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Original Article

OPTIMIZATION OF FORMULATION OF FLOATING HYDROGELS CONTAINING GAS FORMING AGENT USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Objective: The objective of present study was to determine the optimum gas forming agent concentration to enhance the floating ability and drug release pattern of floating hydrogels by using factorial design.

Methods: In this article calcium carbonates as gas forming agents was synthesized in modified floating kappa carrageenan hydrogels. Ranitidine Hydrochloride was used as drug. 3^2 full factorial design was utilized for the optimization of gas forming agents. Polymer ratio (X_1) and calcium carbonates concentration (X_2) were used as independent variables. The floating hydrogels were characterized and the results obtained are swelling ratio study, drug entrapment efficiency, floating lag time and cumulative drug release.

Results: From the factorial batches, it was observed that formulation containing 0.5% of calcium carbonates with 80:20ĸC : NaCMC showed the optimum floating properties, swelling ratio, drug entrapment efficiency and cumulative drug release.

Conclusion: Thus, formulation containing 0.5% of calcium carbonates with 80:20ĸC :NaCMC appear to be a potential approach to develop sustains drug release in gastrointestinal condition.

Keywords: Floating hydrogels, Calcium carbonates, Kappa carrageenan, Sodium salt of carbomethylcellulose, Ranitidine hydrochloride, Optimization, Factorial design.

INTRODUCTION

A floating drug delivery system is one of the approaches for achieving prolonged and controlled drug release in the gastrointestinal tract[1]. FDDS has lower density than gastric fluid that remains float in the stomach for a long period of time with continuous release of drug [2]. The FDDS use matrices prepared with swellable polymers such as kappa carrageenan, sodium alginates and gas forming agents such as sodium bicarbonates or calcium carbonates [3, 4]. Once the carrier reached the stomach, the carbon dioxide is released from the matrix thus decreasing its specific gravity which causes an upward motion of the dosage form to float on the top of the chime. The carbonates present in the matrix enhance the floating properties of the dosage form and also render the initial alkaline microenvironments for the polymers to gel [5].

In normal conventional optimization process, a single independent variable is varied while all others are kept constant at specific set of conditions. During the formulation development, it is very hard to change more than one parameter at a time. This approach may lead to unpredictable result and incorrect conclusion as well as wastage of material and time. Response surface methodology (RSM) is an alternative method to overcome this problem, which can be used to optimize the formulation with suitable experimental design. RSM has important applications like optimization and establishing the robustness of that product [6,7].

In order to achieve a good floating carrier, optimization of gas generating agents is an essential element. In addition, concentration of gas forming agents is also important for sustain drug release. Hence response surface methodology is the best method to determine the optimal concentration of gas forming agents. Jagdale *et al*. (2011) [8] used 3^2 full factorial designs to determine the optimum concentration of sodium bicarbonates for maximum floating ability along with HPMC K4M as independent variables.

Kappa carrageenan is a natural polymer that consistsof sulfated polysaccharide with a repeating d-galactose and 3, 6-anhydro-D-galactose units[9, 10]. ĸC has numerous applications in food, non-food, pharmaceutical and other industries including indrug delivery system[11], wound dressing[12], tissue engineering[13]and others. Currently kappa carrageenan hydrogel has been widely used as drug carrier for delivery of active substances. This is due to its low toxicity, biodegradability and high stability at biological environment. ĸC works well in the presence of cellulose derivatives such as sodium salt of carbomethyl cellulose. NaCMC has exhibited very good swelling behaviorsand has been shown to be both pH-sensitive and ionic [14, 15]. Ranitidine hydrochloride (RHCl) also known as zantac is a competitive, reversible inhibitor of the action of histamine at histamine H_2-receptors, including receptors on gastric cells with a minimal effect on H1-receptors. Ranitidine is used to treat duodenal ulcers, gastric ulcers, gastro esophageal reflux and erosive esophagitis. This drug has a short biological half-life of approximately 2-3 hours and an absolute bioavailability of only 50% [16, 17]. The objective of the present investigation is to optimize the gas forming agents using RSM. In this study kC:NaCMC and calcium carbonates concentration were selected as independent variables while swelling study, floating lag time and cumulative drug release were selected as dependent variables.

