Quality by design of curcumin-loaded calcium alginate emulsion beads as an oral controlled release delivery system.

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Received: March 21, 2015; *Accepted:* May 1, 2015

Original Article

ABSTRACT

The aim of this study was to prepare a curcumin floating bead system to act as an oral controlled release delivery system. The methodology included the use of calcium alginate emulsion beads containing oleic acid and Tween® 80. The effect of these ingredients on curcumin release and gel stability was determined. The formulations with greater concentrations of oleic acid were more physically stable and selected for further analysis. The drug release mechanism was evaluated in simulated gastric fluid. Response surface methodology was used to determine the optimum conditions for preparation using floatation time and curcumin release as the dependent variables. Two independent variables were assessed i.e., the cross-linking time and the Tween® 80 concentration. Both factors affected the floatation time and drug release. The optimum conditions for the preparation of curcumin beads were determined and tested. The observed and predicted responses of the optimum curcumin bead formulation were similar.

KEY WORDS: Floating beads, calcium alginate, controlled release, curcumin, response surface methodology

INTRODUCTION

Curcumin is an active ingredient obtained from turmeric rhizomes widely cultivated for cooking and medicinal purposes. It has many medicinal applications, the most important of which is, its tumoricidal property (1). In addition, it has been used in the treatment of a wide variety of diseases including cystic fibrosis, inflammatory bowel disease, psoriasis, rheumatoid arthritis, Alzheimer's disease, diabetes and metabolic syndrome. (2). Due to its wide range of therapeutic effects and good safety profile, curcumin has attracted the attention of many researchers, for example, it is a component of pharmaceutical preparations, such as, Theracurmin® developed by Theravalues Corporation, Japan (2-4). However, there are multiple reasons that make curcumin difficult to develop and manufacture as a pharmaceutical dosage form. For example, it is photosensitive and requires special precautions during its handling to avoid degradation (5). Its highly hydrophobic nature makes it practically water-insoluble. Moreover, curcumin is rapidly metabolized and eliminated regardless of the route of administration. Thus, the compound is not readily bioavailable (6).

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Formulation scientists have adopted different strategies to overcome these shortcomings, the objectives being to enhance water-solubility, permeability and bioavailability. For example, some studies have focused on the modification of the chemical structure of curcumin (7), curcumin conjugation (8) and curcumin complexation (9). Other studies have used metabolic inhibitors to act as absorption enhancers (10). In another interesting approach, curcumin was incorporated into various delivery systems such as liposomes (11), nanoparticles (12, 13), micro-emulsifying systems (14) and controlled release delivery systems (15).

Oral controlled release of curcumin can also be achieved by using carriers that are able to target the drug to a specific region in the gastrointestinal tract such as the stomach in a gastro-retentive floating system. Floating systems could be the most effective approach for treating local gastrointestinal diseases such as stomach or colon cancer (16). In addition, floating systems are better due to their ability to increase the duration of curcumin release in the stomach. Furthermore, curcumin is more stable in acidic conditions and relatively unstable in the alkaline conditions of the intestines (17). In an alginate bead carrier system, researchers used a pore former composed of polyvinylalcohol-polyethylene glycol copolymer in bead formulations (18). Another alginate system utilized oleic acid for curcumin topical delivery (19).

Stomach indigestibility and palatability were improved when using a non-ionic emulsifier i.e., Tween® 80 in an 'emulgel' (20). The system contained an oleic acid/edible oil/Tween® 80 mixture that could dissolve curcumin and enhance its permeation through the gastrointestinal tract. Based on this 'emulgel' system the aim of the study reported here was to produce curcumin floating beads by cross-linking the alginate with calcium ions. The optimal conditions for the preparation were determined using response surface methodology. The *in vitro* curcumin release mechanism out of 'emulgel' beads was determined.

MATERIALS AND METHODS

Materials

Curcumin from *Curcuma longa* (Turmeric) powder ≥65% (HPLC, determined by Sigma-Aldrich), alginic acid sodium salt (viscosity 2% solution at 25°C, about 250 mPa.s), olive oil (Ph.Eur.), polyoxyethylenesorbitan monooleate (Tween® 80) were purchased from Sigma-Aldrich Co., USA. Calcium chloride (anhydrous) and oleic acid was purchased from Fisher Chemicals, UK. Calcium chloride 2-hydrate was obtained from PRS, Panreac Quimica SA, Spain. All chemicals and reagents were analytical grade and used without further purification.

Methods

Preparation of the curcumin loaded calcium alginate beads

Different formulations of sodium alginate, olive oil, oleic acid, Tween® 80, curcumin and distilled water were prepared as summarized in Table 1.

