FORMULATION DEVELOPMENT AND IN-VITRO EVALUATION OF ORAL SUSTAINED RELEASE FLOATING TABLETS OF CHLOROGENIC ACID

Aditi Mool*, Dr. Archana Moon², Dr. Veena Belgamwar³, Dr. Sheelpriya Walde¹ and Dr. Sunil Gupta⁴

¹Department of Quality Assurance, Gurunanak College of Pharmacy, Rashtrasanta Tukdoji Maharaj University Nagpur (RTMNU), Maharashtra, India.
²Department of Biochemistry, LIT, (RTMNU), Maharashtra, India.
³Department of Pharmaceutics, RTMNU, Maharashtra, India.
⁴Research and Development Department, Zym Laboratories Limited, Nagpur, Maharashtra, India.

ABSTRACT

The purpose of the study was to develop sustained release floating tablet of chlorogenic acid (CGA) and optimize its drug release for enhanced oral bioavailability. A relatively new approach, \(3^2\) full factorial design, was used to formulate floating sustained release tablets of CGA and to systematically optimize its drug release using varying levels of sodium bicarbonate and hydroxypropyl methylcellulose (HPMC) K 15 M polymer. Sodium bicarbonate was used as gas generating agent. After setting the levels by preliminary trials, nine tablet formulations (F1 - F9) were prepared by wet granulation method using Design Expert Software® - suggested combinations. The concentrations of HPMC K15M (X1) and sodium bicarbonate (X2) were chosen as control variables. Conversely, the response variables selected were percentage (%) of drug released within 12 hours (Y1) and time taken by the tablet to float (Y2). Fourier transform infrared (FTIR) spectroscopy was used to assess compatibility between the drug and the excipients. The response surface and 3D plots drawn demonstrated the suitability of the hydrophilic matrix forming agents for formulating sustained release floating tablets of CGA. FTIR and DSC spectra showed no noticeable incompatibility between drug and polymers in both physical mixtures and in
formulations. Floating lag time remained was $< 3.00$ min with floating duration of $> 12$ h. Considerable agreement was observed between predicted and actual release parameters. Formulation batch (F9) showed satisfactory release profile and floating lag time and thus was selected as the optimized formulation.

**KEYWORDS:** Chrologenic acid (CGA), sustained release floating tablets, hydroxy propyl methyl cellulose K 15 M (HPMC K 15 M), Sodium bicarbonate, $3^2$ full Factorial design.

**INTRODUCTION**

Gastric retentive drug delivery technologies have been reviewed extensively in recent years. The present review mostly considers the technologies for which human in vivo data are available. Numerous studies in dogs shows that gastric retention have failed to produce similar effects in humans, possibly due to differences in the size of the pylorus aperture in dogs or sensitivity to the presence of food in the stomach. In addition, because four-legged animals do not maintain floating dosage forms higher than the pylorus, human studies are needed to validate this concept. Although dogs or other species may represent reasonable screening methods, convincing evidence for gastric retention requires human data because of significant species differentiation. At this point in time, there are three technological approaches under most active investigation in the gastric retentive drug delivery field. These approaches include 1) Mucoadhesion, where the dosage form adheres to gastric or intestinal walls such that motility is limited; 2) Density modification (flotation), where the dosage form cannot leave the stomach because of its orientation to the pylorus; and 3) Expansion, where the dosage form becomes too large to pass through the pyloric sphincter. One common element for all the technologies that needs to be considered, and the element most often weak in the reported clinical studies, is the selection of appropriate controls. Part of the problem with controls is that gastric retention, especially on a fed stomach, can be significant and highly variable for any nondisintegrating dosage form.

In the present study mucoadhesion technology has been used to avoid the issues like gastric emptying and maintain the upright stance. Mucoadhesive drug delivery dosage forms are based on extensive research carried out to find polymers that bind mucosal membranes in vitro and ex vivo. The fundamental challenge with this approach is that mucus is constantly secreted and removed from the surface of the stomach and intestines: successfully binding mucin in vivo does not necessarily imply that this mucin is actually attached to the walls of the GI tract and will consequently change the transit time. As will be discussed below, in
most clinical trials there was little or no benefit of mucoadhesion found. Although the authors of many articles have attributed favourable dosage form behaviour (e.g., increased bioavailability) to gastric retention, the results could as easily be rationalized in terms of other factors in the particular experiments making substantiation of the concept yet to be fulfilled.[1-2]

