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Der Pharmacia Lettre, 2015, 7 (12):338-343
(http://scholarsresearchlibrary.com/archive.html)

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ISSN 0975-5071
USA CODEN: DPLEB4

Evaluation of alginate beads of an antiulcer drug using experimental design: Formulation and *in vitro* evaluation

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ABSTRACT

Rabeprazole sodium is a proton pump inhibitor widely used as an antiulcer drug in gastric ulcers and various gastro-esophaegal diseases. Rabeprazole sodium is absorbed in the gastrointestinal tract and because it is unstable under acidic conditions, enteric delivery systems are required. The aim of the present work was to study the effect of formulation variables e.g., sodium alginate concentration (X1), calcium chloride concentration(X2), on drug entrapment efficiency (Y1), in-vitro release profiles (Y2) and particle size (Y3) of multi-particulates system of Rabeprazole sodium. Experiments were designed according to 3^2 factorial design. Alginate beads were formulated using ionotropic gelation method after preparation the beads were coated with Eudragit RS 100. Developed formulations were characterized and evaluated on the basis of particle size, entrapment efficiency and drug release studies. The formulation variables were optimized by response surface methodology (RSM)

Key words: Rabeprazole sodium, factorial design, alginate beads, sodium alginate

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INTRODUCTION

Proton-pump inhibitors (PPIs) have been widely used for the management of a variety of acid-related disorders. However, as PPIs are acid-labile, they need to be protected from the destructive effects of gastric acid when administered orally. Various types of enteric coating have been developed to protect the PPIs, but they all delay PPI absorption. [1] The hydrogel systems for controlling the release of drugs respond to surrounding conditions such as pH, ionic strength, temperature and frequent changes of environment in the gastrointestinal tract, which has a variation of pH from the stomach to intestine. Hydrogels from natural polymers, especially polysaccharides have been widely used of their advantageous properties over synthetic polymers such as non- toxicity, biocompatibility, biodegradability ability to modify the properties of aqueous environment, capacity to thicken, stabilize, encapsulate, swell and to form gels.[5, 6] Sodium alginate is a salt of alginic acid and it is a linear polymer of ß (1-4) mannuronic acid and (1-4) guluronic acid residues in varying proportions and arrangements. Alginate microbeads have the advantages of being orally nontoxic, high biocompatibility, and inability to reswell in acidic environment, whereas they easily reswell in an alkaline environment. [7, 14] So acid sensitive drugs incorporated into the beads would be protected from gastric juice. The aim of the present work is to develop and evaluate an enteric coated oral hydrogel bead of Rabeprazole sodium using ionotropic gelation method.

MATERIALS AND METHODS

Materials

Rabeprazole Sodium was obtained from Metrochem API Private Limited, Hyderabad and Eudragit RS100 was obtained as gift sample from Evonik India Private Limited, Mumbai. All other materials, reagent and chemicals used were of analytical grade.

Methods

A response surface method 3^2 factorial design was applied to evaluate the relationship between the independent variables and their responses. Two variables and three responses were involved in the experimental design. The dependent response factor variables measured were entrapment efficiency, particle size and % cumulative drug release. The independent variables are the concentration of alginate (X1) and the concentration of calcium chloride (X2). The formulation variables and the high and low levels of each variable were defined based on preliminary experiments. Center points were repeated three times to estimate the experimental error.[4] The Independent and Dependent Variables are shown in table 1 and process variables and their levels for full-factorial design are given in table 2

Table 1: Various Independent and Dependent Variables

INDEPENDENT VARIABLES	DEPENDENT VARIABLES
X1- Sodium alginate concentration (% W/V)	Y1- Entrapment efficiency
X2- Concentration of calcium chloride(%W/V)	Y2- % Cumulative Drug release
	Y3- Particle size

Table 2: Process Variables and Their Levels for Full-Factorial Design

FACTORS	LOW LEVEL	MIDDLE LEVEL	HIGH LEVEL
Concentration of Sodium alginate(%W/V)	2.0	2.5	3.0
Concentration of Calcium chloride(%W/V)	1.0	1.5	2.0

Preformulation Studies

Drug-excipients Compatibility Studies

FT- IR Spectroscopic Analysis

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of Rabeprazole Sodium alone or mixture of drug and polymer were weighed and mixed properly with potassium bromide uniformly. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR- spectrum of the pellet from 450-4000cm^{-1} was recorded and compared to study any interference.

