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Statistically-Based Optimization of Verapamil Hydrochloride Loaded Gastroretentive Alginate Beads

Aliaa Ismail*, Eman Mohamed Raafat, Wedad Sakran

Pharmaceutics and Industrial Pharmacy Department, Faculty of Pharmacy, Helwan University, Ain Helwan, Cairo 11795, Egypt

***Corresponding author:** Aliaa Ismail, Pharmaceutics and Industrial Pharmacy Department, Faculty of Pharmacy, Helwan University, Ain Helwan, Cairo 11795, Egypt. Tel. +201224328984*
Email address: aliaa_adly@pharm.helwan.edu.eg

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ABSTRACT

Objectives: A gastroretentive drug delivery system is one of many oral drug delivery systems developed to improve drug bioavailability and control drug release. It allows prolongation of drug gastric residence period for several hours. Verapamil Hydrochloride (VerHCl) is a good candidate for gastro retention because it has narrow absorption window and short half life. The goal of this study was formulation and evaluation of optimized VerHCl loaded gastroretentive alginate beads for increasing drug bioavailability and decreasing its dosing frequency. **Methods:** VerHCl loaded alginate beads were prepared according to 2^3 full factorial design using ionotropic emulsion gelation method. The effect of three formulation variables; oil concentration (%w/v) (X_1), polymer concentration (%w/v) (X_2) and drug to polymer ratio (X_3) was investigated on mean diameter (Y_1), drug loading % (Y_2), entrapment efficiency (EE%) (Y_3), % drug released at 1 (Y_4), 5 (Y_5) and 8 hr (Y_6). The optimized formula was further assessed in terms of *in vitro* floating, *in-vitro* drug release, *in vivo* gastro retention in rats and flowability. **Results**: Design-Expert® numerical optimization revealed that optimum formulation levels for VerHCl loaded alginate gastroretentive beads were; oil concentration (X_1) = 17.2 %w/v, polymer concentration (X_2) = 4.34 %w/v and drug to polymer ratio=1.2. The optimized formula exhibited yield% (85.63± 2.65%), Drug loading% (13.60±0.89%), EE% (60.11± 2.52%), prolonged floatability with no initial lag time, sustained *in vitro* drug release over 8 hr, gastroretention in rats over 8 hr and good flowability. **Conclusion:** The optimized formula of VerHCl loaded alginate beads could be promising for retaining VerHCl in stomach for a prolonged time which could possibly be advantageous in terms of bioavailability and patient compliance.

Keywords: *Verapamil Hydrochloride; Gastroretentive systems; Bioavailability; Alginate beads; Controlled drug release.*

INTRODUCTION

Oral route is the most favored pathway for drug delivery due to ease of administration and greater patient compliance[1]. However, some drugs have low oral bioavailability due to narrow absorption window in gastrointestinal tract (GIT). Gastroretentive drug delivery systems can provide controlled delivery for drugs that have an absorption window in the gastric tract by continuously releasing the drug for an extended period of time in the stomach, thus guarantee its optimal bioavailability[2].

There are several gastroretentive devices developed according to various technologies such as:(a) low density systems that induce buoyancy in gastric fluids[3] (b) high density systems that are retained in the stomach [4] (c) bioadhesive systems that adhere to stomach [5](d) expandable systems that increase in size to extent prevent their emptying through the pyloric sphincter [6].

Recently, low density floating systems are the most applicable and extensively studied gastroretentive dosage forms. These systems have the ability to float in the gastric fluid for a prolonged period and eventually increase the gasroretention time [7].

Although, single-unit floating systems are more popular, but such systems have a disadvantage due to their (all-or-nothing) pattern of emptying process, leading to high variability of the gastrointestinal transit time. On the other hand, multiple-unit particulate dosage forms have the advantage of passing uniformly through the gastrointestinal tract avoiding the varieties of gastric emptying, thereby reducing the inter-subject variability in absorption [8].

Floating alginate beads are multiple-unit gastroretentive systems composed of sodium alginate polymer. Alginate is a safe and economic linear anionic co-polymer made up of D-mannuronic acid and L-guluronic acid. Upon preparation of alginate beads using ionotropic emulsion gelation technique, sodium alginate could decrease the interfacial tension between oil and water phases leading to successful emulsification. Sodium alginate is capable of forming rigid gels by the action of calcium ion. The gel is formed by a chemical reaction in which calcium displaces sodium from alginate [9].The major advantage of ionotropic emulsion gelation technique is the lack of use of any organic solvents.

The first calcium channel blocker, verapamil hydrochloride (VerHCl), was licensed by the FDA in 1981 for the treatment of a variety of cardiovascular conditions, including angina pectoris, supraventricular tachycardia, and hypertension. VerHCl has low solubility at intestinal pH [10] and short half-life of 4-6 hr[11].Due to the abovementioned properties, VerHCl was reported in earlier literature to be a good model drug for incorporation into gastroretentive systems. VerHCl has been previously formulated into single unit gastroretentive devices such as floating matrix tablets [2, 12, 13] and solid foam capsules [10]. Moreover, it has been loaded into various multiple-unit floating devices such as microspheres[14], alginate beads[15], chitosan beads [16], nanocomposite beads [17], foam-based, microparticles[18]and pellets[19].

Because VerHCl is mostly used to treat chronic conditions, the incorporation of this drug in controlled release floating systems could improve patient compliance by reducing dosing frequency. Furthermore, it was reported that slow release dosage forms of VerHCl provided better therapeutic response with minimized side effects compared to rapidly absorbed VerHCl dosage forms [20]. Also, Pharmacokinetic studies showed that floating dosage forms of VerHCl were superior over the traditional VerHCl dose forms [19].

To the best of our knowledge, VerHCl was previously formulated into alginate beads using ionotropic emulsion gelation technique[15], but the formulation variables were not optimized according to design of experiment approach. Therefore, this study aimed to exploit 2^3 full factorial design to statistically analyze the effect of oil concentration, polymer concentration and drug to polymer ratio as independent formulation variables and subsequently, optimize the formulation of VerHCl loaded alginate beads.

MATERIAL AND METHODS

Materials

Verapamil Hydrochloride (VerHCl) was kindly gifted from Kahira Pharmaceuticals & Chemical Industries Co. (Cairo, Egypt). Sodium alginate, Light Liquid paraffin, Calcium chloride (anhydrous) were obtained from Chemajet Chemical Co. (Alexandria, Egypt). All other ingredients used were of analytical grade.

Methodology

Experimental Design

Design Expert® software Version 7.0.0 (Estat-Ease, Inc., Minneapolis, Minnesota, USA) was used for construction of a 2^3 full factorial design. This design was applied to optimize the formulation of VerHCl loaded gastroretentive beads through investigating the effect of three formulation independent variables (factors); oil concentration (%w/v) (X_1), polymer concentration (%w/v) (X_2) and drug to polymer ratio (X_3) on a number of evaluated characters of the prepared beads. The investigated factors along with their levels and corresponding responses (dependent variables) were shown in **Table 1**. The independent variables (formulation factors) and their levels were selected based on literature survey and conduction of number of preliminary trials before the design application (data not shown). Combinations of different factors at studied levels were constructed by Design Expert ®and the composition of the developed eight formulations of alginate beads (F1-F8) were shown in **Table 2**.