Floating dosage forms may provide gastric retention by floating on gastric contents and thereby avoiding the pylorus. From the large number of such studies conducted (over 20 human clinical trials and many animal studies reported in the open literature), it would appear that such concepts as hydrodynamically balanced systems (HBS), micro balloons, and the like are now well established. For floating systems to operate there needs to be gastric contents to float on, because liquids empty rapidly, this means that the subject must take the dosage form on a fed stomach and may need to take multiple meals. However, most dosage forms larger than a few millimetres in diameter are retained while the stomach is in the fed state, such that one must demonstrate that providing a buoyant dosage form provides an additional benefit. In addition, because whether the person is upright or lying down could affect the dosage form performance, studies need to adjust for this parameter. Such restrictions as specific feeding schedules and maintaining an upright stance hinder patient compliance and would limit the usefulness of the technology. Floating dosage forms can be formulated into two broad types: large tablets or capsules, and multiple-unit systems such as multiparticulates and minitablets. In the present work tablet dosage form was developed. The floating tablets can be formulated as either effervescent or non-effervescent dosage form.[3]

Conventional dosage forms, which are immediate release in nature, have been used from decades for the treatment of acute and chronic diseases. The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. According to literature survey it was found that the CGA has very poor oral bioavailability and gets metabolized very easily,[4] therefore it was concluded that it should be formulated as a sustained release oral floating tablet. The real challenge in the development of a controlled drug delivery system is not just to sustain the drug release, but also to prolong the presence of the dosage form in the stomach or the upper small intestine until the drug is completely released in the desired period of time.

Sustained release pharmaceutical dosage forms may offer one or more advantages over conventional (immediate release) dosage forms of the same drug, such as improved patient
compliance and decreased incidences of adverse drug reactions. One of the approaches is gastro-retentive drug delivery systems, which target the drug release in stomach for those drugs which are absorbed from the stomach or specific site of action. Gastro retentive drug delivery devices are primarily controlled release drug delivery systems, which gets retained in the stomach for longer period of time, thus helping in absorption of drug for the intended duration of time. This in turn improves bioavailability, reduces drug wastage, and improves solubility of drugs that are less soluble at high pH environment.[5]

The drug chosen for the present investigation was Chromogenic acid (C16H18O9; mol. wt. = 354.31), an ester of caffeic acid and quinic acid (CGA) having antibacterial activity that can be used for the treatment of urinary tract infection. The structure of Chlorogenic acid is shown in figure 1.

In the present investigation, efforts were made to develop a gastro retentive sustained release formulation of CGA for the treatment of urinary tract infection. According to the literature it was concluded that the CGA has very poor oral bioavailability and gets metabolized very easily[6-7], therefore it was concluded that it should be formulated as a sustained release oral floating tablet. [Patent filled on date 17/03/2017, The application No: 201721009454; TEMP/E-1/9452/2017-MUM.].

MATERIALS
The ingredients which were used in the formulation of oral sustained release floating tablet of chlorogenic acid are Hydroxypropyl methylcellulose (HPMC k15 M) as matrix forming polymer, sodium bicarbonate as gas generating agent, talc was used as glidant and magnesium stearate was used as lubricants. Other solvents and materials used were of analytical grades. Iso-propyl alcohol was used as a solvent for the granulation. CGA was obtained from CHEMSWORTH, Surat-394230, India. HPMC K 15 M and sodium bicarbonate were obtained from ALKA Pharmaceuticals, Nagpur-440036, India. Iso-propyl alcohol (IPA) was obtained from Zym Laboratories limited, Nagpur, India. All the polymers and excipients received were of pharmaceutical grade and were used as received. Other
materials and solvents used were of analytical grade. Distilled water was prepared in laboratory using all glass distillation apparatus.

**METHOD**

The oral sustained release floating tablets of chlorogenic acid were prepared using low density matrix forming polymer i.e. HPMC K 15 M and effervescent agent like sodium bicarbonate by using the wet granulation technique for all batches. The composition for all formulations (F1-F9) as per applied factorial design is given in [Table No.1]. Hydroxylpropylmethyl cellulose, talc, magnesium stearate, sodium bicarbonate and the active ingredient were weighed accurately. First HPMC K15 M is passed through the sieve no:30# along with loading dose of chlorogenic acid and mixed in rapid mixer and granulator for 10min at 100rpm. The solution (60ml) of isopropyl alcohol was used as a binder which was then added to rapid mixer and granulator slowly for 1min at 100rpm. The granulation is done for further 2min and collected. The wet granules were dried in a conventional hot air oven at 105°C until 4.71% of LOD was riched. The dried granules were sieved through the sieve No: 12# to get even size granules and then through 20# after LOD has riched. For extragarnulation talc was passed through sieve no: 60# whereas sodium bicarbonate and maintenance dose of chlorogenic acid was passed through sieve no: 30#. Then the extra granulation and prepared granules were blended for 10 min at 20 rpm in blender. After that magnesium stearate was added for lubrication and blending was continued for further 5 min. The prepared mixture was then compressed on Rinek mini press tablet compression machine using 11mm of biconvex punch. The prepared tablets were then evaluated for various parameters.

