of gastric mucosa and food interactions[32].

3. GRDDS based on polymer swelling (expandable system):

This system based on the principle of swelling and expansion of the polymer when it reaches to the gastric juice that leads to an enhancement of size bigger than the size of the pyloric sphincter and remained lodged there and also called as "plug type system"[33-35] as shown in figure 4. Human pylorus diameter is about 12.8 ± 7.0 mm, so to retain the dosage form at pylorus the swelling and expansion should be more than the diameter of the pylorus[36]. The proper selection of polymer or combinations of different polymers with molecular weight and its viscosity determines the swelling and expansion capacity of the dosage form.

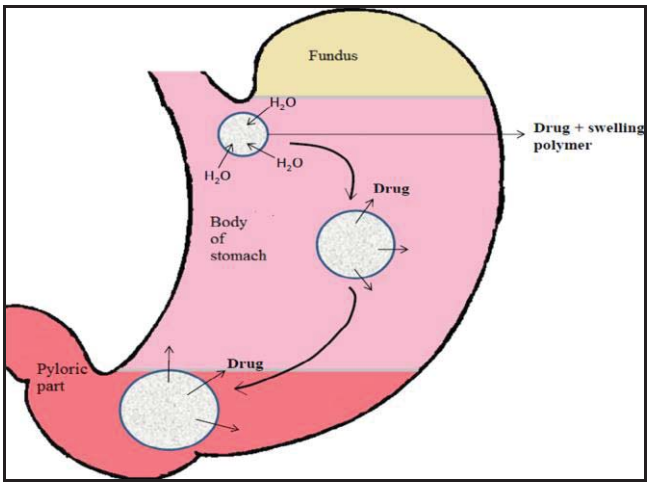


Figure 4: GRDDS based on polymer swelling (expandable system)

The integrity of the formulation during peristaltic movement plays a very important role in the expansion and swelling of the polymers and thereby determines the residence time in GIT. The dosage forms with smaller size, non disintegrating in nature are advisable due to ease of administration and greater swelling capacity than the opening of the pylorus that leads to retention of the formulation for a longer period. However, the the rate and extent of swelling and erosion of the polymer have an impact on the system's efficiency which avoids unwanted side effects. The capacity of these devices to extend gastric retention regardless of whether the stomach is fed or fasted is a significant advantage of this system. However, because of their large size, this system face the risky condition of having retention in intestine permanently, which could result in life-threatening complications after repeated administrations leading to drug-induced mucosal surface injuries[32].

4. GRDDS based on low density (floating system):

In this type of system, the formulations which are having density minimum than gastric fluid (1.004 to 1.01 g/cm^3) is formulated that remains floated for an extended period to provide continuous drug release. The capacity of floating for formulation is determined by the proper selection of polymers or combinations of polymers with

sufficient molecular weight and viscosity[37, 38]. The effervescent components are also added to this system to improve the floating behaviour depicted in figure 5. $NaHCO_3$, citric acid, are mixed into dosage forms to liberate carbon dioxide when it mix with gastric juice gets entrapped in a gellified hydrocolloid system[39, 40].

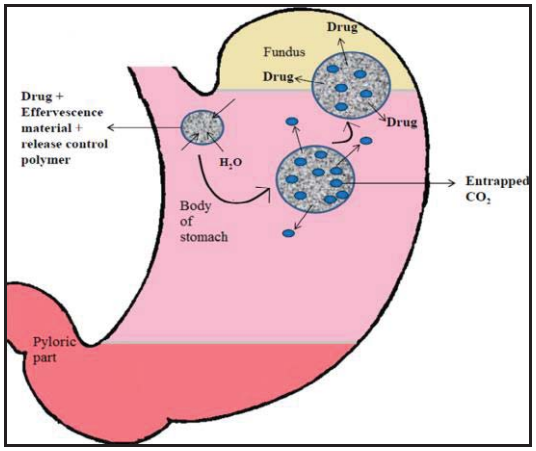


Figure 5: GRDDS based on low density (floating system)

These floating delivery systems are widely accepted as it does not have adverse effect on movement of GIT[41, 42].The vast number of floating drug formulations that have been commercialized and sold globally demonstrates their superiority over other varieties of GRDDS[43].

5. GRDDS based on in situ gelling (raft forming system):

Another patient compliance design for gastroretention are in situ gel preparations (called as raft formation system) mixing with carbon dioxide bubble entrapment. Sodium alginate, which is the polymer widely used in in situ gel formulation which is generally mixed with bicarbonates and these are responsible for generation of efferevescence. The system swells when combines with gastric fluids, they form gel which is viscous and cohesive in nature and it trapped CO_2 bubbles and these allow allows the drug to float. Raft forming systems are often used to treat gastroesophageal reflux since they create a coating on the upper part of the gastric fluid[44, 45].

Factors affecting gastric retention of the formulations:

Formulation and Idiosyncratic factors are mainly responsible for the gastric retention of the developed dosage forms.

1. Formulation factors which affects gastric retention:

Formulation factors like size, shape, density of the dosage form greatly affect gastric retention.

a) Size of the formulation (tablets):

The size of the tablets has a significant impact on their retention in the stomach. Smaller tablets undergo quick emptying from stomach during digestion, however, tablets with large size are expelled at the time of housekeeping waves[46]. Diameter of human pylorus is 12.8 ± 7.0 mm and which acts as a strainer and a mechanical structure to keep large particles out. As a result, its diameter can be employed as a critical criterion for dosage form emptying. Particles with a diameter of less than 7 mm are easily evacuated, but it is widely agreed if diameter of more than 15 mm is required for essential prolonged retention, specially at the time of fasting state [47].The floating dosage systems stayed floated on the gastro intestinal contents during their tenure in stomach, only if their size, and when non-floating units saturated throughout the lowest part of the stomach. The GRT of extra small as well as medium-size of floating units was also found to be prolonged by buoyancy, although there was no substantial difference between floating as well as non-floating large units (9.9mm diameter)[48].

b) Shape of the formulation:

Owing to its ability to reach a scale wide enough to be held in the stomach and their versatility to avoid to empty it by the powerful propulsive powers of the stomach, tetrahedron and devices with ring shape provided great retention and prolong gastric

retention than another forms (i.e., cloverleaf, loop, pellet, and disk)[41, 49]. The gastric retention time of a device with a diameter of 9.9 mm is longer than that of a device with a diameter of 7.5 mm, and the residence time of a dosage type with a tetrahedron shape is longer than that of another device of the same dimension.

c) Density of the formulation:

The key factor influencing the residence time of formulation is density. Due to having pyloric sphincter away from it, a buoyant formulation and formulation with minimum density of gastric juice (1.0 g/cm^3) floats and is stored in the stomach for a longer period, whereas formulations with high density settled at the bottom level of stomach. The dosage mechanism can be isolated from the pylorus in any location[50].

d) Viscosity and grades of the polymers:

The viscosity of polymers and their interactions have a significant impact on release of drug as well as properties of floating. Polymers whicha are having low viscosity (e.g., HPMC K100 LV) are more effective in enhancing floating properties than the polymers with highest viscosity (e.g., HPMC K4M)[51].

2. Idiosyncratic factors affecting gastric retention:

Idiosyncratic factors like gender, age, posture, food effect, and concomitant intake of drugs greatly affect gastric retention.

a) Gender:

Gastric emptying takes longer for women than it does for men. Regardless of weight, height, males have a lower average ambulatory gastric retention time (3.4 ± 0.4 hrs) than their age (4.6 ± 1.2 hrs)[52]. The elders have a slower gastric emptying time than younger people. Gastric and intestinal transit times show intrasubject and intersubject variability. GRT of the elderly, especially those over the age of 70 is considerably longer[53].

b) Food effect:

In the presence of food GRT increases, resulting in increase in dissolution of formulation at most absorbable site. After having food containing fats and proteins, a GRT of 4 to 10 hours has been recorded[54].

c) Concomitant intake of drugs:

Prokinetic drugs such as metoclopramide and cisapride, anticholinergic drugs such as atropine or propantheline, and opiates such as codeine will also influence the GRT of the formulations being administered. The use of GI motility-reducing drugs together will extend the time it takes for the stomach to empty[25].

d) Effect of posture:

In the case of floating systems, for example, in an upright state, the system having ability to float at the top of the gastric material and remains there for a prolong time, resulting in a prolong gastric retention time (GRT), while in a supine position, the GRT is reduced[25,55].

e) Feeding frequency:

Due to the low level of MMC, gastric retention time increases by 400 minutes when repeted meals are offered instead of a single meal[50].

Ideal drug candidates for GRDDS[56, 57]:

Drugs delivered in a continuous and controlled manner have fewer adverse effetcs and have their effects without requiring frequent dosing. Slow release of drug is also beneficial for medicinal drugs where stomach is not always ready to absorb since it extends the agent's contact time in the stomach or upper small intestine, where absorption begins and its contact time is reduced. Molecules with low colonic absorption but improved properties of absorption in the topmost sections of the GIT are suitable agents for controlled release gastroretentive dosage formulations.

Following are the drug candidates which can be considered ideal for the development of GRDDS in various formulations like tablets, capsules, microspheres, beads, etc.

1) GRDDS is ideal for drug candidates with alkaline pH stability issues e.g captopril, ranitidine HCl, and metronidazole.

2) Medicinal agents which are having narrow absorption window in the stomach or small intestine e.g. riboflavin and levodopa, p- aminobenzoic acid

3) Antibiotics used to eradicate *Helicobacter pylori*, such as tetracycline, clarithromycin which disrupt regular colonic bacteria.

4) Diazepam and verapamil are examples of drugs which are having very poor solubility at high pH are also ideal candidates for GRDDS.

5) Drug which acts in the stomach locally e.g. misoprostol, antacids.

6) Medicinal agents which decompose in the colon e.g. Ranitidine HCl and metronidazole.

7) Drugs with high absorption ability mainly from stomach and upper GIT e.g.,chlordiazepoxide and cinnarizine.

8) Drugs that quickly get absorbed from GIT e.g.tetracycline, ranitidine, metronidazole, metformin HCl.

9) Following table 2 represents the potential candidates for the GRDDS based on the type of formulation.

Table 2: Potential drug candidates for GRDDS[58, 59]

Sr. No	Dosage form	Potential drug candidates
1	Tablets	Aspirin, Verapamil, Amoxicillin, Atenolol, Captopril, Famotidine, Furosemide, Ibuprofen, Ciprofloxacin, Diltiazem, Nimodipine, Riboflavin, Metoprolol, Ampicillin, Acetyl Salicylic acid, Acetaminophen, Fluorouracil, Domperidone, Satalol, Theophylline, Cefuroxime Axetil, Griseofulvin
2	Capsules	Levodopa, Misoprostol, Nicardipine, Verapamil, Diazepam, Propanolol, Pepstatin, Chlordiazepoxide HCl.
3	Powder	Sotalol, Riboflavin, Theophylline, Basic drugs
4	Granules	Indomethacin, Diltiazem, Cinnarazine, Diclofenac sodium, Isosorbide mononitrate
5	Films	Peritanide, Prednisolone, Quinidine, P-amino benzoic acid, Cinnarizine
6	Microspheres/ Multiparticulate system	Aspirin, Famotidine, Cefpodoximeproxetil,Griseofulvin, Ibuprofen, Ketoprofen

Advantages of GRDDS[60-62]:

1) GRDDS enhances patient compliance by reducing dosing frequency.
2) Floating GRDDS is beneficial for drug candidates which are essential for the action in stomach and acts locally e.g. antacid.
3) The therapeutic efficacy of the drugs having a shorter half-life can be enhanced using GRDDS.
4) Drug delivery which is site specific to the stomach can be possible with GRDDS
5) GRDDS reduces the dose dumping by formulating a floating drug delivery system in microspheres form.
6) It can enhance the oral bioavailability of the drugs by allowing maximum absorption through the stomach.

7) It minimizes the adverse effects on the colon by preventing the drug that can reach the colon.

8) GRDDS can reduce the fluctuations in the drug concentrations as it produces a narrow range comparable to the immediate release dosage forms.

9) GRDDS reduces the concentration-dependent adverse effect by a slow and gradual drug release at a constant rate within a narrow limit.

10) It improves selectivity in receptor activation. as GRDDS causes the reduction in fluctuation in drug concentration

This technique may be a useful for the prevention of gastric and duodenal cancers as this dosage form prolongs the gastric retention time and increases the concentration of drug at target site and hence improves therapeutic effeicacy and avoid side adverse effects.

Disadvantages or limitations of GRDDS[63, 64] :

1) A very high amount of gastric fluid is necessary for the floating drug delivery technique to work efficiently.

2) Medicinal agents with less stability and poor solubility in gastric fluidsGIT are generally not ideal candidates for GRDDS. Such drug molecules can not be formulated in GRDDS.

3) Drugs having first-pass metabolism are also not appropriate agents for the development of GRDDS e.g. Nifedipine.

4) Drugs causing gastric mucosal irritation are not ideal for the GRDDS.

5) Drugs which are not stable in acidic conditions of the stomach are also cannot be formulated into the GRDDS.

6) Drugs having absorption wimdow in the colon cannot be formulated into GRDDS

7) High-density GRDDS can be waived out during housekeeping waves.

8) GRDDS in the form of bioadhesive technique may change the efficiency over the period as the mucosal lining of the stomach may undergo constant renewal.

9) The success of this system is dependent on the physical integrity in the gastric environment.

10) The drugs having an absorption window at the stomach are only considered ideal candidates.

11) The use of a single large dosage form may lead to the problem of permanent retention in patients suffering from bowel obstruction, gastropathy, or having a narrow pyloric diameter.

Comparison of conventional and GRDDS[60-64] :

GRDDS was found very beneficial as compared to conventional dosage forms. The comparative effectiveness is presented table 3.

Table 3: Comparison of conventional and GRDDS

Sr.No	Feature	Coventioanl dosage froms	GRDDS
1	Adverse effects	High	Low
2	Patient complience	Less	Improved
3	Dose dumping chances	High	No
4	Colon degrading drugs	No benefit	High benefit
5	Drugs with rapid absorption through GIT	No benefit	High benefit
6	Locally acting drugs in the stomach	No benefit	High benefit
7	Narrow absorption window in the small intestine	Not useful	Useful
8	Poorly soluble drugs in alkaline pH	No benefit	High benefit

Marketed formulations of GRDDS [65-67] :

GRDDS is one of the innovative drug delivery technologies that are gaining attention these days. The regulatory authorities have also approved a wide number of formulations. GR formulations are typically developed for agents which are having absorption window in the upper GIT, to improve bioavailability by extending the period spent in the stomach. Several active GR formulations have been licensed and are available on the market presented in table 4.

Table 4: Commercialized GRDDS products [65-67]

Sr.No	Brand name	Drug	GRDDS technology	Compony
1	Cipro XR	Ciprofloxacin HCl and betaine	Erodible matrix	Bayer, USA
2	Tramadol LP	Tramadol	Floating	Galenix, France
3	Liquid Gaviscon	Alginic acid and sodium bicarbonate	Floating with effeverescence	ReckittBenckir Healthcare, UK
4	Oflin OD	Ofloxacin	Floating with gas generation	Ranbaxy, India
5	Conviron TR	$FeSO_4$	Colloidal gel-forming floating	Ranbaxy, India
6	Prazopress XL	Prazosin HCl	Effervescent and floating by swelling	Sun pharma, Japan
7	Xifaxan	Rifaximin	Bioadhesive	Lupin, India
8	Valrelease	Diazepam	Floating capsules	Roche, UK
9	Coreg CR	Carvedilol	Gastro retention with osmotic system	GlaxoSmithKline
10	Glumetza	Metformin HCl	Polymer-based swelling	Depomed Inc, USA
11	Baclofen GRS	Baclofen	floating and swelling with multilayer coating	Sun Pharma, India
12	Cytotec	Misoprostol	Bilayer floating capsules	Pharmacia/Pfizer Inc., USA
13	Madopar	Levodopa and Benserazide	Floating capsules	Roche, UK

LITERATURE REVIEW

The study involved the preparation and characterisation of the gastro retentive Mucoadhesive tablets of cephalexin. This formulation was formulated by wet granulation technique. HPMC K4M, HPC HF, Carbopol 934P, chitosan and Sodium CMC were used in different concentrations as polymers in the formulation. Physicochemical charachteristics were determined like weight variation and drug content. Formulated tablets were obsrved in limits of their respective parameters. For adhesive property, swelling property of Mucoadhesive polymers is essential factor. If polymer swelling is fast, diffusion is fast, it will increases the bond formation and adhesion increases. The formulation which containing hydroxy propyl cellulose, Carbopol 934P and chitosan showed highest swelling index at 24 hr as compard to other formulation. Drug release is dependant on dissolution and swelling of the polymers used in the formulation. The formulation which containing hydroxy propyl cellulose, Carbopol 934P and chitosan was found maximum % cumulative drug release (85.55%) at 10 hour. In vitro release data showed krosmeyers-peppas release kinetic for all formulation. Disintegration test apparatus was used to determine in vitro residence time. F2 formualtion showed maximum in vitro residence time than F5 formulation. F2 formualiton was selected as a optimised formulation and in vivo GRT was evaluated by X-ray radiographic techniques on rabbit. F2 fomulation sticked in the stomach and can be seen there even after 10 hrs. Acclerated stability studies were also performed according to ICH guidelines. Drug content and drug release studies were performed at regular intervals and no any significant change in colour, drug content and release of drug was observed. Hence, F2 formula was found to stable after accelerated studies[68]

Development of cefuroxime axetil was done by utilising polymers like HPMC K4M, HPMC K100M, and the combining polymers like HPMC K4M and Polyox WSR 303. The formulation was prepared by slugg method and then all physicochemical properties were evaluated. F10 formualtion was selected as optimised formulation which is formulated by HPMC K4M polymer. All the physicochemical parameters were found to be within range. The action of drug release obeys the Peppas model. radiographic studies were done , it was found that, tablets stayed in the stomach for 225±30min. For in vivo bioavslaibility study, optimised formulation were compared with marketed formulation (Zocef tablet). The developed tablets showed superior bioavailability than marketed formulation. The relative bioavailability of test was increased and it was compared with reference and it was 1.61 fold when compared to reference[69].

Using a combination of mucoadhesion and floating systems, a gastroretentive dosage type (GRDF) for allopurinol (ALP) was developed. A 3^2-full factorial architecture was used to refine GRDF. The texture analyzer was used to assess GRDF for gastroretentive parameters like FLT and TFT, MF, and in vitro residence time by use of the disintegration test apparatus. Albino rabbits were used in a roentgenography trial to test *in vivo* gastro retentive action of the optimized formulation.After being placed in simulated gastric fluid, developed tablets produced in situ gas and had a FLT of 1.68 s, resulting in buoyancy, and monitored release of drug for 24 hours to follow zero-order drug kinetics. The optimised formula was chosen based on drug release properties *in vitro*. Roentgenography experiments confirmed *in vivo* preservation of the optimized formulation. According to the findings, combining mucoadhesive and floating properties for GRDF helps gain expected gastroretentive efficiency and drug release properties for ALP[70] .

Sodium alginate, gellan gum, sodium citrate, and calcium $CaCO_3$ were used to develop sustained release metronidazole (Mz) floating raft system (FRS) by use of polymers which ion-sensitive. Glyceryl monostearate, Precirol®, and Compritol® were among the lipids used in the formulations. For existing formulations, buoyancy, gelation potential, viscosity, drug release, and kinetics were assessed, followed by accelerated stability testing. Gum Gellan FRSs were able to achieve floating gastroretention, but not the gelation capability needed. GMS increased the lag time of gelatin and length, ensuring long-term release of drug and stability of formulation. The chosen FRS have enhanced characteristics, making them appropriate agent for gastric targated drug to eliminate *Helicobacter pylori*[71] .

A GDDS for ofloxacin was designed for increasing retention time, therapeutic activity and its bioavailability in stomach using a clear and flexible electrospinning technique. The drug encapsulation efficiency and *in vitro* drug release of the fabricated nanofibers in the simulated gastric medium were assessed (p^H 1.2). The *in vitro* release profile and kinetic tests revealed that ofloxacin was released continuously from nanofibers using Fickian diffusion kinetics. In an animal model, the tailored ofloxacin-loadedgellan/PVA nanofibers showed a drug release profile which is biphasic with significant adhesion and retention in GIT .According to the findings, the developed formulation can theoretically increase the pharmacological action of drug and can also be used to improve drug bioavailability through the oral path[72] .

To extend gastric residence time, GRDDS of carvedilol cocrystals were formulated using hot-melt extrusion and optimizition done by use of Box-Behnken Design. Direct compression was used to formulate the tablets, which included HPMC K4M, Carbopol 934P, $NaHCO_3$, microcrystalline cellulose, talc, and magnesium stearate, among other excipients. The optimized formulation was subjected to tests such as

overall floating time, swelling studies, dissolution studies, and parameters pre-compression such as bulk mass, tapped density, compressibility index, and angle of repose. Tablets were place in 0.1 N Hydrochloric acid having pH 1.2 for 12 hours, the optimized tablets (F2) displayed 11 s of floating lag time, indicating that the process of release of drug was a Non-Fickian form of diffusion. In contrast to a formulation containing only carvedilol, the improved formulation (F2) had an *in vitro* drug release profile of 81.4 percent at 12 hours (65.55 percent release of drug at 12 h).It was found that HME's successfully prepared carvedilol cocrystals can be utilised to formulate and refine floating tablets to gain expected gastroretentive efficiency while maintaining a reasonable drug release profile for carvedilol[73] .

To improve bioavailability, a mucoadhesive tablet of lafutidine was formed using a natural polymer, sodium alginate and karaya gum. Buoyancy lag time, and % drug released were all measured in the formulated tablets. Korsmeyer–Peppas reported non-fickians release transport from action of drug release. The mucoadhesive power of the optimized formulation (B3) was greater than 35 g. X-ray imaging was used to do an *in vivo* analysis on rabbits. According to radiological proof, a tablet was sticked well in the rabbit's stomach for > 10 hours. After 3 months of storage at 40 °C temperature and 75 % RH, optimized tablets displayed no substantial difference in appearance, drug quality, mucoadhesive properties, or *in vitro* dissolution pattern[74] .

The use of raft formation systems as a possible drug delivery mechanism for delaying Gabapentin’s (GBP's) gastric residence time was studied. An optimal formula with zero-order release profile appropriate for once-daily administration was accomplished using a 2^3full factorial design to determine the effect of formulation variables on the percent of GBP released at different time intervals as well as the gel pressure. The gel shaped was assessed in rats *in vivo* to see how long it stayed in their stomachs.

Furthermore, the oral bioavailability of GBP was compared to the currently available marketed formulation IR oral solution. *In vivo* testing was carried out on rats to determine the gel's gastric residence. Cmax, AUC(0–t), and AUC(0–∞) all saw a significant improvement. The refined formula increased GBP's relative bioavailability by 1.7 times[75].

Microsphere formulation of the Carvedilol was fromulated by emulsion solvent diffusion method and various prosess parameters of the formulation were analysed with the help of full factorial design. The polymers were used in the different ratio. Ethyl cellulose, Hydroxypropyl methyl cellulose polymers were used. Tween 80 was used as the surfactant. Methanol and dichloromethane was used in varying concentration. The emulsion was prepared by using propeller type agitator for about 45 min for 800 rpm. At this period, solvent gets evaporated and microsperes of the Carvedilol drug was obtained. All the physicochemical properties of the formulation like, surface morphology, percentage yield of microsphere, drug entrapment efficiency, floating ability evaluation was done in in vitro and drug release study etc. The optimised formulation showed the spherical hollow microspheres having good floating capacity and entrapment efficiency. The percentage yield of the microsphere was found in the range of 64.43%- 84.43% and it was depending upon increase in concentration of polymer. Drug entrapment effecicncy was found in the range of 60.53% - 88.71%, it was found because of concentration of polymer ethyl cellulose was increased. In in vitro floating capacity study, it was found that, larger is the particles size longer is the time required for floating. In vitro drug release study was determined in 0.1N Hydrochloric acid having pH 1.2 for 12 hours. USP dissolution test apparatus was used for this test. At 10^{th} hour 78.95%- 82.05% drug release was

found and at 12 hrs, it was found above 80%. Because of increase in concentration of ethyl cellulose sustained release of the drug was achieved.[76]

Hollow alginate beads of gliclazide from combination of methoxyl pectin and hydoxypropyl methyl cellulose were formulated by simple ionotropic gelation technique. These are sodium alginate based beads where HPMC K 100LV or pectin LM was used in the ratio of 9:1. In this polymeric solution, calcium carbonate was added and stirring was done for about 2 hrs and solution was placed for 3 hrs to remove air bubbles. The beads were prepared from resultant solution by dropping it from syring in 1% calcium chloride solution and acetic acid. Beads were collected and all physicochemical properties were determined. The optmised beads was found to be spherical and having plain surface which contains cavity internally. HPMC was the polymer used to sustained the drug delivery and due to use of pectin, it causes cross linking with $CaCO_2$ and prevents release of drug. Therefore it was used along with other polymer with sodium alginate. The drug incorporation efficiency was found in the range of 65 to 81.02%. In pectin containing formulation, drug incorporation efficiency was more and drug loading was found to be increased with increased in calcium carbonate concentration. It was also found that, floatation was good in formulation containing higher concentration of $CaCO_3$. Hence the buoyancy percentage was totally depending on concentration of $CaCO_3$ as well as polymer's nature. Swelling study was done in 0.1N Hydrochloric acid having pH 1.2 and buffer solution having pH 5.8 and it was found that, there was increase in swelling as the pH of medium for both polymer composition increased. In in vitro drug release study, it was obsrved that, formulation showed prolong release of the drug. Pectin containing formulation showed slow release of drug than formulation containing HPMC.[77]Floating microspheres of atenolol was prepared for prolong release of

drug.Preparation method for the microspheres was solvent evaporation method. Ethyl cellulose and Eudragit S100 were utilised as polymers in varios concentrations. The drug and polymer solution was mixed in solution of ethanol and dischloromethane and this solution was introduced into the polyvinyl alcohol. This emulsion was stirred about 1 hr at 300 rpm. The resultant solidified droplets filtered and dried to produce microspheres. All the physicochemical parameters were checked. Efficiency of Entrapment was observed to bein the range of 53 to 69% and percentage buoyancy was found to be in the range of 72 to 96%. Phosphate buffer solution was used for in vitro release of optimised formulation pH of buffer was 2.5, 4.5, and 6.5. The formulation showed 96% of release of drug in buffer of pH 2.5, 85% in pH of 4.5 and 76% in pH of 6.5 buffer solution. So , it was obsrved that, release of drug decreased p^H value increases. While comparing the drug release of optimised formulation with marketed formulation, prolong drug release was found because of presence of polyemrs like Eudragit S100 and ethyl cellulose.[78.]

The floating alginate beads of Dipyridamole (DP) were prepared with the help of method that is ionic cross linking method where calcium carbonatewas used and which acts as the gas generating. The method of preparation was devided into two phase, First, preparation of drug loaded solid dispersion. It is done by solvent evapouration method. Eudragit polymer was used for preparation of solid dispersion of (DP). Second step, formulation of floating alginate beads where this solid dispersion was added into the alginate solution and $CaCO_3$ solution. The resultant solution were poured in to the $CaCl_2$ acetic acid solution. The beads were formed which were stirred with magnetic stirrer. Beads were washed, collected and dried. All the physicochemical parametres were analysed like, particles size, drug loading, in vitro drug release, and which fornd withing range. After 9 hr, maximum of 92% of

theformulated beads were floating. In vivo study were also performed on healthy beagle dogs. In in vitro release of drug, it was observe that Eudragit RLPO and Eudragit L100 showed sustain drug release in stomach and it was observed that drug release lowered with increase in Eudragit L100 concentration. In in vivo study, bioavailability of the alginate beads was increased by 2.25 fold as compare to the marketed formulation.[79]

Cefuroxime Axetil (CA) which is the broad spectrum antibiotic and having poor solubility and in gastro intestinal tract it undergoes enzymatic degradation. Hence gastro retentitive floating drug delivery of CA was developed by using hot melt extrusion method (HME) to improve absorption. Gelucire 43/01 was used in the formulation which is the hard fat mixture of glycerides and PEG esters, it is highly hydrophobic in nature and having the low melting point which is beneficial in extrusion process. The floating formulation were analysed for the physicochemical parameters and also effects on various properties of grnaules were investigates. Flow property of the granules were good and floating was found to be more than 8 hrs. It was found that release of drug was in sustained manner for up to 12 hrs which indicates controls release and absorption of the CA. Due to the process applied i. e. HME, it allow the improvement in the absorption of the drug. Pharamcokinetic properties of the granules, marketed formulation and pure form of drug in rat were analysed. The optimised formulation found to have good bioavailability and longer residence time in the stomach and also it decreases the enzymatic degradation due to presence of lipid. Hence it was concluded that due to use of HME method, this drug delivery improves performance in vivo as well in vitro.[80]

Multiple unit floating drug delivery sytem was developed which is depending on the gas generation technique by which there is increase in residence time in GI tract and

bioavailability of formulation also increases. In this system, drug contains the small pellets which are manufactured by extrusion spheronization technique. Anhydrous theophylline was used as model compound. These were having double layers, inner layer was made up of effervescent material for which $NaHCO_3$ was used and outermost layer gas entrapped polymeric membrane which was made up of with colloidal polymer dispersion like Eudragit RL 30 D. By this system, optimised formulation floated within 3 min and buoyancy was maintained over 24 hours. All the physicochemical parameters like particles size, drug content, floating ability and dissolution study were evaluated. The floating capacity of the granules were depend upon the concentration of the effervescent agent which was the inner layer on the core pellets, and also on the type of the coating level on outer level. Due to the high water and low CO_2 permiabilities of Eudragit RL 30D and also with higher flexibility this systmen have ability to float completely in 3min and buoyancy over 24 hours[81]

Floating tablet of the famotidine was formulated which was consisted of floating pellets which are having coating with resin which is acrylic and has ability to delay resistance time in gastric region and improves the oral bioavailability. For preparation floating pellets, extrusion speronization technique was utilised. Which was formulated with steryl alcohol and microcrystalline cellulose. It was then made coating of resin. These pellets were undergo compression to form tablets Avicel PH301 and cross linked polyvinylpyrolidone. Eudragit RL 30D and RS 30D were utilised as coating fluids. Physicochemical parameters were evaluated. Tablets immediately disintegrates into pallets in 0.1N HCl solution and it was remained floating and released drug in sustain action over 12 hours. In vivo study was done on rats and it was compared with the marketed formulation of famotidine. The bioavailability of the optimised formulation was found to be significantly higher than that of the standard formulation.

The experimental results of this study indicated that retention in GIT and sustain release action of the famotidine is the best approach for enhancement of oral absorption of famotidine[82]

Ciprofloxacin Hydrochloride has narrow absorption window in gastro intestinal tract hence, GRDDS was developed to delay the gastric emptying time and thereby sustain release of the drug. In this formulation HPMC was used as polymer which gives sustain release action. Crospovidone, sodium starch glycolate and croscarmelose sodium was applied as swelling agents. $NaHCO_3$ was used as a effervescent substance. Ciprofloxacin HCl tablets were formulated by wet granulation technique. The formulated tablets undergo evaluation for hardness, friability, weight variation, thickness, water uptake, in vitro drug release, in vitro floating lag time, in vivo buoyancy study on human volunteers. Floating lag time was dependant on concentration of sodium bicarbonate and was optimised as 20 % in the formulation. All the parameters were found within range. Formulation containing HPMC K100M showed good tablet integrity, swelling and sustain release of drug. The simillaritiy factor for the optmisied formulation was found to be 26.71 and it was not similar to marketed product. In 5 hours about 92.8% of drug was released. In in vivo study, gastric retention was observed in few hours. The buoyancy time of the tablet in stomach was found to be 320 min and it was occurred due to gastric retention and swelling of the tablet[83.]

