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# Development and evaluation of gastro retentive drug delivery system of monoammonium glycyrrhizinate for the management of gastric ulcer

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**ABSTRACT**: Monoammonium glycyrrhizinate (MAG), a salt of glycyrrhizin, is reported for effective treatment of gastric disorders. The work was aimed to design and develop a gastro-retentive drug delivery system for MAG to delay its release in stomach by developing a stable raft with sufficient strength and acid-neutralizing potential. Preliminary, *in-silico* molecular docking study of MAG with the native ligand (Vonoprazan, a potential proton pump inhibitor) present in the gastric proton pump was performed. Docking studies predicted that MAG could bind to Vonoprazan binding site, indicating its ability to inhibit the gastric proton pump. The most desirable optimal formulation of raft forming tablets of MAG was anticipated with the desirability (0.819). The optimized formulation showed raft strength (8.61 ± 0.06 g), acid neutralizing capacity (11.19 ± 0.03 mEq) and *in vitro* release of MAG (69.11 ± 0.61% over 8h) indicating its suitability as a potential Gastro-retentive raft forming delivery system. The optimized formulation decreased gastric acid production and elevated gastric pH ($p < 0.001$.) in pylorus ligation induced gastric ulcers in animal model and demonstrated significant decrease in ulcer index ($p < 0.001$.) The developed raft-forming tablet of MAG could be a promising alternative to the existing synthetic agents to treat gastric ulcers.

**KEYWORDS**: Gastric ulcers; Gastro retentive drug delivery system; Monoammonium glycyrrhizinate; Raft forming tablets, pylorus ligation induced gastric ulcers.

## 1. INTRODUCTION

Gastric ulcers damage the tissue lining of the stomach which further causes gastric disturbance, heartburn, nausea, vomiting, indigestion and burning pain [1]. In severe cases, if left untreated it may lead to stomach cancer and bleeding. The current treatment approaches for gastric ulcers includes proton pump inhibitors like omeprazole; antibiotics like metronidazole, amoxicillin etc. Despite of their efficacy in treating the gastric ulcers, they are reported to be associated with various side effects like stomach pain, nausea-vomiting, antibiotic resistance which limits its therapeutic applications [2]. Recently, trends have been changed from the use of synthetic medicines to natural products, phytoconstituents etc. for the treatment of various diseases and disorders. Several studies have reported the efficacy of natural products in treating gastric ulcers by improving the quality of ulcer healing and also preventing its recurrence [3]. Moreover, the preclinical and clinical studies have demonstrated the efficacy of natural products in the management of gastric ulcers with minimum side effects [4].

In the traditional system of medicine liquorice root (*Glycyrrhiza glabra*) is widely reported for its anti-ulcer activity and its safety [5]. Glycyrrhizin (GA) (triterpene saponin) is a major bioactive component obtained from the liquorice root. It promotes the normal defence mechanism of the gastric cells, improves its life span and exerts anti-pepsin effect. Studies have also reported the effectiveness of GA against Helicobacter -pylori infections by exerting antibacterial and anti-adhesive effect against it and preventing its adhesion to the gastric mucosa [6, 7]. Hence, the development of gastro retentive drug delivery system of GA would prove to be beneficial in treating gastric ulcers. However, the poor absorption of GA in rats and in humans limits its

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use. Monoammonium glycyrrhizinate (MAG) which is a salt form of glycyrrhizin is reported to exert high acid solubility and could overcome the issues of solubility, concerned with the use of GA. Hence, MAG was selected in the present study.

Very few studies are reported on formulation and development of MAG in treating gastric ulcers by oral administration. The studies report the efficacy of MAG in reducing the score of ulcer index and inhibit further ulceration in pylorus ligated-induced gastric ulcer in rats [8]. However, an insight study is required to understand the action of MAG at the molecular level in terms of its interaction with the native ligands acting as proton pump inhibitors in the gastric proton pump. Interestingly, till date no reports on the design and development of raft forming Gastro-retentive drug delivery system (GRDDS) of MAG using sodium alginate and HPMC as a polymer, by applying the concept of QbD and DOE is available, making this work unique. The efficacy of raft forming systems of some phytoconstituents such as quercetin, curcumin has been proved to enhance the retention of drug in stomach to improve its effectiveness in the management of gastric related disorders [9]. Hence an attempt was made to further investigate and design an optimized raft forming GRDDS of MAG for the management of gastric ulcers. Studies have proven that after oral administration, MAG is converted to 18 β glycyrrhetinic acids by bacterial metabolism. 18β glycyrrhetinic acids exhibit multiple therapeutic properties including antiulcer, anti-inflammatory, anticancer etc. [10]. Hence, the docking studies of 18 β glycyrrhetinic acid, an active metabolite of MAG was carried out against gastric proton pump (PDB5YLU) referred from RCSB Protein data bank. The molecular docking studies represent an effective way to study the interaction of the actives with the protein molecule at the molecular level. This interaction helps to determine and characterize the performance of the actives at the binding site of the receptor protein and to explain the basic biochemical processes occurring due to the interaction [11]. Hence, the molecular docking was carried out to assess the ability of 18 β glycyrrhetinic acid to bind with the gastric proton pump to inhibit it and prove its effectiveness. Gastric proton pump is considered as a novel target to treat gastric acid related disorders as it is responsible for secretion of gastric acids. The inhibition of the proton pump is one of the approaches to manage gastric acid related disorders. Hence, the proton pump inhibitors (PPI) are considered as the choice of drug in treating gastric ulcers, but their use is associated with side effects which again limit their use. Vonoprazan is a more potent and longer acting proton pump inhibitor than traditional ones and can inhibit the enzyme H+K+ATPase in a K+-competitive and reversible manner and is expected to be superior to the existing PPI [12]. The native ligand1-[5-(2-fluorophenyl)-1-pyridin-3-ylsulfonylpyrrol-3-yl]-*N*-methylmethanamine (Vonoprazan) in 5YLU was identified and used to study its interaction with 18 β glycyrrhetinic acid at molecular level via *in silico* molecular docking in order to predict the ability of MAG to acts as an effective proton pump inhibitor to treat gastric ulcers. Till date, *in silico* molecular docking of 18 β glycyrrhetinic acid with 1-[5-(2-fluorophenyl)-1-pyridin-3-yl sulfonyl pyrrol-3-yl]-*N*-methylmethanamine is not reported.

MAG, being a salt form of GA exhibits high acid solubility, but to make it effective in the treatment of gastric ulcers, its retention in the stomach is desired. GRDDS can hold the drug in the stomach for a longer duration of time, thereby increasing the therapeutic efficacy of the drug and making it suitable for targeted delivery in the stomach for local effect [13]. Additionally, as the preferred route for the delivery of GRDDS is the oral route, it offers various advantages such as ease of administration and better patient compliance with reduced frequency of dosage administration as compared to the conventional oral dosage forms. Raft forming gastro retentive drug delivery systems represents an effective approach to retain the drug in the stomach by allowing it to float on the surface of the stomach content after oral administration. The raft forming system contains the gelling polymers like alginates, pectin and gas generating agents like bicarbonates or carbonates [14]. This system forms a viscous, gelatinous continuous layer known as raft. On contact with the gastric fluids, the system forms gel and liberates carbon-dioxide gas which gets entrapped in the gel causing the system to float on the stomach content [15]. This tends to improve the time of contact of drug with the mucus lining of the stomach, thereby improving the therapeutic efficacy of the drug for local action in the stomach. To better understand the formulation variables with respect to its interaction and its effect on the quality of the product, the concept of design of experiments (DOE) was applied. The DOE approach represents an effective way to accomplish statistically optimal results to arrive at the product with desired characteristics. Raft forming tablets of MAG were formulated using sodium alginate as raft forming agent and sodium bicarbonate and calcium carbonate as gas generating agents by direct compression method. The formulations were optimized by applying Box behnken design by varying the concentration of sodium alginate, sodium bicarbonate and calcium carbonate to design the formulation with sufficient raft strength, acid neutralizing capacity and prolong release to achieve targeted delivery of MAG in the stomach and improve its effectiveness.

Thus, formulating a raft forming GRDDS of MAG presents a potential approach to achieve site specific delivery of MAG to the stomach with prolonged retention time to exert anti-ulcer effect. Hence, the aim of the

current research work was to first understand the interaction of MAG with the gastric proton pump at the molecular level and then develop an optimized raft forming GRDDS with desired characteristics to further improve the delivery of MAG at the target site with prolonged retention in managing gastric ulcers. The *in silico* molecular docking studies predicted the ability of MAG to acts as an effective proton pump inhibitor to treat gastric ulcers. The optimized raft forming GRDDS when evaluated for the *in vivo* therapeutic efficacy on pylorus-induced acute gastric ulcers in rats, demonstrated a significant reduction in ulcer index and gastric acid secretions, thereby proving its site specific delivery to the stomach and exerting beneficial effects in treating gastric ulcers.

## 2. RESULT AND DISCUSSION

### 2.1. Target Validation

Target validation studies were conducted using the selected targets and native co-crystallized ligands, indicated low RMSD values within runs confirming the accuracy and repeatability of the docking procedure. The docking results of native ligands with targets are shown in Figure 1.