MATERIALS AND METHODS

Material

Ranitidine hydrochloride were obtained from Pusat Kesihatan UTM, Kappa-Carrageenan ĸC() was purchased from Sigma-Aldrich,(Malaysia), Sodium Carboxymethyl Cellulose (NaCMC) (average molecular weight of 250,000) was purchased from Acros Organic,(Malaysia), Calcium Carbonate ($CaCO_3$)was purchased from QRec, (Malaysia). Distilled water in used in hydrogel synthesis and all chemicals are used as received with no additional purification.

Methods

Preparation of sample

Preparation of floating hydrogels

Floating hydrogel was prepared by traditional mixing method. A solution was prepared by dissolving 150 mg of drug in 5 ml distilled water. The above solution was added to the hot solution of

NaCMC/ $CaCO_3$ in 25 ml at 80℃ under reflux. The solution was stirred for 1 h to obtain a clear, viscous and homogenous solution with no bubbles. Then, the resultant hot solution was poured into ceramic moulds to form the hardened hydrogel of a desired shape. Samples were equilibrated with ambient temperature (25 °C) for 24 hours prior to drying at 37 °C in over-night. Table 1 lists the synthesis condition for the preparation of floating hydrogels.Table 1 lists the synthesis condition of floating hydrogel in this experiment.

Table 1: Synthesis condition of Floating Hydrogels

Sample Designation	κc (g)	NaCMC (g)	$CaCO_3$ (g)	Water (ml)
κC70-0.5%	0.42	0.18	0.15	30
κC70-1.5%	0.42	0.18	0.45	30
κC70-2.0%	0.42	0.18	0.6	30
κC80-0.5%	0.48	0.12	0.15	30
κC80-1.5%	0.48	0.12	0.45	30
κC80-2.0%	0.48	0.12	0.6	30
κC90-0.5%	0.54	0.06	0.15	30
κC90-1.5%	0.54	0.06	0.45	30
κC90-2.0%	0.54	0.06	0.6	30

Measuring swelling ratio

To study the swelling properties of hydrogels, modified gels were immersed in different pH buffer solution of pH 1.2 at room temperature (25 °C). Synthesized gels were placed in a Petri dish filled with 50 ml of each buffer solution. Prior to weighting, filter paper was used to remove the surface water of swollen hydrogel. The swelling ratio (%) was then determined using Eq. (1)

$$\text{Swelling ratio (\%)} = \left[\frac{Wt-Wo}{Wo}\right] X\ 100\% \quad \ldots\ldots\ldots\ldots (1)$$

where W_0 is the initial weight of samples and W_t is the weight of swollen gels at predetermined time t. To allow hydrogels to reach their highest swelling ability, they were immersed in fresh buffer solution after weighting. The test was conducted in triplicate and reported as mean values to maximize accuracy.

Drug entrapment efficiency

For entrapment efficiency hydrogels (150 mg) were powdered and dissolved in 10 ml of 0.1 N HCl. It was sonicated for 30 min and then diluted to 100 ml with 0.1 N HCl. It was then filtered through Whatman filter paper no. 41. Suitable dilutions were made and the filtrate was analyzed spectrophotometrically at 313 nm. Entrapment efficiency can be calculated by Eq. (2)

$$\text{Entrapment Efficiency (\%)} = \left[\frac{\text{Mass of drug in the carrier}}{\text{Mass of drug used in formulation}}\right] X\ 100\% \ldots\ldots\ldots (2)$$

***In vitro* buoyancy study**

Floating properties of κC :NaCMC:$CaCO_3$hydrogels were evaluated with 50 ml of 0.1 N HCl at 37 °C ± 0.1℃. The time required for hydrogel to rise to the surface and float (floating lag time) were measured by visual observation[18].

***In vitro* Release**

The samples were immersed in beakers filled with 50 ml of 0.1 N HCl and placed in an incubator at 37 °C ± 0.5℃. 20 ml of the sample was withdrawn at time intervals, filtered, diluted suitably and analyzed spectrophotometrically at 313 nm. Equal amount of fresh medium was replaced immediately after withdrawal of the test sample. The amount of Ranitidine hydrochloride was calculated by interpolation from the Ranitidine hydrochloride standard curve at 313 nm.

Experimental design

In this full 3^2 factorial experiment design were evaluated, each at 3 levels, and experimental trials were performed on all 9 possible combinations. The polymer to κC:NaCMC ratio (X_1) and gas generating agents (X_2) were selected as independent variables. Swelling degree (%), drug entrapment efficiency (%), in vitro buoyancy and in vitro drug release (%) were selected as dependent variables.Table 2 shows translation of coded levels in actual units.

