FORMULATION AND EVALUATION OF FLOATING IN SITU GEL OF RANITIDINE USING NATURAL POLYMERS

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ABSTRACT
Objective: To formulate and evaluate floating in situ gel of Ranitidine using natural polymers like sodium alginate and pectin and calcium carbonate as cross-linking agent.

Methods: The in situ gel was prepared by mixing sodium alginate (SA), calcium carbonate, sodium citrate and ranitidine. The formulated in situ gel was evaluated for physicochemical parameters, in vitro buoyancy study and in vitro release study.

Results: The in vitro drug release profile demonstrated the positive effect of concentration of both sodium alginate (SA) and Calcium Carbonate on comparing various formulations. It was also found that the ratio of SA and CaCO$_3$ also affects the release pattern. The drug release pattern from the formulations was found to follow Fickian diffusion of the drug.

Conclusion: With the suitable concentration of SA and CaCO$_3$, the sustained release of the drug can be achieved by in situ gel along with good floating properties. Thus, this approach provides a potential development of sustained release floating in situ system of the liquid preparations.

Keywords: In situ gel, Floating drug delivery, Sodium alginate, Pectin, Calcium carbonate, Ranitidine.

INTRODUCTION
An in situ gel drug delivery is a novel idea of delivering drugs as a liquid dosage. It achieves sustained release of drug [1]. The in situ gel delivery systems are advantageous because of ease of administration and the reduced frequency of administration, improved patient compliance, sustained drug release [2]. In situ gel delivery systems are in solution form before administration in the body, but once administered, they undergo gelation in situ to form a gel. Gastro retention in situ gel system helps to increase bioavailability of the drug as compared to conventional liquid dosage form. The gel formed in situ gel system being lighter than gastric fluids, floats over the stomach contents and produces gastric retention of dosage form and increases gastric residence time resulting in prolonged drug delivery in gastrointestinal tract [3].

Ranitidine is a H$_2$ receptor antagonist used in the treatment of gastric and duodenal ulceration and gastro-esophageal reflux disease. It is absorbed from the gastrointestinal tract with the bioavailability of about 50% and an elimination half-life of 3 hours. Patients with reflux esophagitis being treated with proton pump inhibitor may continue to produce acid in the night and thus could be benefited by taking a sustained release formulation of H$_2$ receptor antagonist. Several approaches are currently used to prolong gastric retention time [4]. Among them, the principle of floating in situ gel system is a simple approach to achieve increased gastric residence time for the dosage form and sustain the drug release.

In this study, an attempt was made to prepare a formulation of ranitidine in situ gel forming drug delivery system to sustain the release of drug. The polymers sodium alginate and pectin were used as gel forming polymer and calcium carbonate as a cross-linking agent. The screening of these two polymers was done by employing Plackett-Burman design for the design of the experiment.

MATERIALS AND METHODS
Materials
Ranitidine Hydrochloride and Ranitidine Hydrochloride working standard were received from Chemydrugs Pvt. Ltd, Kathmandu, Nepal as gift samples. Sodium alginate and Calcium carbonate were obtained from the Department of Pharmacy, Kathmandu University, Dhuskule, Nepal. Sodium citrate was received from Qmed formulations Pvt. Ltd. Pectin was purchased from the local supplier.

METHODS
Preparation of in situ gel
The solutions of sodium alginate and pectin, alone, or in combination in different concentrations were made in deionized water, in which 0.25% w/v sodium citrate was previously dissolved. The solution was heated to 60°C with constant stirring on a magnetic stirrer. It was then allowed to cool to 40°C and calcium carbonate in different concentrations was dispersed in the previous solution with continuous stirring. Ranitidine hydrochloride was then added to the resulting solution with continuous stirring. The solution was then stored in amber colored bottles.

Experimental design
Preliminary trials
The trials were conducted using different gelling agents like sodium alginate and pectin in different concentrations. The different types of cross-linking agents like calcium carbonate, calcium chloride and sodium bicarbonate were used for trials. The formulations were evaluated on the basis of gel formation and floating properties.