Table 1 Components and their weight percentages

ITEM	F1	F2	F3	F4	F5	F6	F7	F8	F9
Na alginate	3	3	3	3	3	3	3	3	3
Olive oil	5	5	5	5	5	5	5	5	5
Oleic acid	2.5	5	10	2.5	5	10	2.5	5	10
Tween® 80	0.25	0.25	0.25	0.5	0.5	0.5	1	1	1
Curcumin	1	1	1	1	1	1	1	1	1
Water	88.25	85.75	80.75	88	85.5	80.5	87.5	85	80
Total	100	100	100	100	100	100	100	100	100

All materials, except the distilled water, were mixed and homogenized (IKA® T29 digital Ultra Turrax homogenizer, Germany) with the gradual addition of distilled water. Mixing continued at a speed of 1000 RPM for 10 minutes at room temperature. The mixture was then subjected to probe sonication (Branson

sonifier 450, Probe 102 C, EDP 101-135-066K, at 100% duty cycle with the microtip limit setting at amplitude 7) for 5 minutes (20). Using a 10-ml syringe a constant volume of each ingredient of each formulation was withdrawn and the material was dropped into a 100 ml solution of 2% w/v calcium chloride forming calcium alginate beads spontaneously. The beads were washed three times with distilled water to remove any excess surface material and stored in screw capped test tubes in the refrigerator till use (21).

Physical stability of sodium alginate emulsion

Physical stability of the sodium alginate emulsion during storage prior to bead formation was used as a criterion for the initial selection of three preparations. Samples of 10 ml of each sodium alginate emulsion were centrifuged at 21 g for 10 minutes. The resultant aqueous separation was monitored visually. The percent of aqueous separation was calculated by dividing the volume of aqueous separated out of the total volume (22).

Stability of curcumin using different dissolution media

About 10 curcumin beads of F3 were placed inside each of three dialysis cell chambers with a dialysis membrane of molecular weight cut off at 3000 Da. The chambers were placed inside a USP dissolution apparatus II (Paddle) using three different dissolution media i.e., 0.1 M HCl, USP phosphate buffer pH 7.5 and USP phosphate buffer pH 8.5 and kept in the diffusion cell chambers during the dissolution test at 50 RPM at 37°C for 12 hours. The change in color of the curcumin beads inside the diffusion cell chamber was determined visually after 12 hours.

Dissolution test

A drug release study was carried out according to the following conditions 0.5 g of curcumin beads (~20-25 beads) were placed in the USP Paddle dissolution apparatus. The speed was set at 50 RPM and the temperature was kept at 37±0.5 °C. The dissolution medium used was USP simulated gastric fluid TS without pepsin enzyme (SGF). 0.2% Tween® 80 was added to increase the rate of dissolution of curcumin from the beads. At predetermined time intervals, 5 ml was withdrawn and replaced by an equal volume of fresh medium. Blank bead samples (curcumin free) were used as reference for each preparation.

The samples were analyzed using UV-visible spectroscopy (UV-1800, Shimadzu, Japan) at a wavelength of 425 nm. A standard curve was constructed using a methanolic stock solution diluted with the SGF according to a previously validated method (23). To determine the total amount of drug in 0.5 g curcumin beads, an accurate weight of beads were placed in 100 ml methanol and stirred for 12 hours. The solution was centrifuged at 591 g for 10 minutes and analyzed as above.

Data Analysis

Open source software KinetDS 3.0 was used to analyze the dissolution test data. The chosen *in vitro* release studies were evaluated using the release models shown in Table 2 (24).

Model Selection

The best model criteria were selected based on least value of root mean squared error (RMSE) based on Equation 1 (24).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\left(y_i - \overline{y_i}\right)}{N}} \qquad \text{Eq. 1}$$

Where, y_i is the observed value and $\overline{y_i}$ is the model predicted value.

Fit Factors

The difference factor f_1 and similarity factor f_2 are model-independent approaches used to compare two dissolution profiles as shown in Equations 2 and 3 (25).

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum R_t} x100 \qquad \text{Eq. 2}$$

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} x100\right\} \qquad \text{Eq. 3}$$

Where, R_t is the reference formulation at time *t,* T_t is the test formulation at time *t* and *n* is the number of time points.

