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Research Article

Statistical Approach for Assessing the Influence of Calcium Silicate and HPMC on the Formulation of Novel Alfuzosin Hydrochloride Mucoadhesive-Floating Beads as Gastroretentive Drug Delivery Systems

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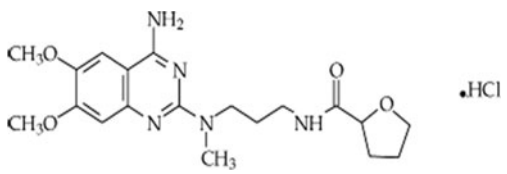
Abstract. Multiparticulate floating drug delivery systems have proven potential as controlled-release gastroretentive drug delivery systems that avoid the "all or none" gastric emptying nature of single-unit floating dosage forms. An objective of the presence investigation was to develop calcium silicate (CaSi)/calcium alginate (Ca-Alg)/hydroxypropyl methylcellulose (HPMC) mucoadhesive-floating beads that provide time- and site-specific drug release of alfuzosin hydrochloride (Alf). Beads were prepared by simultaneous internal and external gelation method utilizing 3^2 factorial design as an experimental design; with two main factors evaluated for their influence on the prepared beads; the concentration of CaSi as floating aid (X_1) and the percentage of HPMC as viscosity enhancer and mucoadhesive polymer (X_2), each of them was tested in three levels. Developed formulations were evaluated for yield, entrapment efficiency, particle size, surface topography, and buoyancy. Differential scanning calorimetry, Fourier transform infrared spectroscopy, *in vitro* drug release, as well as *in vitro* mucoadhesion using rat stomach mucosal membrane were also conducted. Percentage yield and entrapment efficiency ranged from 57.03% to 78.51% and from 49.78% to 83.26%, respectively. Statistical analysis using ANOVA proved that increasing the concentration of either CaSi or HPMC significantly increased the beads yield. Both CaSi and HPMC concentrations were found to significantly affect Alf release from the beads. Additionally, higher CaSi concentration significantly increased the beads diameter while HPMC concentration showed significant positive effect on the beads mucoadhesive properties. CaSi/Ca-Alg/HPMC beads represent simple floating-mucoadhesive gastroretentive system that could be useful in chronopharmacotherapy of benign prostatic hyperplasia.

KEY WORDS: alfuzosin hydrochloride; calcium silicate; gastroretentive; mucoadhesive-floating beads.

INTRODUCTION

There is a strong suspicion among urologists that the prevalence of benign prostatic hyperplasia (BPH) is higher than that reported in clinical studies. In a large community-based survey, the prevalence rate of lower urinary tract symptoms (LUTS) secondary to BPH were reported in 25% of men aged over 40 years. Such symptoms include increased urinary frequency; nocturia, incomplete emptying, and urinary hesitancy (1,2). Currently, α_1-adrenergic receptor antagonists are considered the first-line therapy for treatment of BPH. Although most of the α_1 blockers show comparable efficacies, newer agents such as alfuzosin tend to demonstrate improved selectivity for the prostate and bladder with fewer vasodilatory effects and tolerability advantages over older α-blocking compounds (3,4).

Alfuzosin hydrochloride (Alf) is a selective and competitive α_1-adrenoceptor antagonist that is approved by the Food and Drug Administration (FDA) for the treatment of symptomatic BPH. Its main advantage over other drugs of the same class is that it distributes preferentially to the prostate leading to decreased sympathetically controlled tone of prostatic smooth muscle, as a result, LUTS related to BPH significantly improves (5). Alf is a highly water-soluble compound with the structure (6,7):



Alf shows absolute bioavailability around 25% under fasting condition and is raised to about 49% under fed condition; which suggests that food has significant impact on the oral absorption and bioavailability of Alf mainly through the prolongation of gastric residence time. In addition, alfuzosin has short half-life (3.8 h) that might be due to either preferential absorption in the proximal part of the intestine, in particular, the duodenum and the jejunum or due to its extensive metabolism into the number of inactive products (6,8–10). Consequently, prolongation of gastric residence time of Alf

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would suggest continuous delivery from the stomach to the absorption sites in the upper part of the intestine, resulting in improved oral bioavailability.