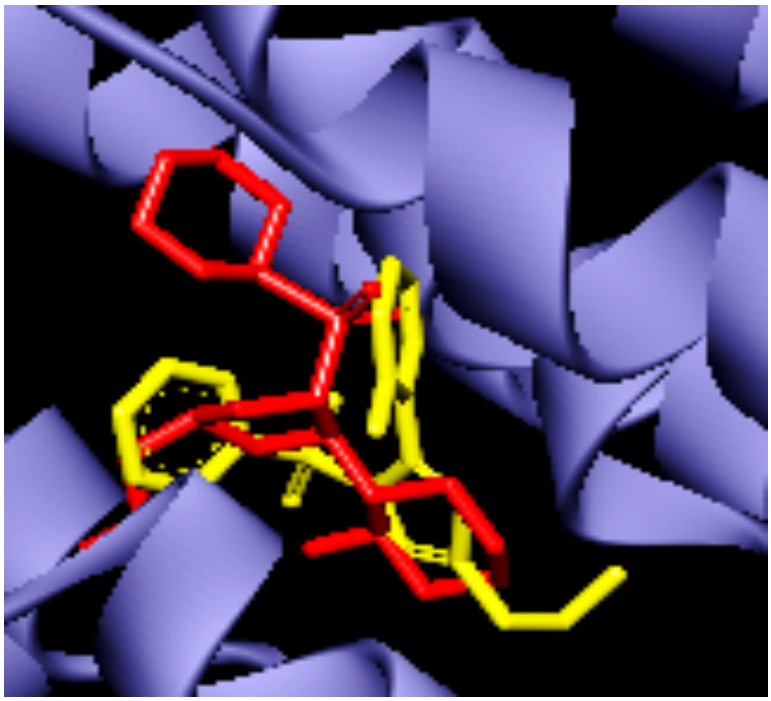

**Figure 1.** Superimposition of re-docked Vonoprazan (red) onto co-crystallized form (yellow) in the active site.

### 2.2. Molecular Docking studies

Table 1 summarizes the outcomes of docking studies with interacting amino acid residues and the types of interactions. The best-docked complexes of these ligands with their interacting amino acid residues are shown in Figure 2A and 2B.

**Table 1.** Docking studies with interacting amino acid

| Compound Name Target 5YLU | Binding Energy (kcal/mol)T | Interacting Amino acids | Bond type |
|---|---|---|---|
| Vonoprazan | -8.2 | Leu 811,<br>Ala 339, Ile 810, Tyr 799, Glu 795 | H- bond<br>π- π stacking<br>π- π stacking |
| 18 BETA | -8.3 | Leu 800, Phe 872, Phe 875, Ile 801, | π- π stacking |

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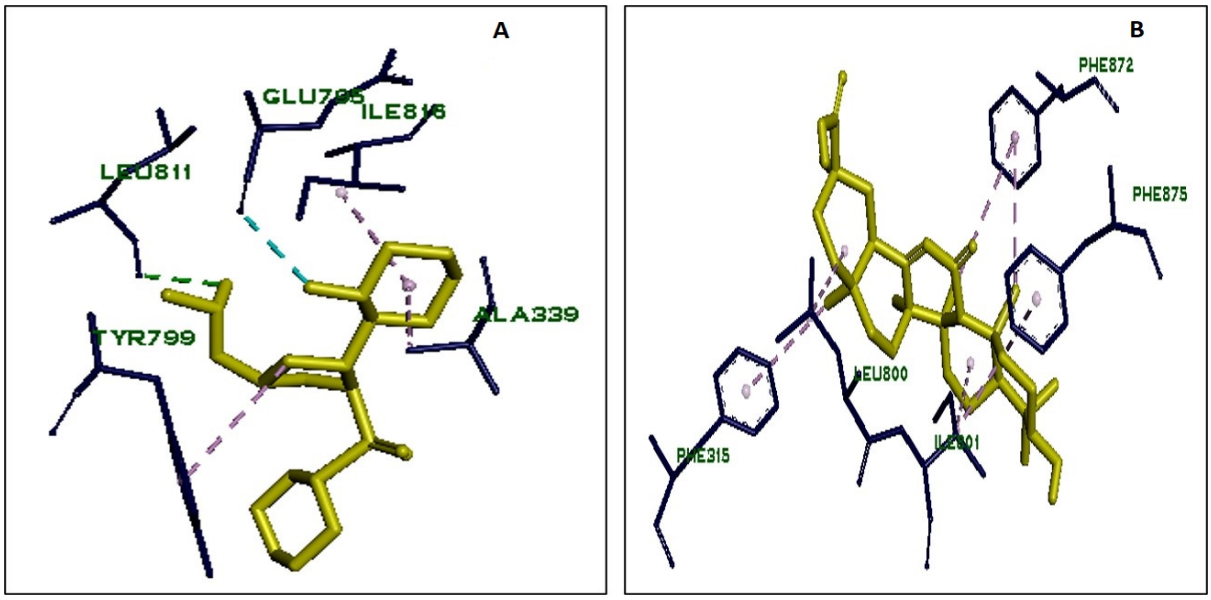


**Figure 2. A)** Molecular Interaction of Vonoprazon with 5YLU. **B)** Molecular Interaction of 18 β glyccheretinic acid with 5YLU.

From the docking studies it was observed that binding affinity of 18 β glycyrrhetinic acid with 5YLU was comparable with the binding of Vonoprazon with 5YLU. 18 β glycyrrhetinic acid exhibited binding energy of -8.3 kcal/ mol while Vonoprazan exhibited binding energy of -8.2 kcal/mol.The binding interaction study confirms that 18 β glycyrrhetinic acid is involved in pi-pi stacked interaction with Leu 800, Phe 872,Phe 875, Ile 801 interacting amino acids and 18 β glycyrrhetinic acid is not involved in hydrogen bonding. The molecular docking studies clearly indicate that 18 β glycyrrhetinic acid showed interaction with gastric proton pump 5YLU ensuring its activity towards this receptor, thus proving its efficacy in acting as a proton pump inhibitor.

**2.3. FTIR**

The FTIR spectra of MAG indicated all the characteristic peaks corresponding to the major functional groups. The peak at 3607.01 cm− 1, represented characteristic stretching vibration of -OH (hydroxyl) group and 1782.29 cm− 1 indicates -C=O Stretch (carboxylic acid). The peak at 1614.47 cm− 1 stands for -C=C- stretching vibration. The peak at 3563 cm− 1 represented N-H symmetric stretching (Figure 3a.) [16]. The FTIR spectroscopy recorded for sodium alginate demonstrated characteristics peak at 3653.01 cm− 1 representing the characteristic stretching vibration of -OH (hydroxyl) group [17]. The peak at 1780.34 cm− 1 indicates -C=O asymmetric stretching for (carboxylic acid) and the peak at 1310.20cm− 1 shows symmetric stretching of C-O (carboxylate salt groups). (Figure 3b.). The FTIR spectroscopy of raft forming tablet of MAG indicated the presence of the characteristic's peaks corresponding to the major functional groups present in MAG, thus confirming the compatibility of MAG with the other excipients (Figure 3c.) indicating the use of excipients would not alter the properties of MAG.

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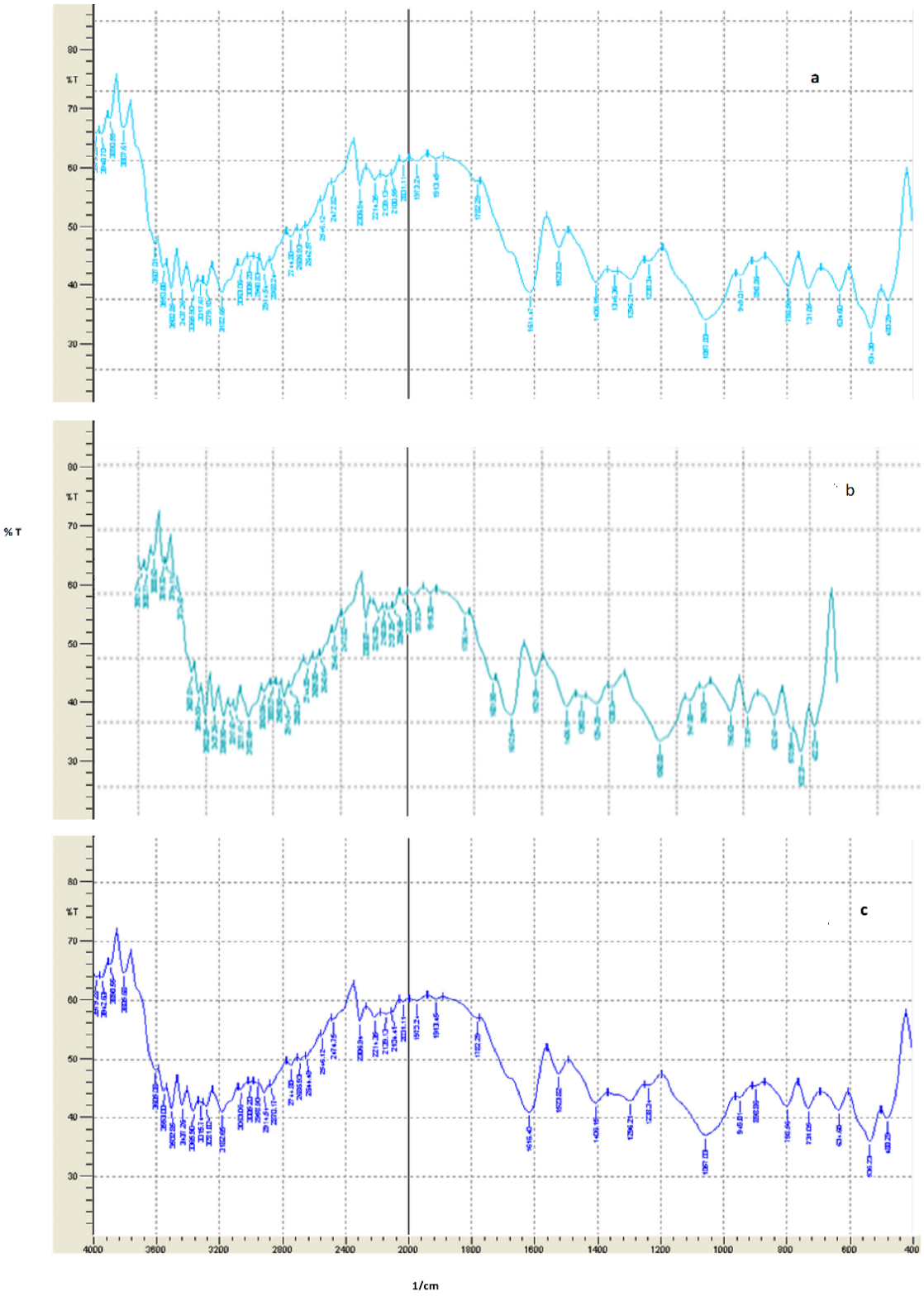


**Figure 3.** FTIR spectra a. MAG, b. sodium alginate c. MAG raft forming tablet

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## 2.4. DSC

The thermogram of the MAG showed a sharp endothermic peak at 110.6 °C and broad peak at 205.1°C. The area of the first peak was bigger than that of the second peak. Studies by [8] reported that more amount of MAG was recovered from liquorice root at 110 °C indicating its melting point at that particular temperature Figure 4a [8]. The thermogram of sodium alginate showed an endothermic peak around 115.6 °C as a result of elimination of water Figure 4b [18]. In the thermogram of the raft forming tablet of MAG, broad peak corresponding to its melting point was observed with a slight shift at 108°C indicating the compatibility of MAG with the tablet components Figure 4c.