The data were subjected to multiple regression analysis using statistical software (Statistica. Inc version 8). The model incorporating first order polynomial terms was used to evaluate the responses and Eq. (3) was:

$$Y = b_0 + b_1X_1 + b_2X_2 + B_{12}\ X_1X_2 + b_{11}\ X_1^2 + b_{22}X^2 (3)$$

where, Y is the dependent variable, b_0 is the arithmetic mean response of 9 runs and b_1 is the estimated coefficients for the related factor X_1. The main effect (X_1 and X_2) represents the average result of changing one factor at a time from its low to high value. The interaction term "X_1X_2" shows how the response changes when the two factors change simultaneously. The polynomial terms ($X_1^2$and X_2^2) are included to investigate non-linearity. Each experiment was conducted in triplicate and the mean determined.

Table 2: Translation of coded levels in actual units

Variables level	Low (-1)	Medium (0)	High (1)
Polymer ratio	70:30	80:20	90:10
$CaCO_3$ Concentration (%)	0.5	1.5	2.0

Data analysis

To compare the differences between the formulations, statistical analysis was carried out using the analysis of variance (ANOVA) using (Statistica. Inc version 8). At the 95% confidence interval, *p* values, less than or equal to 0.05 were considered significant.

RESULT AND DISCUSSION

Swelling Ratio Study

Factorial equation for swelling ratio (%) = $4969.43-103.93X_1-233.664X_2 + 0.625X_1^2 - 162.94X_2^2 - 4.175X_1X_2$

Swelling ratio (%) for all formulas F1 to F9 varied from 488.09% to 143.9% (Table 3) showed good correlation as 0.9256. In the above equation, coefficient of X1 showed negative value sign, so increased polymer concentration decreased the swelling in 0.1 N HCl. Coefficient of X_2 showed negative sign, so increase in calcium carbonates decreased swelling study.

This is because as κC content increased the gel structure became harder and hindered the water absorption into hydrogel network, whereby higher NaCMC content caused the stability of the hydrogel decreases due to matrix erosion[14]. On the other hand hydrogel

might have provided a barrier that hinders absorption of water into hydrogel matrix. Swelling ratio decreases with high calcium carbonates concentration. The presence of calcium carbonates in the hydrogel carrier could neutralize the gastric fluid of swollen region to a neutral pH or even to alkaline pH. In neutral and alkaline environmentthe hydrogel cannot swell well hence the swelling ratio decreases with increased calcium carbonates concentration[19].

It can be concluded that both concentrations of X_1 and X_2 were responsible for the swelling ratio.

Table 3: The 3^2 Factorial design for the formulation (the independent variables are polymers ratio (X_1) and calcium carbonates concentration (X_2)

Formulation	Variables levels in coded form		Real Value		Swelling studies (%)	Entrapment efficiency (%)	Floating Lag time (min)	Cumulative drug release (%)
	X_1	X_2	X_1	X_2				
KC70-0.5%	-1	-1	70:30	0.5	488.09	96.93	3.60	45.61
KC70-1.5%	-1	0	70:30	1.5	370.28	62.01	2.62	60.14
KC70-2.0%	-1	1	70:30	2.0	363.76	56.01	1.30	109.89
KC80-0.5%	0	-1	80:20	0.5	487.31	90.00	2.24	40.70
KC80-1.5%	0	0	80:20	1.5	134.53	54.60	1.68	51.32
KC80-2.0%	0	1	80:20	2.0	126.56	49.21	1.55	88.99
KC90-0.5%	1	-1	90:10	0.5	380.12	81.27	2.94	32.26
KC90-1.5%	1	0	90:10	1.5	125.43	69.42	2.42	47.14
KC90-2.0%	1	1	90:10	2.0	143.9	54.34	1.71	63.07

Table 4: Summary of regression outputs of significant factors for

Coefficient	B0	B1	B11	B2	B22	B12	R^2
Swelling Ratio Study	4969.43	-103.93	0.625	-233.66	162.94	-4.175	0.9256
Drug Entrapment Efficiency	524.64	-9.59	-0.054	-85.28	6.57	0.5593	0.9539
Floating Lag Time	45.8	-1.031	0.006	-2.39	-0.502	0.034	0.89721
Cumulative Drug Release	15.11	1.10	-0.006	15.60	37.04	-0.954	0.9058