Plackett-Burman formulation design
After selection of excipients from experimental trials, the factorial design was used for screening of the excipients by applying Plackett-Burman formulation design in Minitab 16. Sodium alginate 0.5 to 3% w/v and Pectin 1.5 to 3% w/v were used as gelling agent and calcium carbonate 0.25 to 1% w/v was used as cross-linking agent as shown in Table 1.

Central composite formulation design
After Statistical interpretation of data obtained from Plackett-Burman formulation, Sodium alginate 0.5 to 3% w/v and Calcium carbonate 0.25 to 2% w/v were selected as gelling agent and cross-linking agent respectively to design Central Composite formulation in Minitab 16. Twelve formulations were obtained as shown in Table 2.
Evaluation of in-situ gel formulations

The prepared in situ gel was evaluated for the following parameters.

Physical appearance and pH

In situ solutions should be clear and free of any particulate matter. All the formulations were checked visually for clarity. Also the consistency of the gel formed was checked visually. The pH of gel forming solution was measured using calibrated pH meter at 25°C.

In vitro floating ability

The In vitro floating study was carried out using 0.1N HCl (pH 1.2). The medium temperature was maintained at 37±0.5°C. 10 ml of formulation was introduced into the dissolution vessel containing medium with much disturbance. The time the formulation had taken to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on the surface of the dissolution medium (duration of floating) was noted.

In vitro drug release

The release rate of drug from in situ gel was determined using USP dissolution rate testing apparatus I (basket covered with muslin cloth) at 50 rpm. [5, 6, 7] 900 ml of 0.1N HCl was used as the dissolution medium and temperature of 37±0.5°C was maintained. 10 ml samples were withdrawn at the interval of 1 hour for estimating the drug release using UV-Visible spectrophotometer. Same volume of fresh medium was replaced every time the sample had been withdrawn. Dissolution study was carried out for 8 hours.

Mathematical analysis of In vitro drug release studies

The drug release profiles of various formulations were compared mathematically using similarity factor (f2) and dissimilarity factor (f1) given by SUPAC guidelines [8]. The f1 value between 50 to 100 indicates similarity between two dissolution profiles. The drug release kinetics were determined by fitting cumulative amount of drug release at different time intervals into the equation of zero order. First order, Higuchi and Korsmeyer Peppas model.

Table 1: Factorial design of formulations by applying Plackett-Burman Formulation design

<table>
<thead>
<tr>
<th>Std Order</th>
<th>Run Order</th>
<th>Pt Type</th>
<th>Blocks</th>
<th>SA (%v/v)</th>
<th>Pectin (%w/v)</th>
<th>CaCO3 (%w/v)</th>
<th>Dissolution (%)</th>
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Table 2: Factorial design of formulations by applying Central composite Formulation design

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<th>CaCO3 (%v/v)</th>
<th>Q 4hr (%)</th>
<th>Q 8hr (%)</th>
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<td>1.125</td>
<td>68.3</td>
<td>89.9</td>
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</table>

Statistical analysis of data

The software used for statistical analysis of data were Microsoft Excel 2013 and Minitab 16. Also chi-square test had been applied to detect the goodness of fit between optimized batch and predicted values.

RESULTS AND DISCUSSION

Experimental design

Preliminary trials

Preliminary trials were conducted using various gelling agents like sodium alginate and pectin and crosslinking agents like calcium carbonate, calcium chloride and sodium bicarbonate to find out the appropriate gelling agent and cross linking agent by determining the floating properties. With sodium alginate alone gelling and floating were not observed. On addition of calcium carbonate floating took place within few seconds and stiff gel was formed. With sodium bicarbonate, the gel was able to float but the gel so formed was not stiff. Thus sodium alginate and pectin were chosen as gelling agents and calcium carbonate as crosslinking agent for the further studies. On addition of sodium citrate to the previous formulations, no significant effect was observed. But it forms complex with all the Calcium ions present in the formulation maintaining the fluidity of the solution and hence preventing the formation of gel before administration [9,10].