### Table no 1: Composition of Batch F1-F9 as per Applied Factorial Design.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CGA(mg)</td>
<td>305.06</td>
<td>305.06</td>
<td>305.06</td>
<td>305.06</td>
<td>305.06</td>
<td>305.06</td>
<td>305.06</td>
<td>305.06</td>
<td>305.06</td>
</tr>
<tr>
<td>2</td>
<td>HPMC (%)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Sodium Bicarbonate (%)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Magnesium Stearate (%)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Talc (%)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**DRUG–EXCIPIENTS COMPATIBILITY STUDIES (FT-IR)**

The pure drugs and excipients were subjected to IR studies alone and in combination. Pure drugs / pure excipients / combination of drugs excipients were mixed with 100 mg of
potassium bromide. Mixing can be effected by thorough grinding in smooth mortar. The mixture was placed in IR sample holder and analysed by FTIR. The spectra were run from 600 cm\(^{-1}\) to 4000 cm\(^{-1}\) wave number.

**SOLUBILITY STUDIES**

The solubility studies were performed on chlorogenic acid and it was found that 3.5 mg of CGA is soluble per millilitre of water. The solubility of CGA at different pH range shows that it is more soluble in basic buffer than acidic. At pH 4.00 the solubility is 10-15 mg / ml water. At pH 7.2 solubility of chlorogenic acid is 25 mg/ml of water. All the experiments were performed in triplicates.

**Table no 2: Solubility of chlorogenic acid (Mean ± SD, n=3).**

<table>
<thead>
<tr>
<th>Solubility in Distilled Water</th>
<th>3.5±0.2mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility in Acidic Buffer pH 4.00</td>
<td>15±0.91mg/ ml</td>
</tr>
<tr>
<td>Solubility in Basic Buffer pH 7.2</td>
<td>25±0.67mg/ ml</td>
</tr>
</tbody>
</table>

**PRE-COMPRESSION STUDY**

- **Angle of repose**

  The angle of repose of the drug under investigation CGA was determined by funnel method (Repos gram).\(^8\) Accurately weighed powder of CGA was taken in a funnel. Height of the funnel was adjusted in such a ways that tip of the funnel just touches the apex of the heap of the powder. The powder was allowed to flow through the funnel freely onto the surface. Diameter of the powder cone was measured and angle of repose was calculated using the following equation;

  \[
  \tan \theta = \frac{h}{r}
  \]

  Where,

  \(h\) = height of the powder cone and
  \(r\) = radius of the powder cone

- **Bulk density**

  Loose bulk density was determined by placing pre-sieved drug excipient blend in to a graduated cylinder and measuring the volume and weight and recording the same.\(^9\)
• **Tapped density**

Tapped density was determined by USP method II tablet blend was filled in 100 ml graduated cylinder of tap density tester which was operated for fixed number of taps until the powder bed volume has reached a minimum, thus was calculated by formula:

\[ D_t = \frac{M}{V_b} \]

Where,
M = Weight of powder taken
Vb = tapped volume.

• **Compressibility index and Hausner’s ratio**

This was measured for the property of a powder to be compressed; as such they are measured for relative importance of interparticulate interaction. Compressibility index was calculated by following equation:

\[ \text{Compressibility index (\%)} = \frac{(\text{TBD} - \text{LBD}) \times 100}{\text{TBD}} \]