Due to low bioavialabiliy of the propranolol Hydro chloride, gastro retentitve drug delivery system was developed. Various polymers were used in this formulation with different concentration. Hydroxy propyl methyl cellulose (HPMC) K4 M , HPMC E15 LV, HPC, xanthum gum and sodium alginate. Tablet were formulated by direct compression method. $NaHCO_3$ was utilised as a gas producing agent, mannitol acts as

diluent and magnesium strearate was added as a lubrincant. The formulated powder mixture of the tablets were undergo evaluation of different parameters like flow properties which inludes angle of repose, bulk density, tapped density, compressibility index and Hausner ratio. Tablets were evaluated for hardness, friability, uniformity of drug content, in vitro buoyancy study, drug release and in vivo gastrointestinal residence time. Powder flow properties were found to be satisfactory and had acceptable flowing properties. Tablets which formulated with HPMC K4M and xanthum gum found to be good swelling ability and also showed good buoyancy. Floating lag time of this formulation was observed to be less than 1 min. Formulation which consist of 30% HPMC K4M undergo complete disintegration and drug release was obtained in 1 hour. Increase in concentration of polymer, floating property and drug release improved. Dug release was found to be 92% in 18hours. In in vivo evaluation, X ray technique was used which showed that tablet was stayed in the stomach for 4 hours[84.]

By combining approach of swelling and floating, direct compression method was utilised for production of tablets of Valacyclovir hydrochloride for enhancement of gastric residence of the tablet and thereby increases bioavailability. For preparation of this tablet polyethylene oxide (Polyox WSR 303) was used as the swelling agent. Sweliing enhancer i. e. sodium starch glycolate. Sodium bicarbonate was used as gas effervescent agets for floating. Physicochemical parameters of the tablet were tested. Hardness, friability, weight variation, % drug content, % swelling, % water uptake study, buoyancy lag time in in vitro, in vitro drug dissolution and drug release were performed. In vivo studies were performed on healthy albino rabbits. The optimised formulation with 15% polyox WSR 303 and 10% of sodium starch glycolate showed satisfactory results for swelling ability, floating lag time and drug release properties.

X ray imaging study was showing that tablets were floating in the gastric region of rabbit for more than 6 hours hence tablets had good retention ability in gastric region[85].

In gastro retentitive drug delivery system, potential use of natural gum was used in the tablet formulation of ziprasidone HCl. Locust bean gum was used as a natural gum in this formulation. Along with this gum, okragum and HPMC K4M was used as an independent variables. direct compression method was used and all physicochemical parameters of formulation like, hardness, variation in weight, in vitro drug release, drug content estimation etc. Swelling index, in vitro floatability, in vivo floatability and dissolution studies were also evaluated. Floating lag time was depending upon the concentration of polymer used. when level of HPMC K4 M increased in the formulation and okra gum level decreased, There was increase in floating lag time. Swelling index increased by increasing HPMC K4 M level. For total batches, drug release were found to be in range of 80 to 95%. At 8 hours and 24 hours, drug release was enhanced due to increased concentration of okra gum and locust bean gum, gel matrix viscosity increased and water diffusion in to core matrix decreases. In invivo floatability study, the optimised formulation flaotd in the gastric fluid for more than 24 hours. This study was the systemic approach for using natural polymers in the formulation of GRDDS and these sources can be considered to be a promising sustain release polymers[86].

GRDDS of ranitidine hydrochloride was developed and evaluated. For formulation, Guar gum, xanthum gum and Hydroxypropyl methyl cellulose was used as polymers which are having formation of gel properties. Sodium bicarboinate was used as gas forming ingredient in this formulation. In the profile of drug release and to enhance properties like floating, citric acid and stearic acid were used. These two ingredients

were used as an independent variables. Direct compression method was utilised for Tablets formulation. All evaluation parameters were determined. Formulation containing guar gum and xanthum gum, did not find sufficient strength while formulation with HPMC K4M formed tablets with good strength with entrapping CO_2 gas formation and persistant buoyancy. Due to the addition of the citric acid in formulation dissolution enhanced and due to addition of stearic acid, drug release profile improved. The buoyancy lag time of the optimised batch was obsrved to be 2min. In in vitro buoyant study, tablet remained floated for 8 hours[87.]

For furosemide, GRDDS was developed which consist of multiple units of mini tablets. This system is based on the formation of the gas technique. In this system, consist of core units which are made up of thesolid dispersion of drug and other ingredients. Direct compression method was used and then coated with two separate layers where a single layer was efferevescent layer and sodium carbonate was used. Other layer was applied by using polymers which consist of polymethacrylate and Eudragit RL30D. Triethyl citrate 10 % was used as the plasticizer in formulation. Floating study, drug release kinetics, in vitro dissolution study, gastric resisence time were determined on the optimised formulation.As the system consisting of minitablets in the core where coating was done with effervescent layer and then followed by layer of polymer, the system is highly permeable to the water for initiation of effervescent reaction and floating process rapidly occurred. About 12 % of the effervescent coating was done which is required for floating of tablets within minute. In the formulation where efferevescent layer coated with Eudragit RL30D was found to be slow drug release than it was uncoated efferevecent layer. Release of drug was depend on which polymer is used for coating and it was decreased when polymer concentration increased by 5 to 10%. The rapid floating and control release activity of the drug was

achieved by formulating the multiple units which are floating drug delivery system and it was found that these units stayed in the stomach for about 6 hrs[88].

Patents related to GRDDS [89]:

There are several patents reported on the development of GRDDS with various approaches. The details of patents are presented in table 5.

Table 5: Patents related to GRDDS[89]

Sr.No	Patent No	Type of Formulation	Approach
Patents related to high-density GRDDS			
1	US 4938967	Pharmaceutical formulation	Oral dosage form with dimension more than 2 mm comprising multiple subunits having a density between 2.5-2.7 g/ml which was achieved by incorporating 50% by weight of barium sulphate as a weighting agent.
2	US 4193985	Multiple Unit drug dose	Tablet or capsule system with multiple subunits containing a pharmaceutically active ingredient, some of it having higher specific weight when compared to that of the active ingredient.
Patents related to floating drug delivery system			
3	US 95611791	Controlled release floating pharmaceutical composition	Invention with multiple controlled-release coated particles with drug deposited on the surface of floating core and coating which is also controlled release.
4	US 9314430	Floating GR dosage form	It encompasses a cylindrical-shaped elongated device with opposite ends allowing floating owing to its specific shape and size.

5	US8277843	Programmable buoyant delivery technology	The system is made up of core and one additional layer of drug-coated over ore part and preformed hollow spaces and serves as spatially and temporally programmable.
6	US5626876	Floating delivery for oral therapy	It is a floatable, oral and therapeutic system and is light in weight than gastric fluid and so, it tends to float on the latter. It shows the difficulty to goes at lower lying pylorus.
Patents related to mucoadhesive and bioadhesive systems			
7	US8974825	A pharmaceutical composition of gastrointestinal delivery	It comprises at least two entities of which one is controlled in release and the other is bioadhesive.
8	US6306789	Carbomermucoadhesive granules intended for oral administration.	The system is composed of Carbomer granules and salt plus inert diluent and active drug for sustained effect into the GI tract.
9	US5900247	Mucoadhesive pharmaceutical composition for controlled release of API.	It deals with bioadhesive pharmaceutical composition allowing facilitating the prolonged release of API in the buccal cavity or symmetrically at ptelingual, nasal and viginal, rectal membrane.
10	US5472704	Pharmaceutical CR composition with bioadhesive properties	It is related to controlled release mucoadhesive medicinal agent and designed for administration by oral and other means like ocular, vaginal, periodontal.

Patents related to swelling and expandable system			
11	US9801816	GR dosage form for extended-release of acamprosate	Gastro retentive extended-release dosage form of acamprosate which is dispersed in a hydrophilic polymer. Upon swelling of the polymer matrix dosage form gets retained in the stomach under fed condition releasing active moiety for an extended period.
12	US9393205	GR tablets	It consists of monolithic tablets manufactured from drug and swellable polymers. Swelling and imbibitions of the polymer result in the floating of the tablets on gastric fluid and the drug is released in a controlled manner.
13	US7976870	GR oral dosage form with limited release of drug in lower GIT	This system consists of a matrix of biocompatible, hydrophilic, and erodible polymer with the drug which causes swelling as soon as it arrives in gastric fluids followed by a slow and gradual release of drug upon erosion of the polymers.
14	US6723340	Optimal polymer mixtures for GR tablets	This system involves the combination of poly (ethylene oxide) and HPMC which upon swelling causes gastro retention and drug release in a controlled fashion from tablets.
15	US6488962	Tablet shapes to enhance the gastric retention	In this system, the GR is achieved by special-shaped oral swellable dosage form
Patents related to the raft forming system			
16	US0063980	*In situ* gel formation of pectin	It involves in situ floating raft formation upon contact with gastric fluids

17	US01199941	Gastric raft composition	It consists of floating rafts that releases the drug in a controlled manner
Other miscellaneous patents related to GRDDS			
18	EP2575798	GR systems of GABA analogs	This GR system consists of swelling and non-swelling agent as release retardant
19	EP3148514	The expandable GR dosage form	This system unfolds rapidly when it arrives in- gastric fluid.
20	US9119793	GR dosage form of doxycycline	It involves the combination of floating, swelling, and bioadhesive properties.
21	US20160338949	Stabilized GR tablets of Pregabalin	This system contains a blend of swellable polymer and p^H modifier to form stabilized GR dosage form
22	US20150366832	GR dosage form for carbidopa and levodopa	Gastroretention is achieved by the use of polymer which are used in enteric coating are insoluble in GI fluids and swellable polymer which are hydrophilic in nature
23	US6797283	GR dosage form having multiple layers	This system consists of the multilayered active dosage form with swellable layer and drug layer

JUSTIFICATION

Calcium is the most noticeable and very important micronutrient of the body. It is also an integral part of our diet. Calcium's recommended dietary intake (RDA) for children is between 800 and 1,300 mg per day, for adults it is 1,000 mg per day, and for the elderly it is 1,200 mg per day. About 800 million people are malnourished globally, and almost 3.5 billion individuals are at danger of calcium insufficiency due to insufficient dietary availability[90]. Undernourishment and calcium deficiencies are thought to be responsible for global mortality for more than 6%. Calcium deficiency may slow down the growth and cognitive progress, hinder immune function, and raise the risk of diseases which are noncommunicable like skeletal, cardiovascular, and metabolic problems[91]. Lack of calcium in the body may cause fragile or frail bones, bone fractures, growth and development delays in infants, issues with blood clotting, heart complications affecting blood pressure and heart functions, osteoporosis, and many more[92]. Although certain patients experience gastrointestinal symptoms such as constipation, gas, flatulence, and bloating, calcium supplements are usually well tolerated. However, a significant number of elderly and pediatric patients find it difficult to take calcium supplements, resulting in patient noncompliance. Oral calcium is the first line of treatment for calcium deficiency[93,94]. Calcium supplementation is currently provided by calcium salts such as calcium carbonate or calcium citrate in the form of conventional tablets, liquid preparation like syrups, and parenteral preparation[95]. However, since calcium assimilation is pH-dependent, and also depedent on site, and it is restricted by mechanism which is carrier-mediated, these traditional oral formulations have low oral bioavailability[96]. Following oral administration, however, only about 30% of calcium which is available in elemental is absorbed and bioavailable[97]. Because of the activity of the carrier protein ‘calbindin'

at active absorption sites, soluble calcium is usually well-absorbed from the duodenum[98]. Traditional calcium tablets, on the other hand, have a low bioavailability so they can easily pass from the sites of absorption, causing only small portion of dosage form get absorbed. Furthermore, traditional formulations are likely to make the saturation of the duodenal proteins which acts as carriers, preventing maximum absorption of calcium dose and which results in low oral bioavailability. In this case, GRDDS for calcium is needed, with the potential to address the aforementioned limitations of traditional formulations. These techniques are more likely to float and stay close to the active absorption window, where they can release calcium at control rate. Because of its low pH, the gastric media will act as a sink for calcium during dissolution from the system. The new GRDDS will benefit not just oral calcium bioavailability, but also particular populations (elderly and children) who have swallowing difficulties will get benefited. We planed to conduct preformulation, formulation, and assessment of an expandable calcium dosage form based on these possible advantages of GRDDS over conventional formulations.

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Calcium Carbonate

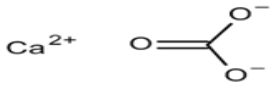
Description[99]:

It is inorganic salt which shows antacid activity by neutralisation of gastric hydrochloric acid. Calcium carbonate can also be used to treat hypocalcemia or as a dietary supplement. It is widely used and mostly prescribed as calcium substitute throughout the world due to its low cost. It is commercially available as chewable tablets, chewing gum, oral liquid, oral suspension, lozenges, and injections.

Physical Appearance[99]:

It is an odourless, colourless, and water-insoluble powder or mineral. It is found in several rocks all over the world. There are various forms available like precipitated and ground form of calcium carbonate.

Table 6: Physico-chemical properties of Calcium Carbonate[99, 100]

SR. No	Physico-chemical property	Description
1	Physical appearance	White colorless, odourless, microcrystalline powder
2	Chemical Name	Calcium carbonate
3	CAS registry number	471-34-1
4	Synonyms	Aragonite, Calcite, Milk of Calcium
5	Molecular weight	100.087
6	Molecular formula	$CaCO_3$
7	Density	2.7 to 2.95 g/cm^3
8	Chemical structure	

Solubility[100]:

Table 7: Solubility of Calcium Carbonate in various solvents

Sr. No	Solvent	Solubility Description
1	Water	Practically insoluble
2	Alcohol	Insoluble
3	Acetic acid	Soluble
4	Hydrochloric acid	Soluble
5	Nitric acid	Soluble

Pharmaceutical uses: antacid, abrasive, nutritional supplement, filler

Mechanism of action (MOA)[99, 100]:

It is inorganic salt which shows antacid activity by neutralisation of gastric hydrochloric acid that leads to form $CaCl_2$, CO_2 and H_2O. The generated $CaCl_2$ reduced to form salts of calcium including calcium phosphate. Pepsin activity is also inhibited through increase in pH and adsorption. It also shows the cytoprotective activity due to increase in prostaglandin and bicarbonate ion (HCO^{3-}) concentrations.

Pharmacodymanics[99, 100]:

The gastric peptic ulcer is caused due to the imbalance between defensive factors like bicarbonates, mucus, prostaglandins and offensive factors including pepsin, hydrochloric acid and *H. pylori.*

Antacids restore acid-base equilibrium by inhibiting pepsin production and increases the bicarbonate as well as prostaglandin secretions. Calcium carbonate has an acid-neutralizing potential of 58 meq/15 ml. Calcium carbonate is used as dietary supplement by specifically rising calcium deposits in the body.

Pharmacokinetics[99-100]:

Absorption:

The maximum absorption of Calcium carbonate happens at the dose of 500 mg or less. Oral bioavailability is influenced by the pH of the intestine, food presence in stomach, and the dose.

Distribution:

Calcium is readily absorbed by tissues of skeletal muscles after absorption and diffusion into extracellular fluids. About 90% of the calcium in the body is found in bones, with the remaining 1% distributed equally across intracellular as well as extracellular fluids.

Metabolism:

Calcium is made available for absorption after ingestion of tablets of $CaCO_3$ which forms conversion of soluble calcium salts in the stomach.

After consumption of $CaCO_3$ tablets, soluble calcium salts are produced in the stomach, and calcium becomes accessible for absorption.

Excretion:

It is found to be mainly excreted in the feces. The renally diluted Calcium is mainly absorbs from proximal and distal convoluted tubules, and ascending limb of loop of Henle. Sweat glands also produce this substance.

Indication:

Calcium carbonate is used for the treatment of acidity and heartburn. It is also used to treat hypocalcemia or as a dietary supplement. It can also be used in calcium deficiency, calcium metabolism disorders, dyspepsia, osteoporosis, gastric ulcers.

Role in protein binding:

It serves as a co-factor to various enzymes.

Drug interactions:

When Calcium carbonate is supplemented with Abciximab, its therapeutic effectiveness can be reduced. Calcium carbonate can reduce acetaminophen absorption, that leads to decrease in serum concentration and therefore reduction ineffectiveness. When Calcium carbonate is supplemented with Acenocoumarin, its medicinal effects can be reduced. Calcium carbonate's thrombogenic properties can be enhanced by an alpha-1 proteinase inhibitor.

Food interactions:

Calcium carbonate is more easily absorbed when consumed with food and can be beneficial when used as a calcium substitute.

2.1.2 Hydroxypropyl methylcellulose (HPMC) (K 100 M, E15LV)[101]:

Description: It is partly O-methylated and O-(2-hydroxypropyl) cellulose.

Physical appearance: its colour is white or creamy-white and texture is fibrous or granular with no odour or flavour.

Chemical name: Cellulose hydroxypropyl methyl ether

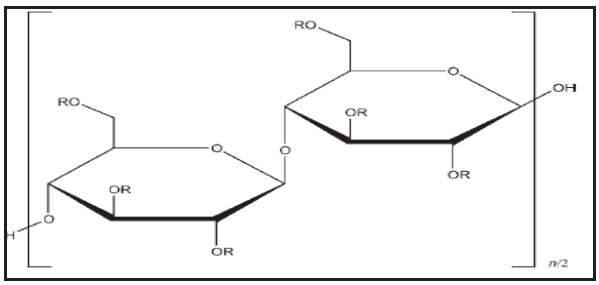
Synonyms: Benecel M HPC, Hypromellosum, Methocel, Methylcellulose propylene glycol ether.

CAS registry number: 9004-65-3

Molecular weight: 10000 to 1500000 g/mol

Empirical formula: $C_{56}H_{108}O_{30}$

Structural formula:



Melting point: 190° – 200°C

BD: 0.341 g/cm^3

Solubility: It is soluble in cold water and DCM mixture, methanol-DCM, mixture, water-alcohol mixture. It is practically not soluble in chloroform, ether, and 95% ethanol.

Pharmaceutical uses: Bioadhesive and control release polymer, emulsifier, film-former, viscosity increasing agent, dissolution enhancer, emulsion stabilizer, mucoadhesive polymer, binder in tablets.

Applications:

HPMC is used in various dental, dermal, nasal, and ophthalmic dosage forms. It is mainly used as suspending as well as a thicknening agent in dermal and topical formulations. The use of HPMC as emulsifying, suspending and stabilising agents in various lotions and gels are well known. It also helps in the prevention of sedimentation in liquid oral formulations. In solid oral tablets it acts as a binder, rate-controlling polymer, film coating agent.

Stability and storage conditions:

It has hygroscopic properties so HPMC powder is practically stable. Its solution is found stable between pH range of 3–11. As HPMC is heated or cooled, it undergoes a reversible sol-gel transition. Depending on the grade and concentration of the substance, the gelation temperature ranges from 50 to 90°C. powder of HPMC must be packed in tightly closed containers in cold and dry places.

2.1.3 Xanthan gum[101]:

Description: Xanthan gum is a polysaccharide gum with a heavy molecular weight with D-glucose, mannose as hexose groups as well as D-glucuronic acid. It is manufactured in various salt forms including calcium, potassium, and sodium. There

are five sugar residues in each xanthan gum repeat unit: 2 glucose, 2 mannose, and 1 glucuronic acid unit.

Physical appearance: It is a free-flowing fine powder with a cream to white colour. It is odourless.

Chemical name: Xanthan gum

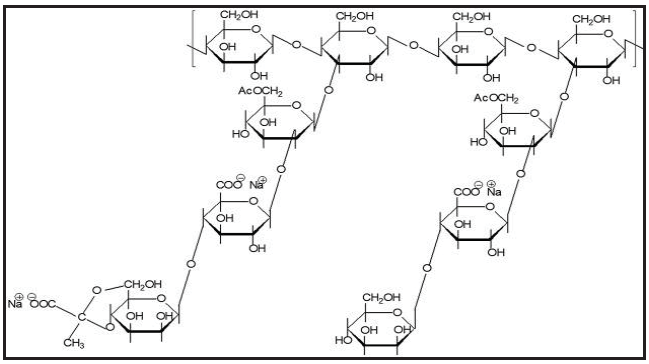
Synonyms: Corn sugar gum, Grindsted, Keldent, Keltrol

CAS registry number: 11138-66-2

Molecular weight: approximately 1 X 10^6 g/mol

Empirical formula: $(C_{35}H_{49}O_{29})_n$

Structural formula:



Melting point: 270°C

Dynamic viscosity: 1200–1600 mPa s (1200–1600 cP) for a 1%w/v aqueous solution at 25°C.

Solubility: It is soluble in cold as well as hot water and practically insoluble in ethanol and ether.

Pharmaceutical uses Stabiliser, viscosity modifying agent, release retarding polymer and suspending agent.

Applications:

Xanthan gum is used as a stabilizer and suspending agent in cosmetic, dermal, and topical formulations. It's also used to thicken and emulsify liquids. It is also act as

suspending agent in dry and suspensions with sustained release action . It is also used in manufacturing sustained-release capsules as primary use as a suspending agent. Along with Carbopol 974P, it is used as a mucoadhesive polymer in the buccal drug delivery system.

Stability and storage conditions:

The aqueous solution of xanthan gum is stable between the pH range of 3–12. But its stability was found greater between pH 4–10 and temperature range of 10-60°C. Higher than ambient temperatures can have a detrimental effect on xanthan gum solutions with a concentration of less than 1% w/v. It is to be kept in a well-closed container in a cool and dry place.

2.1.4 Sodium alginate (Protanal® LFR 5/ 60) [101]:

Description:

The sodium salt of alginic acid is a combination of polyuronic acids comprised of residues of D-mannuronic acid and L-guluronic acid, is the primary component of sodium alginate..

Physical appearance: It is nearly odourless, whitish-yellowish fibrous powder

Chemical name: Sodium alginate

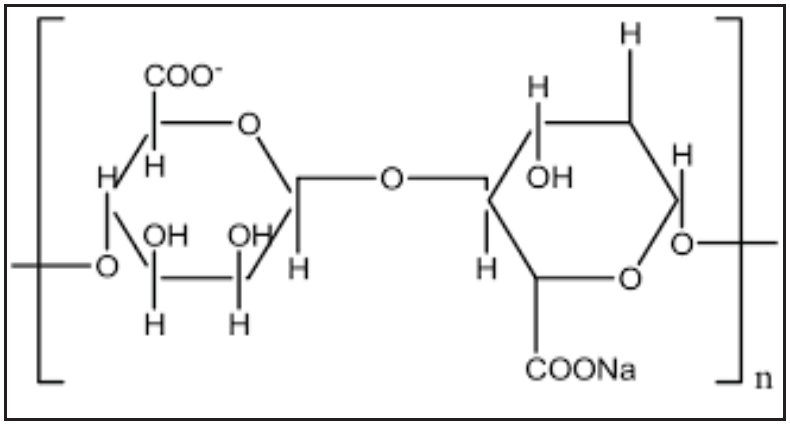
Synonyms: Sodium salt alginic acid, Protanal, sodium polymannuronate

CAS registry number: 9005-38-3

Molecular weight: 216.12 g/mol

Empirical formula: $C_6H_9NaO_7$

Structural formula:



Melting point: >300ºC

Dynamic viscosity: Viscosity of 1 % aqueous solution at 2°C ranges from 20-400 cP

Solubility: It is slowly soluble in aqueous media forming a colloidal viscous solution. Practically insoluble in acidic solution with pH less than 3 and in organic solvents like ether, chloroform water mixture and ethanol 95 %.

Pharmaceutical uses: Stabilizing and suspending agent, disintegrating agent in solid oral dosage forms, binder and viscosity modifying agent.

Applications:

It is often used in oral as well as topical dosage forms. In tablets, it is widely utilised as a binder and disintegrant. In capsules, it acts as diluents. It is also utilised as a sustained release polymer in various dosage forms to extend the dissolution of the dosage forms like suspensions, tablets, and capsules.

Stability and storage conditions:

Being hygroscopic it is to be stored at low temperature and humidity conditions. Aqueous sodium alginate solutions are most stable in the pH range of 4 -10

Alginic acid precipitates below pH 3. Metal containers cannot be used to store solutions. The bulk material should be kept cold and dry in an airtight container.

2.1.5 Sodium bicarbonate [101]:

Description:

It's a carbonic acid monosodium salt with alkalinizing and electrolyte replacement characteristics. It dissociates to form sodium and bicarbonate ions and elevates the pH of the blood due to an increase in bicarbonate and hydrogen ion concentrations.

Physical appearance:

It is crystalline, odourless powder and has a saline, slightly alkaline flavor. Monoclinic prisms are the crystal structure. Commercially available grades range from fine to granules with free flow with varying particle sizes.

Chemical name: Carbonic acid monosodium salt

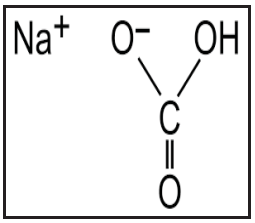
Synonyms: Baking soda, Effer-Soda, Monosodium carbonate

CAS registry number: 144-55-8

Molecular weight: 84.01 g/mol

Empirical formula: $NaHCO_3$

Structural formula:



Melting point: 270ºC

Bulk density: 0.869 g/cm^3

Solubility: It is soluble in water but practically insoluble in organic solvents like ether and 95 % ethanol.

Pharmaceutical uses: alkalizing and therapeutic agent.

Applications:

It is used as a source of CO_2 in effervescent formulations like tablet dosage form, granules, and powders. Apart from this, it is also used as an alkaliser in various formulations. It is also used as a buffering agent in lignocaine, erythromycin, and TPN solutions. It is used in the treatment of metabolic acidosis as an antacid as well as the source of bicarbonates. In raft forming floating alginate systems, it is act as a gas forming agent.

Stability and storage conditions:

It must be kept in a tightly-packed container and a cold and dry place. It is found stable in dry air but gets decomposed in moist air.

2.1.6 Talc [101]:

Description:

Talc is a clay mineral that is composed of hydrated magnesium silicate. Vey trace amounts of aluminum silicate and iron can be present in the talc.

Physical appearance:

Talc is a very fine crystalline powder with white to greyish-white, and odourless. It has excellent sticking properties on the skin having a very smooth touch and free from grittiness.

Chemical name: Talc

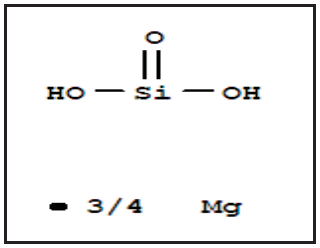
Synonyms: Hydrous magnesium calcium silicate, hydrous magnesium silicate

CAS registry number: 14807-96-6

Molecular weight: 379.27 g/mol

Empirical formula: $H_2Mg_3O_{12}Si_4$

Structural formula:



Melting point: 1500ºC

Surface area: 2.41-2.42 m^2/g

Solubility: It is practically insoluble in water, organic solvents, acids, and alkalies.

Pharmaceutical uses: glidant, lubricant, diluent, and anticaking agent

Applications:

Talc is a lubricant that is commonly used, adsorbent, and glidant in may formulations. Talc is utilised as a dusting powder in dermal and topical formulations.

Stability and storage conditions:

Talc is considered a very stable material but it is to be kept in an airtight, properly sealed container in a cool and dry place. Talc can be sterilised by ethylene oxide and gamma sterilisation techniques.

DRUGS / EXCIPIENT/SOLVENT SOURCE

Table 8: List of drug, excipients, and solvents with sources

SR. No	Name of Ingredient	Source
1	Calcium carbonate	Loba Chemie Pvt. Ltd, Mumbai
2	Barium sulphate	
3	Hydroxypropyl methylcellulose K100 M	Colorcon Asia Pvt. Ltd, Goa.
4	Hydroxypropyl methylcellulose E15 LV	
5	Protanal® (Sodium alginate)	FMC Biopolymer, USA.
6	Xanthan gum IP	Signet excipients Pvt. Ltd, Mumbai.
7	Sodium bicarbonate	S.D. Fine chemicals, Mumbai
8	Potassium dihydrogen orthophosphate	
9	Sodium hydroxide	
10	Sodium acetate	
11	Glacial acetic acid	
12	Hydrochloric acid	
13	Talc	
14	Magnesium stearate	Central drug house Pvt. Ltd, New Delhi.

Table 9: List of instruments and equipments

SR. No	Name of Ingredient	Source
1	FTIR spectrophotometer	Jasco 460 plus, Japan
2	Analytical balance (Model 220A XB)	Precisa, Switzerland.
3	Bilayer tablet rotary press	Ahmedabad, Gujarat
4	Digital Vernier caliper	Mitutoyo digimatic caliper, Japan
5	Monsato hardness tester	M/s Cambell Electronics, India
6	USP friabilator	Electrolab, Mumbai, India
7	Flame photometer	Systronics, Flame photometer 128, Ahmedabad, Gujarat).
8	Dissolution test apparatus-II	Electrolab, Mumbai, India
9	X-ray machine	Skanray Model: Microskan DR
10	GraphPad 5.0 Instat demo version software	GraphPad Inc. CA, USA
11	Rheometer	MCR-92, Austria, Europe

2.2 METHODOLOGY

Pre-formulation studies

2.2.1 Analysis of calcium:

To measure calcium, flame photometry is a well-established sensitive technique[102]. The device is a microprocessor-based device that can perform up to five points of calibration. By pressing the button, you can select a calcium-specific filter. Before use, the instrument was calibrated. The calibration curve was constructed in pH 1.2 using calcium standards with concentrations varying from 10 to 200 g/mL. For reproducibility of the results, the experiments were performed in a triplicate manner. The readings of all working standards were recorded and the graph was plotted against the concentration and the flame photometer reading. To validate the calibration curve's linearity, a regression analysis was done in Microsoft Excel software. All experiments were performed in triplicate to ensure the reproducibility of the results. Until data collection, The *in vitro* samples were sufficiently diluted in such concentrations range that they were within spectrum of beer. The calcium concentrations in the emission response (in ppm) of a flame photometer were converted to g/ml.

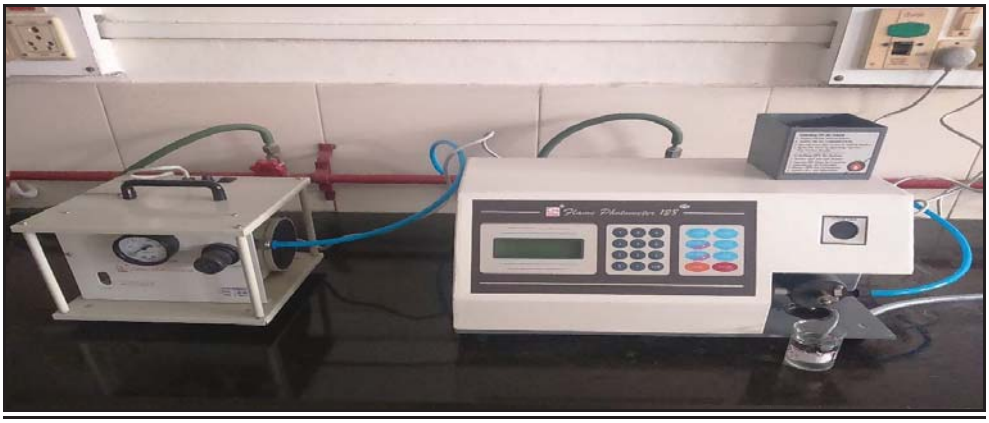


Figure 6: Flame photometer

2.2.2 Solubility studies of calcium salts:

The solubility of both calcium carbonate and citrate was performed in pH range of 1.2 to 7.4. The experiments were carried out in buffers at various pH levels. The excess amounts of calcium salts were added in conical flasks containing 10 ml buffer solutions (1.2, 4.0, 5.5, 6.8, and 7.4 pH). All samples were shaken on rotary flak for 72 hours at RT. The samples were filtered properly with Whatman filter paper No. 12 having a pore size of 0.45 micron to get a clear solution. These samples were diluted and analysed for concentration of Calcium using flame photometric analysis against the appropriate buffer as a blank. Solubility studies were performed in triplicate to ensure consistency.