One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the gastrointestinal tract (GIT) is to control the gastric residence time (GRT). Gastroretentive dosage forms (GRDFs) provide important therapeutic options as they can remain in the gastric region for several hours, therefore, significantly prolong the gastric residence time of drugs (11). GRDFs are especially of particular interest for drugs which are (a) locally acting in the stomach, (b) drugs that are mostly absorbed from the stomach or proximal parts of GIT, (c) drugs having narrow window of absorption, (d) drugs that are poorly soluble at the intestinal alkaline pH, and (e) are unstable in the intestinal or colonic environment (12,13). Various approaches have been proposed for delaying the gastric residence time and thus achieving gastric retention; such approaches include floating systems, mucoadhesive systems that adhere to the stomach mucosal surfaces, swellable and expanding systems that increase in size after swelling and thus retarded from passing through the pylorus, and sedimentation high-density systems (13–15).

Generally, floating drug delivery systems (FDDS) should have a bulk density lower than gastric fluids (density, ≤1.0 g/ml), and thus, they float over the gastric content for prolonged periods of time, without affecting the gastric emptying rate. By remaining buoyant over the gastric contents, FDDS retain the dosage form at the site of absorption slowly releasing the drug at the desired rate, leading to enhancement of the bioavailability. After drug is released, the residual system is emptied from the stomach. Not only do FDDS improve the bioavailability of the administered drug, but they also reduce the variability in bioavailability through reducing the dosing frequency and minimizing fluctuations in plasma drug concentration at steady state by delivering drug in a controlled and reproducible manner (9,11,14–17). Both single-unit systems (tablets or capsules) and multiple unit systems (multiparticulate systems such as beads, microspheres, and microbaloons) have been reported in the literature (13,15); however, multiple-unit FDDS offer several advantages over monolithic ones. Single-unit FDDS such as tablets and capsules are unreliable in prolonging the GRT owing to their "all-or none" emptying process; also, they might be obstructed in the gastrointestinal tract or stick together leading to potential danger. In contrast, multiple-unit particulate dosage forms, such as beads, microspheres, and microbaloons, possess several advantages such as (a) they are dispersed as individual units thus they are not subjected to all or none effect, (b) the risk of dose dumping is reduced, (c) they pass uniformly through the GIT providing an adjustable release, thereby, reducing the intersubject variability in absorption and risk of local irritation, (d) they exhibit longer reproducible GRT, and (e) GI side effects are minimized (12,18–20).

Several techniques were utilized to formulate multiple units FDDS such as inclusion of low density oil, gas forming agents, or highly porous low density polymers (13). Calcium silicate (CaSi) has been utilized as a floating and sustained release carrier for the development of floating multiparticulate systems as it is characterized by highly porous structure with lots of pores and large individual pore volume (12,17,18,21,22). Jain *et al.* (23) achieved a prolonged gastric residence time of over 6 h when prepared Orlistat floating microspheres utilizing calcium silicate as porous carrier, and Eudragit S as polymer by solvent evaporation method. Also, preparation of floating repaglinide microspheres containing calcium silicate as porous carrier and Eudragit S as polymer resulted in more than 80% of the particles kept floating for at least 10 h (12). Javadzadeh *et al.* (17) compared $NaHCO_3$ gas-forming beads and silicate-based beads and observed that although the beads containing $NaHCO_3$ were more buoyant than those of calcium silicate, the silicate-based beads showed slower release pattern, compared to the gas-forming based beads probably owing to the fact that the $NaHCO_3$ produced larger pores than those of silicate-treated ones.