Response surface methodology (RSM)

Design-Expert software (DX9) for the design of experiments (DOE), version 9 (Stat-Ease, Inc., USA), Windows®-compatible program, was used to optimize the process of bead preparation of the initially selected three preparations. RSM uses the quadratic polynomial model based on Equation 4

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_1^{\,2} + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j \qquad \text{Eq. 4}$$

Where, β_o, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linear, second order and interaction terms, respectively. X_i and X_j are the independent variables (19). In order to optimize the conditions of the bead preparation, RSM of the three most promising formulations were evaluated for optimal cross-linking time ($X_i = T_c = A$) and optimal concentration of emulsifier i.e., Tween® 80 ($X_2 = C_t = B$) that could result in optimal responses in terms of floating time (Y1) and release behavior described by mean dissolution time, MDT (Y2). Central composite design type of fourteen runs of two blocks with quadratic polynomial model, where, cross-linking time (Y1) and release behavior described by mean dissolution time, MDT, (Y2). Central composite design type of fourteen runs of two blocks with quadratic polynomial model, where, cross-linking time T_c 5 to 15 minutes and concentration of Tween® 80 C_t 0.25 to 0.75% w/w were analyzed taking into consideration the response constraints of minimizing the floating time (or increasing the buoyancy), while keeping T_c and C_t set in the range.

Table 2 Release models for evaluating *in vitro* release studies

MODEL	EQUATION
Zero order	$Q = k.t. + Q_0$
First order	$\frac{1}{Q} = k.t. + \frac{1}{Q_0}$
Second order	$\frac{1}{Q^2} = k.t. + \frac{1}{Q_0^2}$
Higuchi	$Q = k.\sqrt{t}$
Korsmeyer-Peppas with lag time	$Q = k.\left(t - t_{lag}\right)^n$
Weibull with lag time	$Q = 100\left(1 - \exp\left[\frac{-\left(t - t_{lag}\right)^b}{a}\right]\right)$
Hixson Crowell with lag time	$Q^{\frac{1}{3}} = k.\left(t - t_{lag}\right) + Q_0^{\frac{1}{3}}$
Michaelis-Menten	$Q = \frac{Q_{\max}.t}{k + t}$
Hill	$Q = \frac{Q_{\max}.t^n}{k^n + t^n}$

Q is the amount or percent of drug released at time t
Q_0 is the initial amount of the drug
Q_{max} is the maximum amount released or percent released
t is the time
t_{lag} is the lag time
a, b, k are equation constants
n is the order of the equation

There equations were generated using KinetDS v 3.0 software

Experimental design

The experimental design, face centered cubic (FCC) design was selected since it is one of the

simpler models that can predict the best conditions for all factors affecting the process (19). The design has its axial points set at ± 1 unit away from the center. Once a determination of the relationship between the input parameters (C_t and T_o) and the output responses (mean floating time and mean dissolution time) was determined from the FCC, an optimization of the results could be made. The predicted response was compared with the experimental value for confirmation.

Floating time (Y1)

Floating time was taken as a measure of the floating ability of the preparations. The time required for the beads to become buoyant was directly related to the floating ability of the beads. Five beads were simultaneously added to a 25-ml graduated cylinder filled with 0.1 M HCl and kept in a water bath at 37±0.5 °C. The beads were observed to settle down and then started to float to the top of the 25 ml cylinder. The time required for each bead to reach the surface and become buoyant was recorded. The average floating time of the five beads was used for the RSM analysis (26).

Mean dissolution time (Y2)

Mean dissolution time (MDT) was selected as a model independent measure to describe the *in vitro* dissolution process based on Equation 5 (24).

$$MDT = \frac{\sum_{j=1}^{n}\left[\left(t_i + t_{i-1}\right)/2\right]_j \, x\left(Q_{t-}\, Q_{t-1}\right)_j}{\sum_{j=1}^{n}\left(Q_{t-}\, Q_{t-1}\right)_j} \qquad \text{Eq. 5}$$

Where, t is the time and Q is the amount or % of drug released at different time intervals

Surface Morphology of beads

The beads were squeezed on a flat surface by pressing them between two microscopic slides. The beads were then placed on a filter paper and dried in an oven at 40°C for 48 hours. The water and organic liquids diffused in the filter. The bead surface was scanned using an atomic force microsope AFM using Force Modulation mode (Dimension Edge, Bruker, Germany). The scanning software was NanoDrive V8.01 (Build R1.65039) Veeco© Inc, Germany, 2010.

RESULTS AND DISCUSSION

Preparation of curcumin beads

Different emulsion-gel preparations were formulated by changing the concentration of emulsifier and oleic acid, as shown in Table 1. Curcumin was dispersed in organic solutions and emulsified systems. Low density calcium alginate beads containing the curcumin emulsion were prepared by dropping the prepared alginate curcumin emulsion into a calcium chloride solution. Other techniques, such as polymer foam powder (27), non-aqueous emulsification, solvent evaporation technique (28) and microporous carriers (29) have also been used to prepare low density stomach-targeted floating systems.