Plackett-burman design

Twelve formulations were developed from Plackett-Burman design in Minitab 16 constituting sodium alginate, pectin and calcium carbonate as variables and ranitidine and sodium citrate as constants. The formulations were evaluated for In vitro drug release at 4 hours. The effect of sodium alginate was found to be 7.428 while the effect of pectin was found to be 6.892. Also from main effect plot figure 1, it was observed that the effect of sodium alginate was found to be more than that of pectin. Thus pectin was dropped and sodium alginate and calcium carbonate were chosen for further studies.
release at 4 hours (Q4hr) and 8 hour (Q8hr) was used as dependent variables. alginate and 0.25% w/v of sodium citrate as constant. The drug crosslinking agent. All the formulations contained 1.68 g of sodium alginate. Calcium carbonate demonstrated effervescence effect releasing carbon dioxide and calcium carbonate in latter. In these two formulations, the gel network resulting in buoyant formulation and then calcium ions react with alginate to produce a crosslinked three dimensional gel which restrict the further diffusion of carbon dioxide and drug molecules and thus resulting in extended period of floating and drug release, respectively [7, 11, 12]. The excellent floating time of calcium carbonate was found to be nearly same. Although as discussed previously that calcium carbonate plays an important role in floating of the gel, it was observed that the concentration of calcium carbonate did not affect the floating lag time of the formulations. As shown in figure 2, the floating lag time for the formulations F2 and F8; F3 and F9; F11 and F13 that contained equal amount of SA but different concentrations of calcium carbonate was found to be nearly same.

Central composite design

The central composite design was used in Minitab 16 to develop 13 formulations using two independent variables sodium alginate (0.5-3% w/v) as gelling agent and calcium carbonate (0.25-2% w/v) as crosslinking agent. All the formulations contained 1.68 g of sodium alginate and 0.25% w/v of sodium citrate as constant. The drug release at 4 hours (Q4hr) and 8 hour (Q8hr) was used as dependent variables.

In vitro evaluation of buoyancy

When the formulations comes in contact with an acidic medium, gelation and cross-linking by Ca^{++} ions occurs to provide a gel barrier at the surface of the formulation. Calcium carbonate demonstrated effervescence effect releasing carbon dioxide and calcium ions. The released carbon dioxide is entrapped in the gel network resulting in buoyant formulation and then calcium ions react with alginate to produce a crosslinked three dimensional gel network which restrict the further diffusion of carbon dioxide and drug molecules and thus resulting in extended period of floating and drug release, respectively [7, 11, 12].

Effect of Sodium alginate concentration on floating of gel

On comparison of F2 and F9 where both contained 2% w/v of calcium carbonate, the floating lag time was more in F2 than in F9. This was due to higher concentration of sodium alginate in F2 which made the gel heavier and thus increasing the floating lag time. Similar effect was observed in between F1 and F13 and then in F3 and F8. This might be because the gel formed from in situ gelling system, being lighter than gastric fluids, floats over the stomach [3].

In vitro drug release studies

The release profiles of Ranitidine in situ gel showed initial rapid release (burst effect) followed by slow and gradual release. The initial release ranged from 24% to 53.2% within one hour which might be due to high solubility of ranitidine in 0.1M HCl. Another reason might be that the formulation being in solution form, the surface deposited drugs dissolves in the HCl before formation of gel. Similar result was discussed by Bihami et. al. where the release of drug from the gel was characterized by an initial phase of high release (burst effect). As gelation progresses, the remaining drug was released at slower rate followed by a second phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics [12].

The formulation F12 that contained only sodium alginate showed the lack of any sustained release owing to the complete release of drug 101.4%; at the end of one hour indicating that sodium alginate alone cannot control the release of drug. This might be because in absence of calcium carbonate there was no crosslinking resulting in no gel formation. Similar effect was observed in the study done by S. Miyazaki et. al. where lack of sustained release of theophylline by the soft algic acid gels formed in absence of Ca^{++} ions [9]. The formulation F10 also showed complete release of ranitidine (110.6%) that contained calcium carbonate only and deprived of sodium alginate.