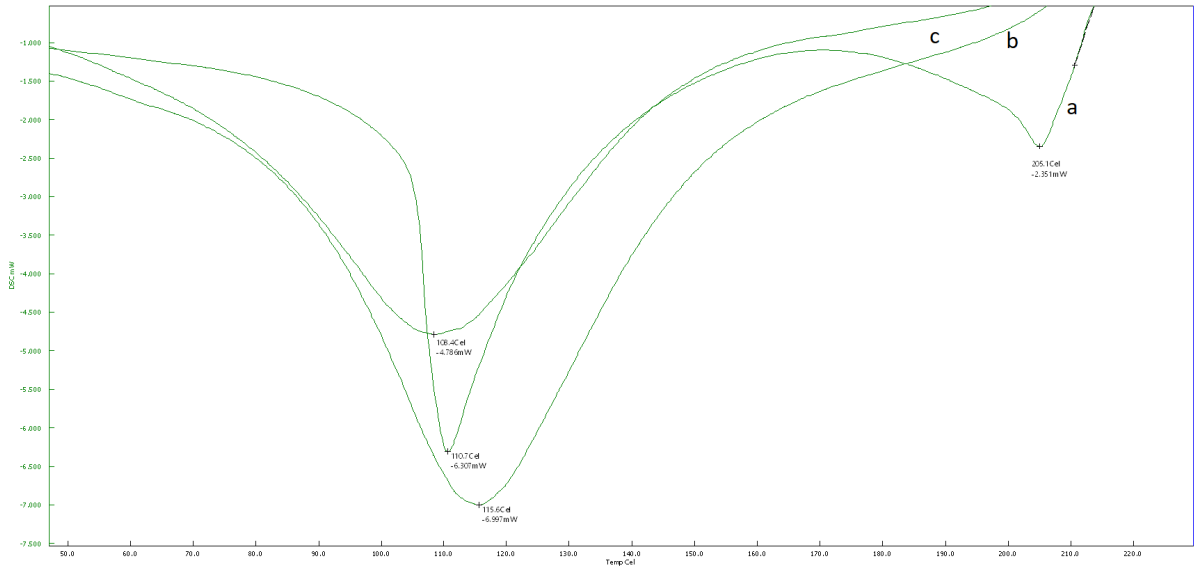


**Figure 4.** Differential Scanning Calorimetry of a. MAG, b sodium alginate c. MAG raft forming tablet.

## 2.5. Evaluation of Tablet

The hardness of the tablet was in the range of 5.0 ± 0.12- 7.2 ± 0.36kg/cm$^2$. The formulated tablets were within the limits of weight variation test as prescribed by USP as no individual weight of the deviated from the average weight by more than 7.5%. The friability values of the tablets were low which indicated their ability to be resistant to the mechanical shocks. The drug content of the tablet was found in the range of 90.22±1.15 to 98.32±2.64 %. The floating lag time of the raft forming tablet was 60±0.3 sec to 65±05 sec and its total floating time was in the range of 8±0.2 to 10±0.5h. A representative HPLC chromatogram for analysing pure MAG is shown in Figure 5. The developed HPLC analytical method for estimating the content of MAG was validated as per the ICH guidelines with respect to linearity, accuracy, precision and robustness. The method showed linearity over the range of 10 to 60 µg/ml with R2 = 0.9945 as shown in Figure 6. The accuracy of the method estimated in terms of recovery study was 97.23-98.97%. The calculated %RSD for intra-day and inter-day precision were 0.22 and 0.25, respectively. The robustness of the method was evident with respect to change in wavelength, flow rate and column temperature.

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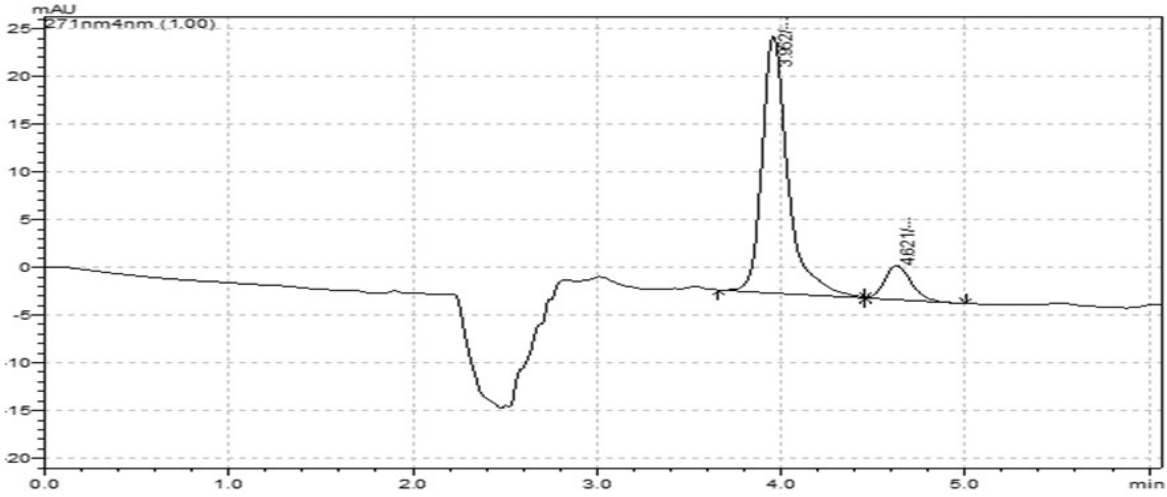


**Figure 5.** Representative HPLC Chromatogram of raft forming MAG tablet to determine the drug content

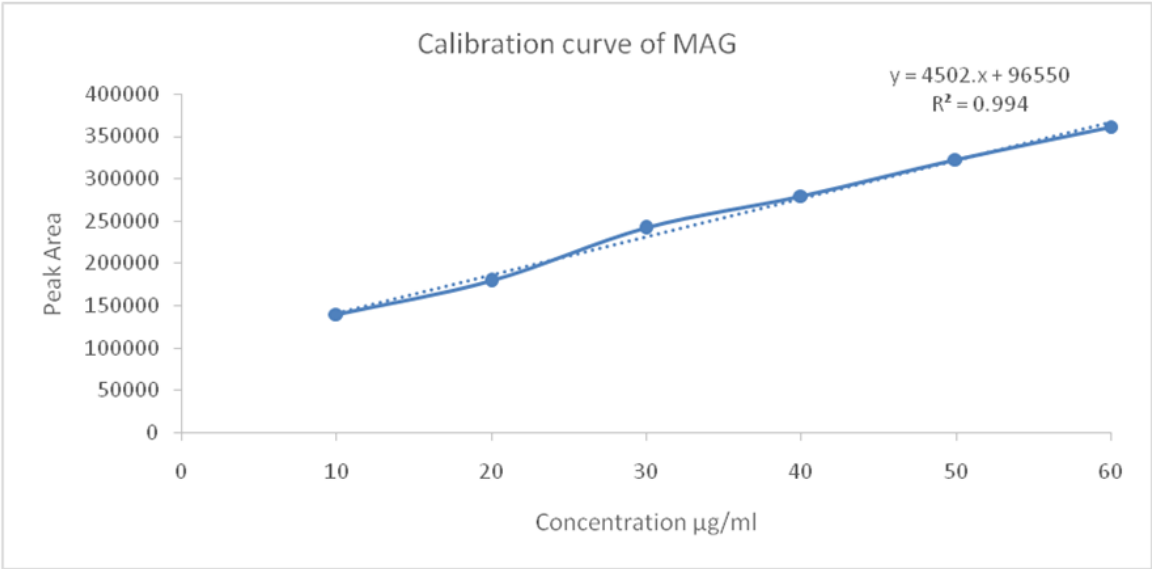


**Figure 6.** Calibration curve for MAG using the developed HPLC method

The SEM image of the tablet showed a dense intact structure as shown in Figure 7a, whereas, the SEM image of the raft showed the presence of pores, viscous, gelatinous structure as shown in Figure 7b.