Preparation of VerHCl loaded alginate beads

Eight formulations of VerHCl loaded gastroretentive beads (F1-F8) were prepared according to the amounts presented in **Table 3**, using ionotropic emulsion gelation method [15]. Briefly, sodium alginate was dissolved in deionized water with aid of magnetic stirring (Genway, UK). Then, liquid paraffin was added

Table 1. Factors and responses of the 2^3 full factorial design for formulation of VerHCl loaded alginate beads

Factors (independent variables)	Levels	
	Low (-1)	High(+1)
X_1: Oil concentration (%w/v)	15	20
X_2: Polymer concentration(%w/v)	3	5
X_3: Drug to polymer ratio*	1	2
Responses (dependent variables)	**Constraints**	
Y_1: Mean diameter(mm)	In range	
Y_2: Drug loading (%)	Maximize	
Y_3: EE (%)	Maximize	
Y_4: *In-vitro* drug release at 1 hr (%)	Target value =10% (Limits 8-12%)	
Y_5: *In-vitro* drug release at 5 hr (%)	Target value =35% (Limits 30-40%)	
Y_6: *In-vitro* drug release at 8 hr (%)	Target value =55% (Limits 50-60%)	

**Drug to polymer ratio is a weight to weight ratio*

Table 2. Composition of the prepared formulations of VerHCl loaded alginate beads

Formulation code	Oil concentration %w/v	Polymer concentration %w/v	Drug to polymer Ratio
F1	15%	3%	1
F2	15%	3%	2
F3	15%	5%	1
F4	15%	5%	2
F5	20%	3%	1
F6	20%	3%	2
F7	20%	5%	1
F8	20%	5%	2

to the polymer solution and then the drug was added while stirring is continued at 500 rpm. The mixture was homogenized at 900 rpm for 10 min using IKA T50 digital ULTRA-TURRAX® homogenizer. The emulsion formed after homogenization was extruded via 23- gauge needle into 5 % calcium chloride solution at rate of 1.5 ml /min, with gentle agitation at room temperature. The beads formed were washed several times with distilled water to get rid of the non-entrapped drug, and then dried in hot air oven (RUMO THERMOSTAT, Egypt) at temperature of 40 ± 0.5° C till reaching a constant weight of beads.

Differential scanning calorimetry (DSC)

The DSC thermograms of VerHCl, sodium alginate, VerHCl / sodium alginate physical mixture(1:1 w/w) and VerHCl loaded alginate beads were recorded using (Shimadzu DSC-50, Tokyo, Japan). The samples were placed in aluminum cell and heated from room temperature to 400°C with heating rate of 10°C/min under nitrogen atmosphere. The flow rate of nitrogen was 25 ml/min.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of VerHCl, sodium alginate, VerHCl / sodium alginate physical mixture (1:1 w/w) and VerHCl loaded alginate beads were recorded using (Spectrum Two FTIR spectrometer, Perkin Elmer, USA). The spectra of samples were recorded over the wavelength region of (4000–400 cm^{-1})[21].

Evaluation of the prepared VerHCl loaded alginate beads

Mean diameter determination

For each formula, the diameter of 10 dried beads randomly picked from three prepared batches was measured individually using digital caliper (SOMET, Czech Republic). Then, the mean diameter from the three batches (n=3) was calculated for each formula [22].

Table 3. The detailed composition (in grams) of VerHCl loaded alginate beads formulations

Formulation Code	Light paraffin oil (g)	Sodium alginate (g)	VerHCl (g)	Deionized water (g)
F1	7.5	1.5	1.5	39.5
F2	7.5	1.5	3	38
F3	7.5	2.5	2.5	37.5
F4	7.5	2.5	5	35
F5	10	1.5	1.5	37
F6	10	1.5	3	35.5
F7	10	2.5	2.5	35
F8	10	2.5	5	32.5

Percent yield

The prepared VerHCl loaded alginate beads were weighed and percent yield was calculated for each preparation using following formula:

$$\text{Yield \%} = (a / b) \times 100 \quad (1)$$

Where, 'a' is the practical weight obtained and 'b' is the theoretical weight.

Drug loading (%) and drug entrapment efficiency (EE %)

Fifty milligrams of VerHCl loaded alginate beads were accurately weighed and then, crushed in a glass mortar using glass pestle. Then, the drug was extracted from the crushed beads in 25 ml of distilled water over a period of 24 hr. The formed suspension was filtered using nylon disposable syringe filter (0.45µm, Zhejiang Aijiren Technology Inc., China) and diluted appropriately with distilled water. The same steps were applied on blank beads (beads without drug) and VerHCl content was determined by measuring UV absorbance of tested sample solution against the solution of the corresponding blank beads. The measurements were performed at λ_{max} 278 nm using UV/VIS Spectrophotometer (Perkin Elmer Lambda Ez 201, USA).

The drug content and the encapsulation efficiency were determined in triplicate using the following equations [23]:

Drug loading (%) = [Amount of drug in sample (mg) / Total sample weight (mg)] x 100 (2)

EE% = [Amount of entrapped drug / original amount of drug added] x100 (3)

***In vitro* floating**

The *in-vitro* floating of VerHCl loaded alginate beads was studied in terms of the visually observed floating lag time and floating duration. Floating lag time is the period between placing the beads in the medium and the beginning of buoyancy. Floating duration is time at which the beads still floating above simulated gastric fluids. The experiment was performed by placing 100 mg of beads in a beaker filled with 50 ml of 0.1 N HCl (pH 1.2) and maintained at 37 ± 0.5ºC. The floating time of beads was observed for 24 hr. The preparation was considered to have buoyancy in the test solution only when all the beads floated in it[24].

Swelling studies

The prepared beads were immersed in 100 ml of 0.1N HCl and at fixed time intervals (1, 3, 5, 8 and 24 hr), the beads were removed, dried using filter paper and then weighed. The weight change of beads was calculated according to the following formula [23]:

$$\text{\% weight change} = [Ws-Wi / Ws] \times 100 \quad (4)$$

Where, Ws is the weight of the beads in the swollen state and Wi is the initial weight of the beads.

***In vitro* drug release studies**

The studies of the *in vitro* release of VerHCl from the prepared alginate beads equivalent to 120 mg VerHCl were conducted under sink condition using USP Dissolution tester, Apparatus II (Rotating paddle) (Hanson Research, Chatsworth, California, USA). The beads were enclosed into cellulose tubing with molecular weight cut off 12,000 - 14,000 Dalton (Dialysis Membrane-150, Code No. LA401, HiMedia Laboratories Pvt. Ltd., India). Then, the filled dialysis membrane was strongly tied to the roatting paddle and immersed in 900 ml of 0.1 N HCl (pH 1.2)(simulated gastric fluid). Throughout the experiment, the temperature of the release medium was adjusted to 37 ± 0.5ºC and the rotation speed of paddle was kept at 50 rpm. Aliquots from the release medium were withdrawn periodically at 0.5, 1, 2, 3, 4, 5, 6, 7, 8 hr. The withdrawn samples were replaced with fresh release medium of the same volume to maintain sink condition. For quantification of released VerHCl, samples were filtered using nylon disposable syringe filter (0.45µm,Zhejiang

Aijiren Technology Inc., China) and measured UV spectrophotometrically against 0.1N HCl as a blank at λ_{max} 278 nm. The release studies were performed in triplicate and the mean values were plotted as cumulative percentage of drug released against time.

In vitro drug release kinetics and mechanisms

To clarify the kinetics of VerHCl release from the prepared alginate beads, the *in vitro* drug release data were fitted to different kinetics models such as zero order (cumulative % drug released versus time), first order (log % drug remaining versus time), Higuchi (cumulative % drug released versus square root of time) and Hixson–Crowell (cube root of initial drug concentration minus cube root of % drug remaining versus time [25].The most fitted release model was selected on based on the highest regression coefficient (R^2) values.