Experimental Design

A number of preliminary trials were conducted before application of the design. The levels of the factors were also determined randomly by evaluating the depended variables (responses) of trial batches. A three square factorial design was used for optimizing the formulations using Design-Expert Software (Version-8.0.7.1). The concentration of sodium alginate (X1) and the concentration of calcium chloride (X2) were classified as low, medium and high values for independent variables as given in Table 1.These studied factors along with their levels and the corresponding responses are summarized in Table 1.The significance of the model was determined by the comparisons of statistical parameters, and the best model (suggested) was decided based on reasonable agreement between adjusted R^2 and predicted R^2, higher values of adjusted R^2 and predicted R^2 and model p value. Three-dimensional (3D) response surface plots resulting from the equations were constructed using Design-Expert software. [4]

Preparation of Sodium Alginate Microbeads

The microbeads were prepared by ionotropic gelation technique. Sodium alginate was dissolved in deionized water at a concentration of 1-3 %w/v. using gentle heat on magnetic stirrer. After mixing of sodium alginate, an accurately weighed quantity of Rabeprazole Sodium was added and dispersed uniformly into the solution. The bubble free sodium alginate-drug dispersion (50ml) were added drop wise via a 18-guage hypodermic needle fitted with a 10 mL glass- syringe into 50 mL of calcium chloride solution (1-2%w/v) and stirred at 200 rpm for 30min. The droplets from the dispersion instantaneously gelled into discrete matrices upon contact with the solution of gelling agent. The drug loaded microbeads were further stirred in the solution of gelling agent for an additional 30 min. After specified

stirring time and stirring speed the gelled beads were separated by filtration, washed with deionized water, finally dried at 60 °C for 2h in a hot air oven. The dried alginate beads were stored in well closed container for further use. [2, 3, 5, 6, 7, 9]

Enteric coating of alginate beads

The beads were transferred into acetone solution of Eudragit RS 100 containing triethyl citrate (20% w/v), and coated for 15 min under stirring. The resulted coated beads were filtered and air dried. This coating process was repeated three times to get uniform coated beads. [9, 10, 11]

Characterization of Microbeads

Study of Size and Morphology of alginate Beads

The diameter of beads was determined by a screw gauge. For this purpose, 20 dried alginate beads were randomly selected from each batch and the mean diameter was determined. The least count of screw gauge was 0.005 mm. Color and shape of dried beads of each batch was noted.[8,10]

Drug entrapment efficiency

Rabeprazole sodium content in the alginate beads was estimated by a UV spectrophotometric method. Accurately weighed 100 mg of alginate beads (100 mg) were powdered and suspended in7.4 pH phosphate buffer. The resulting solution was kept for 24hrs. Next day it was stirred for 20 min using ultra sonicator. The solution was filtered and suitable dilutions were made. Rabeprazole sodium content in the filtrate was analyzed at 282 nm using UV-Visible spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug. Calculating this concentration with dilution factor the percentage of actual drug encapsulated in alginate beads was determined. The drug entrapment efficiency was calculated using following relationship. [8, 10, 11]

% Drug Entrapment Efficiency = [Actual drug content /Theoretical drug content] x 100…….. (1)

Dissolution studies

In vitro drug rselease studies of enteric coated beads were carried out in 0.1N HCl for 2hrs and then in phosphate buffer 7.4 for 10 hrs by using USP Dissolution Test Apparatus II (paddle type) at 37± 0.5^0C and 100 rpm speed. The weighed amount of Rabeprazole Sodium loaded beads were placed in the baskets and then submerged into 900 ml dissolution flask containing 900 ml of dissolution medium. Aliquots of 5 ml were withdrawn and replaced with the same volume of fresh solution at each different time intervals. Aliquots withdrawn were filtered through Whatman filter paper 0.45 µm. The withdrawn samples were suitably diluted and the absorbance was measured at 274 nm spectrophotometrically. The cumulative percentage drug release was calculated for regular time intervals.