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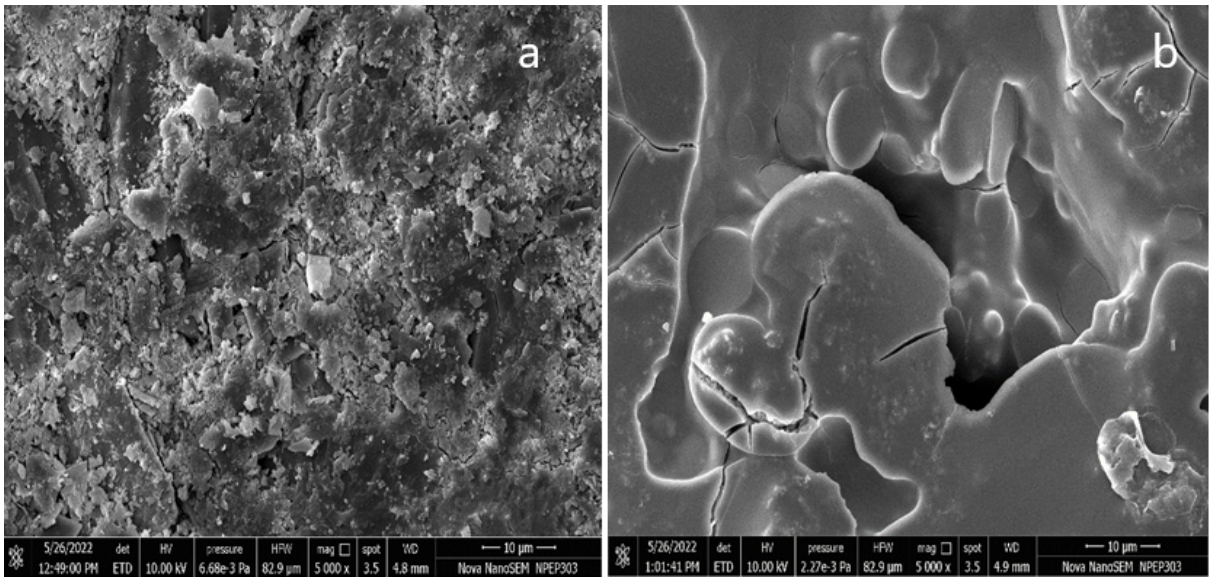


**Figure 7a.** SEM image of the MAG tablet showing dense intact structure **7b.** SEM image of the raft showing the presence of porous, viscous and gelatinous structure

*2.5.1. Density*

Rafts forming systems are required to float on the content of the stomach in order to increase its residence time to achieve sustained release. The raft forming tablets on contact with the acidic medium liberates Ca++ ions from Calcium carbonate which cross links with alginates and forms gel-like matrix. The presence of sodium bicarbonate generates carbon dioxide which gets entrapped in this matrix causing the raft to float. The density of all the raft-forming tablets was less than the density of the gastric medium, thus causing they're floating on the gastric medium as well as preventing their entry into small intestine and increasing gastric residence.

In the formulation of raft forming tablets, the concentration of polymer sodium alginate and the concentration of gas generating agent's sodium bicarbonate and calcium carbonate are critical parameters. As per the literature, the use of sodium alginate as a polymer in the formulation of raft forming tablets is suggested [19]. Even the floating of the raft system depends on the entrapped gas molecules in the gel matrix of alginate. Hence in order to study the effect of concentration of sodium alginate (X1), concentration of sodium bicarbonate (X2) and concentration of calcium carbonate (X3) on the formation of raft forming tablets, Box Behnken design (BBD) was used. The effect of these independent variables were evaluated on the responses raft strength (Y1), acid neutralizing capacity (Y2) and drug release in 8hr (Y3) as shown in the Table 2.

**Table 2**. Response parameters of the formulations as per the Box Behnken design

| Std | Run | Factor 1 A:Sodium alginate (mg) | Factor 2 B: Sodium bicarbonate (mg) | Factor 3 C:Calcium carbonate (mg) | Response 1 Raft strength (g) | Response 2 Acid Neutralizing capacity (mEq) | Response 3 % drug Release |
|---|---|---|---|---|---|---|---|
| 3 | 1 | 100 | 75 | 100 | 6 | 7 | 89 |
| 7 | 2 | 100 | 50 | 150 | 7 | 6 | 88 |
| 4 | 3 | 200 | 75 | 100 | 8 | 11.2 | 65 |
| 8 | 4 | 200 | 50 | 150 | 9.5 | 10 | 70 |
| 17 | 5 | 150 | 50 | 100 | 7.2 | 8.2 | 62 |
| 2 | 6 | 200 | 25 | 100 | 9 | 8.5 | 60 |
| 14 | 7 | 150 | 50 | 100 | 7.3 | 6.3 | 73 |
| 13 | 8 | 150 | 50 | 100 | 6 | 6.3 | 73 |
| 12 | 9 | 150 | 75 | 150 | 7 | 10.9 | 85 |
| 16 | 10 | 150 | 50 | 100 | 7.3 | 7.8 | 73 |
| 5 | 11 | 100 | 50 | 50 | 6.2 | 5.8 | 75 |
| 6 | 12 | 200 | 50 | 150 | 6.5 | 6.3 | 60 |
| 1 | 13 | 100 | 75 | 100 | 5 | 4.9 | 86 |
| 9 | 14 | 150 | 25 | 50 | 6.5 | 5.5 | 79 |
| 11 | 15 | 150 | 25 | 150 | 6 | 8.8 | 77 |
| 10 | 16 | 150 | 75 | 50 | 6 | 7 | 80 |
| 15 | 17 | 150 | 50 | 100 | 7.3 | 6.3 | 73 |

The results obtained from the Box Behnken design (BBD) was fitted in the linear regression analysis and the following equations were obtained

Raft strength (Y1) = +6.93 +1.10 X1+0.062 X2 + 0.54X3 ------- (1)

In the equation 1, the positive co-efficient obtained for X1, X2 and X3 indicates that an increase in the raft strength was observed with an increase in the concentration of sodium alginate, sodium bicarbonate and calcium carbonate. The response 3D surface plots obtained for the effect of factors X1, X2, X3 on raft strength were obtained. The data obtained from the statistical models and ANOVA to evaluate the effect of factors on the raft strength indicated that the linear model was statistically significant model with an F value of 6.06 and P< 0.05 indicated that the model was significant. The model exhibited a non-significant lack of fit of value 2.48. The raft strength for all the batches of the tablets are shown in the Table 2. An increase in the raft strength was observed (5 -9.5 g) with an increase in the concentration of sodium alginate and calcium carbonate. Darwish, et al., 2019 reported the similar findings of raft strength using sodium alginate as a raft forming agent [19]. The formulation F4 with the highest concentration of sodium alginate and calcium carbonate exhibited maximum raft strength of (9.5±0.6g) as compared to the other formulations. In the acidic medium calcium ions are liberated from insoluble calcium carbonate, which converts the alginates into a hydrogel by cross linking with it. The binding of the calcium ions to the guluronic acid blocks present in the sodium alginate polymer, forms a gel like network responsible for the raft strength [20]. The formulation F4 and F6 exhibited a higher cross-linking density, hence maximum raft strength. It was concluded from the study that both the concentration of sodium alginate and calcium carbonate is critical for the raft strength. The concentration of sodium bicarbonate is also critical for raft formation, as it helped to provide a porous structure to the raft to promote its floating. Sodium bicarbonate being a gas generating agent, it helps in the formation of carbon dioxide gas which gets entrapped in the formed gel matrix of the polymer causing its floating. It was also observed that the increase in the concentration of sodium bicarbonate lead to the disintegration and breaking of the raft due to the formation of highly porous structure of the raft due to higher amount of released carbon –dioxide gas which caused the matrix of the raft to enlarge, causing it to rupture [21]. Hence an optimum concentration of sodium bicarbonate was required for the floating and raft formation

*2.5.2. Acid Neutralizing Capacity (ANC)*

ANC (Y2) = +7.46 +1.54 X1+1.05 X2+ 1.39X3 ------- (2)

In the equation 2, the positive co-efficient obtained for X1, X2 and X3 indicates that an increase in the ANC was observed with an increase in the concentration of sodium alginate, sodium bicarbonate and calcium carbonate. The 3D response surface plots were obtained for the effect of factors X1, X2, X3 on ANC. The data obtained from the statistical models and ANOVA to evaluate the effect of factors on ANC indicated that the linear model was statistically significant model with an F value of 13.57 and P< 0.05 confirming that the model

was significant. The model exhibited a non-significant lack of fit of value 1.28.The ANC of all the batches were reported in the range of 4.9±0.3 to 11.2±0.5. The formulation F3 and F9 exhibited higher ANC due to higher amount of sodium bicarbonate and calcium carbonate which acts as antacids to neutralize the acid produced in the gastric ulcers. Similar findings were observed by Dettmar *et al.*, 2017 [22] when studying the chemical properties of the alginate antacid raft forming formulations. It was observed from the responses that increase in the concentration of sodium bicarbonate and calcium carbonate resulted in enhancing the ANC of the formulations. For the acids to get neutralized, an optimum level of porosity should be present in the raft structure to allow the acids to interact with the antacids. Studies by Dettmar *et al.,* 2017 [22] have suggested that antacids are trapped in the raft structure and sufficient time must be given for the acids to interact with the antacids in the raft structure for is neutralization. Hence, the concentration of sodium bicarbonate is critical as it not only acts as an antacid but also controls the porosity of the raft structure.