The present study was investigated with an aim to develop formulations of Alf-loaded calcium alginate (Ca-Alg) mucoadhesive-floating beads containing highly porous CaSi as floating aid and hydroxypropylmethyl cellulose (HPMC) as both viscosity adjusting and mucoadhesive agent. A 3^2 full-factorial design was used in the development of the floating beads in order to evaluate the effects and interactions of the two factors (CaSi and HPMC concentrations) on different dependent variables of the developed beads. The floatable beads were subjected to thorough *in vitro* investigations as well as novel modified *in vitro* muco-adhesiveness evaluation using rat mucosal membrane.

MATERIALS AND METHODS

Materials

Alfuzosin HCl was a kind gift from Amriya for Pharmaceutical industry (Alexandria, Egypt), Calcium silicate was obtained from Riedel-de Haën (Sigma-Aldrich laborchemikalen, GmbH, Germany). Alginic acid sodium salt was obtained from Sigma (St. Louis, MO, USA) and HPMC was a kind gift from COLORCON (METHOCEL™ K100M Premium, COLORCON, UK). Calcium chloride and magnesium stearate were from ADWIC (Elnasr Pharmaceutical Chemicals Co., Egypt). All other reagents and chemicals used were of analytical grade.

Preparation of Alf-loaded Mucoadhesive-floating Beads

Preparation of Alf-Adsorbed Calcium Silicate Dispersion

In order to adsorb Alf into the pores of the CaSi powder, an accurately weighed quantity of alfuzosin (250 mg) was dissolved in 50 ml acetone, then, the calculated amount of CaSi (1, 2, or 4% *w*/*w* of the final dispersion) was dispersed into the drug solution while shaking. The chalky white suspension was subsequently stirred at 500 rpm for 2 h followed by sonication for 10 min to ensure that the drug solution was imbibed into the pores of the porous CaSi carrier and adsorbed on it. The acetone was then allowed to evaporate while stirring until only 10-ml Alf-adsorbed CaSi suspension remained.

Preparation of the Mucoadhesive-Floating Beads

The Ca-Alg beads were prepared by conventional ionotropic gelation method (24–26) with slight modifications. Sodium alginate (Na-Alg, 2% *w*/*w* of the final dispersion) was dissolved in 40 ml distilled water, then, magnesium stearate (MgSt, 3% *w*/*w*) was added with continuous stirring for 5 min. Various HPMC concentrations (0%, 0.15%, and 0.3% *w*/*w*)

were added according to the factorial design with slow stirring for 10 min followed by addition of the previously prepared Alf-adsorbed CaSi suspension. The dispersion was stirred for additional 30 min to ensure homogeneous dispersion of the whole system. The exact compositions of the beads formulations are presented in Tables I and II.

The resulting drug–polymer dispersion was then extruded through a 19 G needle dropwise into 100 ml of stirred 2% calcium chloride ($CaCl_2$) solution with an approximate extrusion flow rate of 5 ml/min. The extrusion and dispersion were carried out with gentle stirring to allow internal gelation. Stirring of the gelled beads was subsequently continued for further 30 min in order to cure the beads, *i.e.*, ensure the formation of insoluble Ca-Alg beads due to simultaneous internal as well as external gelation on the outer surface of the droplets (17,27). Since the reaction between calcium ion and alginate is moderately rapid ($k = 11.6\ min^{-1}$), and completed within approximately 30 min (28,29), the beads curing time was limited to 30 min to reduce the drug loss by diffusion into the surrounding medium (Alf is a highly water-soluble drug), which directly affects the EE. Beads were then collected by filtration, washed twice with distilled water, and then dried at room temperature in desiccators over fused $CaCl_2$ for 24 h. The schematic presentation of the stepwise formulation of Alf-loaded mucoadhesive floating beads is given in Fig. 1.