Selection of formulations

Prior to the preparation of the beads, a phase separation test was performed on the different formulations by subjecting the preparations to centrifugation. Formulations F3, F6 and F9 had the least percent aqueous phase separation (as shown in Figure 1) and were chosen for further optimization. These physically stable preparations contained the greatest concentration of oleic acid. Oleic acid is an amphiphilic molecule, known to act as an emulsion stabilizer (30). Moreover, oleic acid is a good solvent and penetration enhancer for curcumin. A recent study demonstrated the advantage of dissolving curcumin in oleic acid and showed the possibility of absorption of curcumin/oleic acid using mesoporous silica particles (31).

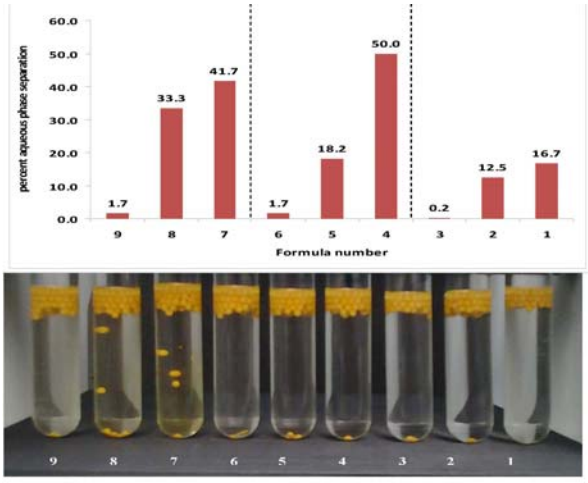

Figure 1 Percentage aqueous separation of curcumin 'emulgel' preparations after 24 hours (top). Floating beads standing in distilled 2% calcium chloride solution and the diffusion of curcumin out of beads (bottom).

Color stability of curcumin in dissolution medium

In order to determine a suitable dissolution medium, the yellow curcumin beads were placed in dialysis containers in separate USP Paddle dissolution apparatus at pH 1.2 (0.1 M HCl), 7.5 (USP phosphate buffer) and 8.5 (USP phosphate buffer). The change in color of curcumin was observed visually after 12 hours. Curcumin showed yellow, orange and red discoloration after dissolution for 12 h at pH 1.2, 7.5 and 8.5, respectively (see Figure 2).

Curcumin (1E, 6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, also known as diferuloylmethane, is known to be unstable. It undergoes rapid hydrolytic degradation in neutral or alkaline conditions to feruloyl methane and ferulic acid (32). In terms of color changes, curcumin in basic solutions produces a red color, while in acidic media it has a yellow color (33). Thus, stability constraints require that a sustained release dosage form containing curcumin should be gastro-retentive.

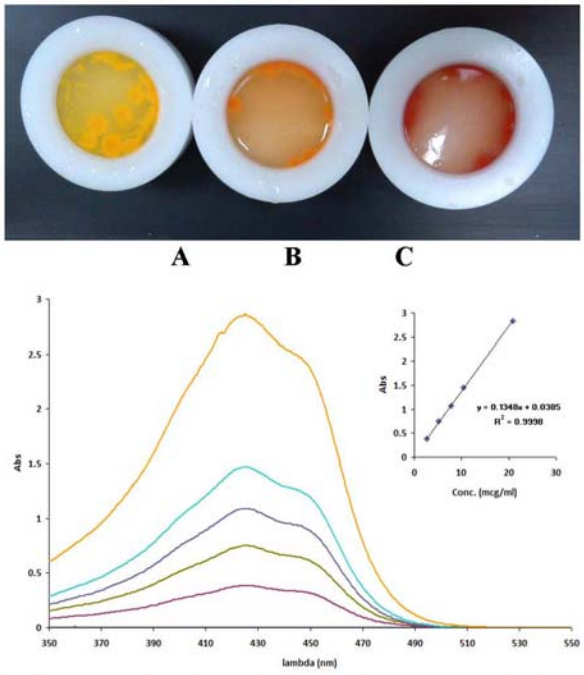

Figure 2 Curcumin beads stored in dialysis containers in dissolution apparatus at 37°C stirring for 12 hours at pH 1.2, 7.5 and 8.5 as shown in A (yellow), B (orange) and C (red) respectively (top). Wave length versus absorbance of different curcumin standard curcumin samples in acidic condition at pH 1.2 and the calbration curve for curcumin (bottom).

In vitro dissolution

Simulated gastric fluid was used for bead dissolution to mimic the acidic condition of the stomach. A constant amount of Tween® 80 was added to the dissolution medium to enhance the rate of curcumin release for all formulations since curcumin has a very low solubility in aqueous medium (0.0004 mg/ml) (34).

The simulation of release behavior of curcumin from the beads was performed using different models as shown in Table 2. In order to evaluate the best fit model, RSME was used as shown in Table 3. The best fit model, with the lowest RSME, was the Weibull model for the F3 formulation, while F6 and F9 were best represented by the Korsmeyer-Peppas model (Figure 3).