*2.5.3. Effect on the Drug Release*

Drug Release (Y3) = +74.59 -10.38 X1+2.13 X2+ 3.25X3 ------- (3)

Equation (3) represents the effect of X1, X2 and X3 on drug release (Y3) after 8 h. The value of negative coefficient for X1 indicated that the drug release decreased, when the concentration of sodium alginate was increased. The positive co-efficient obtained for X2 and X3 indicates that an increase in the drug release was observed with an increase in the concentration of sodium bicarbonate and calcium carbonate. The 3D response surface plots were obtained for the effect of factors X1, X2, X3 on the drug release. The data obtained from the statistical models and ANOVA to evaluate the effect of factors on drug release indicated that the linear model was statistically significant model with an F value of 10.47 and $P < 0.05$ confirming that the model was significant. The model exhibited a non-significant lack of fit of value 1.42. Formulations containing highest amount of sodium bicarbonate exhibited a faster drug release, due to increased amount of carbon–dioxide generated which caused the bursting of the raft due to the formation of pores in it as observed in the formulations (F1, F9, F10, F13). The pores formed in the raft structure caused the faster diffusion of the drug into the dissolution media. From the obtained results, it was seen that the formulations containing highest concentration of sodium alginate (F3, F4, F6 and F12) and high amount of calcium carbonate exhibited minimum drug release. The reason behind this minimum drug release is formation of gel like matrix due to cross linking of Ca++ ions with sodium alginate (23). Also, when exposed to the acidic medium, some amount of Ca++ ions present in the calcium alginate raft were replaced for the protons present in the acidic medium to form alginic acid. Alginic acid being slightly soluble in water, it formed a dense network which could have delayed the release of MAG Another reason for delayed release of MAG could be the presence of HPMC K15 which could have contributed to augment the formation of alginate gel, as in the presence of acidic medium HPMC forms a viscous gel thereby creating a resistant barrier for the diffusion of MAG [24].The percent release of formulated batches of raft forming tablets of MAG (F1- F17) percent is shown in Figure 8.

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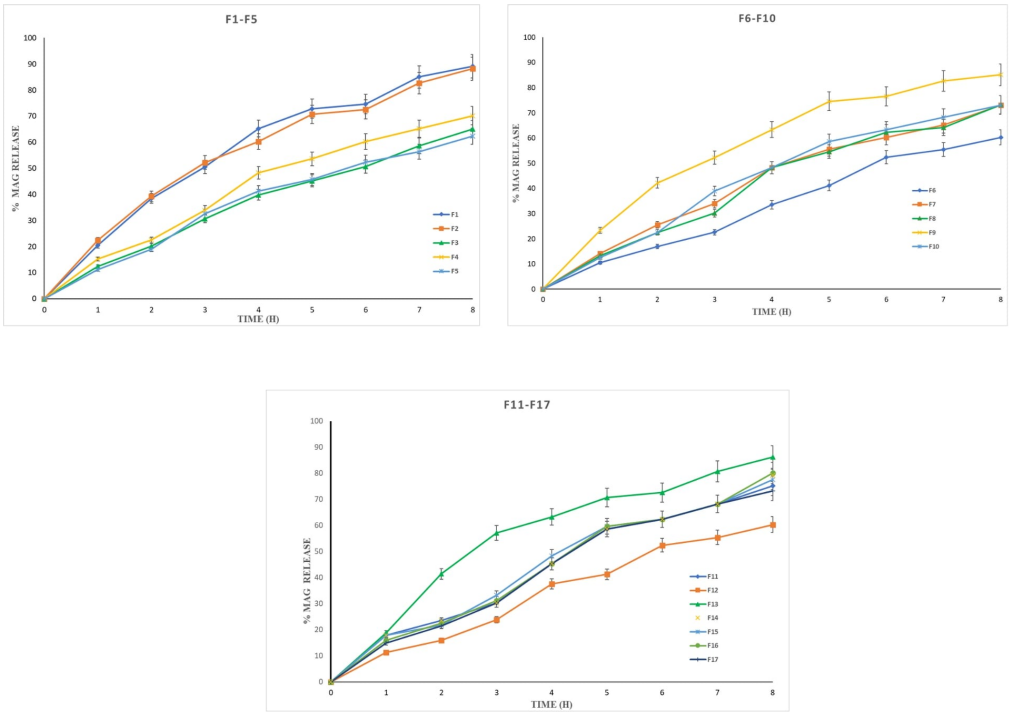


**Figure 8.** Percent drug release of MAG over a period of 8h.

*2.5.4. Determination of design space*

The optimum raft forming tablet formulation was selected based on the higher values of acid neutralizing capacity, raft strength, and sustained drug release. To select the optimized formulation, point prediction of the Design Expert software was used to determine the optimized formula which can give the closeness of desirability factor to 1. The response 3D surface plots obtained for the effect of factors X1, X2, X3 on raft strength, ANC and percent drug release is shown in Figure 9a, 9b and 9c respectively. Figure 9d shows the overlay graph, which predicts the optimized concentrations of the selected factors to give the desired responses: X1 (sodium alginate) = 200 mg, X2 (sodium bicarbonate) = 69.43 mg and X3 (calcium carbonate) = 150 mg. To validate the process, the two optimized formulation was prepared as per the input variables suggested by the design expert software, the obtained values of the experimental responses for Y1, Y2 and Y3 were compared with the predicted values and the percent relative error was determined. The percent relative error between the predicted values and experimental values was below 5%. This low magnitude of relative error confirmed the predictability and indicated the validation of the experimental design used. The optimized formulation demonstrated the raft strength, ANC and percent release of MAG as 8.61g, 11.19mEq, 69.11%respectively.

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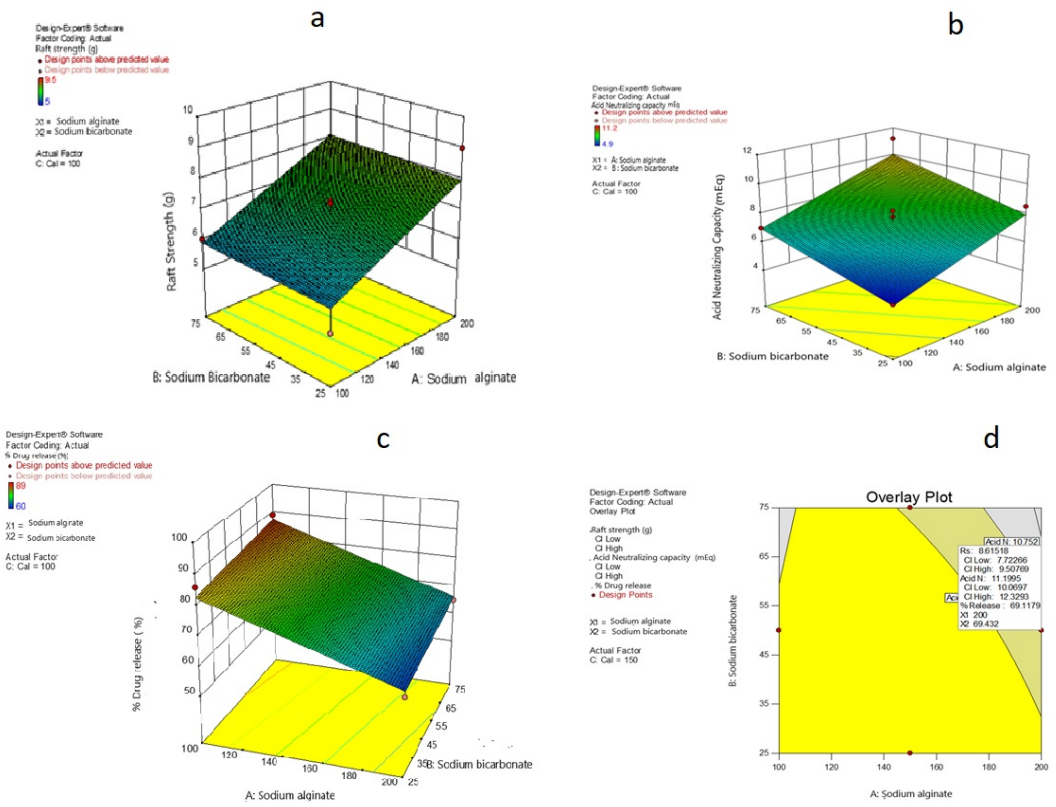


**Figure 9a.** Effect on the raft strength of the tablet **9b.** Effect on ANC of the tablet. **9c.** Effect on the drug release of the tablet. **9d.** Overlay plot indicating the design space.

## 2.6. In-vivo Pharmacodynamic study

In-vivo study in rats was carried out to determine the effect of MAG for its ulcer healing properties which was evaluated by estimating the UI and percent inhibition of ulceration. Ulcers were induced by ligating pylorus of the rats, as the pylorus is obstructed due to increase in the production of acid content which leads to the destruction of gastric mucosa. This method is suitable for the screening of the agents which can act as ulcer healing agents by reducing the acid secretion and enhancing the production of mucus. The gross appearance of the gastric mucosa is show in Figure 10 for all the groups. In the Group II more number of ulcers were observed as compared to the number of ulcers observed in Groups III and IV after 5 h of the treatment. The value of ulcer index in the group IV was 4.3±0.6 which was less as compared to the group II which was 14.9±0.25 and the standard group III 7.4±0.5 Figure 10. The percent inhibition of ulcer for the group IV was 71.11%, and for the group III was 50.33%. The protective effect of MAG on the stomach ulcers was evident from the smaller number of ulcers observed in the Group III, the reason for this would be the due to the increase in the local concentration of prostaglandins that stimulates the mucous secretion and cell proliferation in the stomach. Also, the presence of sodium alginate might have imparted a muco-protective effect on the surface of the gastric mucosa by providing a protective covering on it. The platelet aggregation effect elicited by sodium alginate is also reported to reduce bleeding and prove effective in treating the gastric ulcers. Ranade *et al.*, 2012 [25], observed the similar pattern of the ulcer index and percent ulcer inhibition. Studies also report the effectiveness of GA against H-pylori infections by exerting antibacterial and anti-adhesive effect against H-pylori and preventing its adhesion to the gastric mucosa.

The effect of raft forming tablets of MAG on the macroscopic appearance of the stomach mucosa in pylorus ligation method induced mucosal injuries in Male Wistar rats. Group I: Normal control group showed no mucosal damage as shown in Figure 10a. Group II: Disease induced group: noticeable ulcers, haemorrhagic streaks, and mucosal damage was observed as shown by black arrows as shown in Figure 10b. Group III Standard control group, here animals were administered with omeprazole pellets (20 mg/kg), very minor damage were observed in the gastric mucosa and no haemorrhagic streaks as compared to the group II Disease control group as shown in Figure 10c. Group IV served as a drug treated group received 200 mg/kg mini tablets of MAG where a significant reduction was observed in gastric mucosal damage and the mucosa seemed

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to be flat. Furthermore, the absence of haemorrhagic streaks and no injury to the gastrointestinal mucosa was observed as shown in Figure 10d.