Experimental Design

Ca-Alg beads containing Alf-adsorbed CaSi were prepared based on the 3^2 full-factorial design allowing a synchronized assessment of two formulation variables and their interactions. Concentration of CaSi as Alf-adsorbing agent and floating aid (X_1) and concentration of HPMC as viscosity adjusting agent, drug dispersing agent, and mucoadhesion facilitator (X_2) were selected as two independent variables. Three levels of each variable (determined from preliminary studies) were selected and nine formulations were prepared using different levels of variables (details outlined in Table I). A polynomial equation (Eq. 1) was used to study the effect of variables on different evaluation responses (Y), where the coefficients in the equation (β_0, β_1, β_2, and β_{12}) were related to the effects and interactions of the factors.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \qquad (1)$$

Where β_0, the intercept, is the arithmetic average of all quantitative outcomes of the nine runs, β_1 and β_2 coefficients of factor X_1 and X_2, respectively, and β_{12} the coefficient of interaction of X_1 and X_2. The interaction (X_1X_2) shows how the dependent variable changes when two or more factors are simultaneously changed.

All experimental data were analyzed statistically according to the established factorial design using Design-Expert software (Version 7, Stat-Ease Inc., Minneapolis, USA). The responses for statistical evaluation assessed various responses such as percentage yield; beads size and buoyancy; percentage Alf released after 3, 6, and 9 h; and *in vitro* mucoadhesion of the beads.

Table II. Formulation Composition of the Investigated Alf-Floating Beads (percent *w*/*w*)

Formula number	Percent composition (% *w*/*w*)[a]					
	Alfuzosin	Ca Si	HPMC	Na-Alg	Mg-stearate	$CaCl_2$
A1	0.125	1	0	2	3	2
A2	0.125	1	0.15	2	3	2
A3	0.125	1	0.3	2	3	2
A4	0.125	2	0	2	3	2
A5	0.125	2	0.15	2	3	2
A6	0.125	2	0.3	2	3	2
A7	0.125	4	0	2	3	2
A8	0.125	4	0.15	2	3	2
A9	0.125	4	0.3	2	3	2

[a] Percentage of the final formulation

Table I. 3^2 Factorial Design Showing Different Formulations with their Respective Percent Yield, Percent Entrapment Efficiencies, and Average Bead Diameter

Formula number	Independent variables levels in coded form		Results		
	X_1	X_2	% Yield±SD[a]	%DEE[b]±SD	AD (mm)[c]±SD
A1	−1	−1	58.67±3.9	58.24±2.9	1.46±0.13
A2	−1	0	59.91±6.9	65.41±6.5	1.40±0.14
A3	−1	+1	70.48±6.9	70.51±3.7	1.35±0.18
A4	0	−1	57.03±6.1	74.03±3.1	1.68±0.3
A5	0	0	62.51±10.9	83.26±3.5	1.74±0.17
A6	0	+1	74.97±12.4	73.05±6.5	1.85±0.22
A7	+1	−1	62.18±2.3	49.78±5.4	1.85±0.26
A8	+1	0	72.57±4.7	73.78±12.1	1.88±0.14
A9	+1	+1	78.51±4.5	63.46±1.9	2.11±0.2

The codes in the table symbolize Factor	Level used		
	−1	0	1
X_1: CaSi concentration (%)	1%	2%	4%
X_2: HPMC concentration (%)	0	0.15%	0.3%

[a] *SD* standard deviation from the mean, [b] *DEE* drug entrapment efficiency, and [c] *AD* average beads diameter