2.2.3 Drug -excipients interaction study (FTIR Studies):

Infrared spectrophotometry was a valuable analytical technique for determining the chemical interaction within the formulation. The calcium carbonate along with other excipients was grinded and mixed with dry potassium bromide in a porcelain mortar and pestle. In an FTIR spectrophotometer, the sample in powder form was kept in the sampler and analysed in 4000-400 cm^{-1} wavelength. IR spectra of calcium carbonate were compared with spectra of the mixture to ensure no interaction between Calcium carbonate and excipients.

2.2.4 PREFORMULATION STUDIES FOR GASTRORETENTIVE TABLETS:

Selection of manufacturing process:

A comparative study was carried out for the selection of manufacturing process. Preliminary batches were formulated by utilizing different binders and binder concentrations using dry (direct compression) and wet granulation techniques. The wet granulation process produced preliminary batches with satisfactory hardness and tablet strength.

Preparation of gastroretentive tablets:

The wet granulation technique was used to manufacture calcium carbonate tablets without any controlled-release polymers. Calcium carbonate was mixed properly in mortar & pestle and granulated using a 5% w/v aqueous solution of HPMC. By using sieve no 20, damp mass was slightly pressed from it and the granules were subjected to dry in dryer at 60°C. The formulated granules were forcefully sized through sieve no 30. Lubrication was done with magnesium stearate and talc. Granules were compressed by using caplet-shaped punches on Mini tablet press - I.

Screening of the polymers:

For the formulation of gastroretentive tablets, several viscosity grades of HPMC were chosen, including HPMC K4M, HPMC K15M, HPMC K100 M, and HPMC K100 M LV. In addition, the developed formulations were tested in vitro to check floating time and calcium release characteristics to determine the best polymer combination for the dosage forms. The formula composition with different grades of HPMC is represented in Table 10.

Table 10: Ingredients of gastroretentive calcium carbonate tablets with various HPMC grades.

SR. No	Excipients	F1	F2	F3	F4
1	Calcium Carbonate	500	500	500	500
2	HPMC K4M	50	-	-	-
3	HPMC K15M	-	50	-	-
4	HPMC K100M	-	-	50	-
5	HPMC K100MLV	-	-	-	50
6	Magnesium Stearate	5	5	5	5
7	Talc	5	5	5	5

Manufacturing of gastroretentive tablets:

Wet granulation was used to develop the calcium carbonate gastroretentive tablets. Calcium carbonate was granulated with a water-based polymeric solution. By using sieve no 12 the damp mass was slightly pressed from it and the granules were subjected to dry in an oven at 60°C for 30 minutes. The sizing of the dried granules was done through the same sieve. The dry calcium carbonate granules were lubricated for 2-3 minutes with magnesium stearate and talc in a polybag. The lubricated blend was undergo compression and further used for characterization.

Evaluation of tablets

The manufactured tablets were characterized for hardness test, friability, *in vitro* buoyancy, and *in vitro* Calcium release.

Formulation of bilayer gastroretentive tablets:

Bilayer tablets were formulated by using – i) Buoyant layer (swellable polymer + effervescence substance) and ii) Calcium layer.

Buoyant layer:

Various grades of HPMC (K4, K15, K100 M) were employed as swellable polymers to increase the floatability of the dosage form. HPMC K100 M was used because it is often used as a low-density hydrocolloid system that forms a hydrogel layer upon contact with water and swells up to 40.32% after 6 hours. Carbon dioxide is produced as a consequence of a chemical reaction when sodium bicarbonate and HPMC come into contact with the GI contents, and it is kept inside the gellified hydrocolloid system, decreasing the flotation lag time. As a result, the dosage form has a lesser density than stomach fluid, resulting in an upside movement of the formulation that keeps buoyancy for a longer period of time.

Buoyant layer optimization:

This layer was optimized by varying ratios of the HPMC K100M: Sodium bicarbonate. The ratios are presented in the following table.

Table 11: Optimisation of HPMC K100M: Sodium bicarbonate ratio

S. No	HPMC K100M	Sodium bicarbonate
1	1	1
2	2	1
3	2	2

Drug layer optimization:

For the complete release of the calcium from the calcium carbonate layer within 6 hrs of dissolution, calcium carbonate granules were prepared with different granulating agents like PVP K30, HPMC 15cps, and HPMC E15 LV. The concentrations of granulating agents are presented in the below table no 12.

Table 12: Concentration of granulating agents

SR. No	Granulating agent	Concentration
1	PVP K30	8 % w/v
2	HPMC 15 cps	8 % w/v
3	HPMC E15 LV	8 % w/v

Bilayer tablet manufacturing procedure:

All ingredients were weighed accurately and sifted using a 250 µ sieve. Direct compression technique was used to manufacture floating layer using various grades of HPMC and sodium bicarbonate. The layer of calcium was manufactured by the wet granulation technique by use of granulating agents mentioned in Table 12. Calcium carbonate was mixed with a aqueous solution of granulating agent. The sieve no 12 was used to pass the moist stuff and these prepared granules subjected to dry in oven at 60°C for the period of 30 minutes. The sizing of the dried granules was done

through the same sieve. The floating layer and dry calcium carbonate granules were lubricated separately for 2-3 minutes with magnesium stearate. The lubricated blend was undergo compression using bilayer tablet rotary press machine using die of 9 mm diameter at a weight of 420 mg with hardness of 5-7kg/cm^2.

2.2.5 FORMULATION STUDIES OF GASTRORETENTIVE FLOATING BILAYER TABLETS:

Formulation of gastroretentive floating bilayer tablets of calcium carbonate using 3-factor 2-level d-optimal mixture design:

Design of experiment (DoE)

A 16-run, three-factor, two-level D-optimal mixed design was created in this investigation using design expert software (Version.11). The goal of this study was to see how three independent variables interacted. These variable are: X_1 a swellable polymer (HPMC K100 M), X_2 a gas-forming agent (Sodium bicarbonate), and X_3 a binder (HPMC E15 LV) – on calcium carbonate release rates, swelling indices, and floatation lag times, as well as to develop an optimized double-layer gastro retentive preliminary experiments guided the selection of each component's collection.

Table 13: Details of dependent and independent variables with ranges in D-optimal design.

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

Bilayer tablet manufacturing procedure:

All ingredients were weighed accurately and sifted using a 250 µ sieve. Direct compression technique was used to manufacture floating layer using sodium bicarbonate and HPMC K 100 M. Wet granulation was used to develop the calcium carbonate layer. Calcium carbonate was mixed with a water-based HPMC E15 LV solution. By using sieve no 12 damp mass was slightly pressed from it and the granules were subjected to dry in an oven at 60°C for 30 minutes. The sizing of the dried granules was done through the same sieve. The floating layer and dry calcium carbonate granules were lubricated separately for 2-3 minutes with magnesium stearate and talc. The lubricated blend was undergo compression using bilayer tablet rotary press machine using die of 9 mm diameter at a weight of 420 mg with hardness of 5-7kg/cm^2. The following table no 14 shows the formula composition of 16 batches as per design.

Table 14: Formula composition (in mg) of the bilayer tablets as per D-optimal design.

Run	Weight X1*	Weight X2*	Weight X3*	$CaCO_3$	Mg. Stearate	Talc	Total wt.
F1	77.00	73.41	3.59	250	6	10	420
F2	98.95	52.75	2.33	250	6	10	420
F3	119.61	30.80	3.61	250	6	10	420
F4	121.66	30.80	1.55	250	6	10	420
F5	97.79	51.59	4.65	250	6	10	420
F6	77.00	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	3.10	250	6	10	420
F12	119.61	30.80	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	3.10	250	6	10	420
F16	77.00	73.41	3.61	250	6	10	420

X_1= HPMC K100 M, X_2= Sodium bicarbonate, and X_3= HPMC E15 LV. $X_3$was used at 8% w/v as a binding solution in formulations

Evaluation of granules:

The bulk density (BD), tapped density (TD), compressibility index (CI), Hausner's ratio (HR), and angle of repose were evaluated by use of lubricated blend of Calcium carbonate.

Angle of repose (θ):

For determining the angle of repose, the funnel method was utilised. In the funnel, a weighed quantity of lubricated granules was kept and regulated at a certain height such that the heap of powder just reached the funnel's tip. The heap's diameter was calculated and the following formula was used to calculate the angle of repose.

$$\tan\theta = \frac{h}{r}$$

Where,

h = Height of the pile

r= radius of the base

Bulk density (BD):

Accurately weighed lubricated granules were slowly poured into a measuring cylinder of 50 ml, and the bed was made uniform without disturbing. The volume was measured in millilitres, and the BD was calculated using the formula below.

$$BD = \frac{\text{mass of sample in g}}{\text{volume occupied by sample in ml}}$$

Tapped density (TD):

Lubricated granules were taken and weighed accurately and poured in a measuring cylinder placed in a bulk density tester. The initial volume occupied by the sample was noted it was tapped (50-100-250 times) till no change in the volume was observed and noted as tapped volume.TD was determined using the following formula

$$TD = \frac{\text{Mass of sample in gm}}{\text{tapped volume occupied by sample in ml}}$$

Compressibility Index (CI):

The CI was calculated using the formula below.

$$CI = \frac{TD - BD}{TD} X\ 100$$

Hausner's Ratio (HR):

HR was calculated with the help of below formula.

$$HR = \frac{TD}{BD}$$

Evaluation of bilayer tablets:

Thickness and diameter:

For the determination of diameter and thickness Digital vernier caliper was used. For this test, 10 tablets were randomly selected[102]. The dimensions were calculated in millimeters. The diameter, thickness, and standard deviation were measured.

Hardness:

The hardness of the tablets was measured on randomly selected ten tablets using Stokes Monsanto hardness tester[103]. The average, as well as standard deviation, was calculated.

Friability:

For this test, 20 tablets were chosen at random from individual batches, and the test was run for 100 rotations on an automatic friabilator. The weight of dedusted tablets was recorded and friability was determined and calculated as the mean of three determinations. The tablets with weight loss of less than 1 % were usually deemed to be suitable[104].

Content uniformity:

A content uniformity test was conducted according to USP protocol on 10 tablets randomly selected from each batch.[105]. these tablets were subjected to crush and kept in a buffer having pH 1.2 for 24 hours to equilibrate. Filtration was done through 0.45µm filter followed by appropriate dilution to estimate Calcium carbonate content with flame photometer.

Weight Variation:

The official procedure was used to calculate the weight variation of each batch[106]. Twenty bilayer tablets were chosen at random and their weights were measured accurately in milligrams. The mean, as well as standard deviations, were determined.

Floating lag time:

Floating lag-time is the amount of time it takes for the tablet to rise to the surface of the medium and stay there[107]. For determination of this, dissolution test apparatus was used (USP type II) where pH 1.2 buffer (900 ml) was used and temperature maintained at 37°C ± 0.5°C at 50 rpm. The results were determined in a triplicate manner for every batch of formulated bilayer tablets.

***In-vitro* release studies:**

Dissolution test apparatus was used (USP type II) to determine release of calcium where it contains pH 1.2 buffer (900 ml; 37°C ± 0.5°C) at 50 rpm for the period of 6 hours[108]. The samples of 5 ml were taken out at predetermined time interval (for 6 hours at interval of 1 hour) and replenished with same amount of freshly prepared buffer for maintaining the sink condition. By using 0.45 μm filters the samples were filtered to get clear solutions and Calcium content was determined using a flame photometer.

***In vitro* release kinetics:**

The release mechanism of Calcium from developed tablets was determined by employing zero order[109], first order[110,] and Higuchi kinetic models[111]. Zero-order release kinetics was determined by plotting a graph between the cumulative amount of calcium release and time. Graph plotting was done between log cumulative Calcium remained and time for first-order release kinetics while percentage of Calcium released against square root of time was plotted for Higuchi kinetic.

Stability study:

The stability study of optimised tablets was performed at real-time stability conditions for 6 months. The samples were packed in aluminum foil and loaded for stability testing. The samples were taken out at 1, 3, and 6 months to check the floating lag time, dissolution, and physical characteristics. The results were compared with initial data and the stability of the samples was predicted.

***In vivo* X-ray Imaging Studies:**

The gastroretentive floating behaviour of the optimised formulation was assessed using X-ray imaging in New Zealand white strain rabbits. The institutional animal ethics committee (IE-52, dated 12 Oct 2019) of Biosciences, Bengaluru, India, approved all animal studies. The rabbits, weighing 2 to 2.5 kg, two groups were prepared, each with four rabbits, and were kept at a temperature of 25°C±2°C and a RH of 55±5%, with normal feed and free access to tap water. The animals fasted overnight before the commencement of the experiments to avoid problems at the time of imaging. The first group of animals received an optimised formulation including barium sulphate which serves as a marker, whereas the second group (Control) received a regular tablet containing the marker. At time intervals of 0, 2, 4, and 6 hours, the animals were kept upright to image the position of both control as well as GR tablets in the GI tract using X-ray equipment.

Statistical analysis:

ANOVA was used to statistically interpret the data collected during the in vitro and *in vivo* trials using GraphPad 5.0 Instant demo edition software. The data so obtained was considered statistically significant when $p < 0.05$.

2.2.6 PREFORMULATION STUDIES FOR ORAL RAFT FORMING IN-SITU GELLING SYSTEM:

Screening of the polymers:

Various polymers were screened for the development of raft system and selection was done on the ability of gelation at lower concentrations and pourability of the preparations. The polymers were evaluated at higher and lower concentrations for pourability, gel strength, and gel consistency. The details of polymers and concentrations used are presented below the table.

Table 15: Screening of polymers at different concentrations for raft system

S. No	Polymer	Conc. of polymer (%)	
		Low	High
1	Sodium alginate	2	5
2	Gellan gum	0.5	2
3	Karaya gum	1	10
4	Guar gum	0.5	2.5
5	Xanthum gum	0.5	2.5

Screening of calcium release retardant polymers:

HPMC with varying viscosity grades like HPMC (K4M, K15M, K100M) and polyethylene oxide was procured and selected for preformulation trials as calcium release retardants at different concentrations. Based on their ability of gelation at lower concentrations and pourability of the preparations, the polymers had been finalized which has been represented in table no 16

Table 16: Screening of drug release retardant polymers

S. No	Polymer	Conc. of polymer (%)
1	HPMC K_{15} M	Low: 0.05
		High: 0.2
2	HPMC K_{100} M	Low: 0.1
		High: 0.2
3	HPMC K_{15} M + HPMC K_{100} M	Low: 0.05
		High: 0.1
4	PEO N-80	Low: 0.1
		High: 0.5

Manufacturing procedure of oral raft forming in situ gelling system:

The polymers under screening were dispersed in deionized water. Individually distributed volumes of release retardant polymers and other polymers mentioned in table 15 weighing 150 mg were calculated and added under continuous stirring until a homogeneous polymeric viscous solution was obtained. In the polymeric solution, calcium carbonate equivalent to 100 mg of calcium was eventually added.

2.2.7 FORMULATION STUDIES OF ORAL RAFT FORMING IN SITU GELLING SYSTEM

Development of oral raft forming in situ gel system for site-specific delivery of Calcium using simplex lattice design

Design of experiment (DoE):

Using a simple lattice design, the effect of independent factors on dependent variables was studied. The approach consists of two models and which are determined by doing the changes in their proportions at a similar time and the total was kept constant. The concentration of HPMC K100M (X1) and Xanthan gum concentration (X2) were taken as independent variables and used at 50 to 90 % and 10 to 50 % respectively so

that the total of the two components was 100%. A series of preliminary trials were used to evaluate the range of each variable. BLT (Y1), Release of Calcium at 1 hour (Y2), and 6 hours (Y3) were considered as dependent variables and represented in table no17.

Table 17: Experimental ranges and Independent variables as per simplex lattice mixture design.

Independent variables	**Low level (%)**	**High level (%)**
X1: Concentation of HPMC K100 M (% w/w)	50	90
X2: Concentation of Xanthan gum (%w/w)	10	50
Dependent variables	**Constraints**	
Y1: Buoyancy lag time (sec)	Minimize	
Y2: release at the end of 1st h (%)	Minimize	
Y3: release at the end of 6th h (%)	Maximize	

The total polymer (150mg), $CaCO_3$ (250mg),sodium alginate (250mg) in 5ml which is constant for all formulations

Manufacturing procedure of oral raft forming in situ gelling system:

Sodium alginate at a concentration of 5 % was dispersed under continuous stirring in deionized water. Individually distributed volumes of HPMC K 100 M and Xanthan gum amounting to 150 mg were calculated as per the design and dispersed with continuous stirring until a homogeneous viscous polymeric solution was obtained. In all batches overall the volume of Calcium carbonate and polymers was kept the same. Table 18 shows the batch compositions as per the selected design.

Table 18: Composition of the model formulations as per Simple lattice mixture design

Run	Sodium alginate*	HPMC K100 M*	Xanthan gum*	Calcium carbonate*	Purified water mL
F1	250	105	45	250	5
F2	250	75	75	250	5
F3	250	75	75	250	5
F4	250	90	60	250	5
F5	250	120	30	250	5
F6	250	135	15	250	5
F7	250	135	15	250	5
F8	250	105	45	250	5

** Each ingredient's weight is expressed in milligrammes.*

Characterization of oral raft forming in situ gelling system:

Gelling capacity:

As per the techniques reported in the literature, all formulations were subjected to determine the in situ gelling capacity[112]. In a test tube, 0.1 N HCl and purified water (5 ml each) were added at 37 ± 0.5°C, water bath was used to maintain temperature. The 1 ml test solution was pippet out and added slowly to the top surface of the solution. The gelation time for all formulations was recorded when complete gel formation took place.

Rheological studies:

Rotational calculations were used to test the rheology of gastro retentive raft forming solution (20 mL) to determine the flow properties using a rheometer attached (MCR 92) with a measuring device and fixture (CP50-1) at a distance of 0.1mm at 37 ± 0.5°C. Samples were maintained for one minute before actual measurements to allow for relaxation of sample stress and temperature homogeneity around the geometry of the double gap. Shear rate sweep analysis was used to examine the behaviour of the

flow of the samples, ranging from 10 to 100 (1/s) with a data collection period ranging from 15 sec to 5 sec on a logarithmic scale at a constant temperature of 37˚C.

Floating behaviour:

The 5 ml GRFS was dissolved in 0.1 N HCl (900 ml at 37°C) in paddle-type dissolution test apparatus and the floating behaviour of all formulations was assessed visually. Buoyancy lag time is the time required for formulation to float on the surface of the medium. The amount of time calculated for which formulation floats on the surface was considered as complete floating time[113].

***In vitro* release study:**

Dissolution test apparatus was utilised for the determination of Calcium release. Where pH 1.2 buffer (900 ml; 37°C ± 0.5°C) was used at 50 rpm for the period of 6 hours[114]. The 5 ml samples of GRFS were added in dissolution media and 5 ml samples were taken out at predetermined time interval (for 6 hours at interval of 1 hour) and replenished with equal amount of freshly prepared buffer solution for maintaining the sink condition. By using 0.45 µm filters samples were filtered to get clear solutions and Calcium content was determined using a flame photometer.

***In vivo* X-ray Imaging Studies**

This method was used for evaluation of the gastroretentive floating nature of the optimised formulation in New Zealand white strain rabbits. The institutional animal ethics committee (IE-52, dated 12 Oct 2019) of Biosciences, Bengaluru, India, approved all animal studies. The rabbits, weighing 2 to 2.5 kg, two groups of rabbits were prepared each with four rabbits and were kept at a temperature of 25°C±2°C and a RH of 55±5%, with normal feed and free access to tap water. The animals fasted overnight before the commencement of the experiments for avoiding problems at the time of imaging. In 1st group, they received an optimized formulation including

barium sulphate as a marker, whereas the second group (Control) received a $CaCO_3$ suspension (Calcimax plus) which contains the marker. At time intervals of 0, 2, 4, and 6 hours, the animals were kept upright to image the position of both control and optimised formulation in the GI tract using X-ray equipment.

DATA ANALYSIS PLAN

DATA ANALYSIS PLAN

This plan of data includes:

Triplicate data was produced for all *in vitro* trials and these were mentioned in mean ± standard deviation (S.D). Microsoft Excel software® was used for determination of Linear regression analysis for calibration studies. A special software i.e. design expert® v-10 was utilised for analysis of in vitro data which is obtained after experiments for production of models based on mathematics to establish the correlation within the dependent and independent variables. The results which were obtained from *in vivo* studies were compared statistically and then determination of level of significance using GraphPad 5.0 Instant demo edition software (GraphPad Inc. CA, USA) was done. Analysis was done for archived data i.e. analysis of variance (ANOVA) and $P < 0.05$ was considered as level of significance. In all *vivo* studies New Zealand white strain unisex rabbits were used and divided in to the two groups with four animals in each group with four animals in each group.

RESULTS

RESULTS

4.1 PREFORMULATION STUDIES OF CALCIUM:

4.1.1 Standard calibration curve of calcium carbonate using Flame photometry:

The calcium carbonate's calibration curve is presented in figure 7.

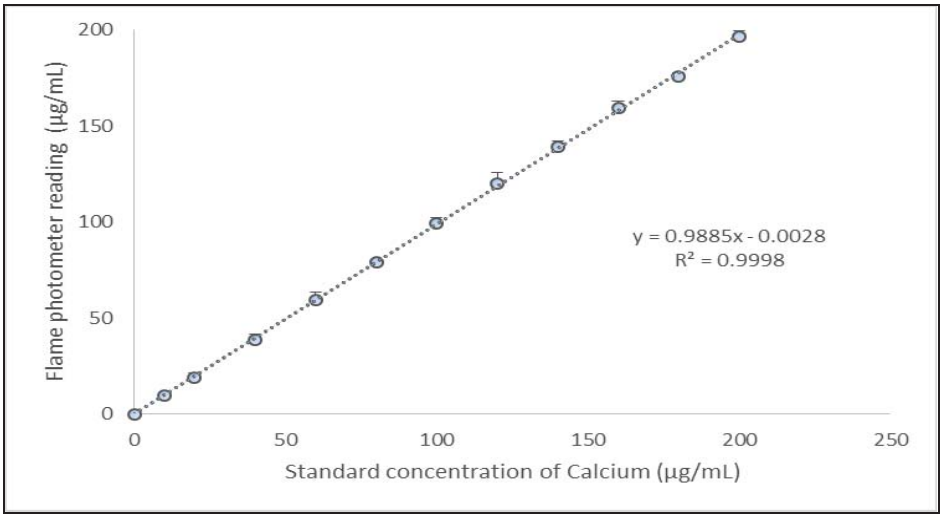


Figure 7: Calcium carbonate's calibration curve in pH 1.2.

Observation: The calibration curve of calcium carbonate in pH 1.2 buffer was observed linear within the range of concentration of 10 to 200µg/mL with the slope of 0.9885 and R^2= 0.998. The regression coefficient equation was presented by y= 0.9885x – 0.0028. Flame photometry analysis method was developed and which was found to be reproducible based on the lower standard deviation value.

4.1.2 pH solubility profiling of calcium salts:

Table 19 represents the comparative solubility of calcium carbonate and calcium citrate.

Table 19: Solubility data of the two salts of calcium in different Buffer solutions.

S. No	pH Buffer	Solubility ± S.D (mg/ml)	
		Calcium salts	
		Carbonate	Citrate
1	1.2 acid	1.602 ± 0.104	5.737 ± 1.299
2	4.0 acetate	10.254 ± 2.737	25.408 ± 11.621
3	5.5 acetate	17.092 ± 0.859	40.713 ± 2.974
4	6.8 phosphate	0.153 ± 0.065	12.557 ± 6.366
5	7.4 phosphate	0.081 ± 0.011	12.296 ± 5.160

Individual data shows Mean ± S.D of three determinations.

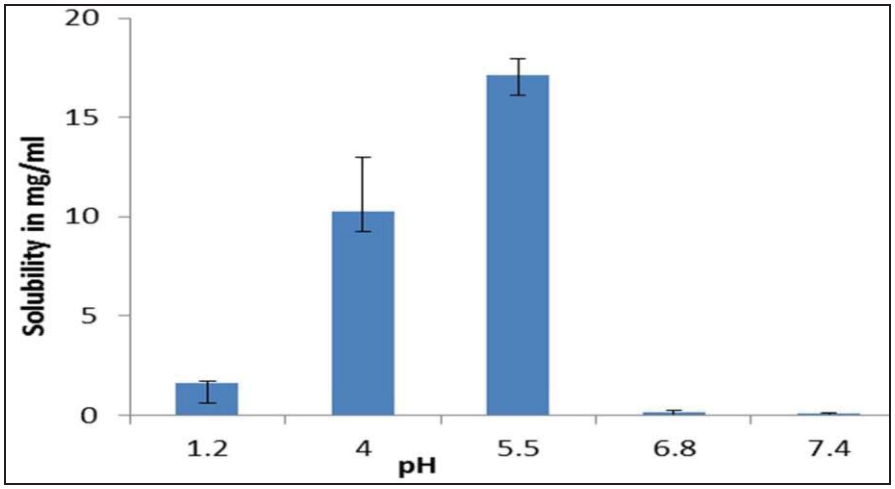


Figure 8: Effect of pH on Solubility profile of Calcium carbonate

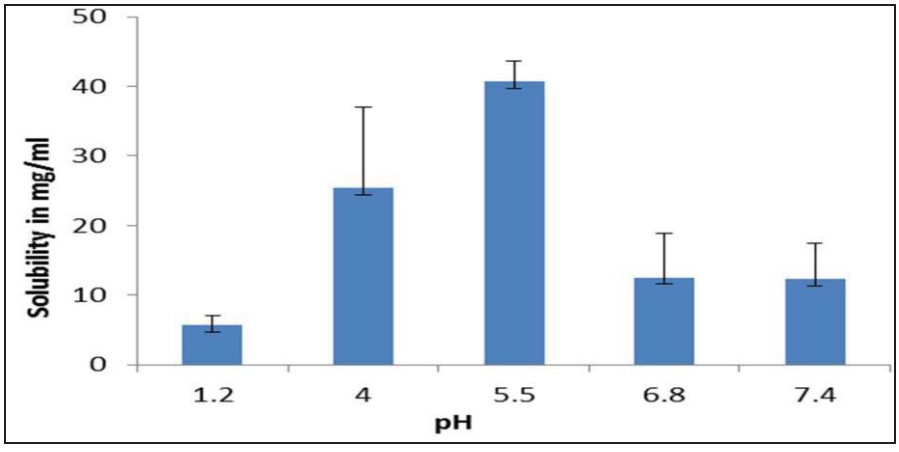


Figure 9: Effect of pH on Solubility profile of Calcium citrate

Observation: The data which is generated in solubility profile in the present study indicated higher solubility of citrate salt as compared to Calcium carbonate in all buffers as shown in Figure 8 and 9.

4.2 PREFORMULATION STUDIES OF GASTRORETENTIVE MATRIX TABLETS:

Table 20: Characterisation of gastroretentive tablets manufactured by wet Granulation method

Sr. No	Parameter	Observation
1	Hardness	6.8 kg/cm^2
2	Friability	< 1%.
3	Buoyancy test	Non floating; disintegrated within 2 hours
4	Calcium carbonate release	92.24% of calcium release over 2 hours

Observation: The *in vitro* Calcium release study showed nearly 92.24% of calcium release over 2 hours indicating the burst release.

Table 21: Characterisation of gastroretentive matrix tablets manufactured with various viscosity grade HPMC (Formulations F1 to F4)

Sr. No	Parameter	Observation
1	Hardness	5-7 kg/cm2
2	Friability	< 1%.
3	Buoyancy test	Non floating

Table 22 shows the Calcium release profile of various viscosity grades of HPMC in 0.1 N HCl for 6 hours. Figure 10 represents the comparative Calcium release from all four formulations

Table 22: Calcium release profile from batches manufactured with different viscosity grade HPMC polymer in 0.1 N HCl

Code	Polymer grade used	% CDR at 6 hours
Dissolution conditions	Appts: USP Type II (paddle), Media: 0.1 N HCl, RPM: 50, Volume: 900 ml, Temperature: 37 ± 0.5°C.	
F1	HPMC K4 M	45.536 ± 2.581
F2	HPMC K15 M	26.680 ± 1.813
F3	HPMC K100 M	21.513 ± 0.515
F4	HPMC K100 M LV	59.206 ± 3.870

Individual data shows Mean ± S.D of three determinations.

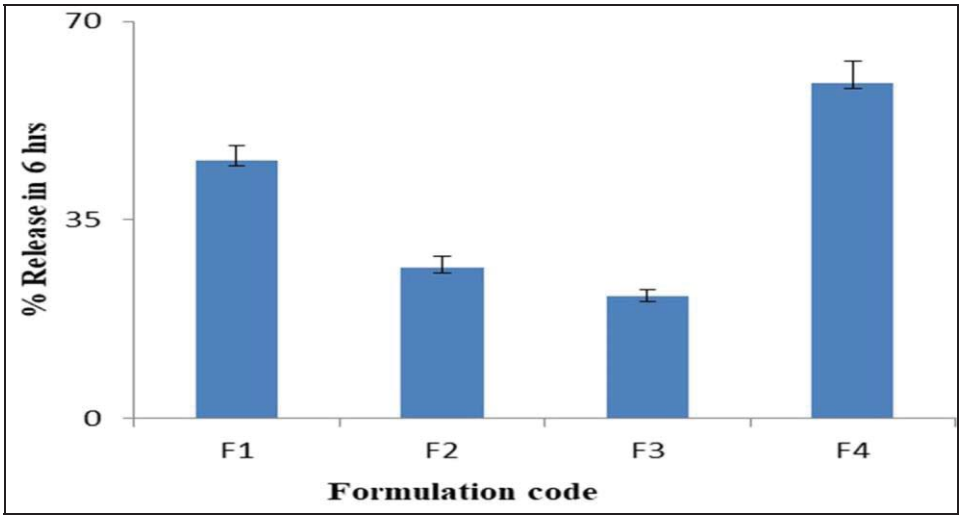


Figure 10: Comparative Calcium release profile of F1-F4 formulations

Observation: The developed set of 4 formulations (F1-F4) failed in terms of floating time and Calcium release. The detailed observations and reasons for the failure of the formulations are presented in table 22.

4.3 PREFORMULATION OF GASTRORETENTIVE BILAYER TABLETS:

Optimisation of HPMC K100M to Sodium bicarbonate ratio:

Table 23: Observation with different ratios of HPMC K100M: Sodium bicarbonate ratio

SR. No	Ratio of HPMC K100 M &Sodium bicarbonate used	Matrix integrity of the tablet	Floating time	Floating lag time
1	**1:1**	Lost	NA	NA
2	**2:1**	Better	Increased	Decreased
3	**2:2**	Good	Increased	Increased

Optimisation of Calcium layer:

Table 24: Observation with different granulating agents

SR. No	Granulating agent (8% w/v)	Hardness	% Release	Remarks
1	PVP K30	2-5 kg/cm^2	90 % within 2 hours	Separation of both layers during dissolution
2	HPMC 15 cps	5-7 kg/cm^2	50 % within 6 hours	Intact tablets during dissolution
3	HPMC E15 LV	5-7 kg/cm^2	80 % within 6 hours	Intact tablets during dissolution

4.4 FORMULATION OF GASTRORETENTIVE BILAYER TABLETS:

Granule's flow property:

Table no 7 and table no 8 shows granules flow property for various batches of model formulations and their post compressional characteristics respectively.