Table 5: Result of two-way ANOVA for dependent variables

	Source	Degree of freedom	Sum of squares	Mean SQUARE	F-value	P-value
Swelling study	X1	1	49858.7	49858.69	10.18	0.050
	X2	1	86711.7	86711.74	17.71	0.025
	Residual	3	14690.5	4896.85		
	total	8	197479.7			
Entrapment Efficiency	X1	1	27.259	27.259	0.742	0.452
	X2	1	1960.8	1960.8	53.35	0.005
	Residual	3	110.26	36.75		
	Total	8	2393.33			
Floating Lag Time	X1	1	0.0642	0.0642	0.42	0.56
	X2	1	2.97	2.97	19.33	0.02
	Residual	3	0.460523	0.153		
	total	8	4.480022			
Cumulative Drug Release	X1	1	766.19	766.19	13.20	0.036
	X2	1	3426.86	3426.86	59.03	0.046
	Residual	3	174.166	58.06		
	Total	8	4929.30			

Drug entrapment efficiency

Factorial equation for entrapment efficiency (%) = $524.64 - 9.59X_1 - 85.28\,X_2 - 0.054X1^2 + 6.57X2^2 + 0.5593X_1X_2$

Entrapment Efficiency (%) for all formulas F1 to F9 varied from 96.93% to 54.34% (Table 3) showed good correlation as 0.9539. In the above equation, coefficient of $X_1$and X_2 showed negative value sign, so increased polymer and calcium carbonates concentration decreased the drug entrapment efficiency in 0.1 N HCl. The result of two way ANOVA presented in Table 5 showed that the concentration of calcium carbonates significantly ($p< 0.05$) affected the floating lag time while the effect of polymers was not statistically significant. Thus factor $X_2$is more responsible than factor X_1 for drug entrapment efficiency. This is because the reaction between gas forming agent and 0.1 N HCl (stimulated gastric fluid) cause carbon oxide generation, which permeates the hydrogel networks, leaving pores. High $CaCO_3$ concentration form many pores in hydrogel matrix and make the internal structure of hydrogel become less dense, with the result that drugs cannotbe retained in hydrogel network [20].

Therefore, as the calcium carbonates concentration increases the drug entrapment efficiency decreases. Calcium carbonates concentration has been found to have a more pronounced effect on drug entrapment efficiency.

Floating lag time

Factorial equation for floating lag time (min) = $45.86 - 1.03145X_1 - 2.39\,X_2 + 0.00612\,X_1^2 - 0.50222\,X_2^2 + 0.02288\,X_1X_2$

Floating lag time (min) for all batches F1 to F9 varied from 3.60 min to 1.71 min (Table 3) showed good correlation coefficient as 0.9647. Floating hydrogel is expected to remain float in the stomach without