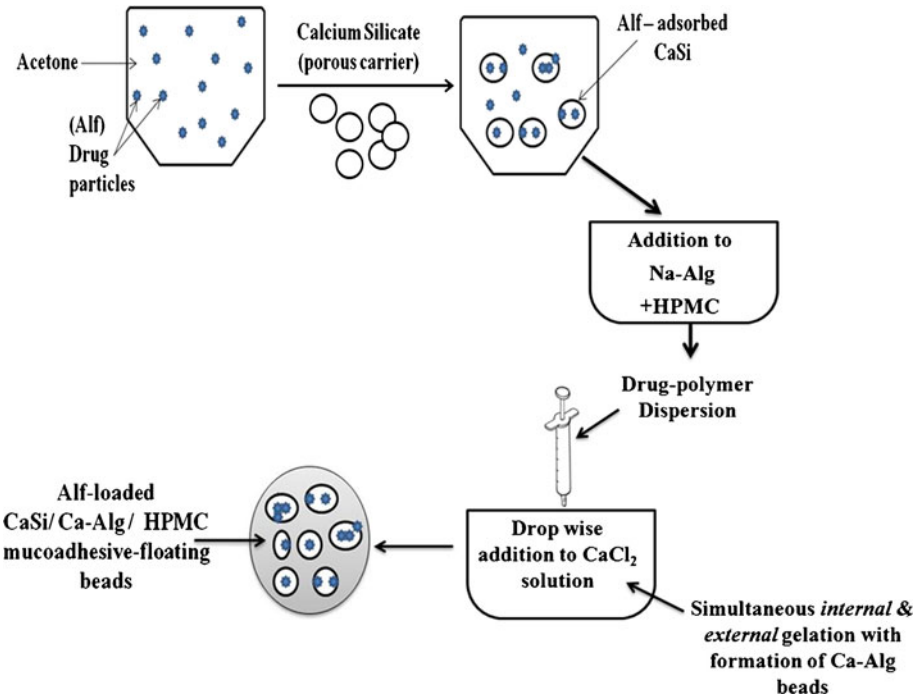


Fig. 1. Schematic presentation of the stepwise formulation method of Alf-loaded CaSi/Ca-Alg/ HPMC mucoadhesive floating beads

Characterization of Alf-Loaded Mucoadhesive-Floating Beads

Determination of Beads Yield and Drug Entrapment Efficiency

Beads were weighed after drying and percent beads yield was calculated. To determine drug entrapment efficiency (DEE), 50 mg of accurately weighed Alf-loaded beads were crushed in a glass mortar, and then dispersed in about 50 ml pepsin-free simulated gastric fluid (SGF) pH 1.2. The dispersion was stirred using a magnetic stirrer at 400 rpm overnight then sonicated for 30 min to ensure complete liberation of the entrapped drug from the beads. The dispersion was then filtered, sufficiently diluted, and spectrophotometrically analyzed at 244 nm (Shimadzu, model UV-1601 PC, Kyoto, Japan). Determinations were done in triplicate then beads percentage yield and DEE were calculated according to Eqs. (2) and (3):

$$\% \text{ yield} = \left(\frac{\text{total weight of dry floating beads}}{\text{total weight of all solid ingredients}} \right) \times 100 \quad (2)$$

$$\% \text{ DEE} = \left(\frac{\text{Actual drug contents in beads}}{\text{Theoretical drug contents in beads}} \right) \times 100 \quad (3)$$

Image Analysis and Particle Size Characterization

Thirty beads of each batch were taken for particle size analysis. The images of the developed beads were captured using digital camera (Samsung TL100 12.2MP, Samsung, USA) and analyzed for their particle size using *Image tool* software and the mean diameter was calculated arithmetically. The readings were average of three determinations±SD.

Morphological Analysis and Surface Topography

Surface topography as well as cross-sections of the beads were observed *via* scanning electron microscopy (SEM) using JEOL (JXA-840A) Electron Probe Microanalyzer (Japan Electron Optical Laboratory, Tokyo, Japan). In preparation of SEM analysis, the whole and cut beads were mounted on metal grids and exposed to high vacuum during a gold-coating process, which was needed to make the samples conductive. The samples were then examined in the SEM using an accelerating voltage of 15 kV at different magnification powers.

Differential Scanning Calorimetry

To characterize the physical status of the Alf inside the beads, the thermotropic properties and phase transition behavior of the following was determined:

- Alf alone
- Ca-Alg beads loaded with Alf adsorbed on CaSi (formulation A3).
- Ca-Alg beads loaded with Alf un-adsorbed on CaSi (control).