Table 25: Granule's flow property

Run	Angle of repose (θ)	Bulk Density (gm/cm^3)	Tapped Density (gm/cm^3)	Hausner Ratio	CI
1	27.528 ± 0.235	0.561 ± 0.032	0.634 ± 0.043	1.13 ± 0.021	11.51 ± 0.041
2	24.512 ± 0.290	0.567 ± 0.045	0.660 ± 0.057	1.164 ± 0.035	14.09 ± 0.019
3	27.210 ± 0.352	0.574 ± 0.058	0.652 ± 0.083	1.135 ± 0.031	11.96 ± 0.054
4	27.050 ± 0.252	0.582 ± 0.026	0.674 ± 0.048	1.158 ± 0.047	13.64 ± 0.027
5	24.625 ± 0.374	0.575 ± 0.048	0.680 ± 0.061	1.182 ± 0.053	15.44 ± 0.055
6	28.561 ± 0.380	0.624 ± 0.043	0.691 ± 0.053	1.107 ± 0.024	9.69 ± 0.043
7	22.605 ± 0.304	0.573 ± 0.045	0.660 ± 0.089	1.186 ± 0.048	13.18 ± 0.025
8	23.112 ± 0.239	0.557 ± 0.015	0.661 ± 0.097	1.175 ± 0.037	15.73 ± 0.049
9	24.840 ± 0.972	0.607 ± 0.057	0.667 ± 0.063	1.098 ± 0.043	8.99 ± 0.036
10	29.653 ± 0.784	0.605 ± 0.086	0.682 ± 0.049	1.127 ± 0.054	11.29 ± 0.028
11	28.462 ± 0.850	0.611 ± 0.048	0.679 ± 0.057	1.111 ± 0.029	10.01 ± 0.039
12	26.875 ± 0.377	0.571 ± 0.057	0.612 ± 0.123	1.129 ± 0.033	6.69 ± 0.042
13	25.912 ± 0.274	0.521 ± 0.079	0.661 ± 0.052	1.146 ± 0.045	21.18 ± 0.051
14	27.389 ± 0.674	0.593 ± 0.053	0.692 ± 0.075	1.167 ± 0.019	9.90 ± 0.012
15	25.427 ± 0.545	0.545 ± 0.039	0.645 ± 0.049	1.152 ± 0.051	15.50 ± 0.057
16	29.528 ± 0.255	0.565 ± 0.039	0.629 ± 0.033	1.139 ± 0.017	10.17 ± 0.030

Evaluation of the compressed tablets:

Table 26: Post-compression parameters of floating bilayer tablets

Run	Weight variation (%)	Diameter (mm)	Content uniformity (%)	Hardness (kg/cm^2)
1	0.422 ± 1.20	8.85 ± 0.01	100.29 ± 1.62	6.13 ± 0.25
2	0.419 ± 2.81	8.80 ± 0.07	100.25 ± 1.55	4.33 ± 0.50
3	0.415 ± 1.05	8.87 ± 0.03	98.17 ± 1.38	6.40 ± 0.43
4	0.417 ± 1.05	8.89 ± 0.06	99.58 ± 2.47	4.27 ± 0.38
5	0.415 ± 1.82	8.82 ± 0.02	99.44 ± 2.64	7.53 ± 0.19
6	0.419 ± 1.60	8.85 ± 0.01	100.53 ± 1.99	4.33 ± 0.09
7	0.420 ± 1.43	8.91 ± 0.05	101.87 ± 2.14	7.53 ± 0.19
8	0.418 ± 0.52	8.87 ± 0.05	99.87 ± 1.88	4.33 ± 0.50
9	0.419 ± 1.76	8.83 ± 0.03	100.91 ± 2.03	4.27 ± 0.19
10	0.420 ± 0.95	8.85 ± 0.08	99.54 ± 0.87	7.13 ± 0.25
11	0.421 ± 1.15	8.82 ± 0.09	99.79 ± 2.09	7.33 ± 0.09
12	0.420 ± 1.53	8.81 ± 0.03	100.66 ± 1.28	6.40 ± 0.43
13	0.420 ± 1.37	8.89 ± 0.04	100.89 ± 1.57	4.33 ± 0.50
14	0.419 ± 1.20	8.87 ± 0.06	100.28 ± 1.22	4.47 ± 0.05
15	0.418 ± 1.40	8.85 ± 0.09	100.63 ± 2.11	5.33 ± 0.09
16	0.419 ± 0.88	8.87 ± 0.01	100.13 ± 2.49	6.13 ± 0.25

Observation: It was found that, content uniformity and uniformity for all batches of bilayer tablets complied with official test.

Calcium excipient compatibility studies using FTIR:

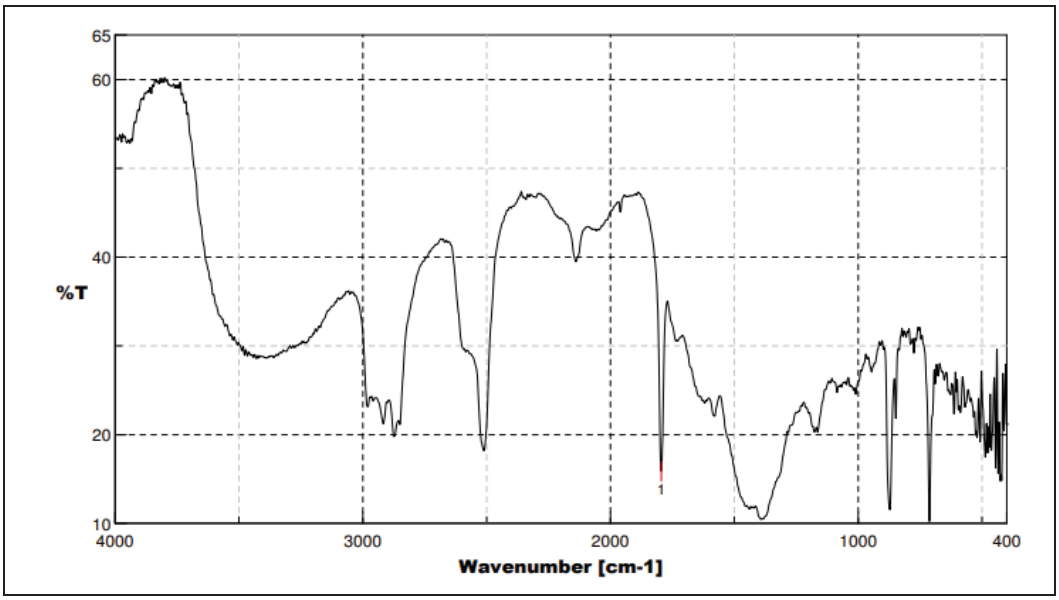


Figure 11: FTIR spectra of Calcium carbonate

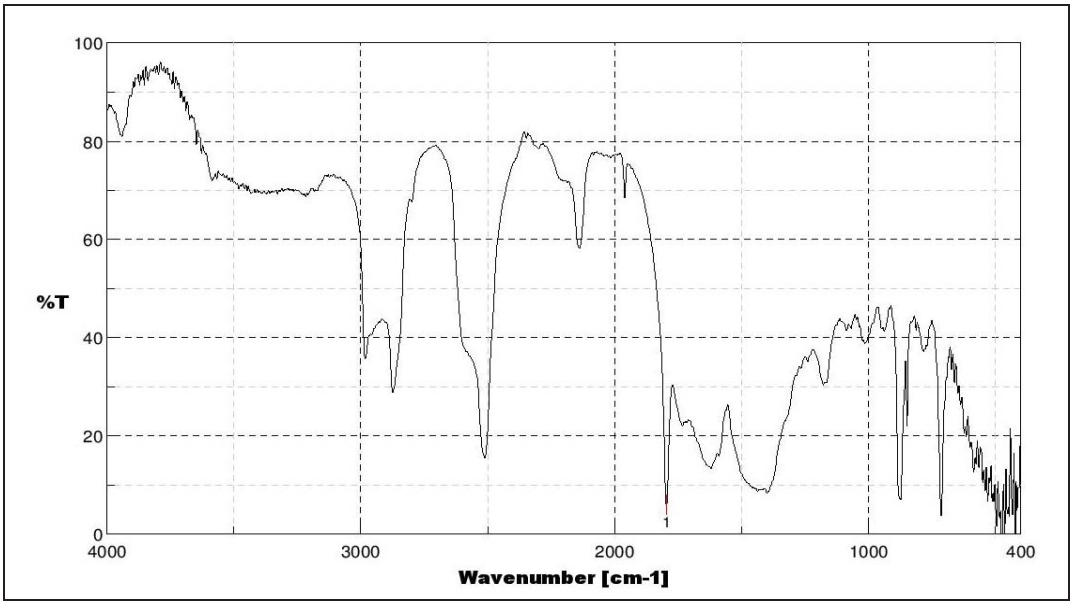


Figure 12: FTIR spectra of combination mixture of CC along with various excipients utilised in the manufacture of gastroretentive bilayer tablets

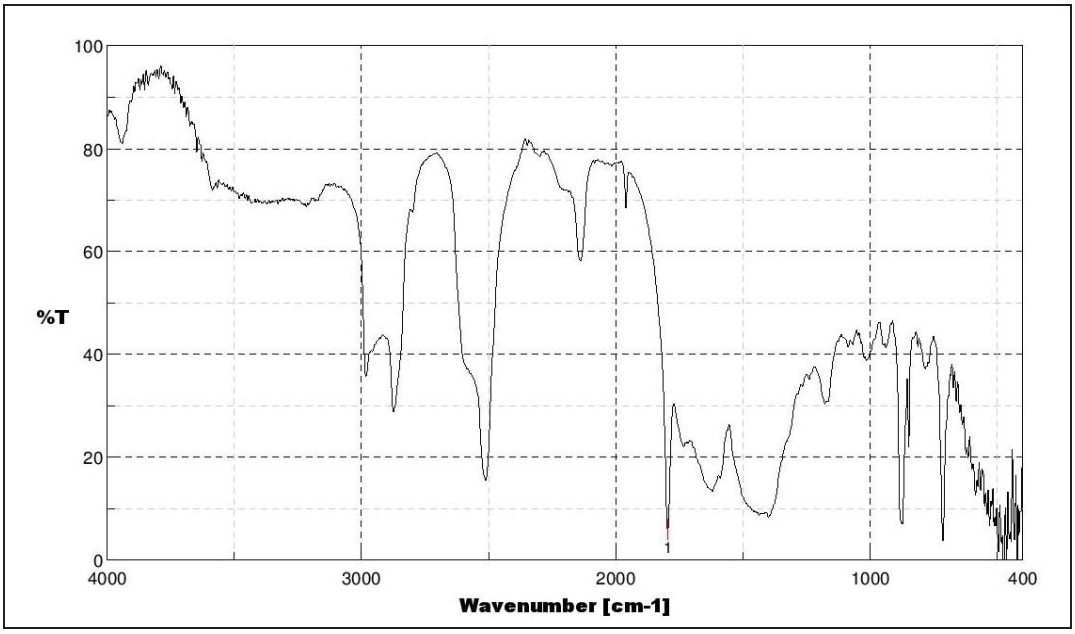


Figure 13: FTIR spectra of bilayer tablet of CC

Model fitting and statistical analysis of dependent variables (Y1, Y2, Y3, Y4):

Data obtained from experiments with varying the concentrations of independent variables are presented in table 27 which shows the collective effect of independent variables on dependent variables.

Table 27: parameters of Response for optimised formulation of calcium carbonate floating bilayer tablets created according to D - optimum mixture design.

Run	X1 (%)	X2 (%)	X3 (%)	Y1 (%)	Y2 (min)	Y3 (%)	Y4 (%)
F1	50.00	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	1.50	0.25 ± 0.03	6.12 ± 0.40	38.75 ± 0.63	87.48 ± 2.41
F3	77.67	20.00	2.33	0.03 ± 0.01	12.47 ± 0.15	22.47 ± 0.35	82.41 ± 1.28
F4	79.00	20.00	1.00	2.53 ± 0.31	3.59 ± 0.21	53.85 ± 2.58	86.33 ± 2.57
F5	63.50	33.50	3.00	0.13 ± 0.02	37.88 ± 0.74	15.87 ± 2.54	54.20 ± 0.58
F6	50.00	49.00	1.00	2.64 ± 0.28	4.14 ± 0.08	54.82 ± 2.13	86.56 ± 1.55
F7	63.50	33.50	3.00	0.15 ± 0.02	37.54 ± 0.42	16.49 ± 0.31	54.08 ± 0.63
F8	64.25	34.25	1.50	0.25 ± 0.03	6.57 ± 0.26	36.07 ± 2.65	87.22 ± 1.19
F9	59.67	39.33	1.00	2.68 ± 0.18	2.85 ± 0.18	56.60 ± 1.59	82.29 ± 0.88
F10	59.00	38.00	3.00	0.10 ± 0.01	6.55 ± 0.57	15.01 ± 0.45	56.12 ± 2.03
F11	71.00	27.00	2.00	0.44 ± 0.10	17.31 ± 0.09	31.95 ± 2.89	86.31 ± 1.75
F12	77.67	20.00	2.33	0.02 ± 0.01	12.76 ± 0.60	21.70 ± 1.26	81.24 ± 0.27
F13	64.25	34.25	1.50	0.25 ± 0.03	4.74 ± 0.81	37.28 ± 1.87	88.72 ± 0.92
F14	69.33	29.67	1.00	2.77 ± 0.64	4.99 ± 0.96	55.61 ± 1.28	88.57 ± 2.80
F15	57.00	41.00	2.00	0.40 ± 0.10	36.55 ± 0.47	26.19 ± 1.54	85.19 ± 1.29
F16	50.00	47.67	2.33	0.03 ± 0.01	36.42 ± 3.59	24.85 ± 1.42	82.08 ± 1.45

X1 is concentration of HPMC K100 M, X2 is concentration of $NaHCO_3$ and X3 represents concentration of HPMC E15 LV. The quantity of X3 was 8% w/v binder solution in the bilayer tablets* individual data point represents mean ± S.D where n=3. The real responses and polynomial equations for Y1, Y2, Y3 and Y4 with respect to the actual factors were used to predict the models and analysis was done statistically with the help of Analysis of Variance (ANOVA) as presented in table 28.

Table 28: Response parameters and its summary of ANNOVA for optimised formulation of Bilayer tablets formulated as per D-optimal mixture design.

Response	F-value	p-value	R^2	Adj R^2	% C.V.
Y1	1544.43	<0.0001	0.9996	0.9989	2.81
Y2	160.02	<0.0001	0.9959	0.9896	4.36
Y3	527.56	< 0.0001	0.9987	0.9968	2.68
Y4	125.72	<0.0001	0.9843	0.9765	2.41
Equations regression for the fitted model which contain significant terms only:					
Friability = + 0.18*X1 + 0.32*X2 – 17897.32*X3 ……. (1)					
In FLT = + 0.24*X1 + 1.19*X2 – 25289.06*X3……(2)					
Rel_{1h} = + 0.40*X1 + 4.38*X2 - 64542.84*X3 ……(3)					
Rel_{6h} = + 0.74*X1 + 0.88*X2 -1539.69*X3…….(4)					

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

Friability Test:

Table 29: Friability results of bilayer tablets

Formulation	Friability Y2 (%)	Result
F1	0.02 ± 0.01	Pass
F2	0.25 ± 0.03	Pass
F3	0.03 ± 0.01	Pass
F4	2.53 ± 0.31	Fail
F5	0.13 ± 0.02	Pass
F6	2.64 ± 0.28	Fail
F7	0.15 ± 0.02	Pass
F8	0.25 ± 0.03	Pass
F9	2.68 ± 0.18	Fail
F10	0.10 ± 0.01	Pass
F11	0.44 ± 0.10	Pass
F12	0.02 ± 0.01	Pass
F13	0.25 ± 0.03	Pass
F14	2.77 ± 0.64	Fail
F15	0.40 ± 0.10	Pass
F16	0.03 ± 0.01	Pass

The surface response plot presented in figure 14 shows the effects of independent variables (HPMC K100 M concentration and binder concentration) on friability.

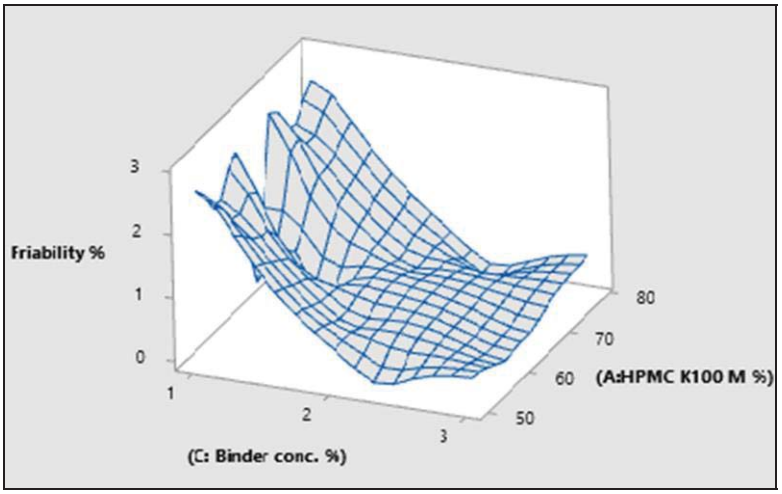


Figure 14: Plots of response surface which shows the effects observed by interaction of dependent Variables on friability

Floating lag time:

The floating lag times of the all batches are presented in **table 30.**

Table 30: Floating lag times of the bilayer tablets

Formulation	Floating lag time Y2 (min)	Observation
F1	38.47 ± 2.16	Increased Floating lag time
F2	6.12 ± 0.40	Acceptable floating lag time
F3	12.47 ± 0.15	More Floating lag time
F4	3.59 ± 0.21	Batch failed in friability
F5	37.88 ± 0.74	Increased Floating lag time
F6	4.14 ± 0.08	Batch failed in friability
F7	37.54 ± 0.42	Increased Floating lag time
F8	6.57 ± 0.26	Acceptable floating lag time
F9	2.85 ± 0.18	Batch failed in friability
F10	6.55 ± 0.57	Acceptable floating lag time
F11	17.31 ± 0.09	Increased Floating lag time
F12	12.76 ± 0.60	Increased Floating lag time
F13	4.74 ± 0.81	Acceptable floating lag time
F14	4.99 ± 0.96	Batch failed in friability
F15	36.55 ± 0.47	Increased Floating lag time
F16	36.42 ± 3.59	Increased Floating lag time

The surface response plot presented in figure 15 shows the effects of independent variables (sodium bicarbonate concentration and HPMC K100M concentration) on floating lag time.

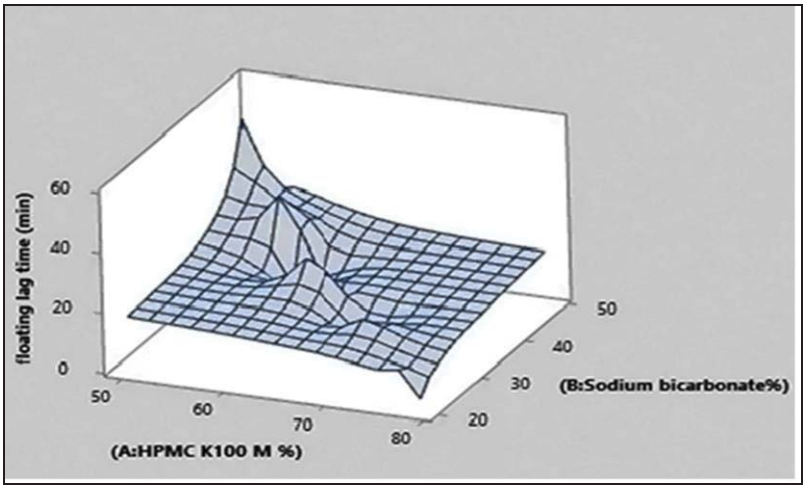


Figure 15: Plots of response surface which shows the effects observed by interaction of dependent factors on floating lag time

Tablet's Floating behaviour:

The optimised bilayer tablet formulation showed excellent floating behaviour up to 6 hours as depicted in Figure 16, 17, 18 and 19.

Figure 16: Bilayer tablet showing floating behaviour in 1.2 p^H buffer at 1 hr

Figure 17: Bilayer tablet showing floating behaviour in 1.2 p^H buffer at 2 hr

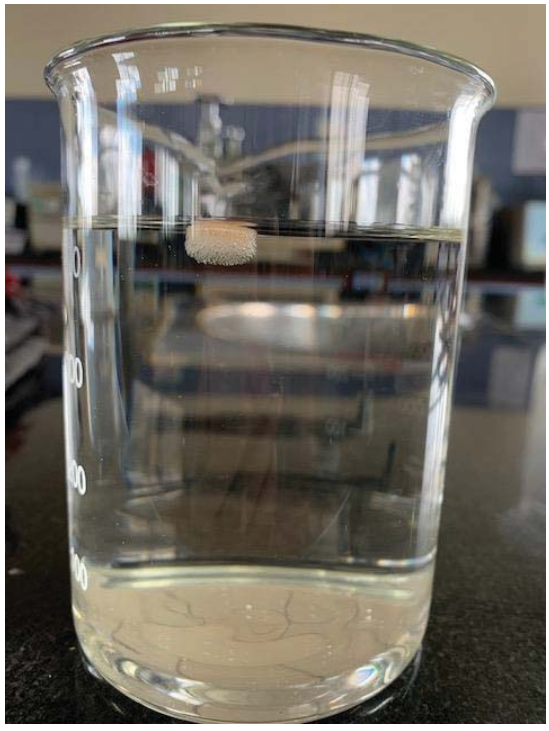


Figure 18: Bilayer tablet showing floating behaviour in 1.2 p^H buffer at 4 hr

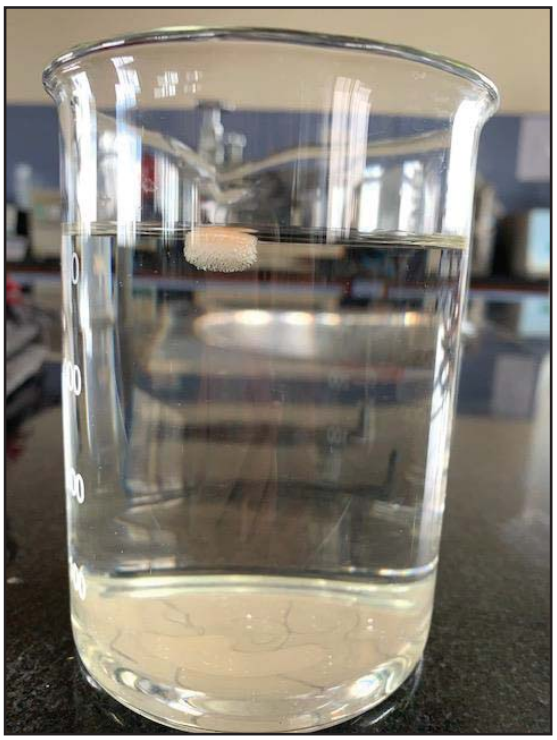


Figure 19: Bilayer tablet showing floating behaviour in 1.2 p^H buffer at 6 hr

***In-vitro* release studies:**

The *in vitro* calcium release profile of Calcium carbonate from model batch in 0.1 N hydrochloric acid is shown in table 31. The calcium release profiles from the model formula in 0.1 N hydrochloric acid are shown in figure 20 and 21 respectively

Table 31: Comparative *in vitro* release profile of Calcium carbonate

RUN	% RELEASE					
	1st Hr	2nd Hr	3rd Hr	4th Hr	5th Hr	6th Hr
1	21.70 ± 0.51	46.98 ± 4.13	59.56 ± 3.85	66.25 ±4.08	73.92 ± 2.82	80.53 ± 2.27
2	39.50 ± 0.67	48.15 ± 0.81	59.36 ± 0.72	65.26 ± 0.60	77.15 ± 2.12	87.97 ± 2.57
3	21.97 ± 0.30	43.65 ± 0.68	59.36 ± 0.72	69.53 ± 5.33	76.19 ± 3.69	81.74 ± 1.79
4	54.08 ± 2.20	62.08 ± 1.73	69.25 ± 0.43	75.06 ± 1.57	80.15 ± 1.09	86.99 ± 2.29
5	14.35 ± 2.67	23.53 ± 0.58	32.36 ± 0.49	39.63 ± 0.36	47.12 ± 1.99	55.81 ± 0.40
6	55.56 ± 2.25	63.10 ± 1.14	69.67 ± 2.12	75.09 ± 3.33	80.20 ± 2.57	85.67 ± 1.91
7	15.35 ± 0.40	22.87 ± 2.81	32.58 ± 3.46	41.01 ± 1.14	45.67 ± 3.80	53.21 ± 0.76
8	37.59 ± 2.19	40.14 ± 2.49	53.74 ± 3.89	66.48 ± 1.69	77.76 ± 1.24	88.32 ± 1.30
9	57.49 ± 1.42	62.29 ± 1.72	67.68 ± 2.43	71.50 ± 1.55	76.17 ± 1.68	80.06 ± 0.74
10	16.73 ± 1.28	22.08 ± 1.19	29.01 ± 1.34	39.04 ± 2.82	44.08 ± 1.04	55.82 ± 2.66
11	30.25 ± 2.01	43.73 ± 1.93	54.08 ± 2.71	66.08 ± 2.07	76.36 ± 2.01	86.41 ± 1.45
12	22.37 ± 1.70	40.22 ± 3.92	59.02 ± 5.60	68.33 ± 4.70	78.24 ± 1.63	80.57 ± 0.53
13	38.50 ± 0.26	50.10 ± 0.60	56.94 ± 1.93	68.95 ± 0.39	77.73 ± 0.69	89.75 ± 0.40
14	57.61 ± 2.10	61.38 ± 1.32	68.12 ± 1.94	74.52 ± 0.76	81.98 ± 0.62	88.37 ± 2.03
15	25.15 ± 1.61	43.28 ± 2.20	55.70 ± 2.30	66.58 ± 2.47	76.83 ± 2.28	84.49 ± 0.88
16	23.81 ± 1.95	44.01 ± 3.25	57.18 ± 2.07	69.76 ± 1.09	77.53 ± 0.54	81.63 ± 1.73

The comparative *in vitro* release of Calcium carbonate batches (F1-F8) is presented in figure 20 and (F9-F16) in figure 21

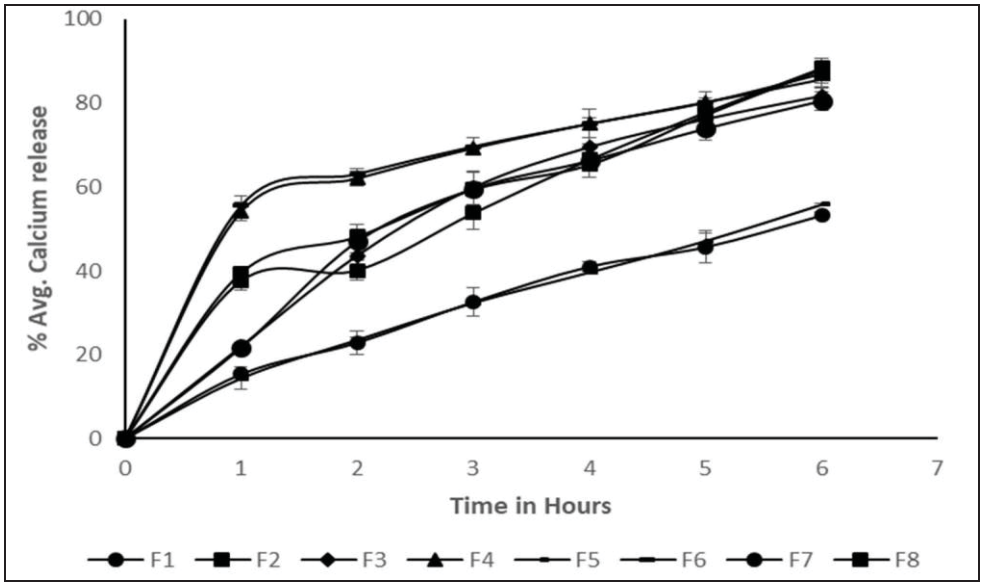


Figure 20: Comparative *in vitro* Calcium release from bilayer tablets (F1-F8) in 0.1 N HCl

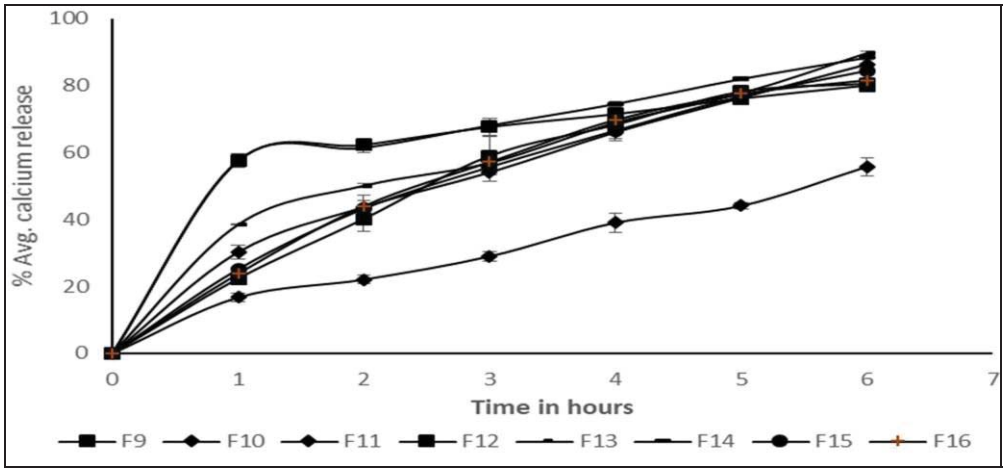


Figure 21: Comparative in vitro Calcium release from bilayer tablets (F9-F16) in 0.1 N HCl

The surface response plot presented in figure 22 and 23 shows the effects of independent variables (sodium bicarbonate and binder concentration) on calcium carbonate release at 1 hr and 6 hr respectively.

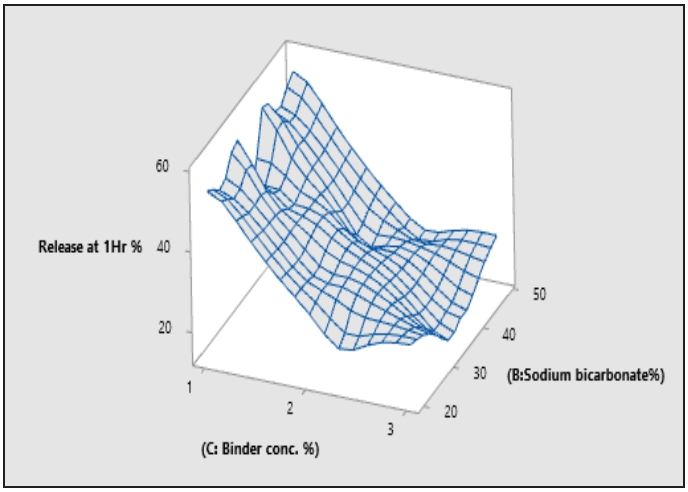


Figure 22: Plots of response surface showing the effects of interaction of dependent factors on calcium carbonate release at 1 hr.

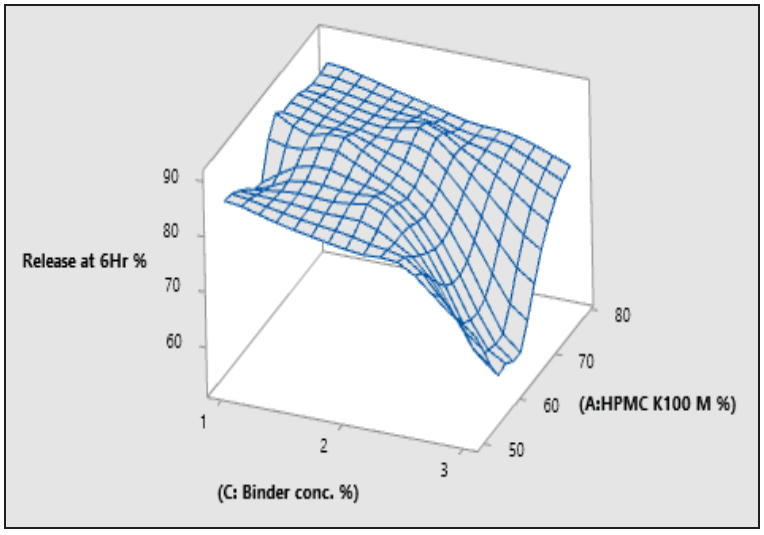


Figure 23: Plots of response surface showing the effects of interaction of dependent factors on calcium carbonate release at 6 hr.