Table 3 Summary of RSME of all preparation showing best model

MODEL	F3 5 min	F3 10 min	F3 15 min	F6 5 min	F6 10 min	F6 15 min	F9 5 min	F9 10 min	F9 15 min
Zero	4.29	4.03	4.07	2.81	3.06	3.66	3.75	2.75	4.03
First	1049.34	1190.73	1299.56	1211.34	1567.97	1817.80	1175.01	1589.66	1749.53
Second	32.50	34.06	36.28	34.15	41.52	46.78	33.26	42.45	46.93
Higuchi	24.28	26.09	27.95	26.41	32.69	37.05	25.54	33.43	36.69
Korsmeyer-Peppas with lag time	6.40	3.49	3.02	2.12	1.09	0.96	2.73	1.84	3.21
Weibull with lag time	2.00	1.65	1.35	2.98	3.84	5.08	3.08	4.98	5.43
Hixon-Crowell with lag time	12.50	13.63	14.54	12.27	15.53	18.02	13.35	14.95	17.24
Michaelis-Menten	15.24	18.60	20.23	19.66	25.06	28.42	18.64	25.40	26.52
Hill	2.75	2.49	2.93	4.15	6.91	9.16	2.77	7.91	8.86

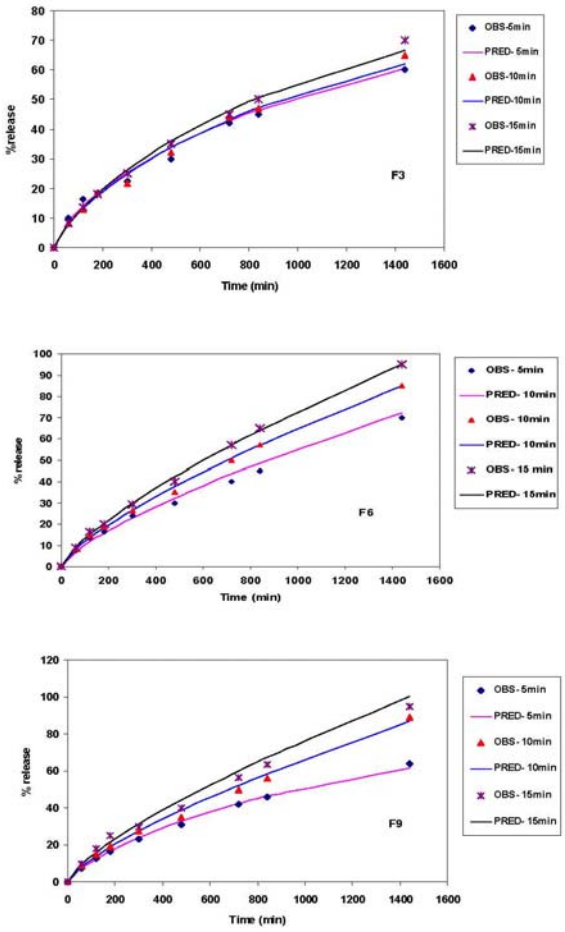

Figure 3 The release profiles of F3, F6 and F9 observed (dots) and predicted (lines). F3 best fitted to the Weibull equation while F6 and F9 best fitted to the Korsmeyer-Peppas model.

Literature reports suggest that there is a significant correlation between the Weibull and Korsmeyer-Peppas power functions (34). The exponent *b* of the Weibull model showed a linear relationship when plotted against the exponent *n* of the Korsmeyer-Peppas model. The established linear relationship indicated not only the mathematical relevance of the exponents *b* and *n* but also the physical significance of the models' parameters and the release mechanism. *n* values in the range of 0.45-0.5 are indicative of pure diffusion controlled release with correlative yields of *b* values in the range of 0.69–0.75 for theoretically anticipated values for Fickian diffusion.

The added Tween® 80 (as a component of the formulation) induced an irregular surface with deeper pores. This was especially evident at greater Tween® 80 concentrations as shown in Figure 4. The increase in cross-linking time allowed calcium ions to penetrate deeply inside the bead structure thus enhancing the formation of a more structured torturous matrix. As Tween® 80 concentration increased, a more porous structure, albeit a structure with a greater cross-link density, could be formed which could encourage water diffusion into the bead, yet decrease the diffusion of curcumin into the aqueous medium due to increased cross-link density. This could explain the lower rate of curcumin release (greater MDT) upon increasing Tween® 80 concentration or upon increasing the cross-linking time as shown in Table 4. In addition, the release mechanism seemed to have shifted to anomalous (non-Fickian) transport rather than to Fickian diffusion when increasing either the concentration of Tween® 80 or the cross-linking time.