In addition, differential scanning calorimetry (DSC) thermograms of CaSi, Na-Alg, HPMC, and physical mixture (PM) were also obtained. The samples were analyzed using Shimadzu differential scanning calorimeter, DSC-50 (Shimadzu, Kyoto, Japan). Samples of about 5 mg were sealed in aluminum pans and heated at constant rate of 10°C/min over a temperature range of 25–320°C. Empty aluminum pans were used as

references and the whole thermal behaviors were studied under a nitrogen purge.

Fourier Transform Infrared Spectroscopy

Drug-excipients compatibility study was performed using Fourier transform infrared spectroscopy (FT-IR). The infrared spectra were recorded with FT-IR spectrometer (Jasco-6100, JASCO, Japan) for Alf, Na-Alg, CaSi, HPMC, as well as mucoadhesive floating beads (formulation A4 and A6). For every sample of the above, the dried sample was ground, mixed with KBr, and dried. The dried mixture was then compressed into a thin disk using hydraulic pressing machine. The FT-IR spectra of the KBr disk was then measured over the wavelength range 4,000–400 cm^{-1}.

Buoyancy/Floating Behavior of the Beads

To evaluate the floating characteristics of the beads, 30 beads were initially placed in a flask filled with 250 ml of pepsin-free SGF (pH 1.2). The time from introducing the beads into the medium till all the beads float to the upper one third of the dissolution vessel is recorded as the *buoyancy lag time* (30). Then, the medium was stirred using magnetic stirrer at 50 rpm while the temperature was kept constant at 37°C. At hourly intervals, stirring was stopped for 2 min and the number of floating beads (percentage) was visually counted. The mean floating time was the average of three determinations. In addition, the time for which the whole formulation remained floating on the surface of the medium (duration of buoyancy) were measured simultaneously (17,19,26,29–31).

In Vitro Alf Release and Similarity Factor Analysis

In vitro release studies were performed for all the formulations in triplicate using USP II dissolution test apparatus; paddle type (Pharma Test, Germany). An accurately weighed sample of the floating beads formulations (equivalent to 10 mg Alf) was dropped into 500 ml of pepsin-free SGF (pH 1.2) maintained at a temperature of 37°C±0.5°C and stirred at a speed of 50 rpm. At predetermined time intervals lasting for 10 h, 3-ml aliquot sample was withdrawn and the volume was replaced with an equivalent amount of fresh dissolution medium. The collected samples were filtered and analyzed at λ_{max} 244 nm using a UV–visible spectrophotometer against pepsin-free SGF (pH 1.2) taken as blank. Three model independent parameters (*i.e.*, the amount of released drug after 3 h, Q3; 6 h, Q6; and 10 h, Q10) were employed to statistically compare the drug release from different formulations.

In order to confirm the similarity of the dissolution profile of the formulated beads, the similarity factors were used. For this purpose, the *in vitro* release profile of the marketed reference sustained release (SR) tablets (Xatral SR, Sanofi-Aventis, France), was performed under similar conditions as used for *in vitro* release testing of the test products for the release of Alf. The similarity factor denoted as f_2 (32) directly compares the similarity between percentage drug dissolved per unit time for the test and reference products, in this case between the floating beads and reference marketed product (Xatral SR). The f_2 is a logarithmic transformation of the sum-squared error of differences between the test T_i and reference product R_i over all time points:

$$f_2 = 50 \log \left\{ \left[1 + (1/N) \sum_{i=1}^{n} |R_i - T_i|^2 \right] \right\}^{-0.5} \times 100 \qquad (4)$$

Where N=number of time points, R_i and T_i=dissolution of reference and test products at time i.