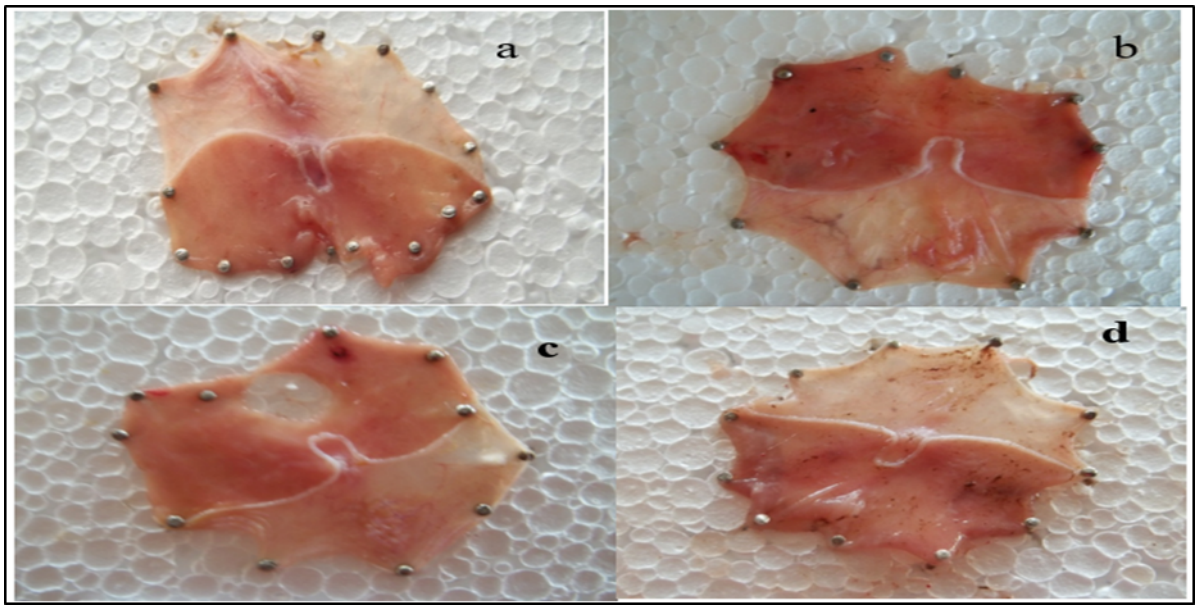


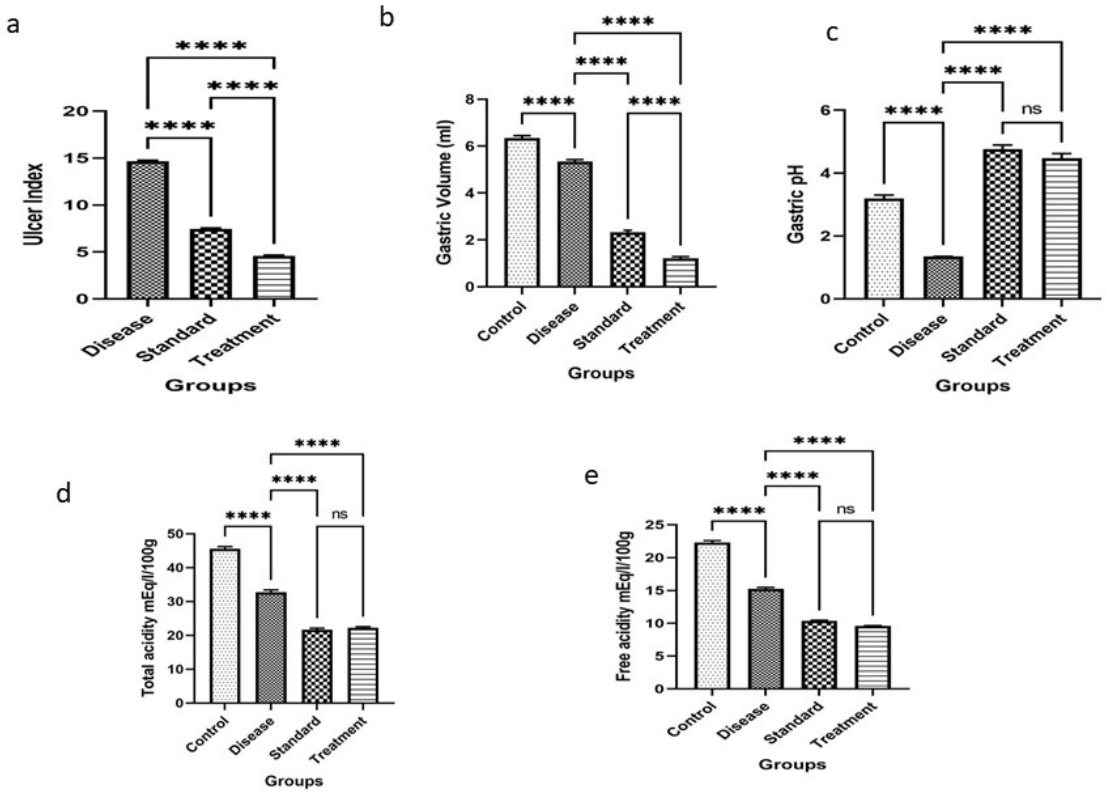

**Figure 10a. Group I:** Normal Control Group (No mucosal damage)

**10b.Group II:** Disease Control Group (Gastric ulcer, noticeable ulcers, hemorrhagic streaks, and mucosal damage)

**10c. Group III:** Standard treatment group with omeprazole pellets **(**very minor damage were observed in the gastric mucosa and no hemorrhagic streaks)

**10d. Group IV:** Treatment group with MAG tablets (significant reduction in gastric mucosal damage and the mucosa seemed to flat and absence of hemorrhagic streaks and no injury to the gastrointestinal mucosa)

The results obtained for the various parameters such GUI inhibition, gastric pH, gastric volume, free and total acidity of the gastric content were expressed as mean ± standard error of the mean (S.E.M) as shown in Figure 11a, 11b, 11c, 11dand 11e.




Results are expressed as mean± S.E.M. (n = 6). Results were analysed by one-way ANOVA followed by multiple comparison test, Group II compared with Group III and IV. Group I compared with Group II ****p< 0.001.;

**FIGURE 11** a. Effect of treatment on **a.** Ulcer Index **b.** The gastric volume **c.**The pH of the gastric content **d.**The gastric volume **e.**The pH of the gastric content

*2.6.1. Effect of treatment on the gastric volume and gastric volume*

The gastric volume in the disease group was found to be 5.33± 6.6 ml. Treatment with raft forming MAG tablet significantly reduced the gastric volume to 1.2 ± 0.8ml as compared to the disease group. In present study, MAG was effective in lowering the gastric acid secretion and thus the gastric volume. The action is similar to that exhibited by the proton pump inhibitors.

The pH of the gastric contents in the disease group was found to be 1.3±4.0. Treatment with raft forming MAG tablet significantly increased the gastric pH to 4.4 ±0.7 as compared to the disease group. In present study, MAG was effective in raising the pH of the gastric acid secretions. The action is similar to that exhibited by the antacids.

*2.6.2. Effect of treatment on the free acidity and total acidity*

The free acidity and total acidity of the gastric contents in the disease group were found to be 15.2±0.5meq/l/100g and 32.7±meq/l/100g respectively. Treatment with raft forming MAG tablet significantly reduced free acidity and total acidity of the gastric contents to 9.5±0.9 and 22.2±4 respectively as compared to the disease group.

*2.6.3. Histopathological evaluation*

The sections of gastric mucosa from each group were carefully stored in 10% buffered formalin and then subjected to processing to prepare a paraffin block for the samples, followed by staining with haematoxylin and eosin. The histological changes in the different groups such as haemorrhage, degeneration, and erosion was compared to the histopathology of rat stomach in the normal control group. The segment of stomach of the animals in group I that were given merely distilled water without ulcer induction (Normal control group) displayed a normal gastric mucosal layer segment with normal tissue architecture and no signs of deterioration. The results were in line with the authors [26]. No abnormality was detected as shown in Figure 12a. The segment of the stomach of animals in the diseased group II demonstrated the absence of gastric pits

and mucosal epithelium degeneration and sloughing of mucosa (as shown by black arrow Figure 12b with a mild reduction in thickness of muscularis propriaris (as shown by yellow arrow). The same results were observed in the ulcer group and are in line with the authors (27). The segment of the stomach of the animals in group III treated with the standard omeprazole (20 mg/kg) showed significant change in histopathology as compared to the diseased group with regeneration of structure and demonstrated a normal intact glandular architecture and prevention of haemorrhage and edema. The results are in line with the authors [28]. The animals treated with standard drug (omeprazole) showed almost normal pattern as shown in the Figure 12c.

The segment of the stomach of the animals in group IV treated with a dose of 200 mg/kg mini tablets of MAG exhibited absence of gastric pits, reduction in ulceration with no abnormality and improvement in the mucosal degeneration and demonstrated the development of normal and intact gastric mucosa as shown in the Figure 12d.