Kinetic modelling of Calcium release:

Table 32: Kinetic modelling of Calcium release of the formulation Run 1 – 16

RUN	Zero order	First order	Hixson crowell	Korsemeyer peppas	Higuchi
1	0.9219	0.9930	0.9792	0.8500	0.9781
2	0.9058	0.9974	0.9652	0.6804	0.9902
3	0.9359	0.9974	0.9866	0.8654	0.9771
4	0.7481	0.9418	0.8930	0.5256	0.9443
5	0.9877	0.9955	0.9966	0.8500	0.9635
6	0.7228	0.9570	0.8693	0.5084	0.9310
7	0.9771	0.9955	0.9921	0.9565	0.9733
8	0.9400	0.9475	0.9711	0.7244	0.9756
9	0.6580	0.8486	0.7863	0.4672	0.8901
10	0.9795	0.9764	0.9816	0.9354	0.9502
11	0.9563	0.9738	0.9901	0.7917	0.9909
12	0.9440	0.9914	0.9848	0.8732	0.9738
13	0.9153	0.9454	0.9685	0.6940	0.9919
14	0.7413	0.9351	0.8904	0.7413	0.9334
15	0.9594	0.9928	0.9981	0.9594	0.9870
16	0.9419	0.9975	0.9981	0.9419	0.9828

Optimisation of the formulation:

Two novel tablet formulations with the expected results were developed utilising a numerical optimization method based on the approach of desirability. Table 33 shows the content of the optimised batches of formulated tablets, as well as predicted and actual values for response parameters.

Table 33: optimized formulations and its composition and their experimental values with comparison between response parameters values and the predicted values

Composition X1:X2:X3*	Responses	Predicted value	Experimental Value	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	27.3	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	12.5
	Y2	8.5	9.13 ± 0.24	6.9
	Y3	34.8	36.41 ± 1.12	4.42
	Y4	88.7	88.05 ± 1.96	-0.74

***In vivo* radiographic studies:**

In vivo X-ray imaging method evaluate the strength of gastroretentive optimized tablet formulation at 0, 2, 4, and 6 hr are presented in Figures 24, 25, 26 and 27 respectively that indicated that the tablets were found to float till 6 hours *in vivo* in rabbits.

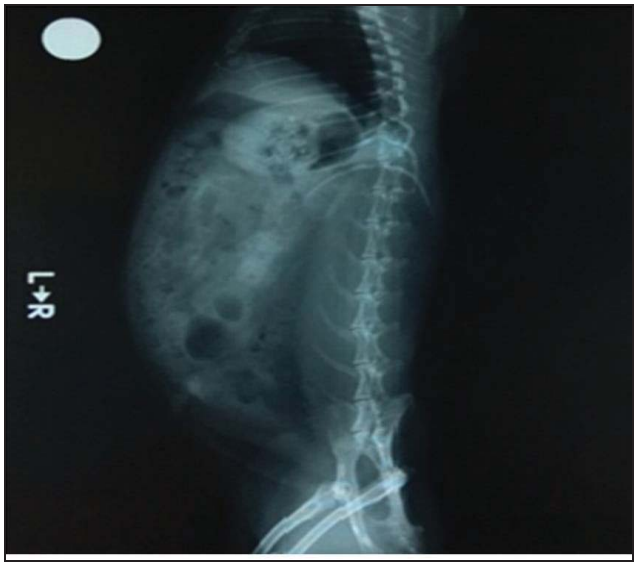


Figure 24: X-ray imaging radiograph at 0 hours

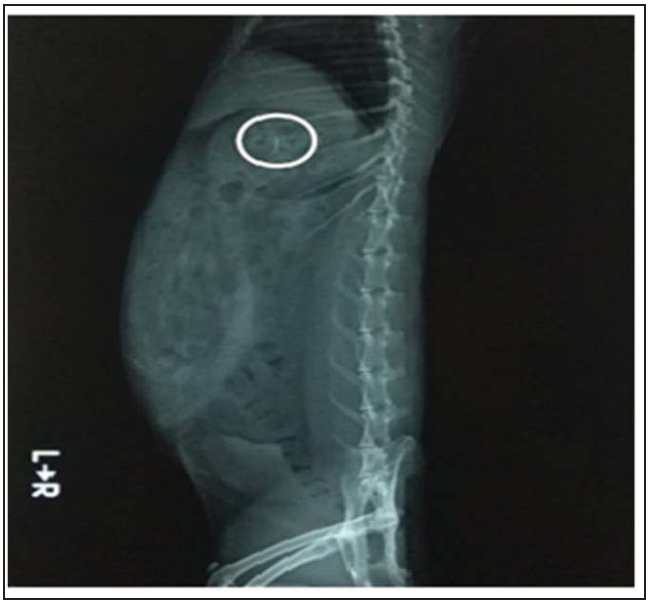


Figure 25: X-ray imaging radiograph at 2 hours

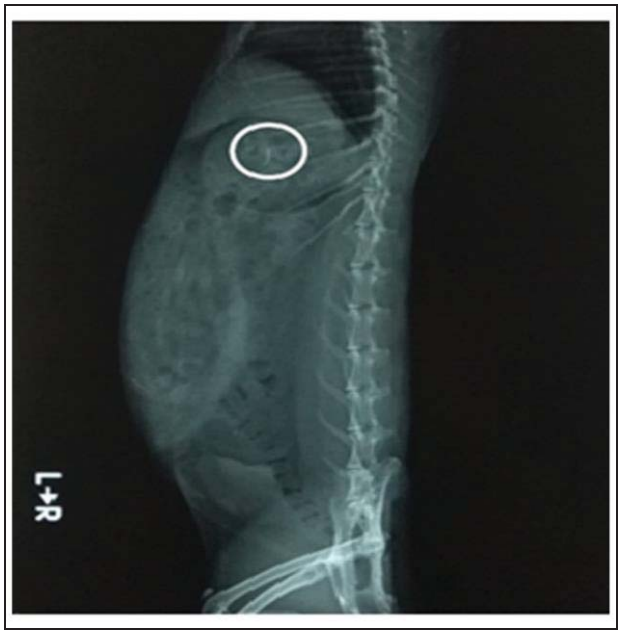


Figure 26: X-ray imaging radiograph at 4 hours

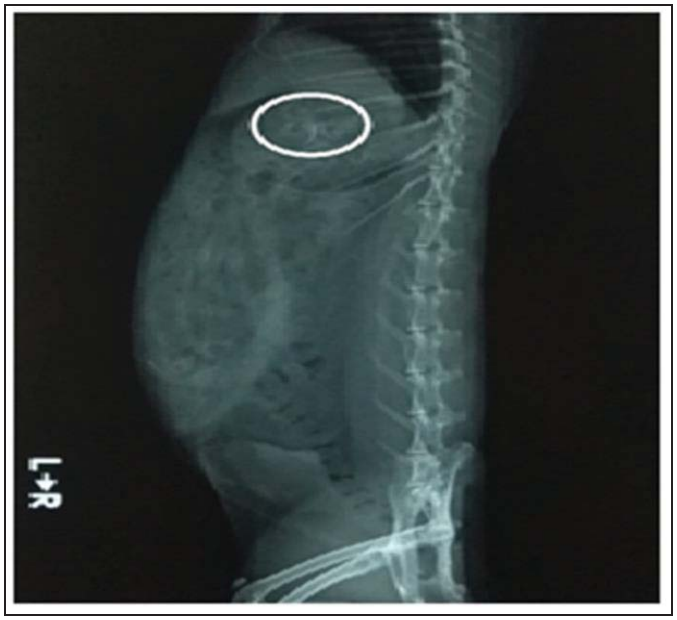


Figure 27: X-ray imaging radiograph at 6 hours

Stability studies:

The content uniformity, floating lag time, burst release, and amount released at 6h showed no significant differences, demonstrating the stability of the formulations as shown in table 34.

Table 34: Results of the optimized formulation on 25°C, 60% Relative humidity

Condition	Time point	Content uniformity * (%)	Floating lag time (min)*	Release at 1 h (%) *	Release at 6 h (%) *
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

*Individual data point shows mean ± S.D (n=3)

PREFORMULATION STUDIES FOR ORAL RAFT FORMING IN-SITU GELLING SYSTEM:

Various polymers were screened for the development of the oral raft forming in situ gelling system. The observations are presented in table 35

Table 35: Screening of the various polymers in oral raft forming in situ gelling system

Sr No	Polymer	Pourability	Gel strength	Gel consistency
1	Sodium alginate	✓	Good	Good
2	Gellan gum	✓	Good	Good
3	Karaya gum	✓	Weak	Thin
4	Guar gum	X	Weak	Thick
5	Xanthum gum	X	Weak	Good

Screening of Calcium release retardant polymers:

Table 36: Selection of Calcium release retardants

Sr No	Polymer	Polymer concentration	Pourability	Strength	Consistency
1	HPMC K15 M	Low: 0.05	✓	Good	Poor
		High: 0.2	✓	Good	Good
2	HPMC K100 M	Low: 0.1	✓	Good	Good
		High: 0.2	X	Good	Good
3	HPMC K15 M + HPMC K100 M	Low :0.05	✓	Good	Good
		High :0.1	✓	Good	Good
4	PEO N-80	Low :0.1	✓	Poor	Poor
		High :0.5	✓	Good	Good

Model fitting and statistical analysis of dependent variables (Y1, Y2, Y3):

The data which is obtained experimentally with varying the concentrations of independent variables are presented in table 37 which shows the collective effect of independent variables on dependent variables.

Table 37: Responses of each variable for the in-situ gelling raft formulations

Run	X1	X2	Y1	Y2	Y3
F1	70	30	15.55 ± 3.51	21.78 ± 2.47	83.50 ± 1.20
F2	50	50	4.52 ± 2.78	16.15 ± 3.46	68.65 ± 0.97
F3	50	50	4.58 ± 0.49	14.51 ± 3.06	65.50 ± 1.98
F4	60	40	10.46 ± 0.20	25.12 ± 1.80	82.90 ± 1.13
F5	80	20	34.43 ± 2.39	27.34 ± 2.01	87.07 ± 0.85
F6	90	10	56.17 ± 3.62	58.56 ± 2.64	80.33 ± 1.68
F7	90	10	50.74 ± 0.44	56.54 ± 3.60	79.30 ± 0.04
F8	70	30	13.46 ± 0.57	19.54 ± 3.30	85.69 ± 2.44

X1 represents amounts of HPMC K100M (%), and X2 represents, Xanthan gum (%) while Y1, Y2 and Y3 represents buoyancy lag time (sec), 1 h % release at and 6 h % release respectively.

The real responses and polynomial equations for Y1, Y2, and Y3 with respect to the actual factors were used to predict the models and analyse it statistically by performing ANOVA as presented in table 38.

Table 38: Summary of ANNOVA for the response parameters of the Model formulations of raft gelling system formulated according to simplex lattice design.

Response	F-value	p-value	R^2	Adj R^2	% C.V.
Y_1	395.04	<0.0001	0.985	0.9825	4.69
Y_2	269.11	<0.0001	0.9951	0.9914	5.44
Y_3	52.54	0.0015	0.9546	0.9364	2.54
Equations of regression for the fitted model which contain only the significant terms:					
Log BLT = + 0.05*X1 – 0.01*X2 …… (5)					
Rel_{1h} = + 1.36*X1 – 8.94*X2 + 0.16*X1X2 …… (6)					
Rel_{6h} = + 0.67*X1 - 0.92*X2 + 0.03*X1X2……. (7)					

Gelling capacity and floating behaviour:

Rapid gelation was observed for each formulation F1 to F8 as soon as it comes closer with the gastro intestinal fluids (0.1 N HCl with pH 1.2). When concentration of alginate increased in the initial trials i.e., from 2% to 5%, gelation ability gets increased when gelation time slows down and gel strength raised up. When HPMC K100 M is incorporated, alginate's gelation capacity gets increased. The gelation and floating properties of GRFS in 0.1 N HCl with pH 1.2 at 0h, 2h, 4h and 6h intervals have been represented in figure 28, 29, 30 and 31 respectively

Figure 28: Gelation and floating property of the GRFS at 0 hour in 0.1 N HCl

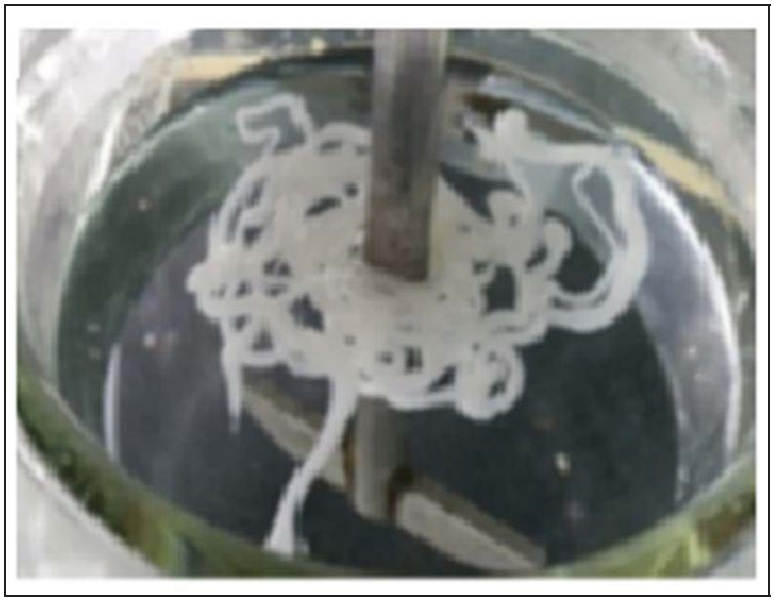


Figure 29: Gelation and floating property of the GRFS at 2 hours in 0.1 N HCl

Figure 30: Gelation and floating property of the GRFS at 4 hours in 0.1 N HCl

Figure 31:Gelation and floating property of the GRFS at 6 hours in 0.1 N HCl

Rheological measurements

Rheological measurements and yield stress for all formulations have been represented in table 39. The rheograms of the same are represented in figure 32.

Table 39: Results of rheological studies

Sr No	Sample	Viscosity at 10 (1/s)	Yield stress (Pa)
1	F1	1250	5.07
2	F2	50063	55.07
3	F4	11703	41.26
4	F5	2474	19.49
5	F6	12978.9	8.22
6	Optimised formulation	9008.1	48.02

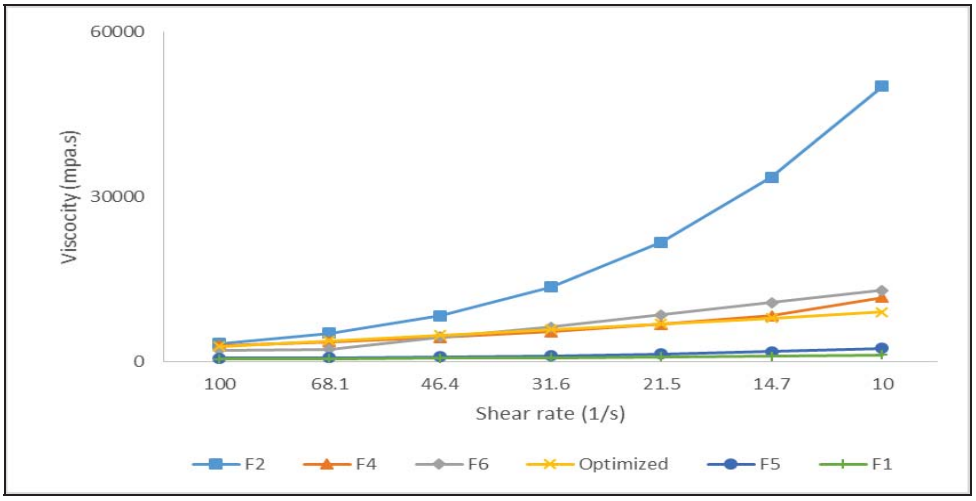


Figure 32: Overlay of Viscosity Curve (Viscosity versus Shear rate)

Floating lag time:

It was observed in the range of 4.52 ± 2.78 sec (F2) to 56.17 ± 3.62 sec (F6) Formulations which are having higher concentration of HPMC K100 M observed highest values of floating lag time. Two components mix plots clearly show the 2 formulation parameters impact on the BLT and which is shown in figure 33.

Table 40: Responses of Individual variables for in-situ gelling raft formulations

Run	X1	X2	Y1	Y2	Y3
F1	70	30	15.55 ± 3.51	21.78 ± 2.47	83.50 ± 1.20
F2	50	50	4.52 ± 2.78	16.15 ± 3.46	68.65 ± 0.97
F3	50	50	4.58 ± 0.49	14.51 ± 3.06	65.50 ± 1.98
F4	60	40	10.46 ± 0.20	25.12 ± 1.80	82.90 ± 1.13
F5	80	20	34.43 ± 2.39	27.34 ± 2.01	87.07 ± 0.85
F6	90	10	56.17 ± 3.62	58.56 ± 2.64	80.33 ± 1.68
F7	90	10	50.74 ± 0.44	56.54 ± 3.60	79.30 ± 0.04
F8	70	30	13.46 ± 0.57	19.54 ± 3.30	85.69 ± 2.44

X1, and X2 represents amounts of HPMC K100M (%), Xanthan gum (%) respectively while Y1, Y2 and Y3 represents buoyancy lag time (sec), 1 h % release and 6 h % release at respectively.

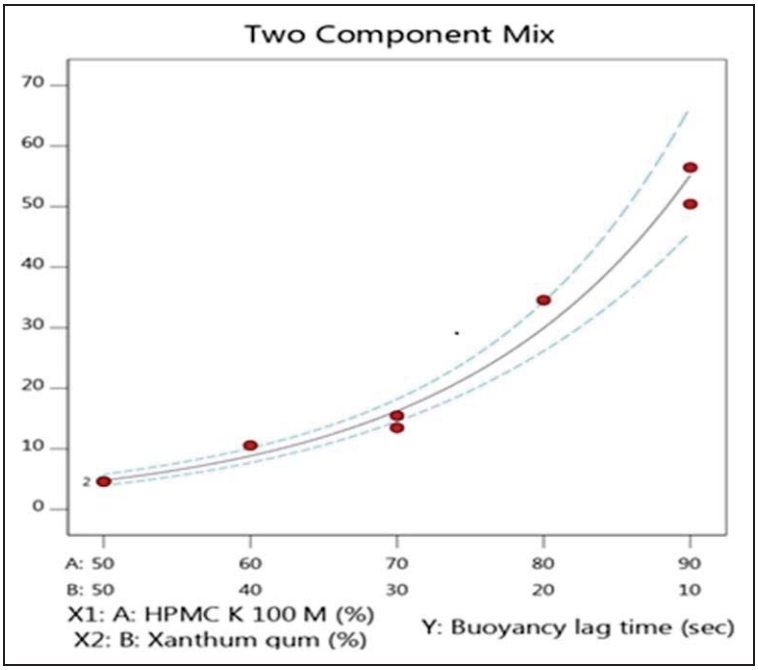


Figure 33: Mix plots of two component showing the effects by interaction of independent factors on a Buoyancy lag time

***In vitro* release study:**

The *in vitro* calcium release profile of CC in 0.1 N HCl is presented in figure 34

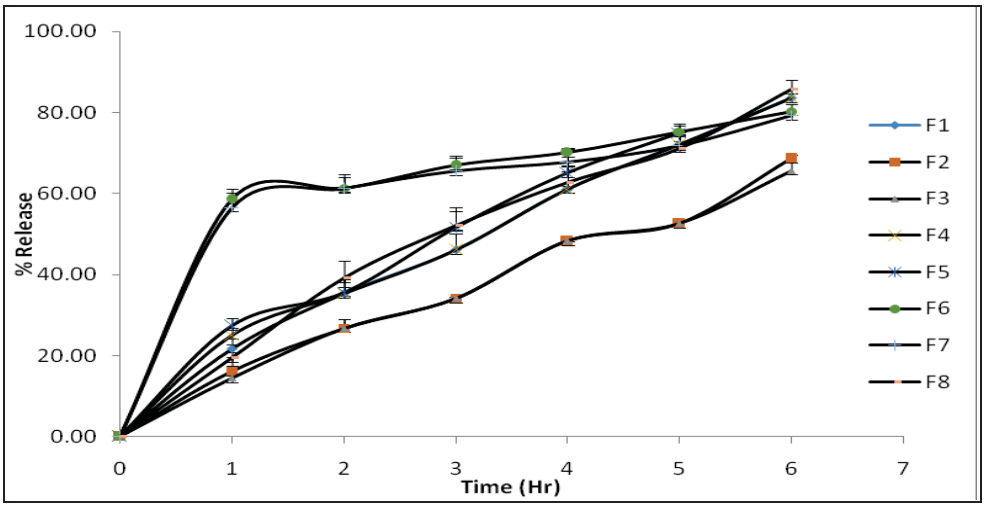


Figure 34: Calcium release from in situ gelling raft formulation of batches F1-F8 and its cumulative comparison

Release at 1hr

The % of release of calcium at termination of 1st hour was determined to be between 14.51% 3.06% for F3 to 58.56 ± 2.64% for F6 as presented in table 40. Figure 35 shows two

component mix graphs that clearly highlight the effects of formulation parameters on the burst effect.

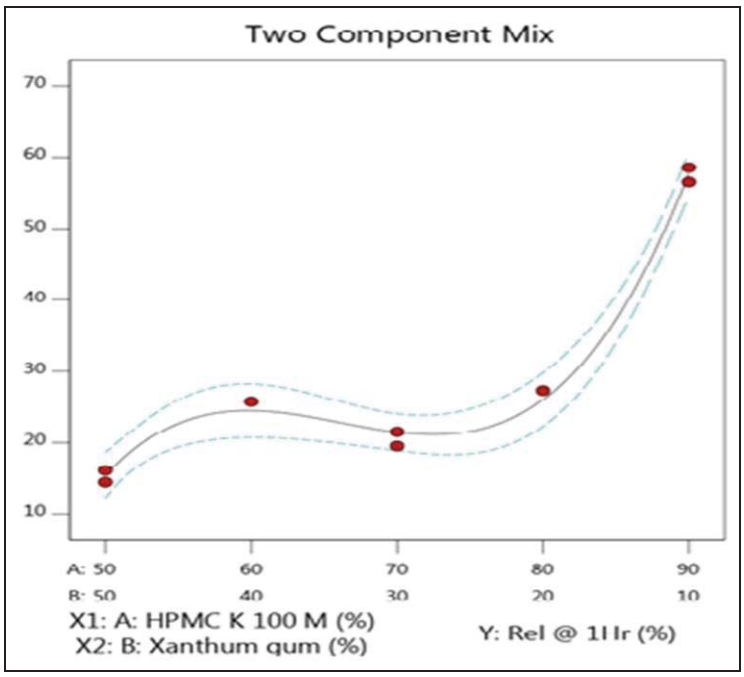


Figure 35: Mix plots of two components showing the interaction effects of independent factors on release at 1 hr

Release at 6hr

By six hours, the calcium release percentage ranged from 65.50 ± 1.98 percent for F3 to 87.07 ± 0.85 percent for F5 as presented in table 40. The calcium release pattern was found to be controlled in the batches F1, F2, F3, and F8 which were free of first burst release in two component mix plots depicted in figure 36, and the effect of formulation factors on release at 6 hours is exactly obvious.

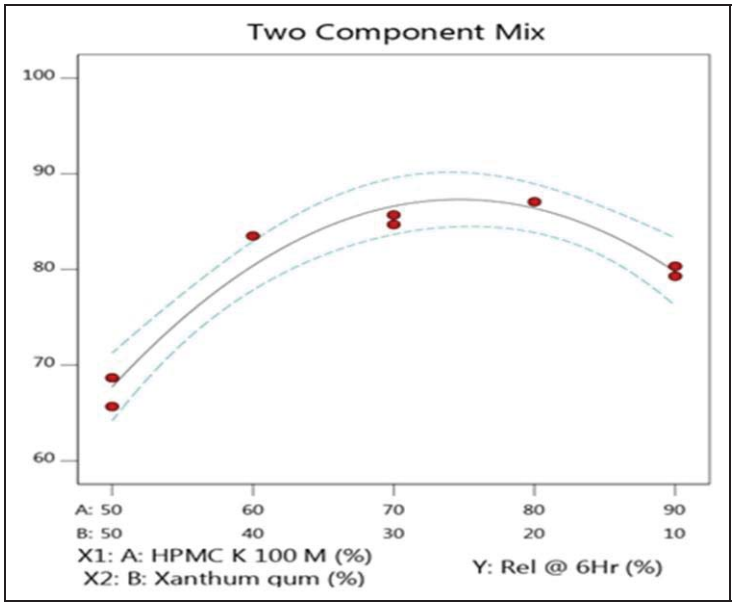


Figure 36: Mix plots of two components showing the effects by interaction of independent factors on release at 6 hr

Kinetic modelling of Calcium release:

Table 41: Kinetic modelling of Calcium release of the formulation Run 1 –8

RUN	Zero order	First order	Hixson crowell	Korsemeyer peppas	Higuchi
1	0.9811	0.9610	0.9844	0.8549	0.9648
2	0.9863	0.9595	0.9762	0.9531	0.9369
3	0.9882	0.9795	0.9885	0.9681	0.9449
4	0.9743	0.9576	0.9801	0.8263	0.9653
5	0.9780	0.9572	0.9856	0.8440	0.9694
6	0.6517	0.8400	0.7787	0.4583	0.8820
7	0.6473	0.8220	0.7654	0.4590	0.8789
8	0.9787	0.9577	0.9856	0.9099	0.9660

Optimisation of the formulation:

For development of new GRFS formulations with the expected results, a numerical optimization strategy based on the desirability approach was used. Table 42 shows the components of the improved formula, as well as anticipated and values obtained in experiments for the response parameters.

Table 42: Content of the optimized formulations, experimental values and its comparison of the response parameters with the predicted values

Factor	Optimized level (D = 0.757)		
X1: HPMC K 100 M	65.88		
X2: Xanthan gum	34.13		
Response	**Predicted value**	**Observed value**	**% Residual Error**
Y1: BLT	12.52	10.9	14.86
Y2: Release at 1Hr	22.95	20.74	-10.65
Y3: Release at 6Hr	84.82	87.25	-2.78

***In vivo* radiographic studies:**

In vivo X-ray imaging technique was utilised for evaluation of potential of gastroretentive tablet formulation at 0, 2, 4, and 6 hr are presented in figures 37, 38, 39 and 40 respectively

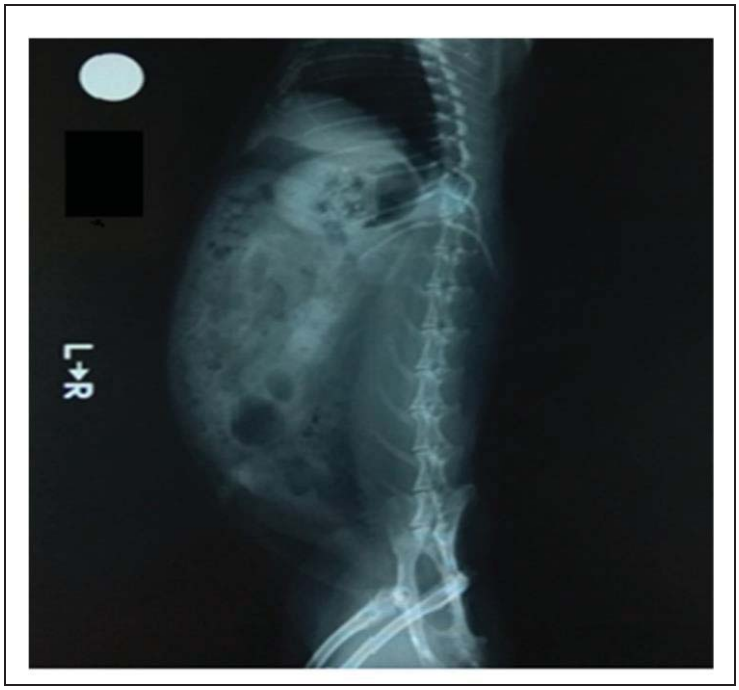


Figure 37: X-ray imaging radiograph at 0 hours

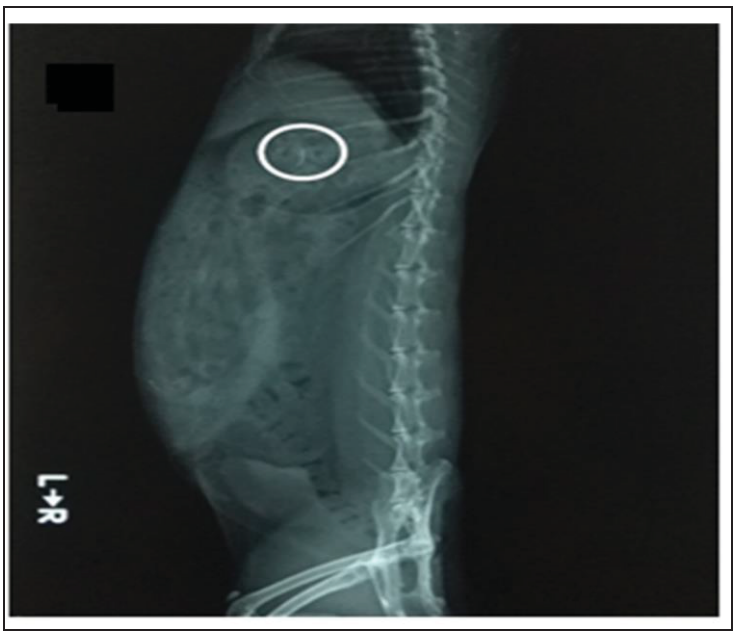


Figure 38: X-ray imaging radiograph at 2 hours

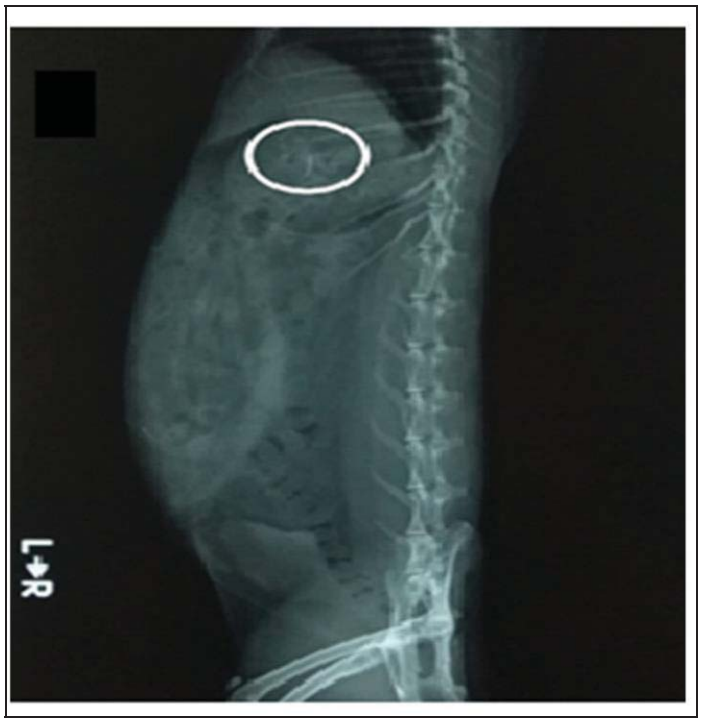


Figure 39: X-ray imaging radiograph at 4 hours

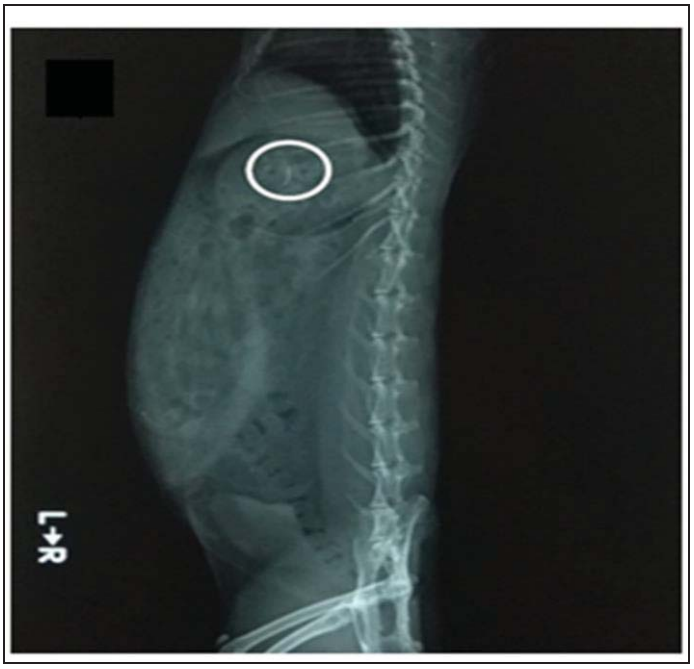


Figure 40: X-ray imaging radiograph at 6 hour

DISCUSSION

DISCUSSION

5.1 Standard calibration curve of calcium carbonate using Flame photometry:

The Calcium carbonate calibration curve in buffer solution having p^H 1.2 was observed linear within the range of concentration of 10 µg/mL to 200 µg/mL having slope of 0.9885 and r^2= 0.9998. The regression coefficient equation was presented by y= 0.9885x – 0.0028. The flame photometry analytical method proposed was obsrved to be reproducible, as evidenced by the low standard deviation readings.