Moreover, the mechanism of drug releasewas elucidated from the analysis of *in-vitro drug* release data according to Korsmeyer – Peppas release modelusing the following equation:

$$M_t/M_\infty = kt^n \qquad (5)$$

Where (M_t) is the amount of drug released at time t, (M_∞) is the amount of drug released at infinite time, (K) is the kinetic constant and (n) is the release exponent which indicates the release mechanism. The (n) values used for elucidation of drug release mechanism from the prepared alginate beads were determined from the slope of the plot of log cumulative % of drug release ($\leq$ 60%) versus log time. The (n) value depends on the release mechanism and the shape of the drug delivery device. For spherical particles, if ($n \leq 0.43$), the release mechanism follows "Fickian diffusion". Values of ($0.43 < n < 0.85$) indicate non-Fickian model (anomalous transport) where release is controlled by a combination of diffusion and swelling and relaxation. If the n value is 0.85, this indicates Case II transport which is controlled by the swelling and relaxation of the drug delivery system matrix and it is independent of time(zero order). If the n value is more than 0.85, this indicates Super Case II where the release is governed by the macromolecular relaxation of the polymeric chains[26].

Analysis of factorial design

Data analysis of factorial design was performed using ANOVA (classical sum of squares type III) for the selected factorial model. For each response, the model selection was based on the highest prediction R^2 which indicates the ability of the model to navigate the design space predicting even unperformed trials [27]. ANOVA test evaluated the level of significance of individual(main) effect of the tested factors and their interactions on the mean diameter(Y_1), drug loading % (Y_2), entrapment efficiency (EE%)(Y_3), % drug released at 1 (Y_4), 5 (Y_5) and 8 hr (Y_6) the percentage drug released from different alginate beads formulations at 1, 5 and 8 hr. A Statistically significant level was considered at $P<0.05$.

Optimization of the prepared VerHCl loaded alginate beads

In an attempt to optimize the characters of VerHCl loaded alginate beads, a numerical optimization technique by the desirability approach was performed using Design- Expert® software according to the constraints listed in **Table 1**. The target values of the constraints of the studied responses relevant to the *in vitro* drug release (Y_4, $Y_5$and Y_6) were extracted from a previously reported study for optimizing the formulation of a controlled release gastroretentive system[28].

Evaluation of the optimized VerHCl loaded alginate beads

Mean diameter, yield%, drug loading (%), EE%, %, in vitro floating and in vitro drug release

The optimized formula (F9) was prepared using ionotropic emulsion gelation method according to the levels of formulation variables recommended by Design Expert® numerical optimization. Then, it was evaluated in terms of mean diameter, yield%, drug loading %, entrapment efficiency (EE %), *in vitro* floating, swelling studies, *in-vitro* drug release studies and in vitro release kinetics and mechanism.

Validation of elucidated factorial models predicted data

The validity of the elucidated factorial models to predict responses for certain trial were tested for the optimized formula (F9). The actual experimental values of responses were tested for lying within the 95%prediction interval range. Moreover, the % deviations of these actual values of responses from the predicted values obtained from elucidated models were calculated using the following equation[29]:

$$\text{Percent deviation} = \frac{|\text{Predicted value} - \text{Experimental value}|}{\text{Predicted value}} \times 100 \qquad (6)$$

Scanning electron microscope

The surface morphology of the optimized VerHCl loaded alginate beads (F9) was examined and imaged using scanning electron microscope (Jeol-JSM5200, Japan).

Investigation of _in vivo_ gastroretention

In vivo gastroretention of the optimized VerHCl loaded alginate beads (F9) was evaluated in rats. The protocol of this experiment was authorized by the Animal Ethics Committee of Faculty of Pharmacy, Helwan University, No. 05A2022. Nigrosin dye was used to stain the F9 beads to identify their presence in stomach. Nine rats (males and females) weighing 250-300 g, were enrolled in this experiment. The rat equivalent dose of VerHCl was calculated by

multiplication of the average VerHCl human dose (2 mg/kg) by the Km ratio. The Km ratio is calculated by dividing human Km by rat Km where Km is a correction factor estimated via dividing the average body weight (kg) of species by its body surface area (m^2). The Km ratio value for converting human dose into rat equivalent dose equal 6.2 [30].Thus, the stained F9 beads equivalent to drug dose of 12.4 mg / kg were administered to the rats after overnight fasting. The beads were administered using orogastric tubes with 6 mL of water and a free access to water is allowed through the experiment. Rats were observed for any signs of nausea, vomiting or spitting of tested beads for about 30 minutes after administration of beads, and observed for their normal behavior and/or any possible adverse effects throughout the experiment. Then, after 1, 5 and 8 hr from administration of the stained beads, three rats were subjected to exsanguination under anesthesia at each time for gastroretention examination. The stomach of each rat was isolated and opened along the greater curvature with the mucosal surface turned upwards. Photos for the opened stomach were captured using digital camera to prove gastroretention of beads.

Estimation of flowability of the optimized VerHCl loaded alginate beads

The angle of repose is the simplest method to express the flowability of powders. The link between flow properties and angle of repose has been established. When the angle of repose is less than 25º, the flow is considered to be excellent; on the other hand, if the angle of repose is more than 40 º, the flow is considered to be poor [31]. The angle of repose is defined as the steepest slope of the unconfined material, measured from the horizontal plane on which the material can be heaped without collapsing [32].

The angle of repose of the optimum formula (F9) was determined using the funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the beads. The beads were allowed to flow through the funnel freely onto the surface. The diameter of the beads' cone was measured and angle of repose was calculated using the following equation [33].

$$\theta = \tan^{-1}(h/r) \quad (7)$$

Where; 'h' and 'r' are the height and radius of the cone, respectively. Three determinations were performed.

RESULTS

Preparation of VerHCl loaded alginate beads

Eight formulations of VerHCl loaded alginate beads were successfully prepared according to full 2^3 factorial design using ionotropic emulsion gelation method. The detailed composition of each formulation was given in **Table 3**. An image for one formula of the prepared VerHCl loaded alginate beads was represented in **Figure 1**.

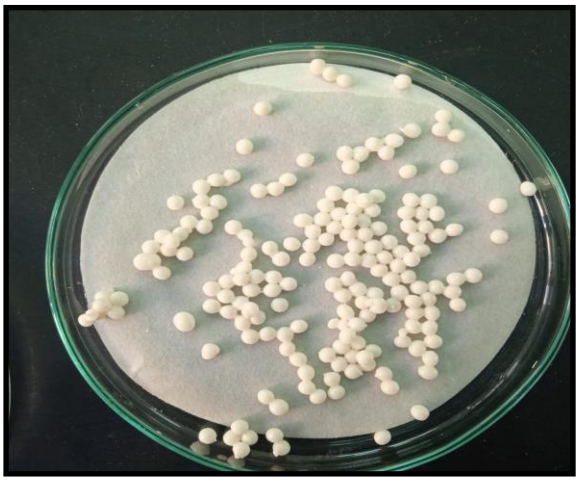

Figure 1. An image for one formula of the prepared VerHCl loaded alginate beads

Differential scanning calorimetry (DSC)

Figure 2, illustrates the DSC thermograms of VerHCl, sodium alginate, VerHCl / sodium alginate physical mixture (1:1 w/w) and VerHCl loaded alginate beads. The thermogram of pure VerHCl showed a characteristic sharp endothermic peak at 146.88ºC which was matched with previous findings [34]. This peak was still appearing in the thermograms of VerHCl / sodium alginate physical mixture and VerHCl loaded alginate beads indicating absence of interaction between VerHCl and sodium alginate**.** The shortening in the drug endothermic peak observed in the thermograms of VerHCl / sodium alginate physical mixture and VerHCl loaded alginate beads might be due to dilution factor.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of VerHCl, sodium alginate, VerHCl / sodium alginate physical mixture (1:1 w/w) and VerHCl loaded alginate beads were illustrated in **Figure 3**. From FTIR spectra, it was observed that all characteristic peaks of VerHCl that present in the fingerprint region (1500-1100 cm^{-1}) appeared in FTIR spectra of VerHCl / sodium alginate physical mixture and VerHCl loaded alginate beads which indicated that there were no interaction involving bond formation between drug and sodium alginate. Strong high intensity peaks appeared in the FTIR spectrum of VerHCl loaded alginate beads at the region (2850-3000 cm^{-1}) could be

due to carbon hydrogen stretching and bending absorption bands of linear saturated aliphatic structure of the paraffin[35].