The release was highest in the formulation F8 which contained 3% w/v sodium alginate and 0.25% w/v calcium carbonate. The amount of sodium alginate was greater than the amount of calcium carbonate (SA: CaCO₃::12:1) due to which insufficient cross linking between sodium alginate and calcium carbonate occurred.
Effect of Sodium alginate concentration on drug release

The release was found to be higher in the formulation F13 containing 1.75% w/v of sodium alginate that F1 containing 3.517% w/v sodium alginate and both formulations contained the same concentration of calcium carbonate. This is because sodium alginate forms gel in the presence of Ca++, and the greater quantity of gelling agent forms more gel.

The decrease in the rate and extent of drug release was observed with the increase in polymer concentration and is attributed to increase in the density of the polymer matrix and also an increase in the diffusional path length which the drug molecules have to cross. Similar effect was observed in the study conducted by Miyazaki S et al. Where the decrease of percentage release of theophylline from alginate gels with the increase of alginate concentration.

Drug release kinetics

In order to investigate the mode of drug release from the in situ gels, the drug release data were analyzed using mathematical models: zero order kinetics, first order kinetics, Higuchi equation and Krosmeyer Peppas model. The R^2 value indicated that all the formulations followed Krosmeyer Peppas model of drug release except F13 which followed Higuchi model.

Krosmeyer Peppas model as the release mechanism was also found in the study done by Patel J. K. et. al.[13] The n value was found to be <0.45 except the formulation F5 where n = 0.518, indicating that all the formulations follow non-fickian diffusion.

Optimization of batch

For the optimization of 50-60% drug release at 4 hours and 80-100% drug release at 8 hours [4, 13, 14] the concentration of sodium alginate (X1) and calcium carbonate (X2) was taken as independent variables and the cumulative percentage release at 4 hr and 8 hr as dependent variables for the experiment design. The overlaid contour plot for the release at 4 hr and 8 hr was plotted as shown in figure 4 by using Minitab 16.

Factorial equation for Q_{4hr}

The polynomial equation developed for drug release at 4 hrs was

\[ Y = 101.015 - 5.986X_1 - 10.654X_2 + 4.85X_1^2 + 5.653X_2^2 - 4.48X_1X_2 \]

The above equation represents the quantitative effect of process variables, X1 (concentration of sodium alginate) and X2 (concentration of calcium carbonate), and their interactions on the response Y (drug release at 4 hr). From overlaid contour plot when the value of X1 and X2 was substituted with 1.898 and 1.852, the value of Y for Q_{4hr} was found to be 80.26%.

Chi-square test of optimized batch

The hypothesis was developed for optimized batch as following,

Chi-square test of optimized batch

The optimized batch was formulated using 1.899% w/v of sodium alginate and 1.852% w/v of calcium carbonate as indicated by overlaid contour plot shown in figure 4. This formulation was analyzed for In vitro drug release and floating time. The floating lag time was found to be 59 sec and the floating time was more than 24 hours which indicated the good floating properties. On comparing the cumulative % release of optimized formulation with the predicted value at 4hr and 8hr, the similarity factor was found to be 88.41 that indicate the good similarity between them.

Chi-square test of optimized batch

The hypothesis was developed for optimized batch as following.

\( \mu_o \) observed value is close to predicted value

\( \mu_e \) observed value is not close to predicted value

The chi-square value was calculated to be 0.047 which is less than critical chi-square value 3.841 (at degree of freedom = 1 and confidence interval = 95%) indicating that the null hypothesis is true. The p-value was found to be 0.99 and the percentage error was calculated to be 0.02%. Since a p-value is greater than the conventionally accepted significance level of 0.05 (i.e., p > 0.05) we fail to reject the null hypothesis. Thus, goodness of fit between the observed value of optimized batch and predicted value is significant.
CONCLUSION

The *In vitro* drug release profile demonstrated the positive effect of concentration of both SA and CaCO$_3$ on comparing various formulations. It was also found that the ratio of SA and CaCO$_3$ also affects the release pattern. All the formulations were found to follow Krosmeyer Peppas model except F13 that followed Higuchi model. The present study showed that the floating in situ gel can be prepared using sodium alginate as gelling agent and calcium carbonate as crosslinking agent. With the suitable concentration of SA and CaCO$_3$, the sustained release of the drug can be achieved by in situ gel along with good floating properties. Thus, this approach provides a potential development of sustained release floating in situ system of the liquid preparations.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

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