5.2 Solubility study of calcium salts in different pH

The comparative solubility profiling of the two salts is presented in table19. The data obtained in solubility profile was produced in the this study indicated higher solubility of citrate salt as compared to Calcium carbonate in all buffers. Both salts showed maximum solubility in an acidic condition in comparison to the basic environment. The highest solubility of 40.713 mg/ml and 17.090 mg/ml was observed at pH 5.5 acetate buffers in the case of citrate and carbonate salts respectively (Figure 8 and 9). Calcium is mainly absorbed from upper gastro intestinal tract and stomach. Because of this particular property of absorption, the residence time in GI tract of formulation which contains calcium must be enhanced to allow calcium to reach the region of active site of absorption at a control rate and to increase bioavailability of calcium by oral route. For individuals with hypohydrochloria syndrome, a controlled calcium delivery system would be useful since calcium would be released at control rate rather than by burst effect of conventional form. Considering solubility profile of Calcium citrate and Carbonate, the solubility of the CC was found to be higher in acidic medium as compared to the citrate salt, which makes it potential candidate for gastroretentive drug delivery. Hence CC was selected forward for the further trials instead of citrate form.

PREFORMULATION STUDIES OF GASTRORETENTIVE TABLETS:

The gastroretentive tablets of calcium carbonate manufactured by using wet granulation method had excellent hardness and strength but failed in buoyancy test. The tablets were not found floating and disintegrated within 2 hours in release media. Moreover, the tablets showed burst release (92.24 ± 1.26 %) of Calcium over 2 hours. So, in order to retard the Calcium release different viscosity grade HPMC polymers had been used to develop the gastroretentive tablets. But the developed tablets were found to be non-floating in nature. The most probable failure causes of the tablets with respect to floating behaviour are presented in table 43. The gastroretentive matrix tablet approach was failed due to non-floating behaviour of the formulations.

Table 43: Formulations with different grades of HPMC and their failure causes

Code	Polymer grade	Floating lag time	Calcium release at 6 hours	Weight gain at 6 hours
F1	HPMC K4 M	Increase in floating lag time approximately 1hr 45 min	45.53% ± 0.59	25.64% ± 0.23
F2	HPMC K15 M	Increase in floating lag time approximetly1hr 43 min	26.68 % ± 0.72	30.19% ± 0.41
F3	HPMC K100 M	Increase in floating lag time approximately 1hr 30 min	40.32 % ± 0.37	21.51% ± 0.54
F4	HPMC K100 M LV	Tablet not floating	59.20 % ± 0.64	No weight gain

5.3 Preformulation of Gastroretentive bilayer tablets:

The bilayer tablet approach with development of buoyant layer and Calcium layer was adopted with the use of sodium bicarbonate as effervescent component along with HPMC to decrease the flotation lag time, when these components come closer with gastric contents, there is occurrence of a chemical reaction, releasing CO2, which is retained within the gellified hydrocolloid system [115]. As a result, the dosage form achieves an effective density lower than that of the stomach fluid, resulting in an upward movement onto a formulation that is responsible for maintenance of buoyancy for a long time. HPMC K100 M and Sodium bicarbonate at 2:1 ratio showed better matrix integrity of the tablets, decreased the floating lag time and increased the floating time in release media. HPMCE15 LV was found to be suitable granulating agent as compared to PVP K30 and HPMC 15 cps due to desirable release profile and intactness of the tablets in dissolution media.

5.4 FORMULATION OF GASTRORETENTIVE BILAYER TABLETS:

Flow properties of the granules:

The granules' flow characteristics were assessed, and the findings are represented in table 8. The angle of repose of the granules was observed between 22.605± 0.304 to 29.653 ± 0.784, BD was ranged from 0.521± 0.079 to 0.624± 0.043 g/cm^3, TD was found between 0.612± 0.123 to 0.692 ± 0.075. HR was ranged from 1.098 ± 0.043 to 1.167± 0.019 and CI was found between 6.69± 0.042 to 21.18± 0.051. The flow property of the granules is considered as a excellent if the angle of repose lies between 25-30. Based on the granules angle of repose obtained in our batches, the flow was found to be excellent. Also, the higher BD values of the lubricated blend was might be due to the wet granulation process employed in manufacturing leading

to excellent compressibility index (less than 10). Overall, the flow properties of the granules were found to be excellent as per the requirement of the compression.

Evaluation of the compressed tablets:

The diameter of the tablets was found 8.80± 0.07 to 8.91± 0.05 mm. The weight variation was ranged from 0.415± 1.82 to 0.422 ± 1.20 % while content uniformity was varied from 98.17± 1.38 to 101.87± 2.14%. The weight variation and content uniformity of the tablets was found within acceptable range due to the excellent flow properties of the granules. The complete die filling was achieved during the compression that led to lesser weight variation in the tablets and that ultimately resulted in to the excellent content uniformity of the CC.

Calcium excipient compatibility studies using FTIR:

In order to determine the physical compatibility between Calcium and excipients FTIR study was performed. The IR spectra of pure calcium carbonate exhibited principal peak at 1796 cm^{-1} confirming –C=O stretching. Also, intense peak characteristic to the stretching of OH was observed because of the presence of moisture. Similarly, the spectrum of IR for physical mixture showed a broad bands which can be attributed to OH stretching, Though the peak intensity differed, the classic absorption peak was seen at the same position, which indicates that there was no interaction between calcium carbonate and another various excipient in the physical mixture. Similarly, the IR spectra of a calcium carbonate bilayer tablet revealed no more shifting in the peaks, despite the fact that the peak's intensity reduced, which indicates that there was no interaction between calcium carbonate and other excipients at the time of tablet manufacturing, demonstrating the integrity of calcium carbonate in the bilayer tablet. Figures 5, 6, and 7 show the FTIR spectra of CC, a physical mixture of CC and excipients, and a bilayer tablet, respectively.

Statistical analysis of Friability (Y1)

The regression equation for the friability can be given by equation (1) presented in table 28. The friability of the formulated tablets was ranged between 0.02 ± 0.01% (F12) to 2.77 ± 0.64% (F14) as presented in table 9. Friability of less than 1% is allowed for compressed tablets, according to the pharmacopoeia [116]. Except for F4, F6, F9, and F14, all batches pass the test of friability. The friability of those batches was found more than 1 % hence didn't pass the test with the friability test. The hardness of these batches, by chance, did not exceed 4.5kg/cm2. The higher friability values observed in the four batches was due to the lower binder concentration of 1% w/w. Indication of statistical analysis was, X3 factor had the biggest impact of the three elements, followed by X2 and X1. HPMC E15 LV (X3) exhibited a high negative coefficient (-17897.32), indicating that this factor has a significant negative influence on friability. The positive coefficient values of HPMC K100 M and $NaHCO_3$ were observed to be 0.18 and 0.32, respectively, indicating that they had very negligible impact on friability of the tablets. The most probable reason for this poor effect was due to the fact that HPMC K100M and $NaHCO_3$ are present in buoyant layer and not in the calcium carbonate layer. Figure 8 shows the effect of independent variables on friability. The values of friability were decreased due to lower amount of binder leading to the formation of low hardness tablets. It has also been observed that the layer with Calcium carbonate was the main factor responsible for the determination of friability rather than the buoyant layer. It is well understood and known fact that the tablets hardness is directly proportional to the binder concentration used in tablet manufacturing [117]. In our study the use of higher concentrations of the HPMC E15 LV could reduce the friability and it was also proved with 3D plot showing negative impact of binder on friability.

Statistical analysis of Floating lag time (Y2):

The regression equation for floating lag time can be given by equation (2) presented in table 28 and table 27 represents the range of the floating lag time of the bilayer tablets (2.850± 0.18 min to 37.54 ± 0.42 min for F9 and F7 batches respectively. Formulation batches F2, F6, F8, F10, and F13 had shown desirable floating lag time of less than 10 minutes. The batches failed in friability (F4, F6, F9, and F14) were not considered ideal even though they showed minimum floating lag time ranging from 2.53 ± 0.31 min to 2.77 ± 0.64 min. Formulation batches F1, F5, F7, F15, and F16 had a high floating lag time of more than 30 minutes, while batches F3, F11, and F12 had a lag time of more than 10 minutes. A small lag time is preferred since a large lag time might lead to failure of system owing to unexpected or unintentional fast gastric clearance caused by the stomach's peristaltic activity and forceful gastric housekeeping waves. In general, batches with a long floating lag time had greater amounts of the binder HPMC E15 LV (≥2%). The three factors studied were shown to have a substantial impact on floating lag times, according to experimental data obtained in mathematical modelling. The influence of X3 was the greatest of the three factors studied, followed by X2 and X1, with the effect of X1 being the least. The HPMC E15 LV amount had the largest negative coefficient value (25289.06), indicating that the factor has the greatest impact on floating lag times. This might be because greater binder concentrations which result in increase in compactness of tablets having lower porosity. The reduced porosity of tablet is anticipated to significantly impede dissolving media penetration into the tablet matrix, delaying the production of carbon dioxide that may be necessary to begin flotation [118]. To summary, the FLT might be reduced simply by applying moderate amounts of HPMC E15 LV. In figure 15, the detrimental influence of the binder on friability is readily

evident in the 3D plots. The levels of $NaHCO_3$ with a positive coefficient value of 1.19, on the other hand, were observed to have a minor effect on the floating lag times. The bicarbonate's positive effect is mostly due to its capacity to produce carbon dioxide via an interaction of $NaHCO_3$ and stomach fluid, which would be easily engulfed in the layers of polymeric gel, resulting in shorter floating lag periods [119]. Of 3 factors which are investigated, the quantity of HPMC K100 M was observed to have less effect with a positive coefficient of 0.24. Figure 15 shows the effect of independent variables on friability. HPMC K100 M, which is having higher viscosity and hydrophilic in nature that may produce a layer of gel matrix in the stomach juices, is responsible for the beneficial impact [120]. This gel barrier is stronger and efficiently traps the CO_2 released in-situ, lowering the density of the tablet below unity and conferring buoyancy [121]. In the current study, however, rather than the floating layer, the carbonate layer composition had a consistent effect on the floating lag time.

Statistical analysis of the Release at 1h (Y3):

The regression equation for Calcium release at 1 hr can be given by equation (3) represented in table 28. Table 9 represents that the calcium release percentage at the end of the 1st hour ranged from 15.87 ± 2.54 % for F5 to 55.61 ± 1.28 % for F14. The three formulation variables studied were shown to have a significant impact on calcium release after 1 hour. The influence of X3 was the most significant of the three variables, followed by X2 and X1, with the effect of X1 being the least significant. The batches F4, F6, F9, and F14 showed the burst release of 53.85 ± 2.58 %, 54.82 ± 2.13 %, 56.60 ± 1.59 %, and 55.61± 1.28 %, respectively as shown in figure 14 and 15**,** which coincidentally correlated well with higher values of friability of 2.53 ± 0.31, 2.64 ± 0.28, 2.68 ± 0.18, and 2.77 ± 0.64 %. Concentration of binder solution exhibited a higher negative coefficient (64542.84), which indicates that it had the greatest impact

on calcium release after 1 hour. Higher binder levels, on the other hand, resulted in more compacted tablets, which successfully prevented the first burst release. If concentration of binder solution increased in the matrix tablets significantly then burst release decreased within 1st hour, according to previous literature citations [122]. Sodium bicarbonate, on the other hand, had a modest beneficial influence on the release at 1h, with a coefficient of 4.38. The higher calcium release at 1h might be due to bicarbonate's tendency to induce effervescence, which makes the tablet porous. Increased bicarbonate levels have been shown in previous studies to increase medication release from matrix tablets [123]. HPMC K100 M was showing no effect (0.40) on the release at 1 hour of the three variables studied. In conclusion, utilising moderate to high amounts of HPMC E15 LV could reduce burst release. The negative effect of the binder on burst release may be seen clearly in Figure 16's 3D plots. The most likely cause for this is that HPMC K100M was not present in the calcium carbonate layer's matrix. The rest of the batches, which did not have an early burst of calcium release, can be regarded more appropriate since they had a more regulated calcium release pattern.

Statistical analysis of the release at 6h (Y4):

The regression equation for Calcium release at 6 hr can be given by equation 4 represented in table 28. Table 27 represents that the percentage calcium release after six hours ranged from 54.08±0.63% for F7 to 88.72±0.92% percent for F13. Figure 14 and 15 shows a controlled manner of calcium release in 12 batches, including F1-F3, F5, F7, F8, F10-F13, F15, and F16, that were free of initial burst release. Because of lower hardness and higher friability, the batches F4, F6, F9, and F14 were observed to have a burst release of more than 50%. The later release of calcium from these pills, on the other hand, appeared to be slowed. The reason for this is the delayed release is

that a dissolution medium which has already been saturated with dissolved calcium carbonate (> 50% calcium carbonate in dissolved state) is having less capacity for creating a sink condition that would allow calcium carbonate to be dissolved from the matrix tablet matrix. The three variables studied were shown to have a significant impact on the release at 6 hours. X3 had the greatest impact of the three factors studied, followed by X2 and X1, with X1 having the smallest impact. The quantity of HPMC E15 LV (X3) showed a large negative coefficient (-1539.68), indicating that the factor had a significant impact on the release at 6 hours. The calcium release from the matrix tablets was effectively controlled by increasing the binder content. Greater binder concentrations resulted in compact and thicker tablets with a regulated calcium release over 6 hours, which could explain the deleterious effect of the binder. The findings also suggest that the release rate may be controlled simply by changing the binder HPMC E15 LV concentration. HPMC E15 LV has been shown to successfully limit medication release from matrix tablets when used alone [124]. To summarise, employing medium concentrations of HPMC E15 LV was sufficient to ensure complete calcium release. The 3D Plots captured in figure 17 showed that the binder has a negative impact on the release at 6 hours. Sodium bicarbonate, on the other hand, was shown to have a little beneficial effect on the release at 6 hours, with a coefficient of 0.88. The propensity of bicarbonate to make tablets porous, particularly in those which having low levels of binding solutions, may be cause of the greater release seen [125]. HPMC K100 M was shown to have very minute effect on release of calcium at 6 hours when compared to the other two variables. As previously stated, HPMC K100 M low effect might be attributed to the fact that the polymers having higher molecular weight did not serve as the matrix material in the calcium carbonate layer. The batches F2, F8, and F13, which contained a medium concentration of

binder, which was observed to be a appropriate formulations, as they met the friability limits as per official test, were free of the initial burst effect, had a short floating lag time, and released calcium in a controlled yet complete manner by 6 hours.

Optimisation of the formulation:

Using a numerical optimization strategy based on the desirability approach, two novel floating bilayer tablet formulations with the expected results were produced. Table 33 shows the compositions of the optimised formulations of floating bilayer tablet, as well as predicted and actual values for response parameters. The response parameter prediction error was observed to vary from -14.29 to +12.50. The validity of the mathematical models created by ANNOVA and regression analysis was demonstrated by the low levels of prediction errors. The calcium release from the optimised bilayer tablet formulation was shown to follow *in vitro* first order kinetics.

***In vivo* radiographic studies:**

According to the in vivo examinations, the mean stomach retention time for the tablets from the optimised batch corresponded well with floating time in in vitro. The optimised batch of bilayer floating tablets stayed in the stomach for an average of 5.5±1.0 h in rabbits, and it was significantly longer ($p < 0.05$) than the conventional tablets, which had average gastric retention duration of less than 2 hours. In comparison to conventional tablets, the bilayer tablets were well stayed in the stomach due to their floating characteristics, despite the effect of peristalsis and forceful housekeeping waves. Figures 18 to 21 show sample images of the in vivo radiography examinations with the bilayer floating tablets.

Stability of the formulations:

The findings of real time stability testing for optimum batch was done according to ICH standards was not indicating any visual changes in the tablets at the time of study

period. The usual peaks of calcium carbonate were also visible in the tablet's spectra, indicating the integrity of calcium carbonate while also ruling out any chemical interactions between calcium carbonate and the other excipients used in the formulation. In, you may see typical spectra of calcium carbonate and tablet combination. The content uniformity, floating lag time, burst release, and amount released at 6h showed no significant differences, demonstrating the stability of the formulations as shown in table 34.

PREFORMULATION STUDIES OF ORAL RAFT FORMING IN-SITU GELLING SYSTEM:

Gel strength and gel consistency of the sodium alginate and gellan gum was good than other polymers and both polymers were found to be pourable. Gel strength and gel consistency of HPMC K15 M, HPMC K100 M and combination of HPMC K15 M with HPMC K100 M and PEO N-80 at high concentration was found to be good. The results are presented in table no 35 and 36

FORMULATION OF ORAL RAFT FORMING IN-SITU GELLING SYSTEM:

Gelling capacity:

The sodium salt of alginate crystallises as a thick, low-density gel (g/ml). The gel's strength is determined by the molecular weight and ratio of D-mannuronic and L-glucuronic acid residues in sodium alginate. In general, sodium alginate rafts with a larger molecular weight and glucuronic acid concentration have higher viscoelastic strength. The ability of calcium to cross-link alginic acid and form a "egg box" structure increases raft strength even more. When in contact with stomach juices, all of the formulations quickly gelled (0.1 N HCl, pH 1.2). As the gelation duration decreased and the gel strength improved, when concentration of alginate increases in the early trials from 2% to 5% enhanced the gelation capacity. Weak gels were produced using formulas including 2% sodium alginate, leaving turbid liquids below. Such devices are unsuitable for use as GRFS because they are prone to peristaltic motions causing fast stomach emptying [126]. To achieve raft compositions with acceptable gel characteristics, the sodium alginate content was kept constant at 5% in all formulations. Addition of HPMC K100 M enhanced the gelation capability of alginate, according to the findings. HPMC K100M was added to the raft systems to enhance sodium alginate gelation capacity and provide sustained release

characteristics [127]. The gelling behaviour of the formulations is presented in figure 28 to 31.

Rheological studies:

Pseudo plastic flow may be seen in the rheograms of dispersions of liquid which contains the natural gums for example, sodium alginate and Xanthan gum as shown in figure 32. Each liquid GRFS showed a fall in viscosity when the shear rate was increased, indicating pseudoplastic flow or it termed as shear thinning behaviour [128]. The RFS viscosities were discovered to rise in the following order: F2 > F4 > F1 > F5 > F6. At a shear rate of 10 sec^{-1}, a ranking of order correlation was discovered in the viscosities. At 37°C, the yield stress of the GRFS is shown in Table 4. When xanthan gum amount in the formulation arose, the viscosity of the GRFS increased. The yield values are utilised to calculate the dispersibility and portability of the GRFS [129] system. The low yield stress values suggest that the systems are able to easily dispersible and pourable with little agitation. The GRFS did not cake when left to stand, but were readily dispersible after 2 months of repose (at negligible shear rates). The profiles of viscosity show a decrease in viscosity as soon as rate of shear increases, which is a common feature of a pseudoplastic system. The systems were discovered to have higher viscosities at rest or at low shear rates, allowing $CaCO_3$ to be evenly distributed for lengthy periods of time. The high viscosity values would allow for a consistent calcium dosage to be withdrawn. The systems, on the other hand, showed large drop in viscosity at high shear rates, allowing for uniform calcium carbonate dispersion with very modest agitation. The viscosities achieved, on the other hand, are dependent on the polymer content in the systems. Furthermore, when developing GRFS, it was necessary to establish a delicate balance between viscosity, yield stress, and sustained release characteristics.

Buoyancy lag time:

The GRFS floating lag time was observed within range of 4.52 ± 2.78 seconds for F2 to 56.17 ± 3.62 seconds for F6. The regression equation for buoyancy lag time can be given by equation 5 as presented in Table 38. A small lag time is preferred since a large lag time which causes the failure of system owing to unexpected or unintentional fast gastric clearance caused by the stomach's peristaltic activity and forceful gastric housekeeping waves. The two parameters studied were shown to have a significant impact on floating lag times, according to mathematical modelling of the experimental data as shown in figure 33. The effect of HPMC K100 M (X1) was showing a good impact on the BLT, whilst the effect of xanthan gum (X2) was observed to have a negative effect on the BLT. Batches with greater amounts of HPMC K100 M were having a longer floating lag time in general. Calcium carbonate serves as a gas producing ingredient soon as it comes closer to acidic dissolving media, releasing carbon dioxide and so speeding up flotation. The CO_2 generated is likely to become trapped within the raft gel, enhancing the raft's buoyancy. Furthermore, GRFS with high amounts of HPMC and strong gel strength which are well known for better entrapment of developed carbon dioxide, allowing for easier flotation. Figure 11 shows two component mix graphs that clearly show the influence of the two formulation variables on the BLT. The figure shows that GRFS with low BLT are always related with high xanthan gum levels. This suggests that HPMC promoted fluid flow into the liquid raft formulation by swelling it and then extending the raft gel surface [130].

Release at 1 hour:

The regression equation for Calcium release at 6 hr can be given by equation 6 as presented in table 38. The two parameters studied were shown to have a significant

impact on the burst release or release at 1h, according to mathematical modelling of the experimental data as shown in table 40. The fast release might be explained in part by the fact that 0.1 N HCl (pH 1.2) provides a sink condition for $CaCO_3$ dissolution. This theory is supported by the solubility profile data produced in this study, which shows that $CaCO_3$ has a high solubility in pH 1.2. The effect of HPMC K100 M on the burst effect was found to be positive, whilst the effect of xanthan gum on the same was found to be negative. Burst effect was shown to be absent in batches with greater amounts of xanthan gum. Figure 35 depicts two component mix plots which affects the formulation variables on the effect of burst. It should be highlighted that by utilising large amounts of xanthan gum, the burst effect from raft compositions might be reduced. High amounts of xanthan gum are likely to result in the development of a more compact gel, preventing the dissolving medium from penetrating and therefore preventing the burst effect. The dissolving liquid must penetrate the gel matrix before the calcium can be broken down and released into the surrounding media [131]. However, when the concentration of xanthan gum was reduced, the burst effect increased, indicating that a gel matrix formed was more porous. The porous matrix was unable to prevent the dissolving medium from penetrating the porous matrix, causing calcium to dissolve and finally explode. Gel matrices with pores are having the capacity for permission of aqueous medium to easily permeate the raft-forming mechanism, allowing dissolution of calcium and thereby diffusion from the raft gel to occur [132].

Release at 6hr:

The regression equation for release of Calcium at 6 hr can be given by equation 7 as presented in table 38. The two variables studied were determined to have a significant impact on the release at 6 hours. The effect of X2 was the greatest, followed by X1,

and the interaction effect of X1X2 was the smaller of the two components studied as shown in table 40. The concentration of Xanthan gum (X2) showed a large negative coefficient (-0.92), indicating that the component had a significant impact on the release after 6 hours. When xanthan gum is exposed to water, it produces a viscous, strong gel network. The ability to avoid the first burst release and better Calcium retarding capacity of Xanthan gum is a therapeutic advantage [133]. As expected, the gel matrix compactness increased while porosity decreased, resulting in a regulated calcium release from the raft. When utilised at the right concentrations, xanthan gum can be utilised as a controlled release carrier [134, 135] the most common Calcium release action from these matrices that expand to a greater extent was discovered to be diffusion. Calcium in the matrix core may take longer to reach the surface in the long run. However, when compared to the impact of xanthan gum, the effect of HPMC K100 M was shown to be less significant. In two component mix graphs recorded in figure 36, the influence of formulation variables on release at 6 hours is clearly evident. The graphs clearly showed that modest amounts of xanthan gum would be required to decrease the burst impact while ensuring a near-complete and regulated calcium release.

Optimisation of the formulation:

Table 42 shows the optimised formula's composition, as well as predicted and experimental response parameter values. The values of experiments and its responses was observed to be quite similar to the mathematical models' predictions. The predicted and observed experimental values were collated to find the mean error. The mathematical models' validity was therefore confirmed by a low amount of mean residual error. The optimised formulation's in vitro Calcium release kinetics were discovered to be first order.

***In vivo* radiographic studies:**

In humans, the average stomach residence duration is 2 hours, although it can be as long as 6 hours depending on the availability of food, posture, and a variety of physiological factors. In light of this, the in vivo experiments were set to last 6 hours, during which time we expected a controlled but almost complete release of calcium from GRFS. Figure 37 to 40 depicts typical pictures from the GRFS in vivo radiography investigations. The mean stomach retention time for the optimised batch of GRFS corresponded well with the in vitro floating time, according to the in vivo experiments. The optimised batch of GRFS retained in the stomach for an average of 5.64±0.43 hours in rabbits, which was significantly longer ($P<0.05$) than the commercial formulation, which had a gastric retention duration of less than 2 hours. Compared to traditional suspension, the GRFS was shown to be well maintained in the GI tract due to its floating property and bioadhesive property. Despite the effects of peristalsis and strong housekeeping waves, this was the case. Because the raft system is well-retaining in the stomach around the absorption window, the contents are likely to be released in a controlled way. We expect a lesser effect of burst of ~ 20% and approximately ~75% of calcium to be released from the GRFS at control rate based on the mean stomach residence time and the *in vitro* dissolution results. Because calcium would be supplied at control rate, it will be less likely to overload the transporters in the duodenal portion of the gastrointestinal system. As a result, as compared to a traditional oral solution, the RFS is expected to have a higher bioavailability.

SUMMARY

SUMMARY

Calcium absorption was found to be dependent on pH, site specific and carrier mediated transport. Also available conventional formulations of calcium can quickly pass the absorption site and hence only small portion of the calcium is absorbed which results in low bioavailability. For this reason there was need for the research on gastro retentive drug delivery system. The present study involved in the development of various formulations of calcium carbonate i. e. gastroretentive bilayer tablets of calcium carbonate and raft forming in situ gelling system (GRFS) inorder to avoid the drawbacks of the existing conventional systems.

Preformulation study was done by determining the pH solubility profile of calcium salts (carbonate and citrate) and calcium carbonate was found suitable due to its higher acid solubility for formulation of the GRDDS. FTIR studies revealed no interaction between the excipients and the calcium carbonate. Screening of polymers were done by selecting various kinds of grades of viscosity of HPMC in the manufacturing of bilayer tablet dosage form and formulation was evaluated for in vitro floating time and cumulative release. For manufacturing of bilayer tablet, wet granulation technique was found suitable rather than direct compression method. HPMC K100 M in combination with sodium bicarbonate (2:1) was found to be suitable as it showed good floating time, floating lag time and calcium release over the period of 6 hour.

Gastroretentive bilayer tablets were formulated using D-optimal mixture design. The effect of formulation factors like levels of HPMC K100 M (X1), $NaHCO_3$ (X2) and HPMC E15 LV (X3) on responses like floating lag time (R1), release of calcium carbonate at 1h (R2) and 6h (R3) were elucidated. The optimized formulations with expected results were produced by numerical optimization method using desirability

approach. The lubricated granules of calcium carbonate were evaluated for granular properties like bulk density, tapped density, compressibility index, Hausner's ratio & angle of Repose. All responses for these parameters were observed in the range indicating excellent compressibility of the granules. Evaluation was done for bilayer tablets for thickness, hardness, friability, content uniformity, weight variation, floating lag time, in vitro release study and kinetics, stability study and in vivo X-imaging study. The optimised bilayer tablet formulation showed excellent floating behaviour up to 6 hours in pH 1.2 buffer. The batches F2, F8, and F13, which contained a medium concentration of binder, which was observed to be a most appropriate formulation, as they met the friability limits as per official test, were free of the initial burst effect, had a short floating lag time, and released calcium in a controlled yet complete manner by 6 hours. The optimised batch of bilayer floating tablets stayed in the stomach for an average of 5.5±1.0 h in rabbits, and it was significantly longer ($p < 0.05$) than the conventional tablets, which had average gastric retention duration of less than 2 hours.

Real time stability testing was done for optimised formulation as per ICH guidelines. The content uniformity, floating lag time, burst release, and amount released at 6h showed no significant differences, demonstrating the stability of the formulations. Overall, the developed bilayer tablets could be potential alternative for the conventional dosage forms.

Similarly, for raft forming in situ gelling system the preformulation study was performed to select release retardant polymer. Among all tested polymers and HPMC K100 was found to be excellent calcium release retardant polymer. Based on the pourability, gel strength and gel consistency in acidic medium sodium alginate was found to be ideal candidate for raft forming gelling system. The influence of

formulation composition on buoyancy lag durations, percent of CC released at 1hr and 6hr was investigated using a basic lattice mixture design. The amount of HPMC K100 M (X1) and Xanthan gum were the two variables or components in the experimental design (X2).

Gelling capacity, rheological studies, floating behaviour, In vitro calcium release study and in vivo X-imaging studies were carried out. All batches showed rapid gelation when come in contact with gastro intestinal fluid (0.1 N Hydrochloric acid pH 1.2). Increase with concentration of sodium alginate (2-5%), gelation ability and gel strength increased and gelation time decreased. All the developed model formulations displayed lowering in viscosity with rising in shear rate. Floating lag time for the formulations was obsrved in the range of 4.52 ± 2.78 sec (F2) to 56.17 ± 3.62 sec (F6). The calcium release ranged from 65.50 ± 1.98 % for F3 to 87.07 ± 0.85 % for F5 at 6 hours. In vivo X-ray imaging technique was utilised for evaluation of potential of gastroretentive gelling system at 0, 2, 4, and 6 hr. The optimised batch of GRFS retained in the stomach for about 5.64±0.43 hours in rabbits, which was significantly longer ($P<0.05$) than the commercial suspension, which had a gastric retention duration of less than 2 hours.

CONCLUSION

CONCLUSION

The present study demonstrated the feasibility for the research on gastroretentive drug delivery system for Calcium Carbonate in the form of bilayer tablets and oral raft forming in situ gel. The results obtained in this research like decreased floating lag time, minimum burst release and controlled yet complete release for 6 hr showed that the developed drug delivery technologies could be potential alternatives to existing technologies suffering from several drawbacks. The experimental data provided was found to be in good accord with the polynomial models' predictions, demonstrating the models' validity. In vivo radiographic studies of the optimized formulations in rabbits revealed that the gastroretentive systems were found to be retained in the stomach. Furthermore, the study showed that gastroretention along with mucoadhesive properties may prove to be a promising platform for improving bioavailability of nutrients with an absorption window in the upper GI tract. However, further preclinical and clinical studies needed to be established for the safety and efficacy of the developed formulations.