Where,
LBD = Loose bulk density,
TBD = Tapped bulk density

**Table no 3: Evaluation of powder blend of chlorogenic acid.**

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Evaluation parameter</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bulk density(gm/cm(^2))</td>
<td>0.47</td>
<td>0.48</td>
<td>0.45</td>
<td>0.43</td>
<td>0.46</td>
<td>0.45</td>
<td>0.47</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td>2</td>
<td>Tapped density(gm/cm(^2))</td>
<td>0.56</td>
<td>0.52</td>
<td>0.57</td>
<td>0.53</td>
<td>0.56</td>
<td>0.52</td>
<td>0.58</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>Carr’s index (%)</td>
<td>8.78</td>
<td>8.23</td>
<td>12.2</td>
<td>12.6</td>
<td>9.41</td>
<td>9.69</td>
<td>9.42</td>
<td>9.68</td>
<td>9.61</td>
</tr>
<tr>
<td>4</td>
<td>Hausner’s ratio</td>
<td>1.29</td>
<td>1.18</td>
<td>1.17</td>
<td>1.09</td>
<td>1.61</td>
<td>1.20</td>
<td>1.11</td>
<td>1.16</td>
<td>1.10</td>
</tr>
<tr>
<td>5</td>
<td>Angle of repose((\theta))</td>
<td>17.2</td>
<td>17.6</td>
<td>18.10</td>
<td>18.09</td>
<td>18.2</td>
<td>18.4</td>
<td>18.2</td>
<td>18.4</td>
<td>18.43</td>
</tr>
</tbody>
</table>

**ACUTE ORAL TOXICITY STUDY (LD\(_{50}\))**

The LD\(_{50}\) studies were performed on chlorogenic acid by Acute Toxic Class method in accordance to OECD guidelines (2001) 423. A stepwise procedure is adopted with the use of 6 animals of a single sex per step. Group of 6 female rats were used for each step among which 1 was taken as control. Normally females (nulliparous and non-pregnant) are used as generally being slightly more sensitive than males. Their age was between 8 and 12 weeks at the time of dosing and having a weight of 180-200 grams with mean intervals of within ±20%. They were housed at 30-35°C with 50-60% Relative humidity. A standard laboratory diet specific to the species and filtered water, free from contamination was given.\(^{[10]}\)
CGA was administered in a single dose orally by Neocate (24) syringe. Dilutions were made by using distilled water. All grouped animals were fasted prior to dosing. The feed was withheld for overnight. However, water is provided *ad libitum*. Following the period of fasting, the animals were weighed and CGA was administered. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance had been administered, food was withheld for further 3–4 hours. As per OECD guidelines starting dose of 300mg/kg body weight of rat was given to all six animals and one was taken as control. No toxic effects or severe symptoms were observed. So the next dose of 550mg/kg body weighed was administered to the test animals As no lethality was observed with previous dose, the next higher dose of 2000 mg/kg body weight was administered to the other group of animals. The animals were critically observed for 1st thirty minutes of dosing and then periodically during first 24 hours for presence or absence of mortality or severity of symptoms. Then once in a day for total 14 days period to observe any late reactions. Observations include condition of skin and fur, eyes and mucus membrane, respiratory, circulatory, autonomic and central nervous system, somato-motor activity and behavioural pattern. Specific observations include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.[11]

**EVALUATION OF TABLETS**

The tablets were evaluated for the following in process and finished product quality control tests i.e. appearance, dimensions (diameter and thickness), weight variation, hardness, friability and drug content.

**Appearance**

The tablet should be free from cracks, depressions, pinholes etc. The colour and the polish of the tablet should be uniform on whole surface. The surface of the tablets should be smooth.

**Dimensions**

The dimensions of the tablets are thickness and diameter. The tablets should have uniform thickness and diameter. Thickness and diameter of the tablets was measured using Vernier calliper. These values were checked and used to adjust the initial stages of compression.

**Uniformity of weight**

Twenty tablets were weighed individually. Average weight was calculated from the total weight of all tablets. The individual weights were compared with the average weight. The
Percentage difference in the weight variation should be within the permissible limits (±7.5%). The percent deviation was calculated using the following formula.

\[
\% \text{ Deviation} = \frac{\text{Individual weight} - \text{average weight}}{\text{Average weight}} \times 100
\]

**Hardness**

Hardness was measured using Pfizer hardness tester, for each batch six tablets were tested.

**Friability**

Twenty tablets were weight and placed in the Roche friabilator and apparatus was rotated at 25 rpm for 4 min. After 100 rotations (4 minutes), the tablets were taken out from the friabilator and intact tablets were again weighed collectively. Permitted friability limit is 1.0%. The percent friability was determined using the following formula.

\[
\text{Friability} = \frac{(W1 - W2)}{W1} \times 100
\]

Where,

- \( W1 \) = weight of the tablets before test
- \( W2 \) = weight of the tablets after test

**Content uniformity**

Twenty tablets were selected randomly and average weight was calculated. Tablets were crushed in a mortar and accurately average weighed amount of powdered tablets was taken for the analysis.\[^{12}\] Samples were transferred to different volumetric flasks and were diluted up to the mark using 0.1 N HCl. The content was shaken well and kept for 30 minutes to dissolve the drug completely. The mixture was filtered and appropriate dilutions were made. The drug content in each tablet was estimated at 324 nm.