The f_2 similarity factor for formulation F3 was greater at 5 min/10 min than at 10 min/15 min as shown in Table 5. In contrast, the f_2 similarity factors for formulations F6 and F9 were lesser at 5 min/10 min than at 10 min/15 min. This suggested that the pattern of release for the F6 and F9 formulations were similar to

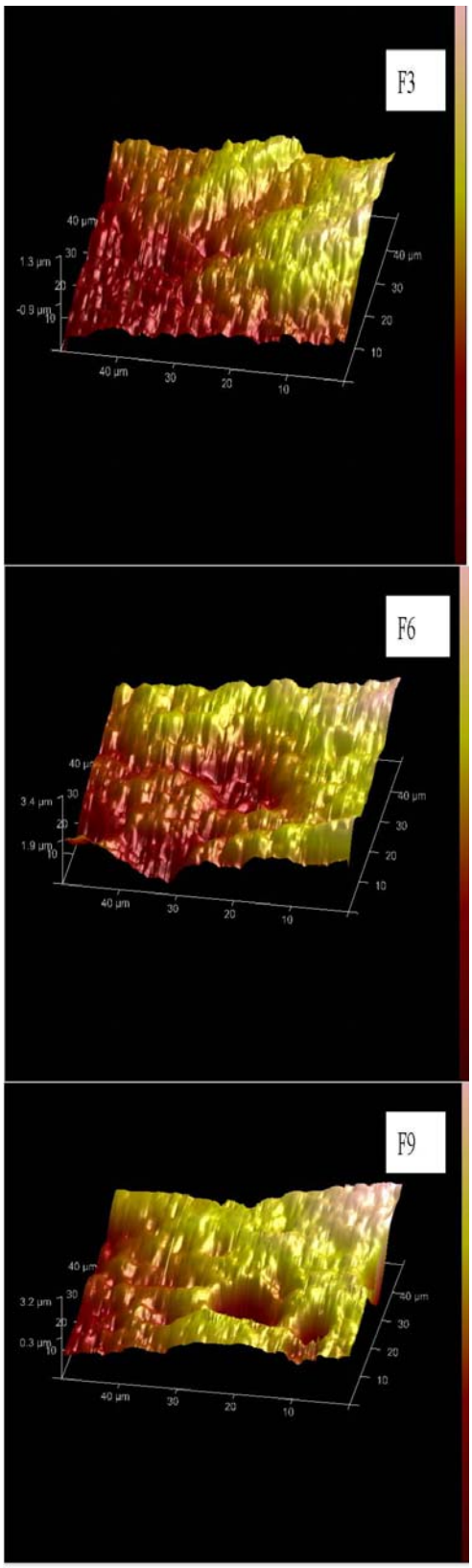

Figure 4 AFM FMM height sensor (50x50 micrometer) of F3, F6 and F9 cross-linked for 5 minutes.

Table 4 Face-centered cube design arrangement and responses for the factors crosslinking time and Tween® 80 concentration.

		RESPONSE	
Cross-linking time (T_c) (min)	Tween® 80 conc. (C_t) (%)	(Y1) Floating time (min)	(Y2) MDT (min)
15 (+1)	0.75 (+1)	5.00	614.4
10 (0)	0.5 (0)	5.33	624.11
15 (+1)	0.25 (-1)	14.30	566.96
5 (-1)	0.75 (+1)	3.33	565.32
5 (-1)	0.25 (-1)	6.66	528.75
10 (0)	0.5 (0)	6.00	619.28
10 (0)	0.5 (0)	4.00	620.98
10 (0)	0.5 (0)	6.00	623.89
10 (0)	0.75 (+1)	3.66	647.65
5 (-1)	0.5 (0)	3.50	438.02
10 (0)	0.5 (0)	5.00	629.74
10 (0)	0.5 (0)	5.50	627.41
15 (+1)	0.5 (0)	6.67	616.26
10 (0)	0.25 (-1)	9.00	559.29

each other but different from the release profile of formulation F3. This difference could be due to the presence of higher levels of Tween® 80 in formulations F6 and F9 than in formulation F3.

Response surface methodology

Multiple regression coefficients were determined using a least squared method to predict the polynomial model for floating time and MDT using the experimental data shown in Table 4. The model fitting was significant as indicated by p-value ($p<0.05$) and the values of coefficient of determination R^2 (linear) of floating time and MDT i.e., 0.952 and 0.80, respectively. The effect of C_t and T_c on floating time (Y1) and mean dissolution time (Y2) were evaluated using RSM (see Table 6).