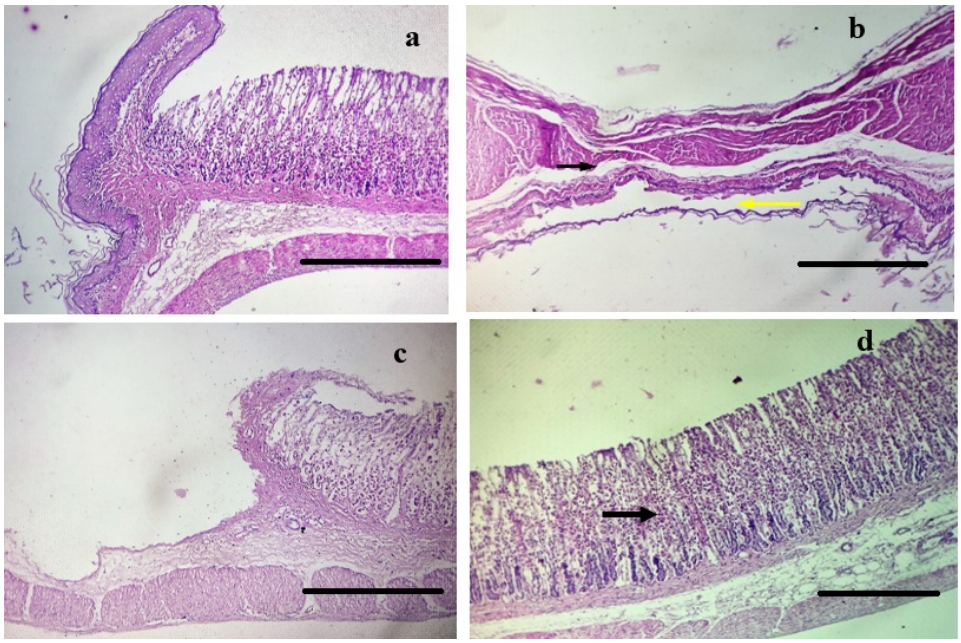



**Figure 12.** Histopathology of the rat gastric mucosa a. Normal Control group. b. Disease group. c. Standard group. d. Treatment group. Scale bar 20 µm.

## 3. CONCLUSION

The design of experiments approach was utilized to formulate gastro-retentive raft forming tablet of MAG using sodium alginate for the management of gastric ulcers. The *in silico* molecular docking studies of MAG demonstrated sufficient binding energy to bind with 5YLU gastric proton pump which was comparable with the binding of Vonoprazon with 5YLU to inhibit the gastric secretions. The developed formulation exhibited sufficient raft strength, acid neutralizing capacity and prolong release of MAG over 8h demonstrating its effectiveness in achieving targeted local action in the stomach for treating gastric ulcers. Furthermore, the therapeutic potential of the developed formulation, evaluated in pylorus induced rat model exhibited significant reduction in the gastric ulcer index with an increment raise in gastric pH and acidity (both free and total) thereby promoting ulcer inhibition and improving the gastric mucosal integrity. Thus, it can be concluded that raft forming tablet formulation of MAG could serve as a better alternative to the existing synthetic agents to treat gastric ulcers and the incorporation of novel drug delivery concept could surely prove to be beneficial in the delivery of herbal phytoconstituents like MAG to improve its therapeutic effectiveness. Even though the raft forming GRDDS of MAG have proven its efficacy in silico and in vivo, but still need arises to resolve the clinical intricacies before commercializing the product.

## 4. MATERIALS AND METHODS

MAG was purchased from TCI chemicals (Mumbai, India). Sodium alginate, calcium carbonate and sodium bicarbonate were purchased from Loba chemie (Mumbai, India), Methanol and water (HPLC grade) was purchased from Loba chemie. All other chemicals and solvents were of analytical grade.

All experimental protocols were conducted in accordance with the ethical guidelines prescribed by C.P.C.S.E. An Animal welfare Division, Ministry of environment and Forest, Govt. Of India and carried out at Central Preclinical Research Facility of Datta Meghe Institute of Medical Sciences (DU), Wardha, (DMIMS/IAEC/2021/10). In all experiments, animals were humanly euthanized by $CO_2$ inhalation in a $CO_2$ chamber.

### 4.1. Molecular Docking

*4.1.1. Selection of protein*

Crystal structure of the gastric proton pump (PDB ID- 5YLU) was selected as the protein targets for the present study. The crystal structure of desired proteins was downloaded from RCSB Protein data bank in .pdb format. The native ligand present in protein 5YLU is 1-[5-(2-fluorophenyl)-1-pyridin-3-ylsulfonylpyrrol-3-yl]-*N*-methylmethanamine (Vonoprazan).

*4.1.2. Selection of ligands*

Gastric H+, K+-ATPase enzyme is present in the apical membrane of the gastric parietal cells and involved in the secretion of acids. The blockers of this enzyme have been widely explored in the design of anti-ulcer drugs [29]. Hence to evaluate the binding efficacy of 18 β glycyrrhetinic acid at the molecular level to exert inhibitory effect on gastric H+, K+-ATPase enzyme, docking studies were carried on the crystal structure of the gastric proton pump (PDB ID- 5YLU)

Chem Bio Draw Ultra 14.0 was used to draw the ligand structures with 3D coordinates and the file was saved as a .mol file. Energy minimization was performed using Avogadro software with steepest descent algorithm which was then converted into a .pdb file.

*4.1.3. Molecular docking studies*

Molecular docking studies were done by using Autodock Vina software [30]. Optimization of the ligands, proteins and grid box creation was carried out by Graphical User Interface program Autodock Tools. Target proteins were optimized using Autodock Tools by adding polar hydrogen groups, removing water molecules, adding kollman and Gasteiger charges and the prepared file were saved as pdbqt file. Ligands were energy minimized and optimized to convert into pdbqt file using Avogadro.

The amino acids that make up the active site of the target proteins were established by visualization of the binding of native ligands using Biovia Discovery Studio 2016. Grid box was generated by arranging the grid coordinates (X, Y, and Z) about the active site of the proteins. The grid size was set to 40 × 40 × 40 xyz points for both targets with grid centre designated at dimensions (x, y, and z): - 47.164, -14.285 and 1.978. During the docking procedure, both the proteins and ligands were considered as rigid structures. The root-mean-square deviation (RMSD) was observed and the pose with the most favourable free binding energy was considered (RMSD value less than 0.1A$^{o}$). Then with the help of Biovia discovery studio, the pose with lowest energy of binding was aligned with receptor structure for further analysis.

*4.1.4. Validation of target proteins*

To understand the accuracy and reproducibility of the docking process and the targets selected for the study, target validation was performed. The native ligand1-[5-(2-fluorophenyl)-1-pyridin-3-yl sulfonyl pyrrol-3-yl]-N-methyl methanamine present in target protein 5YLU was removed from the protein structure. The co-crystal ligand was redrawn using Chem Draw, energy minimisation was carried out and was re-docked into the active sites using Autodock Vina software. Grid was generated about the active site of the target protein and the re-docked complex was superimposed on its respective reference co-crystallized complex and the root mean square deviation (RMSD) was computed.

### 4.2. Formulation of Raft Forming Tablets

The direct compression method was used to formulate the raft-forming tablets of MAG using a rotary punch tablet compression machine (Rimek micro press, MT-II, India) as per the formula in Table 3. [31]. The thickness, drug content, floating time, floating lag time, raft strength, raft density and *in vitro* characterization of the formulated tablets were done.

**Table 3.** Composition of raft forming tablets as per Box Behnken design

| Ingredients | Formulation code | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 |
| MAG (mg) | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Sodium alginate (X1) (mg) | 100 | 100 | 200 | 200 | 150 | 200 | 150 | 150 | 150 | 150 |
| Sodium bicarbonate (X2) (mg) | 75 | 50 | 75 | 50 | 50 | 25 | 50 | 50 | 75 | 50 |
| Calcium carbonate (X3) (mg) | 100 | 150 | 100 | 150 | 100 | 100 | 100 | 100 | 150 | 100 |

**Continued table**

| Ingredients | Formulation code | | | | | | |
|---|---|---|---|---|---|---|---|
| | F11 | F12 | F13 | F14 | F15 | F16 | F17 |
| MAG (mg) | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Sodium alginate (X1) (mg) | 100 | 200 | 100 | 150 | 150 | 150 | 150 |
| Sodium bicarbonate (X2) (mg) | 50 | 50 | 75 | 25 | 25 | 75 | 50 |
| Calcium carbonate (X3) (mg) | 50 | 150 | 100 | 50 | 50 | 50 | 100 |

In all the formulations (F1-F17) the quantity of excipients used was HPMC K15:-170mg, Mannitol:-80mg, Aspartame: - 10mg, Talc: - 10mg, Magnesium stearate: - 5mg, Avicel: - q. s. to produce 850mg.

### 4.3. Characterization

*4.3.1. Fourier- Transform Infrared Spectroscopy (FTIR)*

To evaluate any possible physical and chemical interactions between the drug and excipients, a drug-excipient compatibility analysis was conducted using Fourier transform infrared (FTIR) spectroscopy (Shimadzu, 8400S, Japan). Samples of 1-2 mg and the entire raft forming table constituents were mixed with KBr IR (grade) and compressed into discs in the compression unit under vacuum and scanned from 400-4000 cm-1 with an empty pellet holder as a reference [32].

*4.3.2. Differential Scanning Calorimetry (DSC)*

DSC (PerkinElmer, DSC 4000, USA) study was carried out to study the physical state of the samples (MAG, sodium alginate and raft forming MAG tablet. The samples were accurately weighed (1mg) and placed in a sealed aluminium pan and analysed using a nitrogen gas flow of 50 mL/min and a scanning rate of 10 $^{o}$C per min from 50 to 300 $^{o}$C.

*4.3.3. Optimization using Box Behnken Design (BBD)*

From the preliminary trials of raft forming tablets, the concentration of sodium alginate, sodium bicarbonate and calcium carbonate were identified as high-risk formulation variables. To study the effect of these factors in developing the optimum formulation, Box Behnken design (BBD) by Design-Expert® software version 10 was used. The design consisted of a three level, three factor BBD resulting in 17 runs: sodium alginate (X1) at levels 100,150 and 200mg, sodium bicarbonate (X2) at levels 25, 50 and 75 mg and calcium carbonate (X3) at levels 50,100 and 150mg were used as independent variables. The responses selected for the study included the variables namely; raft strength (Y1), acid neutralizing capacity (Y2) and the percent drug release (Y3) as shown in Table 4. The multiple linear regression analysis was done to study the effect of these variables on the selected responses. The statistical significance of the data was established using analysis of variance (ANOVA). The surface response and contour plots were generated to study the interactive effects of the variables.