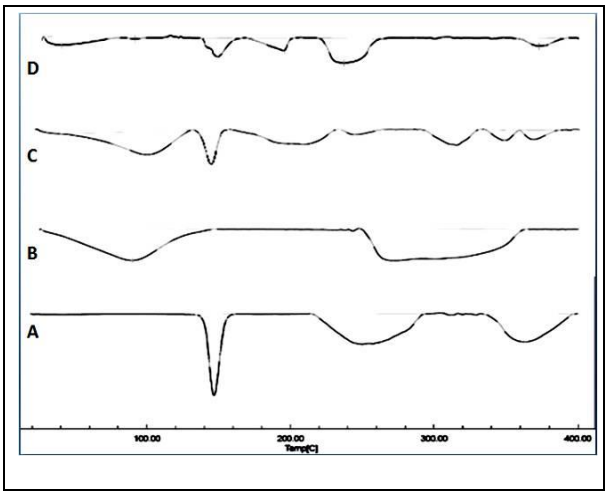

Figure 2. The DSC thermograms of (A)VerHCl, (B) Sodium alginate, (C) VerHCl / sodium alginate physical mixture and (D) VerHCl loaded alginate beads.

Evaluation of the prepared VerHCl loaded alginate beads

Mean diameter, yield%, Drug loading (%), EE%, floating lag time and total floating time

The characters of VerHCl loaded alginate beads namely, % yield, entrapment efficiency; mean particle size, floating lag time and total floating time were summarized in **Table 4**.

Swelling studies

The swelling behavior of the beads was performed in 0.1N HCl. The beads showed no swelling in 0.1N HCl as no noticeable change in the weight of the beads was observed after immersion in 0.1N HCl.

In vitro drug release studies

Figure 4 illustrated the in vitro release profiles of VerHCl from the prepared alginate beads. It was found that the release was faster from the formulae having drug to polymer ratio of 2 (F2, F4, F6 and F8) compared to their counterpart formulae having drug to polymer ratio of 1 (F1, F4, F6 and F8, respectively). F8 formulation showed the fastest release among the eight formulations while F1 showed the lowest release rate. However, it can be observed that all formulations succeeded in providing sustained drug release over 8 hr.

In vitro drug release kinetics and mechanisms

Numerous mathematical models (zero-order, first order, Higuchi diffusion and Hixson-Crowell) were utilized to explore the kinetics of in vitro drug release from various VerHCl loaded alginate beads according to the highest R^2. The results in **Table 5** showed that *in vitro* drug release from (F1, F2, F5, F6, F7 and F8) followed zero order release kinetics. On the other hand, the release data of F3 and F4 were best fitted to Hixson-Crowell and Higuchi diffusion models, respectively. Moreover, the model of Korsemeyer-Peppas was applied to all preparations and showed a good linearity for all the preparations (R^2=0.9383–0.9951). The estimated values of the release exponent (n) varied from 0.6758 to 0.9275.

Analysis of factorial design

For successful design of a new formulation, it is important to identify the influencing parameters which will affect the properties of the final dosage form. The experimental design approach statistically analyzes the influence of different formulation variables on the properties of the drug delivery system [36]. In this study, a full 2^3 factorial design was used to elucidate the relation between the response and the variables. ANOVA test was used to measure the level of significance of the tested factors on mean diameter(Y_1), drug loading % (Y_2), entrapment efficiency (EE%)(Y_3), % VerHCl released at 1, 5, and 8 hr (Y_4, Y_5, and Y_6, respectively). The three-dimensional (3D) plots showing the effect of formulation variables on the studied responses were illustrated in **Figures 5 and 6**.

Analysis of mean diameter (Y_1)

The best fitted model for this response was the main effect model (Predicted R^2= 0.9156) and the regression equation in terms of coded factors was:

$$Y_1 = +2.32 + 0.19\,X_1 + 0.31\,X_2 + 0.087\,X_3 \quad (8)$$

ANOVA test revealed that all factors had significant effect (p<0.05) on Y_1.

Analysis of drug loading% (Y_2)

The best fitted model for this response was the 3FI model (Predicted R^2= 0.9621) and the regression equation in terms of coded factors was:

$$Y_2 = +15.77 - 8.30\,X_1 + 3.05\,X_2 + 4.64\,X_3 - 0.35\,X_1X_2 - 0.89\,X_1X_3 + 0.81\,X_2X_3 - 0.46\,X_1X_2X_3 \quad (9)$$

ANOVA test revealed that all factors and their interactions had significant effect (p<0.05) on Y_4 except the interaction X_1X_2 and $X_1X_2X_3$.

Analysis of EE% (Y_3)

The best fitted model for this response was the main effect model (Predicted R^2= 0.6097) and the regression equation in terms of coded factors was:

$$Y_1 = +65.47 + 4.11\,X_1 + 2.63\,X_2 + 2.09\,X_3 \quad (10)$$

ANOVA test revealed that all factors had significant effect (p<0.05) on Y_3.