Table 5 Fit factors for preparations F3, F6 and F9 using the crosslinking time 5 min as reference in each preparation

Preparation	5 min/10 min		10 min/ 15 min	
	f_1 fit factor	f_2 fit factor	f_1 fit factor	f_2 fit factor
F3	6.95	79.15	32.93	44.19
F6	19.83	56.04	12.44	63.45
F9	23.96	50.62	12.45	65.28

Table 6 Regression coefficients of predicted quadratic polynomial model for responses

	Floating time (min)		MDT (min)	
Variable	Coefficient	ρ-values	Coefficient	ρ-values
Intercept	5.202		620.543	
Linear				
T_c	2.080	0.000447	44.254	0.015
C_t	-2.995	4.512E-05	28.729	0.077
Quadratic				
T_c^2	0.304	0.560	-72.024	0.010
C_t^2	1.549	0.017	4.305	0.840
Interaction				
T_c x C_t	-1.493	0.00840	2.718	0.877
R^2 (linear)	0.954		0.800	
R^2 (linear adjusted)	0.921		0.657	
CV%	13.712		5.746	
Model		0.000156		0.021

R_2 is the coefficient of determination
CV% is the coefficient of variation

The linear terms obtained for the response of C_t and T_c had a significant effect ($p<0.05$) on the beads floating time (Y1), while the effect of T_c on Y1 showed a positive significant effect, C_t showed negative effect (Table 6). Furthermore, the second-order terms of C_t had a positive significant ($p<0.05$) impact, while quadratic terms of T_c had no significant effect on floating time. There were negative significant interactions of experimental variables that could affect Y1.

The response of beads in terms of MDT (Y2) showed positive significant effect ($p<0.05$) for the linear terms of the two factors i.e., C_t and T_c. The quadratic term of T_c had a positive significant effect, while C_t did not show a significant effect. There was also no significant interaction of the experimental variable that could affect Y2.

The interaction of experimental variables and the strong relationship between the Tween® 80 concentration and the cross-linking time could be due to Tween® 80, i.e., emulsification action which can make the beads more porous and

less hydrophobic thereby facilitating the penetration of calcium chloride or hydrochloric acid within the structure of sodium alginate emulsion.

The increase in Tween® 80 concentration for a given cross-linking time could result in a more porous bead structure, allowing water to penetrate i.e., more buoyant beads. The increase in cross-linking time for a given Tween® 80 concentration could increase the cross-linking density, with the density being greatest at the surface and gradually decreasing into the bead's interior thus allowing for less water penetration into the bead i.e., less dispersible beads in aqueous medium and so less buoyant system as shown in Figure 5.

Buoyancy is determined, not only by the relative intrinsic densities of the floating body and the liquid medium but, also by the volume of the liquid medium displaced relative to the volume occupied by the floating body. The presence of lower density oils in the beads is hence not the only determinant of floatation time. The paradoxical situation of a greater water penetration at greater Tween® 80 concentration yielding more buoyancy (lesser floatation time) may be explained by the above. With an increase in cross-linking time at a given Tween® 80 concentration, the volume of the bead is greater than the volume of water it displaces and neither the (relatively smaller) amount of water penetration nor the presence of lower density oils and organic liquids in the bead compensate for this effect. In this study, no attempt was made to determine the amount of oleic acid and olive oil retained in the beads after the cross-linking process.

In addition, RSM was an appropriate model (0.968) to evaluate cross-linking time and Tween® 80 concentration as factors that could affect calcium alginate bead floating time and MDT.

The optimal conditions were determined using the optimizing function of the Design Expert shown in Table 7. The minimum floating time was (3.242 ± 0.822 minutes) and for the MDT it was (559.467 ± 33.990 minutes) when C_t and T_c were 0.331 and 6.168, respectively. Furthermore, confirmatory experimental work was carried out at the optimum conditions. The results indicated a good agreement between the predicted and experimental values.

Table 7 RSM optimization for preparation of calcium alginate curcumin beads

FACTOR	OPTIMIZED LEVEL	LEVEL
T_c (min)	6.168	5-15
C_t (%)	0.631	0.25-0.75
Response	**Predicted value***	**Observed value***
Floating time (min)	3.242 ±0.822	3.581±0.526
MDT (min)	559.467±33.990	580.892±53.554

*mean±standard deviation

The parameters (T_c and C_t) appeared to have a significant effect on both the floating time and the MDT. This could emphasize the importance in considering both cross-linking time and emulsifier concentration upon formulating such systems. The two factors T_c and C_t have a more pronounced effect on floating time (Y1) compared to their effect on MDT. These results agree with other findings where floating systems could be a result of the use of oily vehicle components within the structure of calcium alginate beads (35).