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ANNEXURE

a. Ethical clearance letter

CERTIFICATE

This is to certify that the project title "Pharmacokinetic study of test substance in rabbits" has been approved by the IAEC.

Name of Chairman/IAEC: Mr. Ravindra K.S.

Name of CPCSEA Nominee: Dr. Raveendra Hegde

Signature with date:

Chairman of IAEC: 22/2/18

CPCSEA Nominee: 22/2/18

b. Oral Presentations and publications

Oral presentation:

1) Oral presentation on **"Formulation and optimization of gastroretentive bilayer tablets of calcium carbonate using D-optimal mixture design."** In IQAC Initiated UGC-STRIDE Sponsored two day International-conference on "Applied Materials and Technology-2020". An approach for Trans-Disciplinary research, Organized by KLESociety's S.Nijalingappa College, Bangalore, India-560010 on 9th & 10th October 2020.

2) Oral presentation on **"Oral raft forming in -situ gelling system for site specific delivery of calcium"** in the JNANA CHILUME 2020- National Conference on "Technical and Socio-economic Transformations of robotics and automation in food technology and chemical sciences" organized jointly by the Department of Food Technology, Department of Chemistry, Department of Humanities and Social Sciences at Faculty of Engineering and Technology, JAIN (Deemed to be University), on 25 th November 2020.

Publications:

1) Moganti M, Nanjappa SH. Formulation and optimization of gastroretentive bilayer tablets of calcium carbonate using D-optimal mixture design. e- Polymers. 2021 Jan 1;21(1):057-71.

2) Moganti M, Shivakumar HN. Oral raft forming in situ gelling system for site specific delivery of calcium. Journal of Drug Delivery Science and Technology. 2021 Feb 1;61:102113.

Research Article

Manasa Moganti* and Shivakumar H. Nanjappa*

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 ± 1.18%), and controlled yet near complete release (88.98 ± 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 g/cm^3) that remain buoyant above the gastric contents, high density systems (>1 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 (~2.71 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 4,000–400 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/cm^2. 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 Monsato hardness tester (M/s Cambell Electronics, India) (14). The hardness was measured in terms of kg/cm^2. 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] X_1, X_2, and X_3 represent the amounts of HPMC K100 M, sodium bicarbonate, and HPMC E15 LV, respectively. X_3 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 ± 2°C/60 ± 5% 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 gastroretentive 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 ± 2°C and 55 ± 5% 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: Microskan 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 cm^{-1} that can be assigned to –C=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/cm^2 to 7.53 ± 0.19 kg/cm^2 and 0.02 ± 0.01 kg/cm^2 to 2.77 ± 0.64 kg/cm^2 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_1X_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 gastroretentive tablets was found to range from 0.02 ± 0.01% for F12 to 2.77 ± 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 ± 0.31%, 2.64 ± 0.28%, 2.68 ± 0.18%, and 2.77 ± 0.64%, respectively. Coincidentally, the hardness of these batches failed to cross 4.5 kg/cm^2. 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] X_1, X_2, and X_3 represent the amounts of HPMC K100 M, sodium bicarbonate, and HPMC E15 LV, respectively. X_3 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:

$\sqrt{}$Friability = + 0.18*X1 + 0.32*X2 − 17897.32*X3
In FLT = + 0.24*X1 + 1.19*X2 − 25289.06*X3
Rel_{1h} = + 0.40*X1 + 4.38*X2 − 64542.84*X3
Rel_{6h} = + 0.74*X1 + 0.88*X2 − 1539.69*X3

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

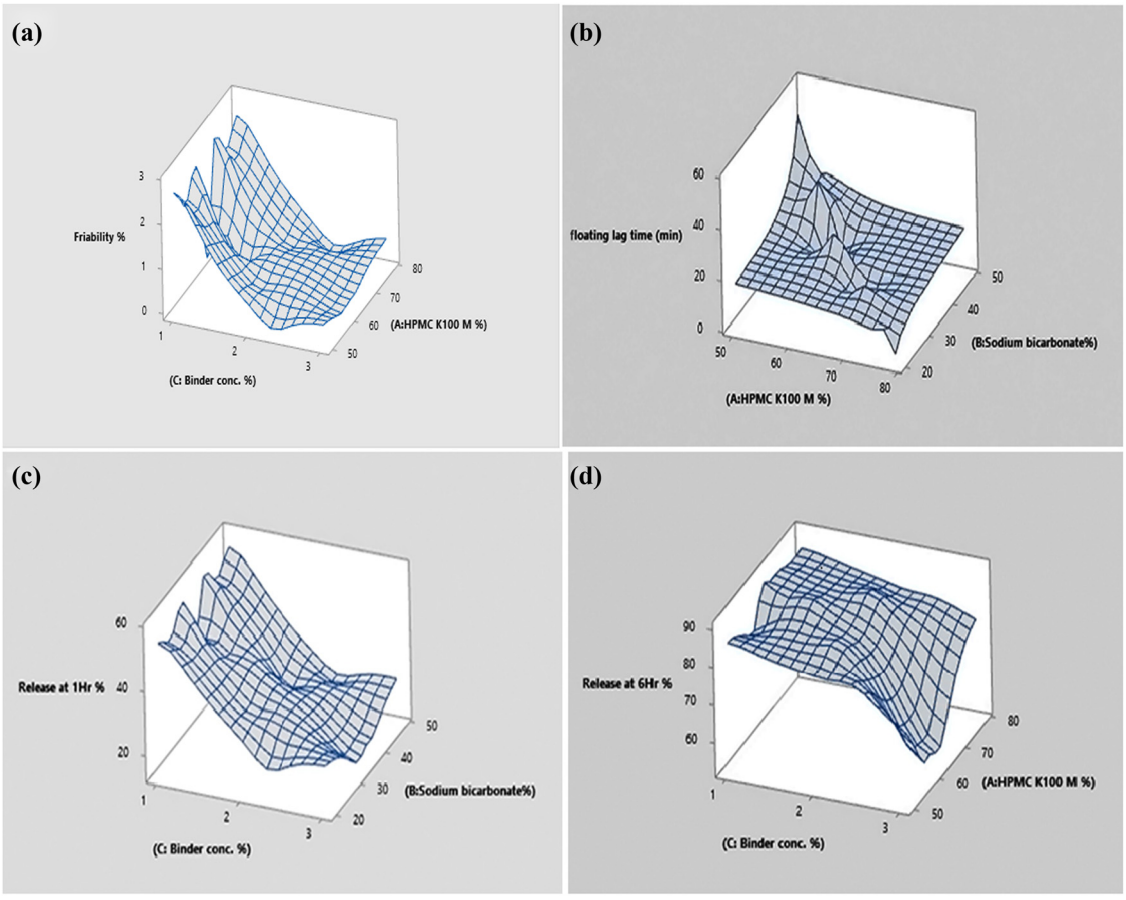

Figure 1: Response surface plots depicting the interaction effects of dependent factors on a Y: friability (a), FLT (b), % release at 1 h (c), and % release at 6 h (d).

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 (≥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 FLTs. 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 FLTs (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

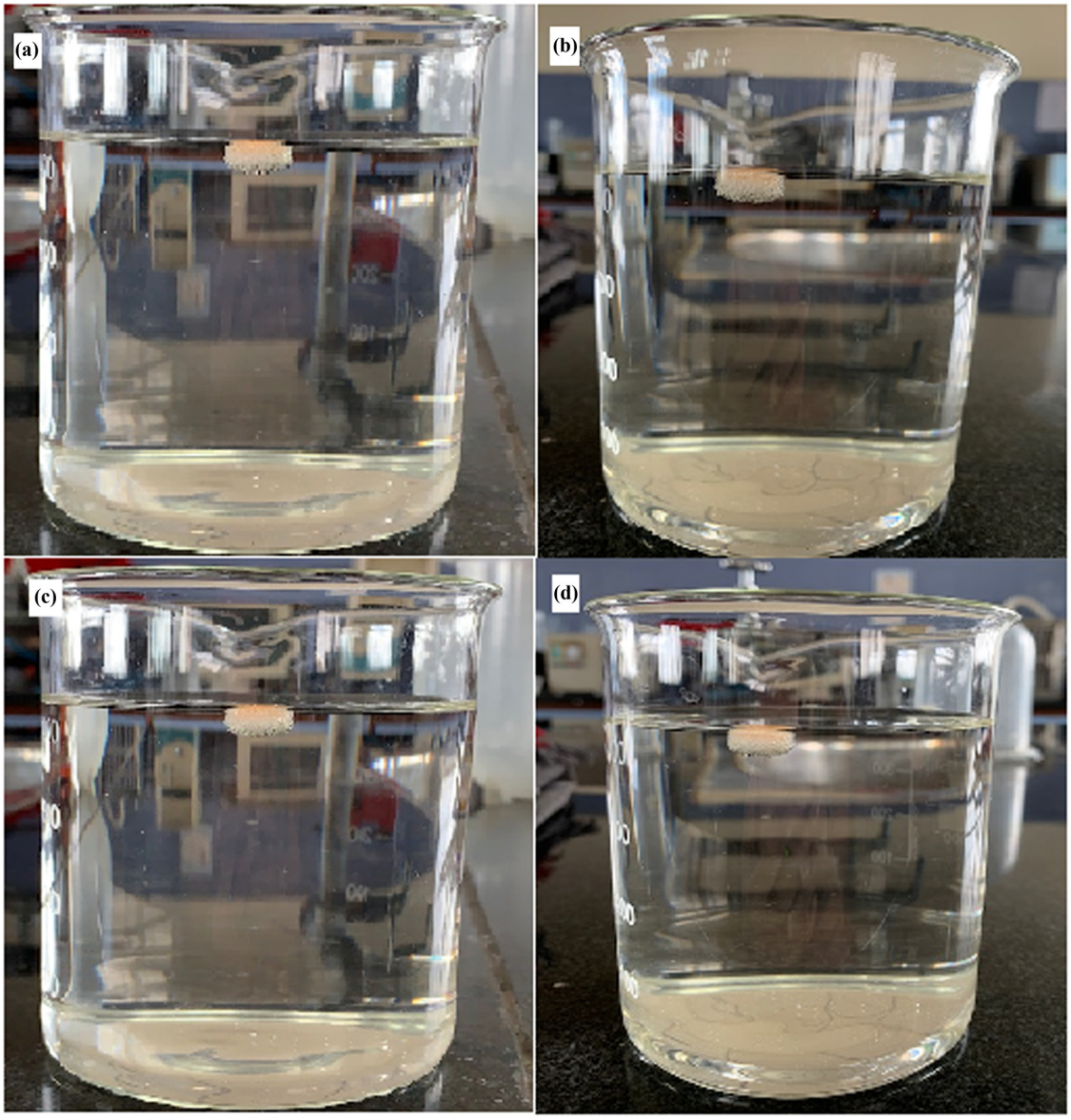

Figure 2: Photographs depicting the side view of bilayer tablets of CC in pH 1.2 buffer at 1 h (a), 2 h (b), 4 h (c), and 6 h (d).

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 ± 2.54% for F5 to 55.61 ± 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 ± 2.58%, 54.82 ± 2.13%, 56.60 ± 1.59%, and 55.61 ± 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.

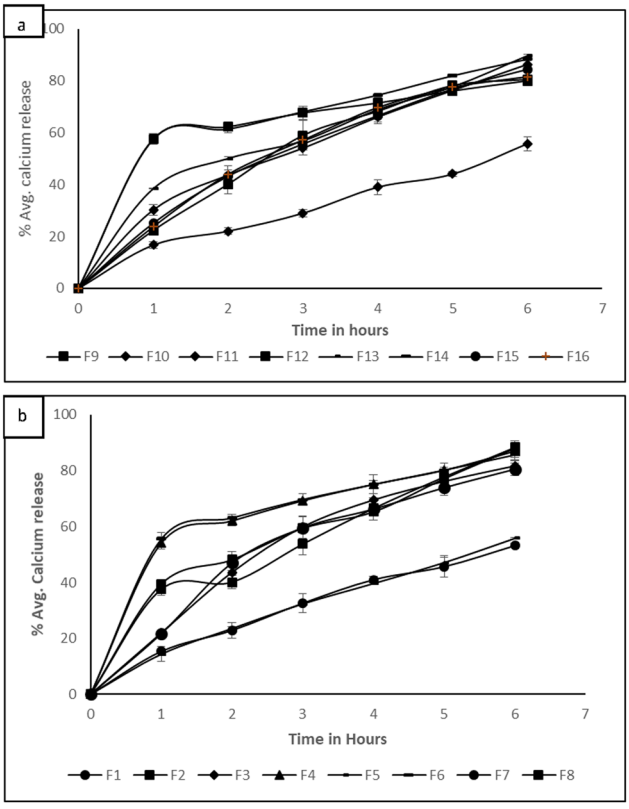

Figure 3: Comparative cumulative amount of calcium release from bilayer tablets of batches F1–F8 (a) and F9–F16 (b).

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 ± 0.63% for F7 to 88.72 ± 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.

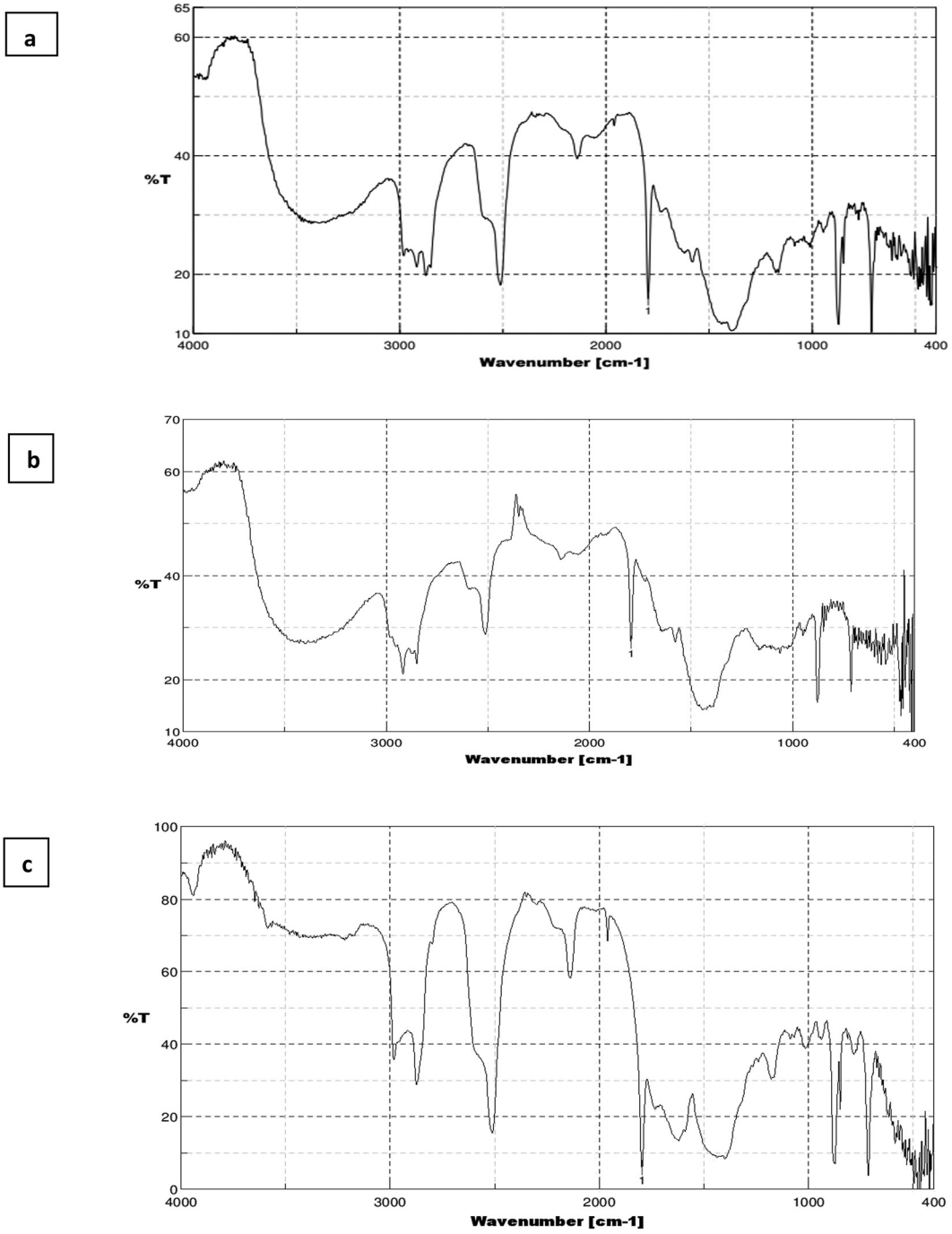

Figure 4: FTIR Spectrum of CC (a), physical mixture (b), and bilayer tablet (c).

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] X_1, X_2, and X_3 represent the amounts of HPMC K100 M, sodium bicarbonate, and HPMC E15 LV, respectively. X_3 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).

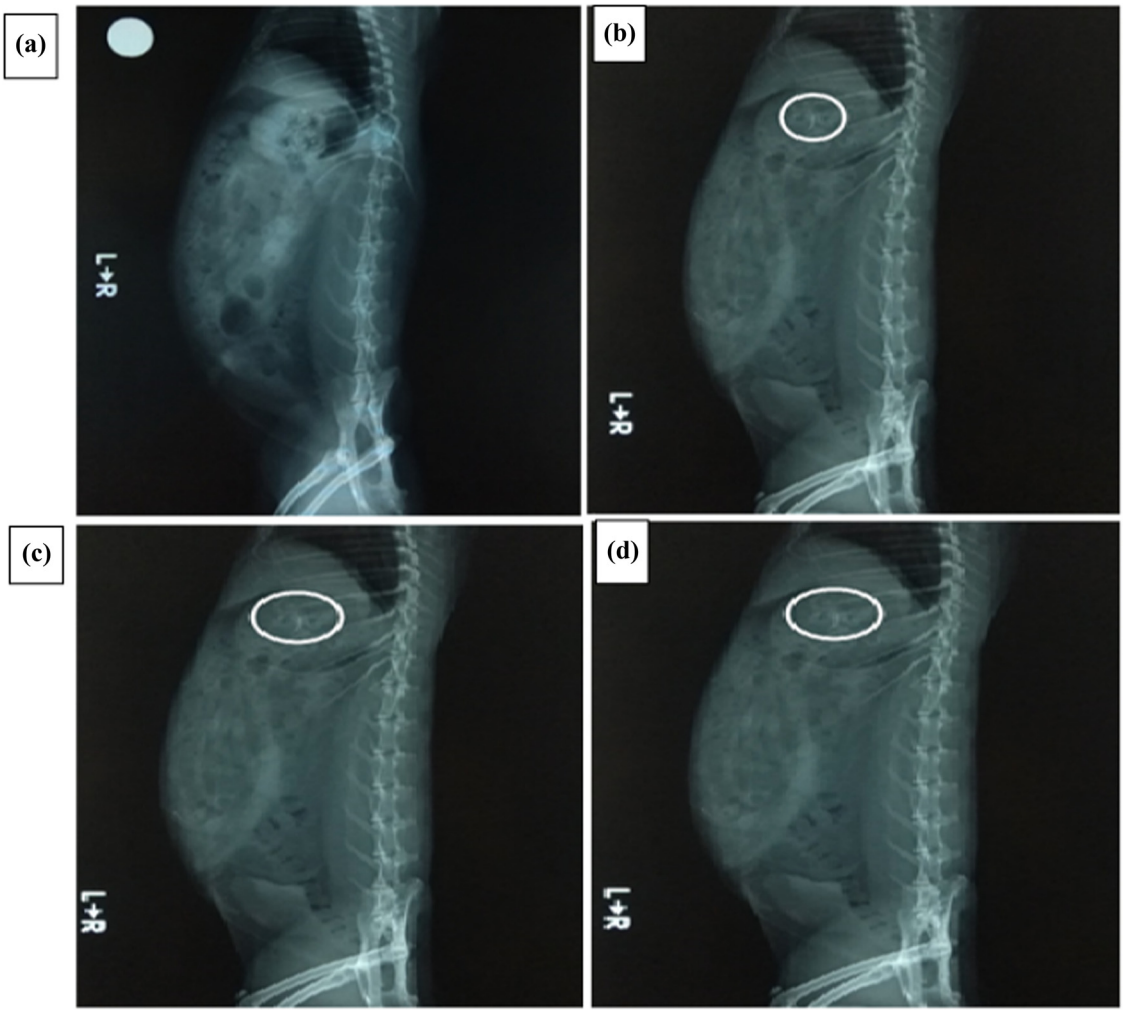

Figure 5: Radiographic images portraying the bilayer floating tablet containing barium sulfate as a radio opaque marker in the stomach of rabbits at 0 h (a), 2 h (b), 4 h (c), and 6 h (d); the tablet is pointed by a circle.

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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Research paper

Oral raft forming *in situ* gelling system for site specific delivery of calcium

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ABSTRACT

In situ gelling Raft forming system (GRFS), a novel sol-gel system of calcium carbonate (CC) was developed with the aim to prolong the gastric residence time and thereby the bioavailability. A simple lattice mixture design was adopted to study the effect of formulation composition (% HPMC K100 M and % Xanthan gum), on buoyancy lag times, percent of CC released at 1hr and 6hr. The mathematical models generated by analysis of variance (ANOVA) indicated that the levels of HPMC K100 M and Xanthan gum were found to significantly affect all the three responses. The optimized formulation developed by numerical optimization technique was found to display short buoyancy lag time (10.90 ± 0.56 s), minimum burst release (20.74 ± 1.08%) in 1 h and controlled yet near complete release (87.25 ± 1.81%) in 6 h. The experimental data for the optimized formulations was in agreement with that predicted by the mathematical models proving the validity of the models generated. *In vivo* radiographic studies in rabbits indicated that optimized batch displayed a mean gastric retention time of 5.64 ± 0.43 h that was significantly higher ($P < 0.05$) compared to the marketed suspension that exhibited a mean gastric retention time of less than 1 h. The studies proved that the GRFS gastroretentive systems can be a promising platform to improve bioavailability of nutrients having absorption window in upper gastrointestinal tract.

1. Introduction –

Calcium is the most obvious and persistent of the micronutrients in the body. Calcium forms an important mineral component of our diet. Calcium supplementation can play a valuable role in bone health throughout the lifecycle. Indian Council of Medical Research (ICMR) recommends more calcium for growing children and adolescents [1]. Even though the recommended dietary allowances for calcium are about 600–800 mg/day, it is desirable to administer higher quantities of calcium for adolescents to achieve high peak bone mass. Calcium Recommended Dietary Allowance (RDA) increases to 1200 mg/d for pregnant and lactating mothers.

Globally, calcium carbonate is the most commonly prescribed and least expensive form of calcium supplement [2]. Calcium supplements are generally well tolerated even though, some subjects complain of gastrointestinal symptoms, including constipation, gas, flatulence, and bloating. However, a sizable population of elderly and paediatrics face big challenge to take calcium supplementation resulting in patient noncompliance [3]. In this context, conventional oral liquid calcium formulations like Calcimax plus are commercialized. However, these conventional oral formulations exhibit poor oral bioavailability because the assimilation of calcium is pH dependent, site-specific and limited by carrier mediated process [4]. It has to be noted that calcium carbonate is practically insoluble in water at neutral pH while it exhibits good solubility in the acidic environment of the stomach. Moreover, calcium absorption is site-specific as it is only absorbed from the duodenum due to the presence of the saturable active transporter protein 'calbindin' at active absorption sites [5]. Owing to the above mentioned absorption issues, the bioavailability of oral conventional calcium dosage forms is limited to 25–35% of administered dose.

In view of the limitation of conventional liquid oral formulations calcium, the aim of the current study is to develop a first of its kind '*in situ* gelling Raft forming (GRFS) systems' for calcium carbonate (CC). These are likely to remain afloat and reside proximal to the active absorption window and release the calcium in a controlled fashion. The gastric media by virtue of its low pH would offer a sink condition for the dissolution of calcium from the system. In view of its strategic location and the ability to release the calcium in a controlled fashion, the GRFS is less likely to saturate the active absorption site in the duodenal region and thereby more likely to improve the oral bioavailability of calcium. The novel GRFS would not only enhance the oral bioavailability of CC but would also benefit the special populations (elderly and the

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paediatrics) who suffer from swallowing dysfunction [6].

Recent technological development have been have been successful to overcome bioavailability issues arising from drugs or nutrients having absorption window located in the upper part of the GI tract. Gastroretentive drug delivery system [7] (GRDDS) is an approach that has been used to resolve the bioavailability issues arising from poor absorption of nutrients or drugs that are absorbed only from a specific absorption window in the upper part of gastrointestinal (GI) tract. These systems are designed to be well retained in the upper part of the GI tract and release the drug in a sustained manner. The floating raft system (GFRS) is a novel GRDDS that would be well retained in the stomach by virtue of floating and bioadhesive properties. Biopolymers like sodium alginate, pectin and numerous others have been used to develop *in situ* gels as they offer advantages in development in terms of low cost, availability, biodegradation and freedom from toxicity and sustainability. Sodium alginate is a biodegradable non-toxic naturally occurring macromolecule that hydrates and swells in water but produces gel on protonation in acidic environment [8,9]. Sodium alginate is a pH sensitive polymer that forms a stable gel at acidic pH but unstable in alkaline medium owing to rapid dissolution.

Xanthan gum is an extracellular polysaccharide produced by bacterium Xanthomonas campestris, with high molecular weight (>2 × 106) and high water solubility. Xanthan solutions show controlled release properties with pseudoplastic behaviour which is very stable over a wide range of temperature and pH and in the presence of salts [30]. Xanthan gum is a GRAS (generally regarded as safe) listed excipient that is used in formulation of extended release liquid orals [33].

Calcium not only serves as a supplement in the present composition but acts as ionic cross linking agents in sodium alginate based GRFS to form three dimensional gel network [12,13]. Calcium carbonate that would be present in the insoluble form in the formulation hitherto would release calcium ions in the acidic medium which cause gelation of system. The released Calcium ions acts as a hardening or a cross linking agent in the present formulation. Divalent (Ca^{++}) ions are known to form a stronger gels than monovalent (Na^{+}) ions causing formation of a rigid gel that helps to control the release too [10,11].

The GRFS is an advanced revolution in liquid oral dosage forms. These systems are liquids or dispersions at room temperature but undergo gelation when come in contact with body fluids containing ions or due to change in pH. These systems display a unique property of either temperature dependent or cation induced gelation, which ensures sustained and site specific drug delivery [14]. The formed gels remain intact within the stomach for several hours resulting in prolonged drug delivery in the upper part of gastrointestinal tract [15]. GRFS could offer a revolutionary solution for liquid oral dosage form containing nutrients or drugs that are absorbed invariably only from a specific absorption window in the upper part of GI tract.

To cut down the cost and time involved in empirical experimentation during product or formulation development, dry lab experimentation utilizing computational modelling is now predominating [16]. This is achieved through developing and optimizing the formulation with few experiments using the concept of Design of experiments (DoE) and Quality by Design (QbD). Optimization of formulations using statistical experimental designs is a powerful and efficient tool in formulation development.

In this study, the main aim is to improve the gastric retention and therefore the bioavailability of Calcium by developing and optimizing the composition of a GRFS that could exhibit an ideal controlled release and floating properties. In this context, a simple lattice design was employed to elucidate the influence of the factors namely the proportion of HPMC K100 M (X1) and Xanthan gum (X2) on the buoyancy lag time (Y1), percent release at the end of 1 h (Y2) and percent release at the end of 6 h (Y3). Further, we plan to optimize the composition of the raft formulation employing numerical optimization technique using desirability approach. Finally, radiographic image analysis of the optimized *in situ* gelling raft formulation was performed in rabbits to assess the *in vivo* gastroretention.

Table 1
Independent variables with experimental ranges as per simplex lattice mixture design.

Independent variables	Low value (%)	High value (%)
X1: Fraction of HPMC K100 M (% w/w)	50	90
X2: Fraction of Xanthan gum (%w/w)	10	50
Dependent variables	**Constraints**	
Y1: Buoyancy lag time (sec)	Minimize	
Y2: Drug release at 1st h (%)	Minimize	
Y3: Drug release at the end of 6th h (%)	Maximize	

The total amount of polymer (150 mg), amount of $CaCO_3$ (250 mg) and sodium alginate (250 mg) in 5 ml are kept constant in all the formulations.

2. Materials and methodology

2.1. Materials

Calcium carbonate (confirming to IP) and Barium sulphate (X-ray grade) purchased from lobachemie Pvt. Ltd, Mumbai. Protanal® LFR 5/60 USP/NF (Sodium alginate) was provided by FMC Biopolymer, USA. Sodium bicarbonate, Potassium dihydrogen orthophosphate, Sodium hydroxide pellets, Sodium acetate, Talc, Glacial acetic acid and Hydrochloric acid were supplied from S.D. Fine chemicals, Mumbai. Hydroxypropyl methyl cellulose K 100 M was supplied by Colorcon Asia Pvt. Ltd, Goa. Xanthan gum IP was supplied by Signet excipients Pvt. Ltd, Mumbai.

2.2. Methodology

2.2.1. pH solubility profiling

Solubility of calcium carbonate in buffers of varying pH was determined in order to find out the most suitable solvent for further studies. The studies were conducted in 5 different buffers of different pH. An excess amount of calcium carbonate was added to10ml of buffer (1.2, 4.0, 5.5, 6.8 and 7.4 pH) in a series of stoppered conical flasks. The samples were shaken for 72 h at room temperature on a rotary flask. After equilibration, samples were filtered using Whatmann filter paper no. 42 (0.45 μm pore size) to separate the undissolved drug, diluted suitably and assayed for calcium content by measuring the concentration against the respective buffer as blank by flame photometry. The solubility experiments were carried out in triplicate to check the repeatability.

2.2.2. Preparation of calcium carbonate GRFS

2.2.2.1. Design of experiment (DOE). A simplex lattice design (*Design Expert Software* version 10.0.6.0) was adopted to elucidate the effect of the formulation factors or independent variables on the responses or the dependent variables. In this design, two factors were evaluated by changing their proportions simultaneously but at the same time maintaining their total constant. The experimental design was containing two factors or components namely the amounts of HPMC K100 M (X1) and Xanthan gum (X2). The proportions of X1 were varied from 50.00% to 90.00%, while that of X2 was varied from 10.00% to 50.00% respectively so that the sum of two components would be 100%. The range of each of the component was determined by carrying out a set of preliminary trials. Pourability and gelling properties on contact with the acidic environment were the two key parameters that were considered during the preliminary trials. The effect of formulation factors on responses like buoyancy lag time (Y1), percent release at the end of 1 h (Y2) and percent release at the end of 6 h (Y3) were systematically investigated. The formulation variables as per simple lattice mixture design and the constraints set on each of the components to obtain the optimized response are shown in Table 1.

Table 2
Composition of the model formulations as per Simple lattice mixture design.

Run	Sodium alginate[a]	HPMC K100 M[a]	Xanthan gum[a]	Calcium carbonate[a]	Vol. of purified water
F1	250	105	45	250	5 mL
F2	250	75	75	250	5 mL
F3	250	75	75	250	5 mL
F4	250	90	60	250	5 mL
F5	250	120	30	250	5 mL
F6	250	135	15	250	5 mL
F7	250	135	15	250	5 mL
F8	250	105	45	250	5 mL

[a] The weight of each ingredient is in mg.

Table 3
Formulation variables for the *in situ* gelling raft formulations and their observed responses.