**In vitro buoyancy studies**

The in vitro buoyancy was determined by floating lag time as per the method described by the Samip et al. The tablets were placed in a 100 ml beaker containing 0.1 N HCl Solutions at room temperature. The time required for the tablet to rise to the surface and float was determined as floating lag time.\[^{13}\]
In vitro drug release studies
In vitro drug release study of the samples was carried out using USP – type I dissolution apparatus (Basket type). The dissolution medium, 900 ml of simulated gastric fluid (without enzyme), was placed into the dissolution flask maintaining the temperature at 37± 0.5°C and rpm of 100. One tablet was placed in each basket of the dissolution apparatus. The apparatus was allowed to run for 12 hours. Samples measuring 1 ml were withdrawn after every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours. The fresh dissolution medium (37°C) was replaced every time with the same quantity of the sample. Collected samples were analysed at 324 nm using 0.1 N HCl as blank. The cumulative percentage drug release was calculated.[14]

Curve fitting analysis
Mathematical models, zero-order, first-order, Higuchi & Peppas were applied to analyse the release rate mechanism and pattern.[15]

EXPERIMENTAL DESIGN
Some possible experimental trials, generated by application of 3² factorial design, were conducted to evaluate each independent factor at 3 levels. Formulation combinations (F1 - F9) using factorial design were shown in Table 2. The percentages of HPMC K15M (X1) and sodium bicarbonate (X2) were chosen as control variables while % release (% release of drug within 12 hours) Y1 and required floating lag time Y2 were selected as response variables.[16]

Table No 4: Factor combinations according to 3² design.

<table>
<thead>
<tr>
<th>Run</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A: HPMCK15M (%)</td>
<td>B: NaHCO₃ (%)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>-1</td>
<td>-1</td>
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<tr>
<td>9</td>
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<td>10</td>
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<td>11</td>
<td>0</td>
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</tr>
<tr>
<td>12</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
The obtained data underwent response surface computations by fitting into the Design Expert Software 10 (Stat-Ease Inc., MN, USA).

Table No 5: Independent variables and their corresponding levels of floating tablet formulation.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>LEVELS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1 0 1</td>
</tr>
<tr>
<td>X1</td>
<td>10 20 30</td>
</tr>
<tr>
<td>X2</td>
<td>10 20 30</td>
</tr>
</tbody>
</table>

**DATA ANALYSIS**

The response parameters examined for optimization in the current study were the percentages of drug released in 12 hours (with an interval of 1 hour) and time taken by the tablet to float on the surface (Floating lag time). Polynomial models comprising quadratic and interaction terms were developed for mentioned release variables using the multiple linear regression analysis (MLRA). The general MLRA model form is shown in Eq 1.

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_1^2 X_1 + \beta_2^2 X_2^2 + \beta_1^2 X_1^2 + \beta_2^2 X_2^2
\]

where \(\beta_0\) is the intercept representing the average value of all quantitative results of nine proceeds, \(\beta_1\) and \(\beta_2\) are the calculated coefficients from actual values of \(Y\), \(Y\) is the response variables (% release, floating lag time) and \(X_1, X_2\) represents the translated levels of the control variables for HPMC K15M and sodium bicarbonate, respectively.\(^{[17]}\)

**STABILITY STUDY**

Tizanidine hydrochloride gastro retentive tablets of optimized formulation (F9) were packed in aluminum-aluminum blister (Alu-Alu blister packing). The packed tablets were placed in stability chamber maintained at 40±2°C and 75±5% RH for 1 month as per ICH guidelines. The samples were withdrawn after one month and were observed for changes in parameters such as change in colour, appearance of spots, bad odour, roughness, and any kind of microbial or fungal growth. Samples were also evaluated for drug content, floating lag time, and in-vitro drug release.

**RESULTS**

**Physical evaluation**

Physical characteristics of the prepared tablets were shown in Table 3. In different formulations, the contents of drug determined varied between 96.21% and 99.17%. Formulation excipients, i.e. sodium bicarbonate, HPMC K15M, magnesium stearate and talc
did not show any interference with the results of drug contents. The tablet’s hardness varied between 4.7 to 5.8 kg/cm², the thickness was found to be between 4.7 and 4.2 mm and the diameter was between 10.80mm and 11mm. Physical parameters of the floating tablets of CGA were within acceptable range.

Table no 6: Evaluation of Floating Tablets of Chlorogenic acid (Mean ± SD, n=3).