Statistical Analysis

Data evaluation was done using stepwise multivariate linear regression analysis. The model predictor equations are estimated for each dependent variable separately. The general type of predictor equation resulting from a three-level experimental design is a second-order polynomial shown in Eq. 2

$$Yi = b_0 + b_1X_1 + b_2 X_2 + b_3 X_1^{\ 2} + b_4 X^2_2 + b_5 X1X2 \text{ …….. (2)}$$

Where y is the response variable, b_0 is a constant, and b_1… b_5 are regression coefficient. X1 and X2 are the independent variables and X1X2 is the interaction term, showing how response changes when two factors are simultaneously changed. X^2 1 and $X^2$2 are quadratic terms of the independent variables to evaluate the nonlinearity. In the final model equations, only the significant factors were included. The statistical analysis data through regression model and plotting the response surface graphs were achieved by design - expert software (Version-8.0.7.1). The developed models were tested for its significance using analysis of variance (ANOVA). All tests were performed at a 95% level of significance (α=0.05). [4, 12, 13]

RESULTS AND DISCUSSION

Drug compatibility studies

Drug compatibility studies using FTIR were conducted for the pure drug, and the formulation and the spectral data are given in Figure 1 and 2. The results indicated no chemical incompatibilities between the drug and the polymer used in the formulation

Particle size

The particle size of Rabeprazole sodium loaded alginate beads were analyzed screw gauge. All the formulations of alginate beads F1-F13 show uniform size distribution. The average particle size of Rabeprazole sodium loaded alginate beads was found to be in the range of 1.00 mm to 1.34 mm. As the polymer concentration was increased, the Microbeads size was also found to be increased. The results are shown in Table 3.The response surface (3 dimensional) is shown in figure1.

Entrapment efficiency

The entrapment efficiency was found to be good and in the range of 65.23to 81.78% for all the formulations. i.e., F1-F13.The entrapment efficiency was higher 81.78 % for F10 (3% Sodium Alginate and 2% calcium chloride formulation. This improved entrapment efficiency simply by due to the greater proportion of polymer with respect to amount of drug. The results are shown in the Table 3. The response surface (3 dimensional) is shown in figure 2.

Drug release studies

The *in-vitro* drug release studies were conducted for all the formulations i.e., F1-F13 in 900 ml phosphate buffer pH 7.4 for 12 hours. The percentage of drug release for formulations F1-F5 was found to be in the range of 87.12 ± 1.72 % to 92.34 ± 0.51%. Maximum drug release was found to be 95.23±0.14 % in formulation F13 in 12 hours due to the initial burst release but in the formulation F11 showed 81.61± 0.22% drug release in 12 hours showing sustained release due to increase the polymer concentration i.e., 3% Sodium alginate. Similarly the formulations, F6- F7, F8, F9 and F10 showed the drug release in the range of 86.9±0.77, 90.67±1.55%, 83.65 ± 0.43, 89.78± 0.56 % and 87% ± 1.07 respectively. Formulations F12 and F13 also showed release in the range of 86.9 and 95.23 in 12 hours when compared to the other formulations. So the formulation F11 is suitable for sustained release of Rabeprazole sodium due to the better entrapment efficiency. The results of *in-vitro* dissolution studies are shown in the Figure 3. The response surface (3 dimensional) is shown in figure 4.

Table (3): Experimental Design and Results for the various measured responses of Rabeprazole beads

Runs	Sodium alginate concentration (X1) %w/v	Calcium chloride concentration(X2) %w/v	%Entrapment efficiency(Y1) %	%Cumulative drug release after 12 hrs (Y2) %	Particle size(Y3) mm
1	2.5	1.5	71.36	87.56	1.24
2	2.5	2	76.23	85.67	1.16
3	3	1	74.18	85.16	1.34
4	2	1.5	66.23	92.34	1.02
5	2.5	1.5	71.45	87.12	1.23
6	2.5	1.5	71.28	86.9	1.24
7	2	2	68.51	90.67	1.00
8	3	1.5	79.76	83.65	1.32
9	2.5	1	70.45	89.78	1.26
10	2.5	1.5	72.56	87	1.22
11	3	2	81.78	81.61	1.28
12	2.5	1.5	71.11	86.9	1.21
13	2	1	65.23	95.23	1.04