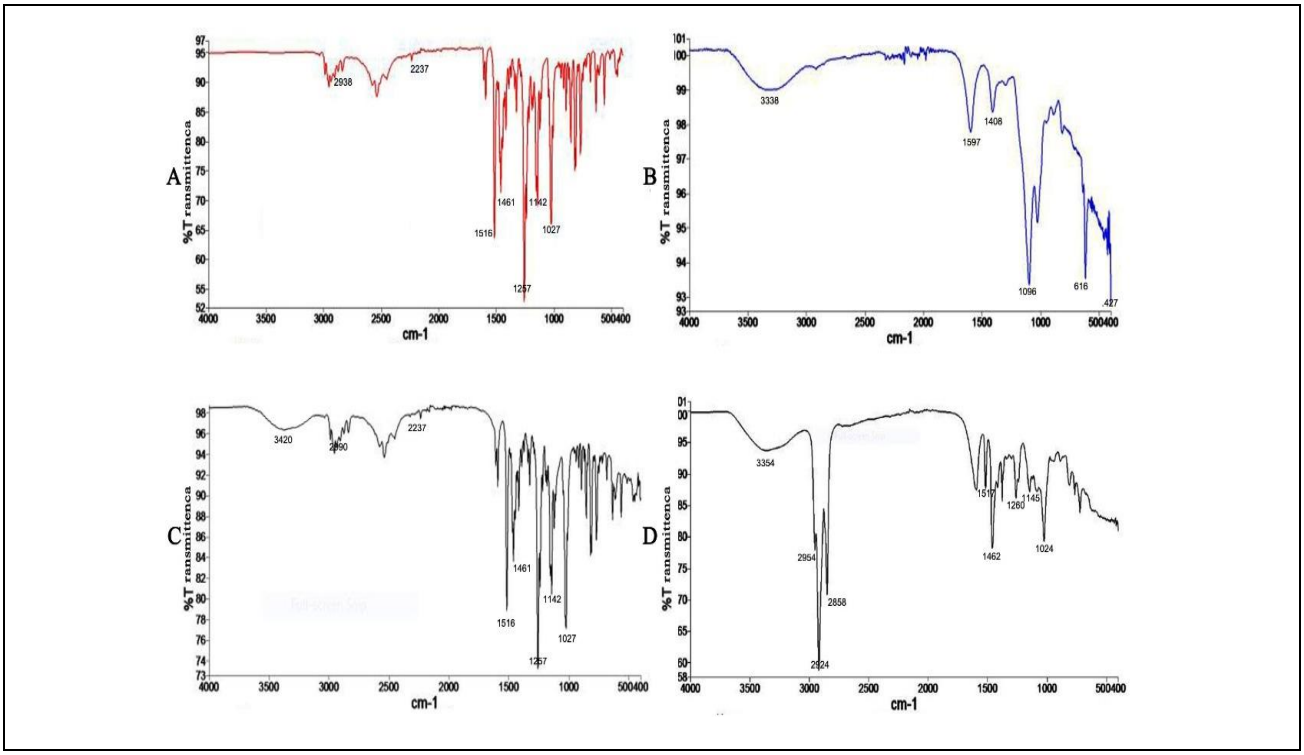

Figure 3. The FTIR spectra of A) VerHCl, B) Sodium alginate, C) VerHCl / sodium alginate physical mixture (1:1 w/w) and D) VerHCl loaded alginate beads.

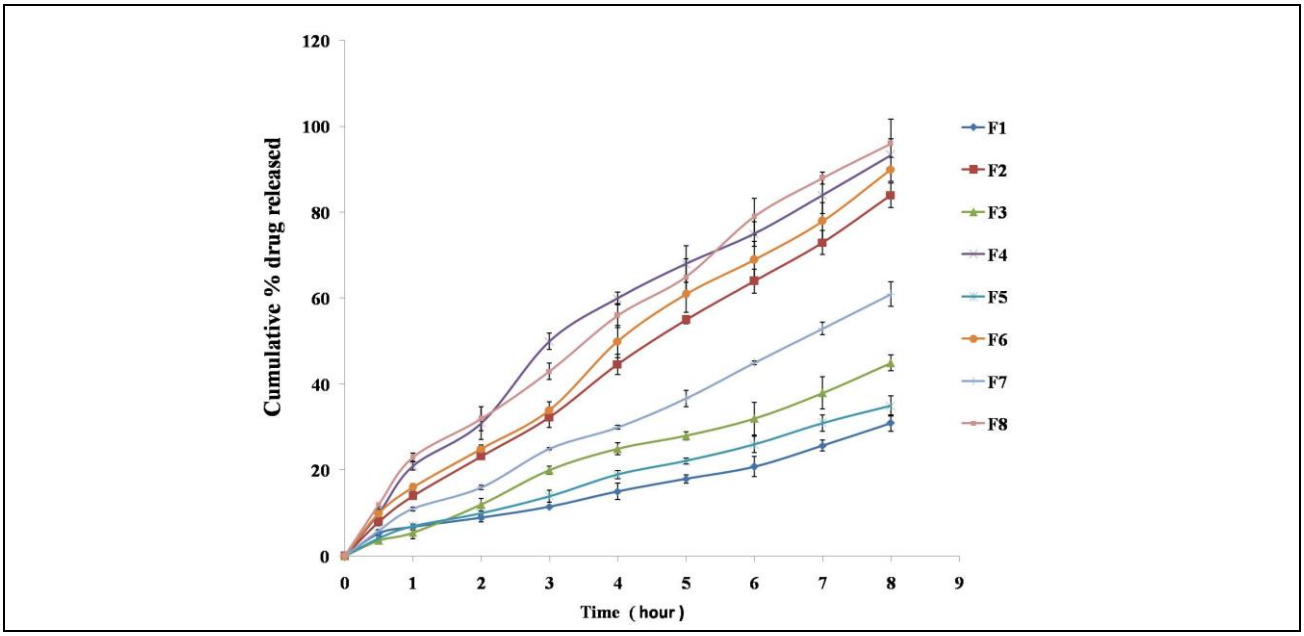

Figure 4. The *in vitro* release profiles of VerHCl from the prepared alginate beads

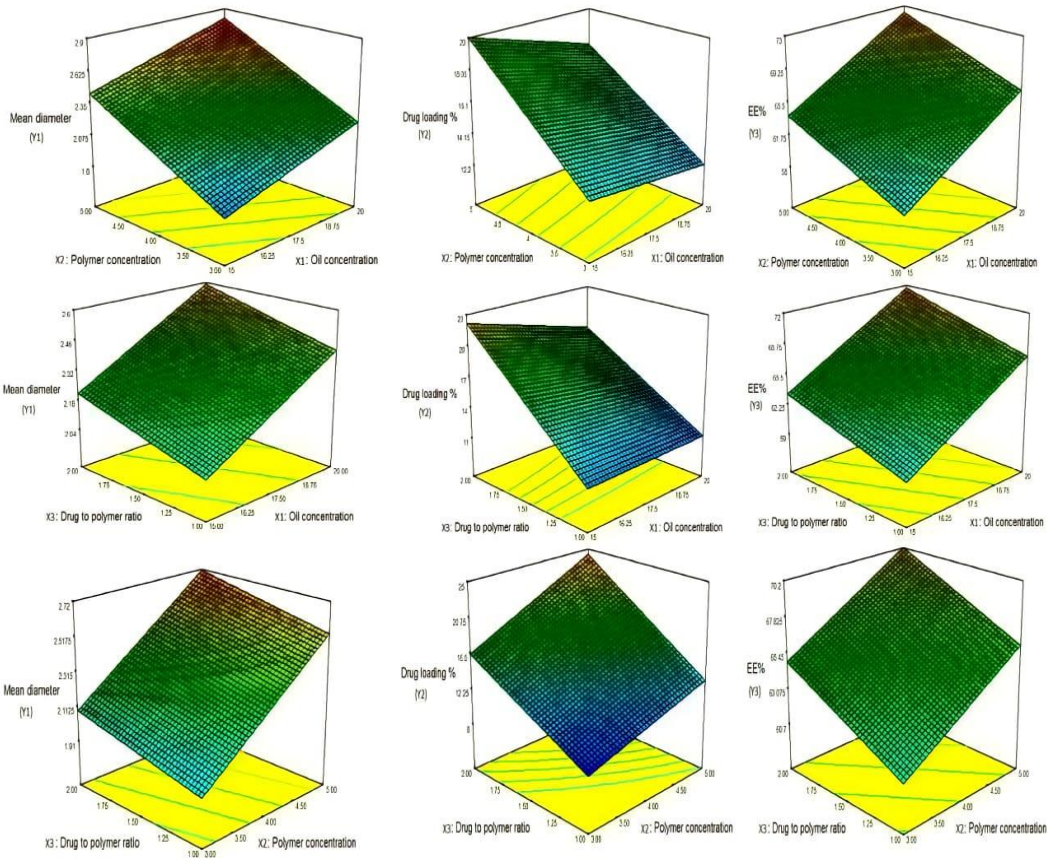

Figure 5. The 3D plots showing the simultaneous effect of two formulation variables on the studied responses Y1, Y2 and Y3 at average level of the third variable

Analysis of % drug released at 1 hr (Y_4)

The best fitted model for this response was the 3FI model (Predicted R^2= 0.9581) and the regression equation in terms of coded factors was:

$$Y_4 = +13.04 +1.21 X_1 +2.08 X_2 + 5.46 X_3 + 0.67 X_1 X_2 - 0.21 X_1 X_3 + 1.42 X_2 X_3 - 0.67 X_1 X_2 X_3 \quad (11)$$

ANOVA test revealed that all factors and their interactions had significant effect (p<0.05) on Y_4 except the interaction X_1X_3.