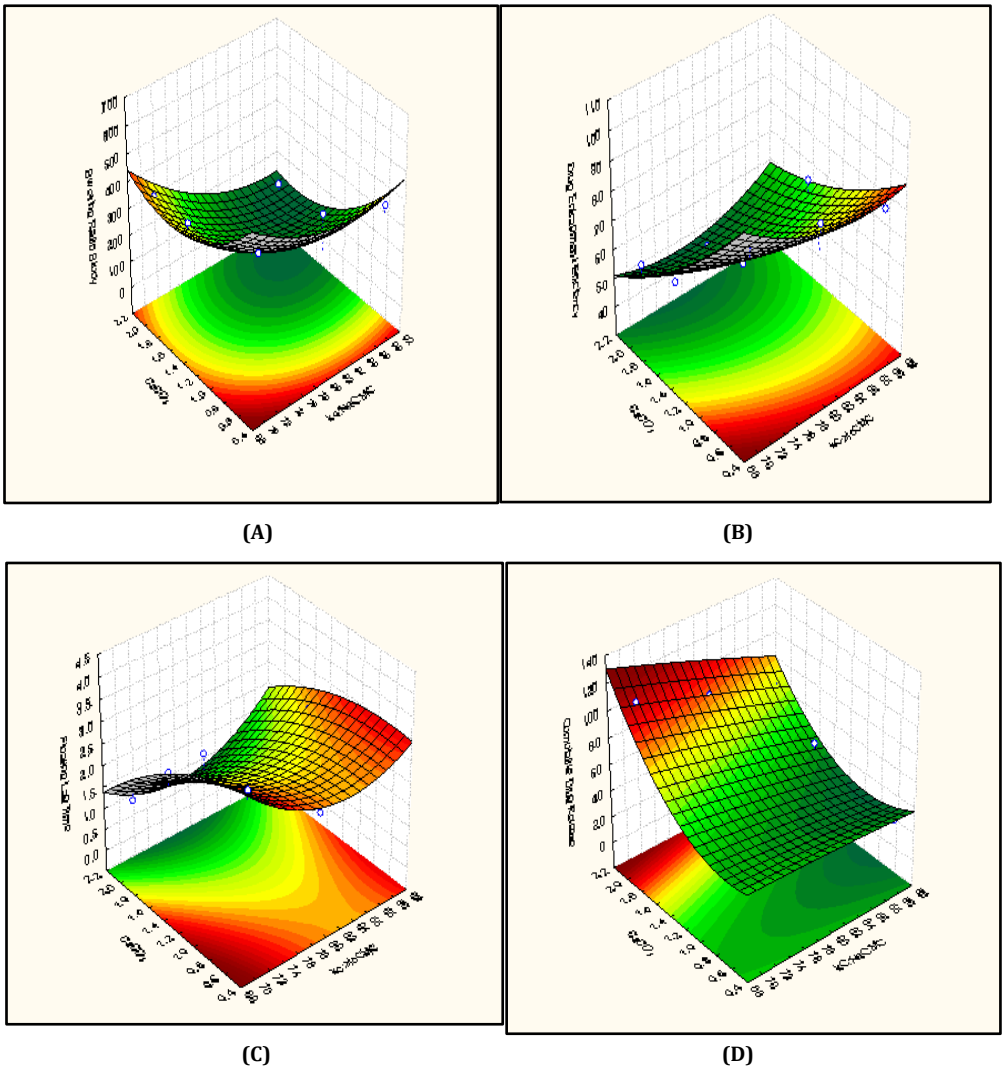
affecting the gastric emptying rate for a prolonged period of time. In the above equation, coefficients of both X_1 and X_2 showed negative value sign, so increased polymer and calcium carbonates concentration decreased the floating lag time of hydrogels in 0.1 N HCl. When the hydrogel comes into contact with acidic medium, calcium carbonates effervesces, releasing $C0_2$ which make the hydrogel remain float over the surface.

The released CO_2 lowers the hydrogel density and makes them float for a prolonged time. The result of two way ANOVA presented in Table 5 shows that the concentration of calcium carbonates significantly ($p< 0.05$) affected the floating lag time while the effect of polymers was not statistically significant. Thus factor X_2 wasmore responsible than factor X_1 for floating lag time.

Cumulative drug release

Factorial equation for cumulative drug release (%) = 15.11 + 1.10 X_1 + 15.60 X_2 – 0.006 X_1^2 + 37.035 X_2^2-0.954X1X2

Cumulative drug release (%) for all formulas F1 to F9 varied from 109.89 % to 32.26 % (Table 3) showed good correlation coefficient as 0.90578. In the above equation, coefficient of X_1 and X_2 showed positive value sign, so increased polymer and calcium carbonates concentration increased the cumulative drug release in 0.1 N HCl. The result of equation indicates X_2 was more responsible than X_1 for cumulative drug release. The result from the two way ANOVA indicates that both polymer and calcium carbonates concentration significantly ($p < 0.05$) affected the cumulative drug release. This is because as the effervescent reaction occurs between acidic medium and gas forming agents, $CO_2$is released and pores are left in hydrogel network as well. Thus drug release mainly depends on pore formation. As the gas forming agent's concentration increases, more pores will be left it is on hydrogel matrix and more drugs will be released within the pores. On the other hand polymers help to make the gel network more porous. The result of two way ANOVA presented in table 5 showed that both X_1 and X_2 factor are statistically significant ($p< 0.05$).



(A) **(B)**

(C) **(D)**

Fig. 1: Surface graphs showing effect of variables on (a) swelling ratio, (b) drug entrapment efficiency, (c) floating lag time and (d) cumulative drug release.

CONCLUSION

In the present study, floating hydrogels containing 0.5% calcium carbonate with polymer ratio 80:20 ĸC:NaCMC showed the optimum result for all variables tested. This formulation shows a good floating ability, high drug entrapment efficiency and also a steady and sustained drug release in gastrointestinal tract.

It can be concluded that 0.5% calcium carbonate is sufficient for appropriate floating propertiesof hydrogelsand controlled drug release as well.

CONFLICT OF INTERESTS

Declared None

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