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Evaluation Parameters</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average wt. (mg)</td>
<td>445.35 ±0.21</td>
<td>439.2 ±0.22</td>
<td>441.4 ±0.20</td>
<td>445.36 ±0.27</td>
<td>439.31 ±0.32</td>
<td>442.36 ±0.27</td>
<td>440.84 ±0.22</td>
<td>451.84 ±0.37</td>
<td>435.35 ±0.21</td>
</tr>
<tr>
<td>2</td>
<td>Hardness (kg/cm²)</td>
<td>5.8 ±0.22</td>
<td>5.1 ±0.23</td>
<td>4.9 ±0.28</td>
<td>4.4 ±0.27</td>
<td>4.6 ±0.23</td>
<td>4.7 ±0.24</td>
<td>4.8 ±0.31</td>
<td>4.8 ±0.21</td>
<td>4.8 ±0.19</td>
</tr>
<tr>
<td>3</td>
<td>Thickness</td>
<td>4.6 ±0.02</td>
<td>4.2 ±0.02</td>
<td>4.4 ±0.03</td>
<td>4.5 ±0.04</td>
<td>4.6 ±0.03</td>
<td>4.4 ±0.02</td>
<td>4.7 ±0.01</td>
<td>4.7 ±0.01</td>
<td>4.7 ±0.01</td>
</tr>
<tr>
<td>4</td>
<td>Friability (%w/w)</td>
<td>0.45 ±0.15</td>
<td>0.43 ±0.17</td>
<td>0.44 ±0.04</td>
<td>0.45 ±0.15</td>
<td>0.45 ±0.19</td>
<td>0.46 ±0.08</td>
<td>0.45 ±0.06</td>
<td>0.45 ±0.18</td>
<td>0.45 ±0.09</td>
</tr>
<tr>
<td>5</td>
<td>Floating lag time (min)</td>
<td>3.21 ±2.1</td>
<td>2.49 ±2.41</td>
<td>3.01 ±3.01</td>
<td>2.62 ±1.20</td>
<td>2.01 ±1.40</td>
<td>1.49 ±2.41</td>
<td>1.89 ±1.29</td>
<td>1.24 ±2.94</td>
<td>1.24 ±0.09</td>
</tr>
<tr>
<td>6</td>
<td>Total floating time (hrs.)</td>
<td>12.08 ±4.32</td>
<td>12.71 ±3.95</td>
<td>12.77 ±4.1</td>
<td>12.58 ±5.74</td>
<td>12.96 ±1.26</td>
<td>11.70 ±4.6</td>
<td>10.64 ±1.29</td>
<td>11.89 ±1.88</td>
<td>11.96 ±1.76</td>
</tr>
<tr>
<td>7</td>
<td>Assay (%)</td>
<td>96.21 ±0.49</td>
<td>98.19 ±0.75</td>
<td>99.17 ±1.28</td>
<td>96.07 ±1.47</td>
<td>95.08 ±2.52</td>
<td>94.22 ±2.11</td>
<td>95.36 ±1.28</td>
<td>97.42 ±1.06</td>
<td>99.73 ±1.09</td>
</tr>
</tbody>
</table>

Thermal characteristics

DSC curves of pure Chlorogenic acid is shown in Figure 1. An endothermic sharp peak at 108.01°C in the spectra of pure Captopril corresponds to the melting point of drug. The Melting Point of Chlorogenic Acid was found to be 207°C To 210 °C. A very sharp and intense peak (negative) was observed indicating fall in temperature due to endothermic reactions (like melting and decomposition).

Figure 1: DSC curve of chlorogenic acid.
FTIR spectroscopy
The IR spectra of pure drug, its physical mixture with sodium bicarbonate, HPMC K15M, magnesium stearate and talc were recorded to evaluate the compatibility profile (Figure 2).

Spectra of physical mixtures of drug and showed no remarkable change in the peaks of drug so we can conclude that there was no interaction found between drug and the selected excipients. There were no extra peaks observed except the drug Chlorogenic acid and the excipients. Hence these excipients are found to be compatible with the drug and can be used for further formulation studies.

Solubility study
According to IP classification of solubility of drug per millilitres of solvent it can be concluded that:

- Chlorogenic acid is freely soluble in water. It shows better solubility at lower temperature.
- At acidic pH (pH 4.00) the drug was found to be soluble.
- Chlorogenic acid is sparingly soluble at basic pH (pH 7.2).
Acute Toxicity Study

Table 7: acute oral toxicity (LD50).