Analysis of % drug released at 5 hr (Y_5)

The best fitted model for this response was the main effect model (Predicted R^2= 0.9513) and the regression equation in terms of coded factors was:

$$Y_5 = +44.23 +1.98 X_1 +5.19 X_2 + 18.02 X_3 \quad (12)$$

ANOVA test revealed that all factors had significant effect (p<0.05) on Y_5 except X_1.

Analysis of % drug released at 8 hr (Y_6)

The best fitted model for this response was the 3FI model (Predicted R^2= 0.9593) and the regression equation in terms of coded factors was:

$$Y_6 = +66.92 +3.58 X_1 +6.92 X_2 + 23.92 X_3 + 1.08 X_1 X_2 - 1.42 X_1 X_3 - 3.08 X_2 X_3 - 1.92 X_1 X_2 X_3 \quad (13)$$

ANOVA test revealed that all factors and their interactions had significant effect (p<0.05) on Y_6 except the interaction X_1X_2, X_1X_3 and $X_1X_2X_3$.

Optimization of the prepared VerHCl loaded alginate beads

Based on the previous in vitro release data, the optimized formula of VerHCl loaded alginate beads was created using Design Expert software. The levels of the optimized formula were; oil concentration (X_1) = 17.20 %w/v, polymer concentration (X_2) = 4.34 %w/v and drug to polymer weight ratio=1.20. The desirability of this formula was 0.677.

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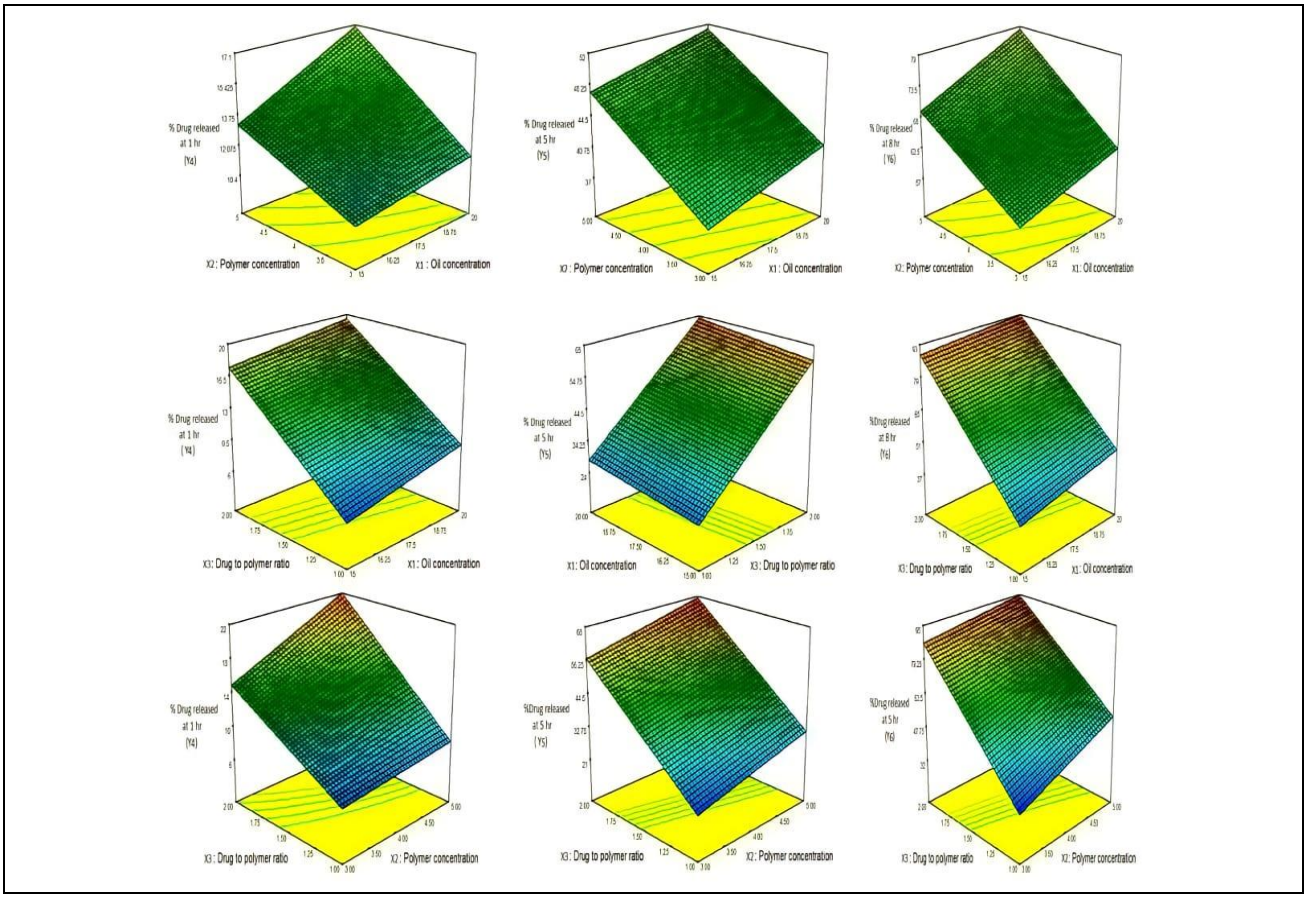

Figure 6. The 3D plots showing the simultaneous effect of two formulation variables on the studied responses Y4, Y5 and Y6 at average level of the third variable

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Evaluation of the optimized VerHCl loaded alginate beads

Mean diameter, yield%, drug loading (%), EE%, in vitro floating and in vitro drug release

The optimized (F9) formula had the following characters: diameter (2.36±0.15 mm), yield% (85.63± 2.65%), drug loading% (13.60±0.89%), EE% (60.11± 2.52%). F9 showed immediate floating in 0.1N HCl without lag time and was able to float over 24 hr. Moreover, no change in F9 weight was observed in 0.1N HCl over 24 hr. As shown in **Figure 7**, the release profile of F9 illustrated the potential of this formula to control drug release over 8 hr. The release kinetics were fitted to be zero order according (R^2 =0.9974) and mechanism of drug release according to Korsmeyer-Peppas was non-Fickian (n=0.8072).

Validation of elucidated factorial models predicted data

As shown in **Table 6**, the two-sided 95% prediction interval test revealed that the actual values fell within the 95% prediction interval of the predicted values. Moreover, the percent deviation of actual values from predicted was low. Thus, the validity of the elucidated models for responses under question could be verified [37].

Scanning electron microscope

Scanning electron microscope image of the optimized VerHCl loaded alginate beads (F9) in **Figure 8**, illustrates the spherical shape of the beads with porous, rough surface.