CONCLUSION

An optimized gastroretentive floating system with controlled release behavior was prepared and evaluated *in vitro* for targeting curcumin to the stomach. The factors affecting the controlled release behavior were the cross-linking time of alginate and the Tween® 80 concentration. The factors were optimized to obtain the minimum floating time and optimal MDT using RSM. Future studies are expected to include the evaluation of controlled release behavior of the optimized curcumin calcium alginate beads using suitable *in vivo* models.

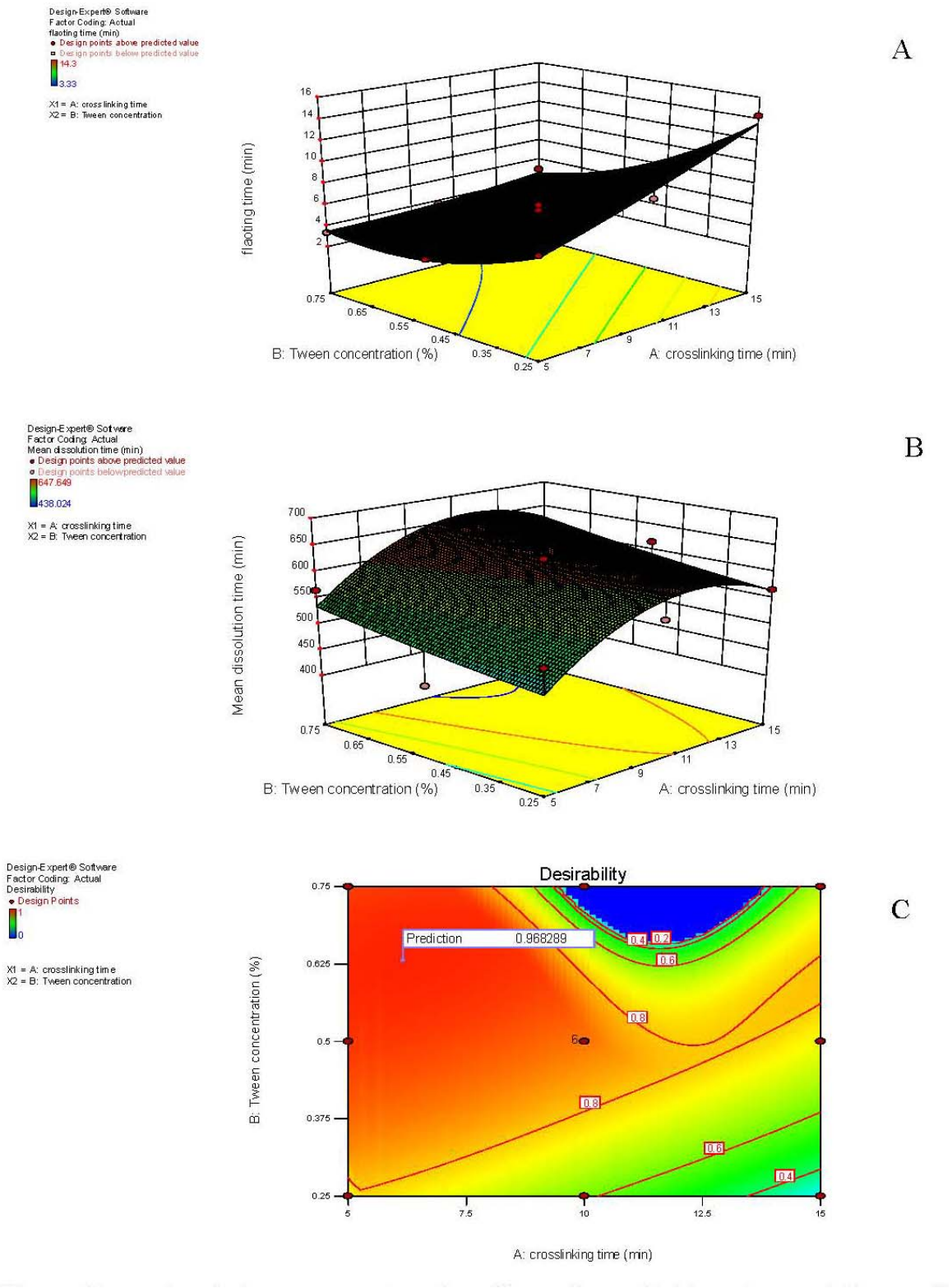

Figure 5 Three dimensional plot reprsenting the effect of cross-linking time and Tween® 80 concentration on the floating time (A) and MDT (B). The desirability plot of RSM of cross-linking time and Tween® 80 concentration on calcium algenate bead preparations (C).

ACKNOWLEDGEMENTS

The author would like to thank Dr. Nisrein Jaber for her assistance in this study. The author also gratefully acknowledges the assistance of Professor Shireesh Apte in providing valuable scientific advice and editorial assistance for this manuscript.

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