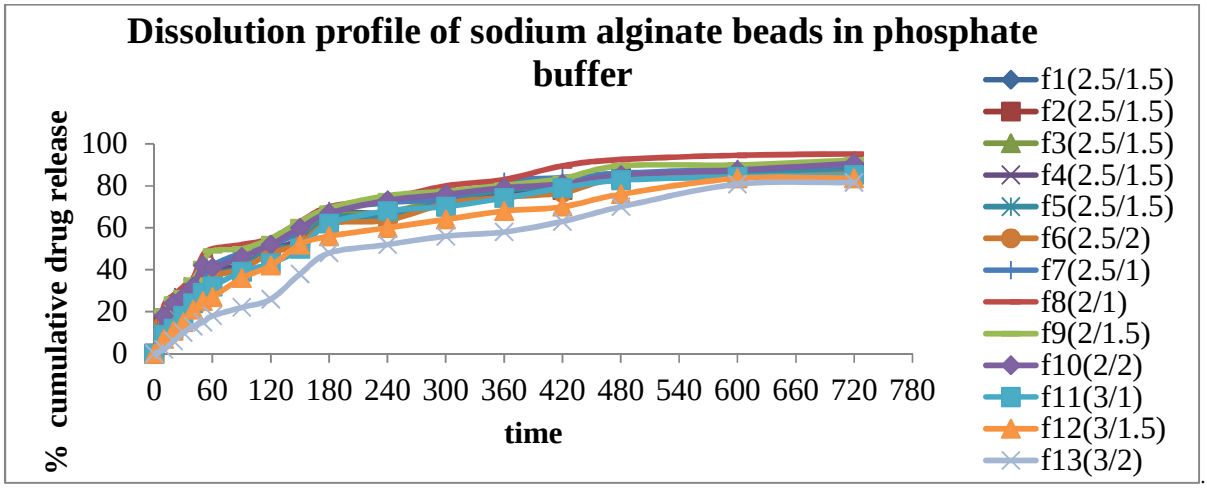

Fig.3: Dissolution profile of sodium alginate beads in phosphate buffer

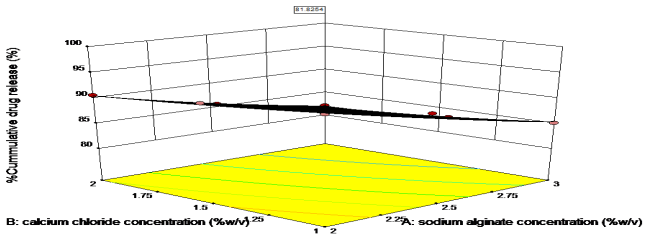

Fig 4: Three dimensional response surface (3 D) plot of % cumulative drug release of optimized formulation (F11)

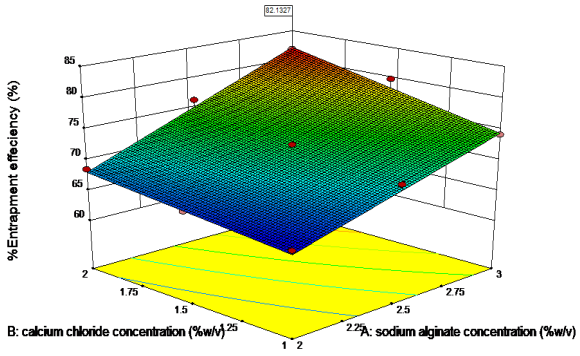

Fig 2: Three dimensional response surface (3 D) plot of % Entrapment efficiency of optimized formulation (F11)

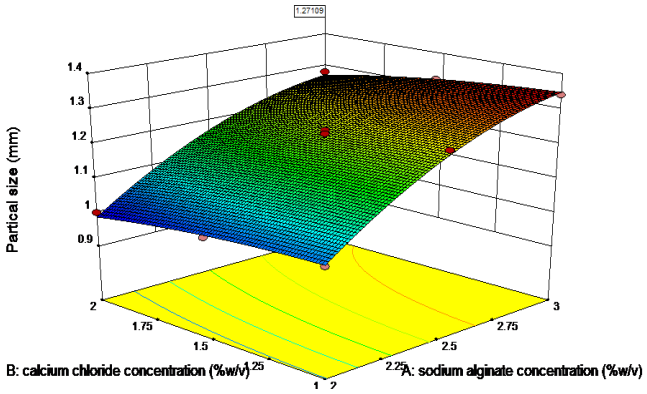

Fig 1: Three dimensional response surface plot (3 D) of particle size of optimized formulation (F11)

CONCLUSION

The Rabeprazole sodium loaded sodium alginate beads were prepared by ionotropic gelation method. In this method, various formulation variables such as amount of sodium alginate and calcium chloride were studied. High entrapment efficiency and sustained drug release were obtained in F11 formulation. The response surface analysis also showed the high entrapment efficiency and sustained drug release in formulation F 11.

Acknowledgment
The author is highly thankful to Chairman, Department of Pharmaceutical Sciences, for providing the necessary facilities.

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