Investigation of *in vivo* gastroretention

All rats showed normal behavior and no signs of nausea or vomiting upon administration of the stained VerHCl loaded alginate beads (F9). The tested beads were hydrated with gastric fluid and were found to adhere to the body region of the stomach as black colored

Table 4. Different characters of the prepared formulations of VerHCl loaded alginate beads

Evaluation parameter	Mean diameter ±SD, n=3 (mm)	Yield % ±SD, n=3 (%)	Drug loading ±SD, n=3 (%)	Entrapment efficiency ±SD, n=3 (%)	Floating lag time (sec)	Floating time (hr)
F1	1.73±0.08	85.7±2.26	8.95±0.35	54.69±2.16	0	>24
F2	1.93±0.08	88.16±2.89	17.47±0.33	63.05±1.20	0	>24
F3	2.28±0.03	88.67±4.72	13.20±0.42	60.72±1.95	0	>24
F4	2.59±0.04	84.42±1.53	26.80±0.85	67.00±2.12	0	>24
F5	2.09 ±0.09	87.18±1.82	8.85±0.64	65.88±4.74	0	>24
F6	2.29 ±0.04	87.93±2.44	15.63±0.18	67.73±0.80	0	>24
F7	2.83±0.05	86.11±3.92	13.55±0.64	72.23±3.44	0	>24
F8	2.81± 0.04	90.45±6.72	21.75±1.77	72.47±5.85	0	>24

Table 5. Fitting of in vitro release data of VerHCl from alginate beads to different kinetic models and Korsmeyer - Peppas model

Formula code	Zero order	First order	Higuchi-diffusion	Hixson-Crowell	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	R^2	n
F1	0.9907	-0.9836	0.9587	0.9862	0.9383	0.6758
F2	0.9995	-0.9725	0.9868	0.9886	0.9951	0.8274
F3	0.9949	-0.9938	0.9888	0.9950	0.9911	0.9275
F4	0.9884	-0.9661	0.9971	0.9906	0.9885	0.8459
F5	0.9991	-0.9967	0.9831	0.9978	0.9918	0.7712
F6	0.9980	-0.9583	0.9855	0.9832	0.9858	0.7404
F7	0.9984	-0.9864	0.9804	0.9922	0.9930	0.8050
F8	0.9977	-0.9408	0.9908	0.9791	0.9866	0.7030

Table 6. Predicted values and actual values of responses of the optimized formula along with the prediction interval and percent deviation

Response	Predicted values for optimum formula (F9)	Two-sided 95% prediction interval		Actual values for optimum formula (F9)	Percent deviation
		Low	High		
Mean diameter (Y_1)	2.35	2.13	2.57	2.36	0.42
Drug loading% (Y_2)	13.92	11.99	15.85	13.60	2.30
EE% (Y_3)	64.63	57.23	72.03	60.11	6.99
%Drug released at 1 hr (Y_4)	10	7.82	12.18	10.16	1.6
% Drug released at 5 hr (Y_5)	35	26.68	43.32	34.28	2.08
% Drug released at 8 hr (Y_6)	55	46.18	63.82	53.97	1.87

gel form. The beads were retained on the mucosal surface of stomach in the three rats at 1 hr and 5 hr. At 8 hr, a mild black stain was still observed in the body region of stomach in the three rats. A Photo of one of the three rats at each time interval was incorporated in **Figure 9** to illustrate the gastroretention behavior of F9 over time.

Estimation of flowability of the optimized VerHCl loaded alginate beads

The angle of repose of the optimized F9 formula was found to be 22.67±1.53º.

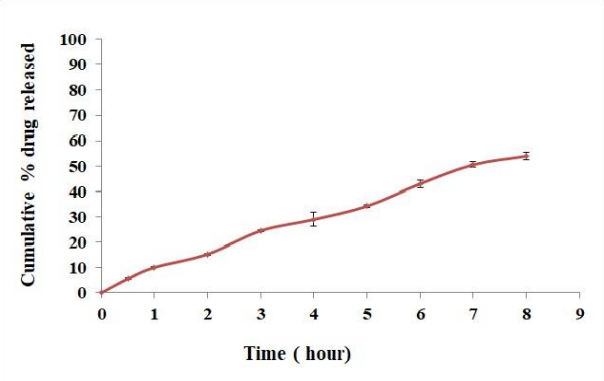

Figure 7. The *in vitro* drug release profile of optimized floating beads in simulated gastric fluids (0.1N HCl)

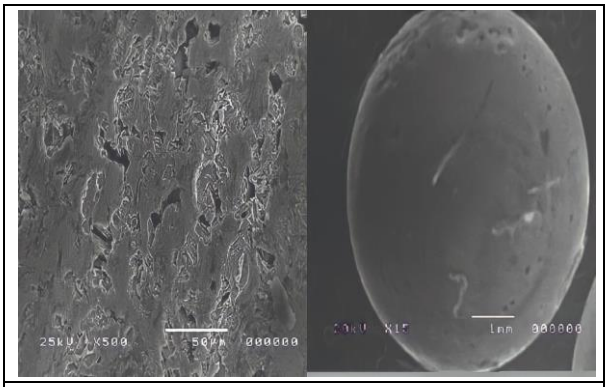

Figure 8. Scanning electron microscope image of the optimum VerHCl loaded alginate beads (F9)

DISCUSSION

In the present study, VerHCl loaded alginate beads were simply prepared without the need of complex machinery using ionotropic emulsion gelation method. When an emulsion of paraffin oil and alginate solution was dropped into calcium chloride solution, spherical beads were then formed instantaneously through formation of intermolecular cross-links between the divalent calcium ions and the negatively charged alginate molecules (egg box model-based gelation) [38].

The 2^3 factorial design recommended a total of 8 formulations for 3 independent factors: oil concentration (X_1), polymer concentration (X_2), and drug to polymer ratio(X_3), all of which differed at low level (-1) and high level (+1).The influences of these independent factors were studied on mean diameter (Y_1), drug loading% (Y_2),EE% (Y_3) and % drug released at 1, 5 and 8 hr (Y_4, Y_5 and Y_6, respectively).

Liquid paraffin was selected as oil phase due to its low density (0.86 g/cm^3) which could help the beads to become buoyant [39] and also due to its superior effect in sustaining the release of a hydrophilic model drug from alginate beads compared to many other vegetable oil[9].It was reported that when light liquid paraffin concentration was increased to 30%w/v, the emulsifying power of alginate would get limited and oil began to leak from the beads. Moreover, it was found that at least 15 % w/wof paraffin oil was required to provide beads with adequate buoyancy without long floating lag time and to avoid drug diffusion in surrounding medium during gelation of beads [39]. Thus, in this study, the two selected levels of oil concentration to be studied were 15%w/v (low level) and 20%w/v (high level).

The eight formulations of beads (F1-F8) were successfully prepared with yield % higher than 80% and showed immediate floating behavior without floating lag time and the floating behavior could extend for a period of time exceeds 24 hr.This could be attributed to the incorporation of paraffin oil which helped in the creation of low dense mass able to float over 24 hr in 0.1 N HCl (pH 1.2)[28].

The behavior of drug release from the prepared formulations followed sustained pattern over 8 hr. The *in vitro* drug release from (F1, F2, F5, F6, F7 and F8) was best fitted zero order release kinetics indicating that these formulations were able to release equal quantity of VerHCl per unit of time which is the best manner of drug release to achieve a delayed effect[40].The release data of F3 was fitted to Hixson-Crowell cube root law which describes the dissolution controlled release from systems where there is a change in surface area and diameter of the particles [41]. The formula F4 was most fitted to Higuchi diffusion model where the drug diffuses at a slower rate as the distance for diffusion increases, which is referred as square root time dependent kinetics. The values of Korsmeyer-Peppas model release exponent (n) indicated that the suggested mechanism of the drug release from all formulations was non Fickian anomalous transport where the release is controlled by a combination of diffusion and swelling and relaxation. The exception was F3 for which the suggested release mechanism was super case II where the release is governed by the macromolecular relaxation of the polymeric chains.