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>ROUTE</th>
<th>SPECIES</th>
<th>DOSE (mg/kg)</th>
<th>OBSERVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oral</td>
<td>Rodent (rat), females</td>
<td>300 mg/kg body weight</td>
<td>No severe symptoms were observed. Animals were behaving totally normal. All animals were survived.</td>
</tr>
<tr>
<td>2.</td>
<td>Oral</td>
<td>Rodent (rat), Females</td>
<td>500 mg/kg body weight</td>
<td>No severe symptoms were observed. Some animals were showing minor irritation reaction. All animals were survived.</td>
</tr>
<tr>
<td>3.</td>
<td>Oral</td>
<td>Rodent (rat), females</td>
<td>2000 mg/kg body weight</td>
<td>No toxic effects were seen. Produces sleep in some of animals. All animals were survived.</td>
</tr>
</tbody>
</table>

As there were no lethality or mortality was seen, thus the drug chlorogenic acid was found to be totally non-toxic with the doses lower than and equal to 2000mg/kg.\[^{18}\]

**In-vitro buoyancy**

Buoyancy characteristics are imparted to floating systems by incorporation of sodium bicarbonate as a gas generating agent. The carbon dioxide is produced as a result of reaction of carbonate with dissolution medium (0.1 N HCl). In this study, it was noticed that even if the amount of sodium bicarbonate used was constant (45.54mg), yet buoyancy lag times possibly changed due to variations in polymer concentrations. However, percentage of bicarbonate achieved optimum buoyancy lag times between 1.24 to 3.00 min. In addition, chlorogenic acid tablets floated for the duration of more than 12 h due to continuous evolution of CO\(_2\) from the tablet surface.

**In-vitro drug release**

Tablets from all the formulations subjected to dissolution tests; the resulting release characteristics as per factorial design are listed in Table 4. The overall results showed that the rate and extent of drug release from floating tablets were controlled by the concentration of polymer used. However, the contribution of HPMC K100M in retarding the drug release was greater as compared with sodium bicarbonate. Developed formulations exhibited the following desired release pattern i.e. 20-25% in 1-2 h, 25-45% in 4 h, 45-65% in 6 h, 65-75% in 8 h and the remaining after 8 h. The formulation F9 was found to be optimum in following the desired prefixed goal for the drug release pattern with floating lag time of 1.24 min. The remaining formulations also showed acceptable results with minor variations from desirability. Release profile of chlorogenic acid floating tablets is shown graphically in.
Experimental Design

The response parameters examined for optimization in the current study were the percentages of drug released within 12 hours and the floating time of prepared formulation. The three-dimensional (3D) response surface graphs for the most statistical significant variables on the evaluated parameters in shown in fig (c & d). The response surface diagrams showed that higher the polymer (HPMC K 15 M) concentration lower the % release and higher the concentration of effervescent agent (NaHCO₃) lesser the floating lag time. The optimized variables showed a good fit to the cubic and quadratic polynomial models. The data was fitted into the general ANOVA model and the following equations were obtained. Eq 1 and Eq 2 representing the % release and FT (floating lag time) respectively:

\[
\begin{align*}
\text{%Release} &= 94.72 - 3.68X_1 + 1.82X_2 - 0.31X_1X_2 + 0.52X_1^2 - 1.70X_2^2 \\
\text{FT (seconds)} &= 1.89 + 0.14X_1 - 0.24X_2 + 0.25X_1X_2 - 0.23X_1^2 + 0.11X_2^2
\end{align*}
\]

The application of ANOVA using the provisions of Design expert software revealed statistically significant \( p < 0.05 \) values in polynomial terms. These include first order main effects, the higher order effects, coefficients for intercepts and interaction terms. Furthermore, useful inferences can be drawn by mathematical equations after carefully looking at the values of the main effects which indicate the effect of each control factor on the response.\[^{19}\]
Figures 4: (a) & (b) shows the corresponding contour plots for the release variables and floating lag time respectively.
Figure 5: (c) & (d) shows the corresponding three dimensional plots (3D) for the release variables and floating lag time respectively.

Table no 9: Analysis of extra designed checkpoints.

<table>
<thead>
<tr>
<th>RESPONSE VARIABLES</th>
<th>PREDICTED VALUE</th>
<th>OBSERVED VALUE</th>
<th>BIAS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Release</td>
<td>99.37</td>
<td>99.71</td>
<td>-0.34</td>
</tr>
<tr>
<td>Floating Lag Time</td>
<td>1.12</td>
<td>1.24</td>
<td>-10.7</td>
</tr>
</tbody>
</table>
The above table shows that the experimental values of batches prepared were within the optimum range and are very close to the predicted values with low percentage of bias, suggesting that the optimized formulation was reliable and reasonable.