**Table 4.** Independent variables displaying the set of experimental ranges as per the Box Behnken design

| Variables | Levels -1 | 0 | 1 |
|---|---|---|---|
| **Independent variables (Factors)** | | | |
| X1= amount of Sodium alginate (mg) | 100 | 150 | 200 |
| X2= amount of sodium bicarbonate (mg) | 25 | 50 | 75 |
| X3= amount of calcium carbonate (mg) | 50 | 100 | 150 |
| **Dependent variables (Responses)** | | | |
| Y1= Raft strength (g) | | | |
| Y2= ANC (ml) | | | |
| Y3= Percent drug released after 8hr. | | | |

*4.3.4. Physical evaluation of tablets*

*Thickness, hardness and average weight*

The thickness of the formulated tablets was evaluated using a vernier calliper (Baker Gauges, India). To measure the hardness of tablets, Monsanto hardness tester was used (Veego, India). The average weights of the tablets were calculated using twenty tablets from each batch. All the tests were performed as per IP.

*4.3.5. Content uniformity*

Three tablets from each of the formulation batches were triturated in the mortar and pestle and the content uniformity of the tablets were evaluated. From the obtained powder mixture, an amount equivalent to 75 mg of MAG was weighed and diluted with 50 ml of hydrochloric acid buffer solution pH 2.0 in a volumetric flask (100ml). The volumetric flask was sonicated for 15 min and the final volume was made with the same buffer pH 2.0. The solution was then filtered using 0.45 mm nylon pore size filter. From the resulting filtrate, 1ml was withdrawn and diluted with hydrochloric acid buffer solution pH 2.0 to make up the final volume to 10ml.The concentration of MAG in the sample was determined using high performance liquid chromatography (HPLC)- Agilent series system. Chromatographic separations were achieved using a Shimadzu Shim-pack GIST C18 LC Column, 5um, 4.6 x 250 mm. The mobile phase used was methanol and 0.1% orthophosphoric acid (85:15% v/v) at a flow rate 1 mL/min. All the readings were recorded in triplicates at a wavelength of 271nm.

*4.3.6. Floating lag time and total floating time*

Floating lag time and the total floating time of the raft was measured using USP type II dissolution test apparatus filled with 900ml of hydrochloric acid buffer solution pH 2.0 as dissolution media maintained at 37±0.5 °C and 50 rpm. The time required for the raft to rise to the surface of the dissolution media was recorded as floating lag time. The total time required for the raft to float on the surface of the dissolution media was estimated as total floating time [24].

*4.3.7. Friability*

Twenty tablets from each formulation were weighed (W1) and taken in drum of Roche friability tester (Erweka D-63150 Heusenstramm, Germany). The drum was rotated at a speed of 25 rpm for a period of 4 min. The tablets were then removed from the drum and reweighed. The percent weight loss of the tablets was calculated and taken as a measure to determine the friability of the tablets [33].

*4.3.8. Scanning electron microscopy*

The images of the formulated tablets and formed rafts after adding it in the hydrochloric acid buffer solution pH 2.0 were recorded using a scanning electron microscope.

*4.3.9. Evaluation of raft (Raft strength)*

Raft strength is an important parameter to assess the capability of the formed raft to endure the effect of peristaltic motion occurring *in vivo*. The raft strength of the tablets was determined using the Texture analyser (CT3 Brookfield). Initially a single tablet was placed in 250 ml glass beaker containing 150ml of hydrochloric acid buffer solution pH 2.0 and kept undisturbed until a stable raft was formed. A stainless-steel L- shaped wire probe was placed vertically on the beaker in contact with the formed raft and not touching the base of the beaker. The wire probe was slowly pulled up in vertical direction through the raft at the rate of 5 mm/s. The maximum force (g) required to pull the probe was determined as the raft strength [34].

*4.3.10. Density*

A single tablet was placed in 30 ml of hydrochloric acid buffer solution pH 2.0 contained in a 50ml measuring cylinder which was kept undisturbed until a stable raft was formed. The cylinder used to form the raft was pre-weighed (W1). The total weight of the cylinder and its contents was calculated after the raft had already been formed (W2). The formula (W2-W1) was used to determine the weight of the formed raft. The graduated marking scale on the cylinder was used to calculate the final volume (V) of the raft. From the calculated weight and the volume of the raft, the density of the gel (raft) was calculated (D= W2-W1/V). The density of each formulation was calculated in triplicates [32, 35].

*4.3.11. Acid neutralisation capacity (ANC)*

Acid neutralisation capacity represents the ability of an antacid formulation to maintain the neutralization of the stomach acidity even in the presence of continuous acid reflux. ANC determines the efficacy of the antacid formulations and have an important role to play in formulation of anti-ulcer drugs. Initially a single tablet was placed in 250 ml glass beaker containing 50ml of water and was stirred continuously on a magnetic stirrer for about 1min. Then 30 ml of 1.0 N HCl was added to the beaker with continuous stirring. The stirring was continued for about 10min after the addition of acid. There was formation of gum base in the beaker which was removed with a long needle after discontinuing the stirring. The needle was washed carefully with 20ml of water and all the washings were again collected in the beaker and the stirring was continued again for about 5 min. Immediately titration was carried out, by titrating the excess of 1.0 N HCl against 0.5N sodium hydroxide to achieve a stable pH of 3.5 (36).

The number of mEq of acid consumed by the raft-forming tablet was calculated by the following formula:

Total mEq = (30× N HCl) – (V NaOH × N NaOH) ---------- (4) [27]

Where, N HCl = Normality of HCl; V NaOH = Volume of NaOH required; N NaOH =Normality of NaOH

**4.4. In –vitro drug release**

The *in vitro* release of the raft forming tablets was carried out with USP dissolution II apparatus using 0.1N HCL (pH 1.2) as the dissolution media maintained at 37±0.5°C and rotated at 50 rpm. Samples (2ml) were withdrawn at a specific time intervals after every one hour. The study was carried out till 8h. The withdrawn samples were filtered through 0.45 µm filter to remove any particulates and then analysed to estimate the content of MAG using HPLC using the same parameters and conditions as described in the section 2.5.

**4.5. Determination of ulcer healing efficacy on acute gastric ulcers induced by pylorus ligation method in rats**

The ulcer healing property of optimized raft forming tablet formulation of MAG was estimated using pylorus ligation-induced ulcer model for the period of one day. Four groups of Wistar rats (six in each group) were used for the study. Before the study the animals were fasted for 24 h and allowed only free access to water. Animals were kept under standard environmental conditions (12:12 h light-dark cycle, a temperature of 22 ± 2 ˚C, and a humidity of 55–65%). The animals were divided into four groups for the study: Group I: Normal control group, Group II: Disease control group (Gastric ulcer), Group III: Standard treatment group received omeprazole 20 mg capsule which contain approximately 120 pellets, so each pellet contain 0.166 mg of omeprazole. The weight of the rats in this group was 300±0.5 g, so the dose of omeprazole administered to the rats were two granules containing 0.33mg of drug using 0.5 % methyl cellulose in purified water through a feeding tube. Group IV Treatment group received mini tablets of MAG containing 0.3±0.005 mg of MAG and sodium alginate (0.3mg) along with the other excipients. The tablets were first crushed and then dissolved in 0.5% methyl cellulose in purified water and administered to rats through a feeding tube. In the treatment groups (Group III and IV) all the dosing were done by oral route. After a period of 30 min of oral dosing, the animals in the treatment group were anesthetized by an intraperitoneal (i.p.) injection of xylazine (6 mg/kg) and ketamine (60 mg/kg), and then their pylorus was ligated and stomach closed by interrupted sutures. Five hours later, all the animals were euthanized dissected and their stomach were removed. The stomach was cut along the greater curvature and the intensity of the gastric lesions was assessed and ulcer index was calculated. The ulcers were characterized using ulcer scoring system: Score 0 = no ulcer, score 10 = denuded epithelium, score 20 = Flank haemorrhages, score 30= 1 or 2 ulcers, and score 40= Multiple ulcers. The gastric secretions were collected and analysed for various parameters namely pH, volume, free and total acidity of the gastric content as per the method described by Aditya et al 2018 [37]. The gastric ulcer index (GUI) and inhibition of ulcer in percentages were estimated by using the following equation

GUI = GUN + GUS + GUP X 10-1

**Percent inhibition of ulcer** $\frac{uic-uit}{uic} X100$ ---------- **(5)**

Where, GUN is the average of number of ulcers per animal, GUS is the mean severity of ulcer score and GUP is the percentage of animals with ulcer prevalence. UIC is control of ulcer index and UIT is test of ulcer index. Also, the sections of gastric mucosa from each group were carefully stored in 10% buffered formalin and then subjected to processing to prepare a paraffin block for the samples, followed by staining with haematoxylin and eosin to carry out the histopathological evaluation. The section was assessed at 10X magnification.

### 4.6. Statistical Analysis

The results obtained for the various parameters such as gastric pH, gastric volume, free and totalacidity of the gastric content and GUI inhibition were expressed as mean ± standard error of the mean (S.E.M). The differences between means were analysed by analysis of variance (ANOVA) followed by multiple comparison for one-way analysis. Statistical analysis was performed using GraphPad Prism 6.01. The differences were considered to be statistically significant when $p < 0.05$ and referred to as statistically highly significant when $p < 0.001$.

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**Conflict of interest statement:** The authors declared no conflict of interest.

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