Analysis of factorial design

Effect of variables on mean diameter (Y_1)

Factorial ANOVA revealed that oil concentration (X_1), polymer concentration(X_2) and drug to polymer ratio(X_3) had significant (P<0.05) positive effects on the mean diameter of beads. The increase in oil concentration from 15% to 20%w/v caused significant (P<0.05) increase in the diameter of beads. This observation was in accordance with the results reported by Choudhury and Kar [9] and Talukader et al [42]. This might be due to the longer time consumed by high

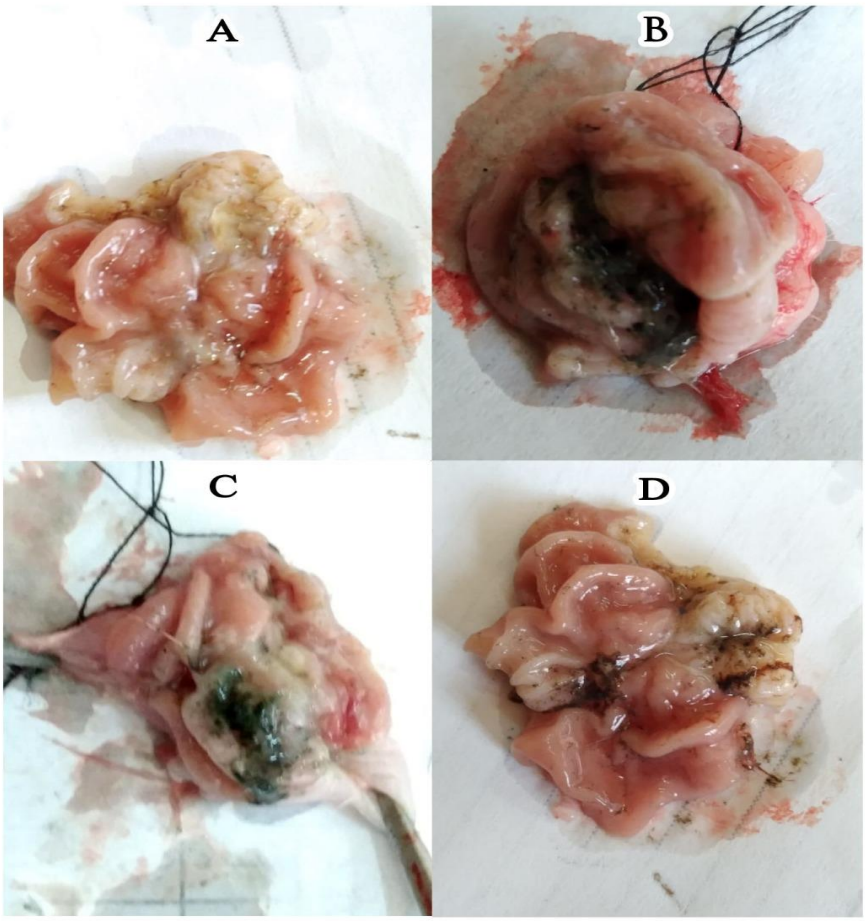

Figure 9. Photos of rat stomach A) without administration of stained F9 (control), B) at 1 hour following stained F9 administration, C) at 5 hours following stained F9 administration and D) at 8 hours following stained F9 administration

oil concentrations for evaporation upon beads drying leading to conservation of the original size of beads [9]. The positive effects of X_2 and X_3 on beads diameter could be attributed to the role of these factors in increasing the entrapment of the drug into the beads. This role will be discussed in details under the section; effect of variables on drug loading (Y_2) and EE% (Y_3).

Effect of variables on drug loading % (Y2) and EE% (Y_3)

VerHCl was successfully incorporated into the prepared eight formulations of alginate beads. The drug loading % ranged from 8.85±0.64 to 26.80±0.85% and the EE% ranged from 54.69±2.16 to72.47±5.85%. The achieved high drug entrapment into the prepared beads could be attributed to the porous nature of the gel bead matrix [43]. On increasing oil concentration, EE% was found to be increased due to the barrier action of oil that prevented water soluble drug diffusion [15].Also, higher polymer concentration led to increased drug loading% and EE% due to increased viscosity of the resulted gel and formation of more rigid polymer network which prevented the drug from diffusing back into the cross linking solution [44].Moreover, the increased drug to polymer ratio played a role in increasing drug loading% and EE% because of presence more available drug to be encapsulated [45]. ANOVA analysis revealed that oil concentration, polymer concentration and drug to polymer ratio were significantly (P<0.05) affecting EE% in a positive manner.

Effect of variables on % drug released at 1(Y_4), 5 (Y_5) and 8 hr (Y_6)

The results of factorial design analysis indicated that the studied formulation factors (oil concentration, polymer concentration and drug to polymer ratio) had main positive effects on % drug released at 1, 5 and 8 hours. In this study, when oil concentration (X_1) increased from 15 to 20%w/v, the % drug released increased (positive effect). This result was

in contrast with previously reported data in literature as it was reported that increasing oil concentration from 15 to 20% w/v would retard drug release (negative effect) because increasing oil would form an additional diffusional barrier for the drug release resulting in slow release of drug from beads [9, 39]. Moreover, the drug might stay saturated and distributed in the oil resulting in a drug-oil distributed network [46].

Also, when polymer concentration(X_2) was increased from 3 to 5% w/v, the % drug released was increased. This result did not match the data found in literature which showed that drug release was slowed down with increasing polymer concentration due to formation of thicker viscous polymer layer around the drug particles leading to more effectively the polymer would hold the drug [39].

Since a significant interaction could often mask the significance of main effects, thus, the deviation of our results from the other publications could be normalized by the presence of significant (P<0.05) interactions between the studied factors (both the two way and the three-way factor interactions) as revealed from polynomial equations of the studied responses.

Optimized VerHCl loaded alginate beads

The optimized formula (F9) suggested by numerical optimization composed of oil concentration (X_1) = 17.20 %w/v, polymer concentration (X_2) = 4.34 %w/v and drug to polymer weight ratio=1.20. This formula showed acceptable characteristics in terms of yield %, drug loading %, EE%, in vitro floating and in vitro drug release. The appearance of black-colored gel of stained F9 beads in rat stomach over 8 hr confirmed the gastroretentive properties of F9. The reduced amount of black colored gel observed in rat stomach at 8 hr compared to amount observed at 1 and 5 hr, could be due to the dissolution of the majority of gel matrix of beads under *in vivo* conditions.

The angle of repose indicated excellent flow properties of F9 beads which could be attributed to their spherical shape. However, it will be difficult to fill F9 beads into capsules because their drug loading % is 13.6±0.89%. Thus, for filling beads equivalent to 120 mg (human single dose for average body weight 60 Kg), this will require large size capsules which will not be suitable for swallowing. Thus instead, it is recommended for F9 beads to be filled in sachets.

CONCLUSION

VerHCl loaded alginate gastroretentive beads could be simply prepared using cost effective iontropic emulsion gelation technique. The optimized formulation F9 showed high % yield and EE%, immediate in vitro floating and sustained in vitro release. The in vivo gastroretention performance of F9 in rats was satisfactory and promising. However, *in vivo* bioavailability studies are recommended to confirm this result.

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Conflict of interest:

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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