Run	X1: HPMC K 100 M (% w/w)	X2: Xanthan gum (% w/w)	Y1:Buoyancy lag time (sec)	Y2:% Rel at 1 h	Y3:% Rel at 6 h
F1	70	30	15.55 ± 3.51	21.78 ± 2.47	83.50 ± 1.20
F2	50	50	4.52 ± 2.78	16.15 ± 3.46	68.65 ± 0.97
F3	50	50	4.58 ± 0.49	14.51 ± 3.06	65.50 ± 1.98
F4	60	40	10.46 ± 0.20	25.12 ± 1.80	82.90 ± 1.13
F5	80	20	34.43 ± 2.39	27.34 ± 2.01	87.07 ± 0.85
F6	90	10	56.17 ± 3.62	58.56 ± 2.64	80.33 ± 1.68
F7	90	10	50.74 ± 0.44	56.54 ± 3.60	79.30 ± 0.04
F8	70	30	13.46 ± 0.57	19.54 ± 3.30	85.69 ± 2.44

X1 and **X2** represent the formulation variables while **Y1**, **Y2** and **Y3** stand for the responses.

The formulations were prepared by dispersing of sodium alginate (5%w/v) in deionised water under stirring. Calculated amounts of HPMC K 100 M and Xanthan gum as per the design amounting to 150 mg were individually dispersed with constant stirring till homogenous viscous polymeric solution was obtained. About 100 mg of Calcium (250 mg of Calcium carbonate) was finally dispersed in the polymeric solution. The total amount of calcium carbonate and polymers including sodium alginate in the formula were retained the same in all the eight formulations. The formulation variables employed to produce 8 batches of Raft forming solutions as per the experimental design is portrayed in Tables 2 and 3.

2.2.3. Characterization of the GRFS -

2.2.3.1. Gelling capacity -. The *in situ* gelling ability of the formulations (as measured by assessing the gelling capacity of each formulation) was evaluated as per the reported procedure [17]. Briefly, about 5 mL each of 0.1 N hydrochloric acid and Purified water was transferred to a 10 mL test tube and maintained at 37 ± 0.5 °C in water bath. Then, 1 mL of the formulation was carefully transferred using a pipette and slowly released by placing the pipette tip at the surface of the above solution. The formulations were assessed visually for the time required to complete the gelation.

2.2.3.2. Rheological measurement –. The Rheology of GRFS (20 mL) was evaluated for flow behaviour by rotational measurements using a Rheometer set up (MCR 92) of measuring system (P-PTD200) with fixture (CP50-1) and gap (0.1 mm) at 37 ± 0.5 °C. Prior to actual measurements samples were kept on hold for 1 min for the sample stress relaxation and temperature homogenization between the double gap geometry. The flow behaviour of the samples were subjected to shear rate sweep analysis ranged from 10 to 100 (1/s) with a data acquisition time varying in logarithmic scale from 15 s to 5 s at a constant temperature of 37° C.

2.2.3.3. Floating behaviour –. The floating behaviour [18] was observed by introducing GRFS (5 mL) into 900 mL of 0.1 N Hydrochloric acid (pH 1.2) maintained at 37 °C in USP type – II (paddle-type) dissolution apparatus. The time taken for the formulation to float to the surface of the medium is considered as buoyancy lag time (BLT). The time the formulation floated on the surface of the medium was recorded as total floating time.

2.2.3.4. Determination of in vitro drug release. The *in vitro* release of calcium from GRFS was determined using USP type – II (paddle-type) dissolution apparatus [8] (Electrolab, Mumbai, India) at a stirring speed of 50 rpm.The formulation of about 5 mL was added to the dissolution medium containing 900 mL of 0.1 N Hydrochloric acid (pH 1.2) and the temperature was maintained at 37 ± 0.5 °C. 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 0.45μ filters and analysed using a Flame photometer to determine the amount of calcium carbonate released at different time points. The dissolution data recorded in triplicate was analysed to calculate percentage cumulative drug released at different time intervals. A graph of cumulative drug released was plotted on the Y axis vs. time on the X axis to obtain the dissolution profiles of raft formulations.

2.2.3.5. Analysis of calcium. The amounts of calcium present in the *in vitro* samples were analysed by photometry in Flame photometer (Model – 128, Systronics, Ahmedabad, India). Flame photometry is an established sensitive technique to quantify calcium [19]. The instrument is a microprocessor based device that enables a maximum of five point calibration. The selection of filter that is specific for calcium is done by actuating the button. The instrument was calibrated before use. Calcium standards ranging in concentrations between 10 and 200 μg/mL in pH 1.2 were used to construct the calibration curve. The experiments were conducted in triplicate (n = 3) to check the reproducibility. The reading of the each working standard solution was measured and a graph of concentration of the solution was plotted against flame photometer reading. The data acquired was subjected to regression analysis in Microsoft Excel software® to check the linearity of the calibration curve. The studies were conducted in triplicate (n = 3) to check the reproducibility. The ***in vitro*** samples were appropriately diluted before data acquisition so that the concentrations lies in the beer's range. The Flame photometer readings in emission response (in ppm) were converted to calcium concentration expressed in μg/ml.

2.2.3.6. In vivo X-ray imaging studies. In vivo animal studies was performed in normal rabbits using X-ray imaging technique for evaluating the gastroretentive potential of the optimized formulation as per the protocol approved by the Institutional Ethical Committee, (IE-52, dated October 12, 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 ± 2 °C and 55 ± 5% 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 GRFS containing X ray grade barium sulphate (15%w/w) as a marker while the control group of animals were orally treated with conventional Calcium

carbonate suspension (Calcimax plus) containing the same the same amount of the marker. The animals were held in the upright position for imaging to locate the position of both control and GRFS in the GI tract. The images of animals were captured using X-ray machine (Skanray Model: Microskan DR) at pre-determined time intervals like 0, 2, 4 and 6 h respectively.

2.3. Data analysis of simple lattice mixture design

2.3.1. Model fitting and evaluation

The experimental data was analysed by fitting to the scheffe [20] polynomial equations. These equations are modified from the general polynomial equations to lack intercept and squared terms in order to fit the mixture designs. The three responses namely buoyancy lag time, Rel_{1h} and Rel_{6h} were simultaneously fitted to linear, cubic and quadratic statistically analysed by performing Analysis of variance (ANOVA). The statistical parameters used to analyse 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.

Numerical optimization was performed using desirability approach to locate the optimal settings of the formulation variables. An optimized formulation was developed by setting constraints on each of the components to obtain the desired response. Constraints were set to minimize the burst release and buoyancy lag time while maximize the release at 6 h. The constraints set on each of the responses are captured in Table 1. The formulation developed was evaluated for each of the responses and the experimental values were compared with those predicted by the polynomial models to assess the residual error.

3. Results and discussion

The aim of the study was to produce floating GRFS that could display minimum floating lag time, float at least for a period of 6 h and at the same time ensure a controlled but near-complete release of the entire dose of CC with minimum burst effect. Considering this, we initially developed floating GRFS of calcium carbonate based on the using sodium alginate and HPMC K100 M as a release retardant. The pour ability, floating and gel properties along with drug release from the raft forming formulations were investigated. Sodium alginate was found to be an essential component of the raft systems that determines the above mentioned physicochemical properties. Even though the raft systems of CC were found to float for the period of more than 6 h, the release of calcium had to be optimized to minimize the burst effect and at the same time ensure a controlled release of calcium in 6 h. Considering this, the concentration of sodium alginate was maintained at a concentration of 5%w/v in all the raft formulations to ensure adequate pour ability. To meet the requirement, different quantities of HPMC K 100 M and Xanthan gum were incorporated to minimize the initial burst release and ensure a controlled release of calcium from the formulation. HPMC K 100 M was added to impart good gel strength to ensure floatation of the formulation. On the other hand, xanthan gum was used to reduce the burst effect and control the release of calcium. Xanthan gum preparations displayed thickening properties owing to its pseudoplastic behaviour [21], which are quite stable over a wide range of temperature, pH and in presence of salts. When used in the right proportions, sodium alginate, HPMC and xanthan gum were found to minimize the burst effect and at the same time ensure a control release of calcium from the GRFS. In this context, there was a need to identify the right proportion of sodium alginate, HPMC K 100 M and xanthan gum that may be necessary to modulate the floating and the drug release properties of GRFS.

In order to systematically accomplish our goals, we planned to develop and optimize the formula or the composition of GRFS of calcium carbonate using a simplex lattice design. Mixture experimental designs are generally used to analyse the impact of formulation variables on the selected responses. Simplex lattice design is a mixture design that is used to evaluate the effect of changes in the composition on the responses and enable statistical optimization of the formulation with least number of experiments.

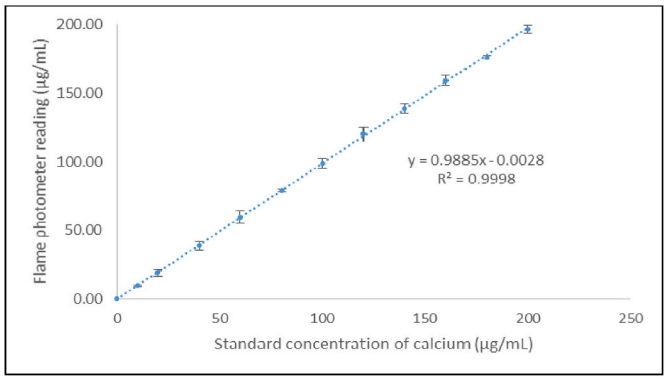

Fig. 1. Calibration curve of calcium carbonate in pH 1.2.

The calibration curve in pH 1.2 was found to be linear in the concentration range of 10–200 µg/mL with a slope of 0.9885 as portrayed in Fig. 1. The calibration curve was found to be linear as indicated as the value of by regression coefficient equation was close to 1.000 $y = 0.9885x - 0.0028$ ($r^2 = 0.9998$). The method for the determination of calcium by flame photometry was found to be reproducible as indicated by the low values of standard deviation (see Fig. 2).

3.1. Characterization of raft forming systems –

3.1.1. Gelling capacity

Alginate sodium salt precipitates to form a low dense (<1 g/ml), but viscous gel. The strength of the gel is dependent on the molecular weight and the ratio of D-mannuronic and L-glucuronic acid residues of sodium alginate. Generally, sodium alginate with higher molecular weight and higher glucuronic acid content form rafts with greater viscoelastic strength [22]. Calcium further increases raft strength through its ability to cross-link alginic acid with the formation of 'egg box' structure.[23]All the formulations showed rapid gelation when in contact with the gastric fluids (0.1 N HCl, pH 1.2). Increasing the alginate concentration in the preliminary trails from 2% to 5% increased the gelation capacity as the gelation time decreased and gel strength increased. Formulations containing 2% of sodium alginate formed weak gels, leaving turbid solutions below. Such systems are not suitable as GRFS as they are prone to undergo rapid gastric emptying by the peristaltic movements [24]. Therefore, the concentration of sodium alginate was maintained at 5% in all formulations to obtain raft formulations with good gel properties. The results also indicated that incorporation of HPMC K100 M improved the gelation capacity of alginate. HPMC K100 M was incorporated to improve the gelation capacity of sodium alginate and impart sustained release properties to the raft systems [25].

3.1.2. Rheological measurements –

The rheograms of liquid dispersions containing natural gums like sodium alginate and Xanthan gum exhibit pseudoplastic flow (Fig. 4) (see Fig. 3). All liquid GRFS displayed a decrease in viscosity with an increase in shear rate, indicative of pseudoplastic flow or shear thinning behaviour [26]. The viscosities of the RFS was found to increase in the order F2 > F4 > F1 > F5 > F6. A rank order correlation was found to exist in the Viscosities at a shear rate of 10 sec^{-1}. The yield stress of the GRFS recorded at 37 °C is reported in Table 4. The viscosities of the GRFS increased as the proportion of xanthan gum increased in the formulation.

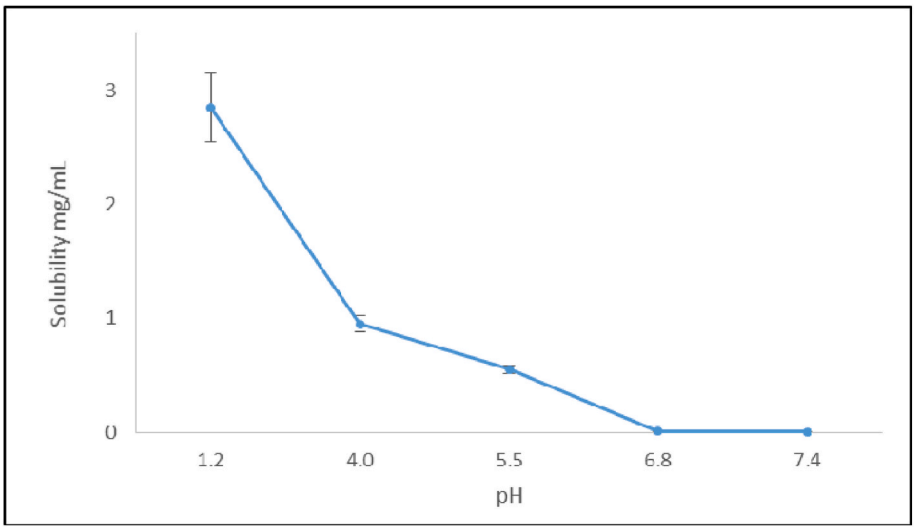

Fig. 2. pH Solubility profile of calcium carbonate.

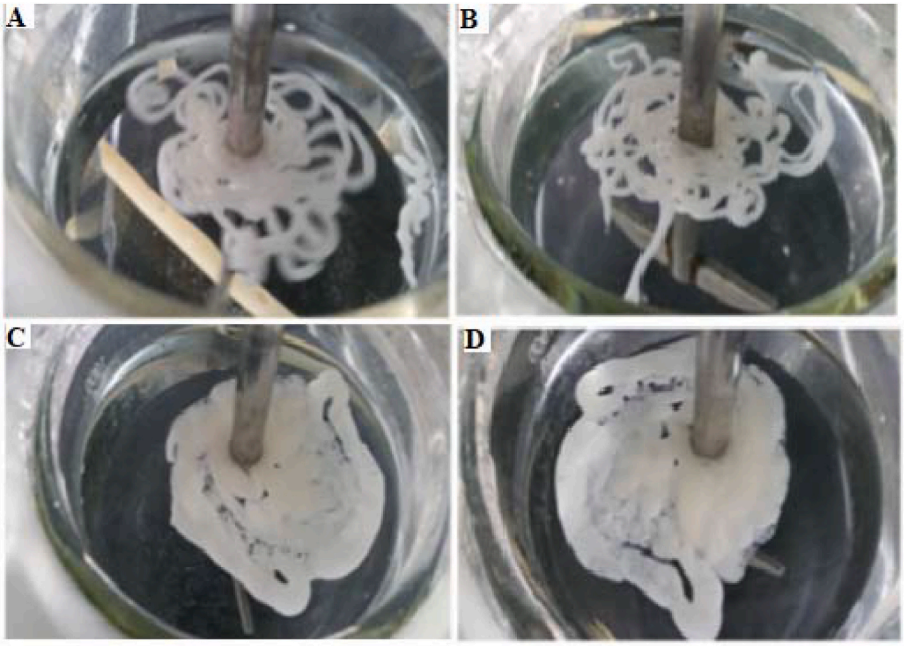

Fig. 3. GRFS in 0.1 N Hydrochloric acid (pH 1.2) at 0 h, 2 h, 4 h and 6 h intervals.

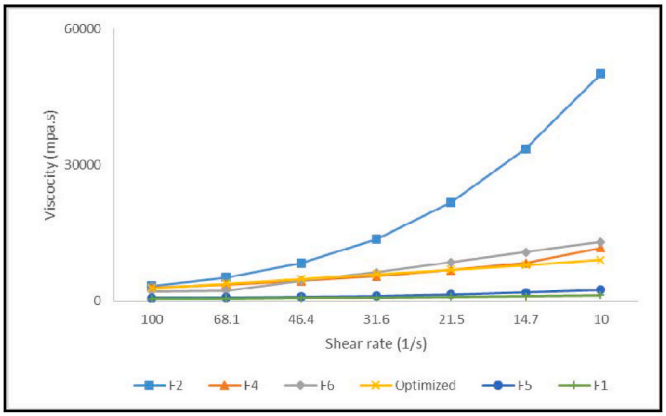

Fig. 4. Overlay of Viscosity Curve (Viscosity versus Shear rate).

Table 4
Results of rheological measurements of the *in situ* gelling Raft formulations.

S.No	Sample	Viscosity at 10 (1/s)	Yield stress (Pa)
1.	F1	1250	5.07
2.	F2	50063	55.07
3.	F4	11703	41.26
4.	F5	2474	19.49
5.	F6	12978.9	8.22
6.	Optimized formulation	9008.1	48.02

The yield values are known to determine the dispersibility and portability of the GRFS [27]. The low values of yield stress indicate the systems are known to readily disperse and pourable just on mild agitation. The GRFS were not found to cake on standing but instead were readily dispersible on aging for 2 months at rest (at negligible shear rates). The viscosity profiles indicate the drop in the viscosity as the rate

Table 5
Summary of ANNOVA for the response parameters of the Model formulations of *in situ* gelling raft formulation prepared as per simplex lattice design.

Response	F-value	p-value	R [2]	Adj R^2	% C·V.
Y1	395.04	<0.0001	0.9850	0.9825	4.69
Y2	269.11	<0.0001	0.9951	0.9914	5.44
Y3	52.54	0.0015	0.9546	0.9364	2.54

Regression equations of the fitted model containing only the significant terms:
Log BLT = + 0.05*X1 – 0.01*X2
Rel_{1h} = + 1.36*X1 – 8.94*X2 + 0.16*X1X2
Rel_{6h} = + 0.67*X1 - 0.92*X2 + 0.03*X1X2

of shear is increased indicating typical characteristic feature of pseudoplastic system. These systems were found to display high viscosities at rest or at low rates of shear enabling $CaCO_3$ to be uniformly dispersed for prolonged time periods. The high values of viscosities would enable one to withdraw uniform dose of the Calcium. On the other hand, the systems exhibited a substantial drop in viscosity at high shear rates enabling uniform dispersion of calcium carbonate on mild agitation. However, the viscosities attained depend on the concentration of the polymers in the systems. Moreover, in the development of GRFS, there was a need to strike a fine balance between the viscosity, yield stress and the sustained release properties.

3.2. Data analysis of the simple lattice mixture design

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

3.2.1. Model fitting and evaluation for the responses: Y1, Y2 and Y3

The experimental data was analysed by fitting the data to the Scheffe [20] polynomial equations. 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 three responses namely BLT, Rel_{1h} and Rel_{6h} simultaneously to linear, cubic and quadratic models and statistically analyse the data by performing Analysis of Variance (ANOVA). The statistical parameters used to analyse 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 four models analysed as the *p values* for Y1, Y2 and Y3 were found to be < 0.0001, <0.0001 and 0.0015 respectively. 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 polynomial equations for buoyancy lag time, Rel_{1h} and Rel_{6h}in terms of the actual factors that are used as predictive models are represented in Table 5.

3.3. Buoyancy lag time (BLT) –

The floating lag time of the GRFS was found to range from 4.52 ± 2.78 s for F2 to 56.17 ± 3.62sec for F6. A short lag time is preferable as 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.

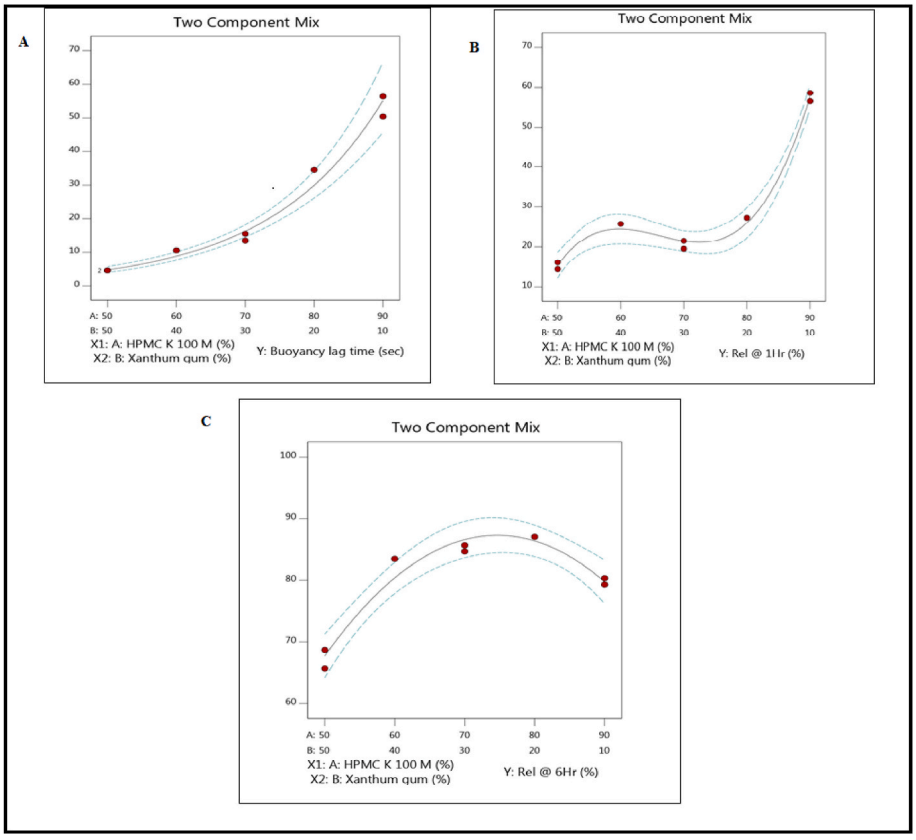

Fig. 5. Two component mix plots depicting the interaction effects of independent factors on a Buoyancy lag time (A), Release at 1hr (B) and Release at 6hr (C).

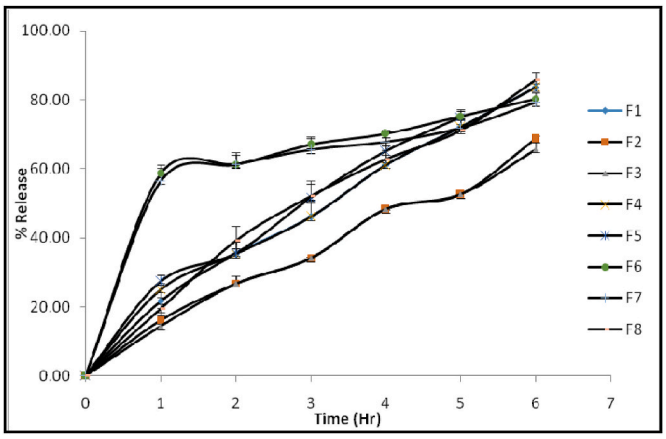

Fig. 6. Comparative cumulative amount of Calcium carbonate release from *in situ* gelling raft formulation batches F1–F8.

Mathematical modelling of the experimental data suggested that the two factors investigated were found to have a substantial influence on floating lag times (Table 3). Among the two factors explored, the effect of HPMC K100 M (X1) was found to have a positive effect on the BLT while xanthan gum (X2) was found to have a negative influence on the BLT. Generally, batches with higher levels HPMC K100 M were found to display high floating lag time. Calcium carbonate can accelerate floatation by acting as a gas generating agent when it comes in contact with the acidic dissolution medium to release carbon dioxide. The produced carbon dioxide is likely to get entrapped within the raft gel, thereby increasing the buoyancy of the raft. Further, it is more likely that the GRFS having high levels of HPMC good gel strength is known to better entrap the evolved carbon dioxide facilitating floatation. The impact of the two formulation factors on the BLT is clearly visible in two component mix plots captured in Fig. 5. The Figure indicates that GRFS with low BLT are invariably associated with high levels of xanthan gum. This indicates that HPMC facilitated fluid movement into the liquid raft formulation through its swelling action, followed by expanding the surface of the raft gel [18] (see Fig. 6).

3.4. Release at 1hr

The percentage calcium release at the end of first hour was found to range from 14.51 ± 3.06% for F3 to 58.56 ± 2.64% for F6. Mathematical modelling of the experimental data suggested that the two factors investigated were found to have a substantial influence on the burst release or release at 1 h (Table 3). The rapid release could be partially attributed to the fact that 0.1 N HCl (pH 1.2) would ensure a sink condition for dissolution of CaCO3. The solubility profile data generated in the present study that indicates high solubility of CaCO3 in pH 1.2 would support this hypothesis. Among the two factors modelled, the effect of HPMC K100 M was found to have a positive influence on the burst effect while xanthan gum was found to have a negative influence on the same. Generally, batches with higher levels xanthan gum were found to be devoid of burst effect. The impact of the formulation factors on the burst effect is clearly visible in two component mix plots captured in Fig. 5. It has to be noted that the burst effect from the raft formulations could be minimized by using high levels of xanthan gum. It is likely that high levels of xanthan gum could result in formation of more compact gel that would prevent the penetration of the dissolution media and therefore the burst effect. It is mandatory for the dissolution media to penetrate the gel matrix prior to dissolution of the calcium and subsequent release into the surrounding media [28]. However, the burst effect increased with decrease in the amount of xanthan gum indicating the formation of a more porous gel matrix. The porous matrix failed to impede the penetration of the dissolution media and the subsequent dissolution of the calcium and eventually the burst effect. The more porous gel matrices are likely to allow the aqueous medium to easily penetrate the raft-forming system, and facilitate the calcium dissolution and subsequent diffusion from the raft gel [29].

Table 6
The observed and predicted values for the optimized *in situ* gelling raft formulation.

Factor	Optimized level (D-0.757)		
X1:HPMC K 100 M	65.88		
X2:Xanthangum	34.13		
Response	**Predicted value**	**Observed value**	**% Residual Error**
Y1:BLT	12.52	10.90	14.86
Y2:Release at 1Hr	22.95	20.74	−10.65
Y3:Release at 6Hr	84.82	87.25	−2.78

$$\%\ \text{Residual error} = \frac{\text{Predicted value} - \text{observed value}}{\text{observed value}} \times 100$$

3.5. Release at 6hr

The percentage calcium release by 6 h was found to range from 65.50 ± 1.98% for F3 to 87.07 ± 0.85% for F5. The batches F1, F2, F3 and F8 that were devoid of initial burst release was found to display a controlled pattern of calcium release. The two factors investigated were found to significantly influence the release at 6 h. Of the two factors investigated, the influence of X2 was the most, followed by X1 whereas the interaction effect of X1X2 was found to be the least (Table 3). The amount of Xanthan gum (X2) had a high negative coefficient (−0.92) that implies the factor was found to have the considerable influence on the release at 6 h. Xanthan gum on coming in contact with aqueous medium forms a very viscous strong gel network. The pharmaceutical advantage of Xanthan gum is its ability to evade the initial burst release and higher drug retarding ability [30]. As anticipated, on increasing the concentration of xanthan gum, the compactness of gel matrix increased while the porosity was reduced that resulted in a controlled release of calcium from raft. Xanthan Gum when employed at appropriate concentrations can be used as a carrier for controlled release of drugs [31]. Diffusion was found to be the predominant drug release mechanism from these matrices which swell to a higher extent. Calcium present in the matrix core may ultimately be requiring more time to traverse towards the surface. However, the effect of the level of HPMC K100 was found be less influential compared to the effect of xanthan gum. The effect of the formulation factors on release at 6hr is clearly visible in two component mix plots captured in Fig. 5. The plots clearly indicated that moderate levels of xanthan gum would be necessary to reduce the burst effect and at the same time ensure a near-complete and controlled release of calcium.

3.6. Optimization

A numerical optimization technique using the desirability approach was employed to develop new GRFS formulations with the desired responses. The composition of the optimized formula along with predicted and experimental values for the response parameters are portrayed in Table 6. The experimental values of the responses were found to be in close agreement with those predicted by the mathematical models generated. The mean error was tabulated from the predicted and the observed experimental value. A low value of mean residual error thus proved validity of the mathematical models generated. The *in vitro* drug release from the optimized formulation was found to follow first order kinetics.

3.7. In vivo radiographic studies

Rabbit model is considered to be a good preclinical model to assess the gastric residence time of gastroretentive drug delivery systems [32].

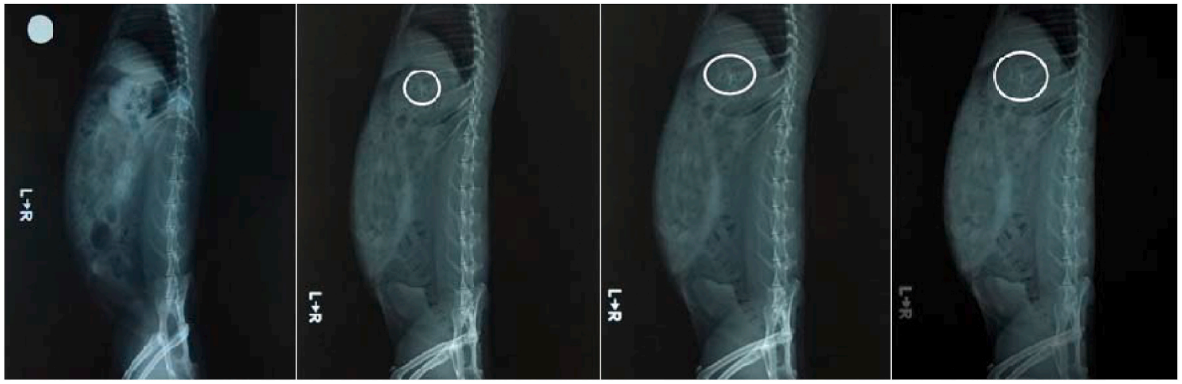

Fig. 7. Radiographic images portraying the gastroretentive GRFS containing barium sulphate as a radio opaque marker in the stomach at 0 h, 2 h, 4 h and 6 h (The position of the formulation is pointed by an circle).

The standard gastric residence time in humans would be 2 h while it can extend up to 6 h depending on presence of food, posture and number of physiological variables. Considering this, the *in vivo* studies was planned for a duration of 6 h during which we anticipated controlled but at the same time a near complete release of calcium from GRFS. The representative images of the *in vivo* radiographic studies with the GRFS are captured in Fig. 7. The *in vivo* studies revealed that the mean gastric retention time for the GRFS from the optimized batch correlated well the *in vitro* floating time. The studies indicated that the GRFS from the optimized batch remained in the stomach for an average period of 5.64 $\pm$ 0.43 h in rabbits that was significantly higher ($P < 0.05$) than the marketed formulation that displayed a mean gastric retention time of less than 2 h. The GRFS by virtue of the floating and the bioadhesive properties were found to be well retained in the stomach despite the action of peristalsis and forcible housekeeping waves compared to the conventional suspension. As the raft system are well retained in the stomach proximal to the absorption window and are likely to release the contents in a controlled manner. Based on the observed mean gastric residence time and the *in vitro* dissolution data, we anticipate a minimum burst effect ~20% and nearly ~75% of calcium to get released from the GRFS in a controlled manner. As the calcium would be released in a controlled fashion, it is less likely to saturate the transporters situated in the duodenal region of the gastrointestinal tract. Therefore the RFS is likely to exhibit a superior bioavailability compared to the conventional oral suspension.

4. Conclusion

In situ gelling raft forming systems of calcium carbonate were successfully developed employing simple lattice design. Of the two formulation factors investigated, the levels Xanthan gum used as a release retardant in the raft forming solutions significantly affected the buoyancy lag time and release of calcium. Numerical optimization technique was successfully employed to develop optimized formulations by setting constraints on the responses. The optimized formulation developed displayed a short buoyancy lag time, minimal burst effect and a controlled yet near-complete release of calcium by 6 h. The experimental data for the optimized formulations was 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 liquid formulations in rabbits revealed that the GRFS were found to be retained in the stomach for 5.64 $\pm$ 0.43 h. The studies collectively proved that gastroretentive GRFS possessing floating and mucoadhesive properties would be highly promising drug delivery platform for nutrients and therapeutic agents with absorption window in the upper part of the gastrointestinal tract.

Author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Journal of drug delivery science and technology.

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