**Drug release kinetics**

To analyse the release mechanism of chlorogenic acid as well as to select the sustained release floating formulation for *in vivo* studies, the *in vitro* release data were fitted into various release equations and kinetic models i.e. first order, zero order, Higuchi and Korsmeyer and Peppas (K-P plots). The F9 batch was chosen as the optimized formulation because it showed more linearity between the cumulative percentage of chlorogenic acid released *versus* time, as indicated by the highest value of the correlation coefficient $R$ or $R^2$ in all the selected models.

Among all formulations, it was best fitted to Higuchi ($R^2 = 0.9563$) model. In the optimized sustained release floating formulation (F7) the immediate release floating layer was found to be: HPMC-K15M 10%, loading dose (140mg) of chlorogenic acid whereas sustained release layer was found to be: maintenance dose (164mg) of chlorogenic acid, sodium bicarbonate 30%, talc 5% and magnesium stearate 1%.

As indicated by the value of $R^2$, the Higuchi model was found to be efficient in describing the kinetics of chlorogenic acid release from the sustained release floating formulation, with drug release being proportional to the square root of release time. To explore the release pattern, results of the *in vitro* release data of optimized F7 batch were fitted to the Korsmeyer and Peppas equation ($Mt/M\infty = k t^n$, where $Mt/M\infty$) is the fraction of drug released after time $t$ in respect to amount of drug released at infinite time, $k$ is the rate constant and $n$ is the diffusional exponent which characterize the transport mechanism. The value of $n$ was 0.306 ($R^2=0.9244$), indicating release governed by Fickian diffusion.
Figure 6: Release pattern of formulation (F9) sustained release floating tablets of chlorogenic acid.

Stability study
The gastro retentive floating tablets of optimized formulation (F9) were packed in aluminum-aluminum blister (Alu-Alu blister packing). The packed tablets were placed in stability chamber maintained at 40±2°C and 75±5% RH for 6 month as per ICH guidelines. The stability studies are currently going on. The sample will be withdrawn in an intervals of one month and observed for changes in parameters such as change in colour, appearance of spots, bad odour, roughness, and any kind of microbial or fungal growth. Samples will also evaluated for drug content, floating lag time, and in vitro drug release.

CONCLUSION
The drug chlorogenic acid possesses commendable antibacterial activity against various microbes making it useful for the treatment of various types of infections (specifically UTI causative organisms eg. E.coli, E.fecalous). It had shown to have poor absorption through GIT and also get easily metabolized, therefore, floating gastroretentive sustained release tablets for sustained drug release of CGA were formulated and evaluated. The solubility
studies were performed on chlorogenic acid and it was found that 3.5 mg of CGA is soluble per millilitre of water. The solubility of CGA at different pH range shows that it is more soluble in basic buffer than acidic. Tablets were prepared by direct compression, using HPMC K 15M as matrix forming polymer and sodium bicarbonate as effervescent agent. Talc is used as glidant, magnesium stearate was added for lubrication. All parameters were found to be within pharmacopoeial limits. Also formulations were evaluated for floating behavior, which showed floating lag time less than 1.24 sec, and total floating time more than 12 hrs. All the data was fitted into Design expert10 software. The obtained formulations were tried and results were significant. Regression analysis showed that by increasing HPMC concentration the drug release was decreased and by increasing the concentration of effervescent agent floating lag time was decreased whereas the drug release was increased.

The data obtained by in-vitro drug release study of floating tablets of CGA was fitted to various kinetic models (zero order, 1st order, Higuchi and Kessmey’s and Peppas (K-P) plot for the determination of release mechanism of chlorogenic acid. The data was best fitted to higuchi model indicated by the highest $R^2$ value = 0.9563. The $R^2$ value obtained from K-P plot indicates that release is governed by the fickian law of diffusion. In-vitro drug release study was performed in simulated gastric fluid i.e.1.2 pH buffer. Optimized batches (F7, F8 and F9) showed that the drug was released at floating sustained manner for 12 hrs. Stability study also showed no changes in results. The tablets were evaluated for pharmacopoeial and nonpharmacopoeial (industry specified) tests. Based on the results, formulation F9 was identified as better formulation amongst all formulations for floating tablets.

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**Conflict Of Interest**

Aditi Mool, Dr. Archana Moon, Dr. Veena Belgamwar, Dr. Sheelpriya Walde, Dr. Sunil Gupta are full time involved in the study.
Figure 7: Photographs taken during in-vitro buoyancy study of formulation F9 in 200 ml of 0.1 N HCL at different time intervals.

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