Novel In Vitro Evaluation of the Mucoadhesiveness of the Beads

A novel method was established for evaluating the beads mucoadhesiveness based on combination and modification of the falling liquid film technique (rinsed channel method; 29,31,33–35) and the method described for measuring mucoadhesiveness of water-soluble polymers by Nakamura *et.al.,* (36) and Tadros (37). The study was conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations. The study protocol was approved by the Research Ethics Committee for experimental and clinical studies at Faculty of Pharmacy, Cairo University, Cairo, Egypt; as per the spirit of Association for Assessment and Accreditation of Laboratory Animal Care International's expectations for animal care and use/ethics committees.

Overnight-fasted male Wistar rats (200–250 g) were kept at constant temperature and humidity at the animal facility; then, the rats were sacrificed and freshly excised stomach mucosa were washed with about 500 ml of physiological saline. After 15 min, for each formulation, the rat stomach mucosa was fixed to a glass Petri dish with the cynoacrylate glue and about 30 beads (N_i) were hydrated with small amount of the pepsin-free SGF, dispersed on the mucosal tissue, and fixed by gentle by applying a light force with a fingertip for 30 s. The whole system was then placed in a constant humidity chamber adjusted to 97% relative humidity for 20 min to give time for the interaction of the beads with the mucosal surface (29,31).

The Petri dish systems were attached to USP disintegration test apparatus (DST-3, Logan Instruments Corp., NJ, USA) and moved up and down in pepsin-free SGF at 37± 0.5°C. At the lowest point, the adhering beads on the mucosal surface on the plate were immersed into the solution and got out of the solution at the highest point. At predetermined time intervals, the number of beads detached from the mucosa (N_s) were observed visually and mucoadhesion strength was calculated as percentage using the following equation (31,34):

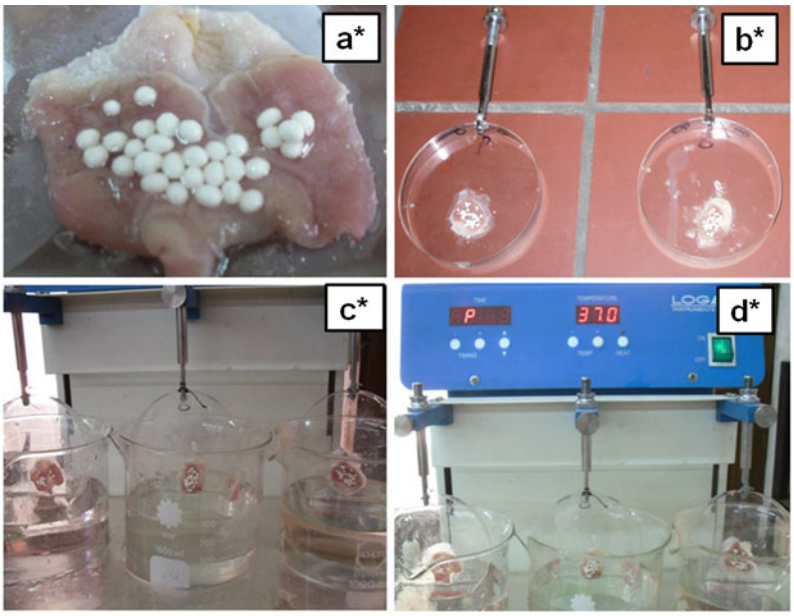
$$\text{Mucoadhesion strength}\,(\%) = \frac{N_i - N_s}{N_i} \times 100 \qquad (5)$$

The stepwise workflow of the novel *in vitro* mucoadhesion study is presented Fig. 2.

RESULTS AND DISCUSSION

Process Design and Preparation of the Mucoadhesive-Floating Beads

Multiple unit FDDS have better potential for sustained delivery of many drugs when compared with their nonfloating counterparts; as with floating multiparticulate systems, it is



***The mucoadhesion test work-flow: a) Beads were dispersed on the rat stomach mucosal tissue, and fixed by gentle finger press for 30 seconds. b) The rat stomach mucosa was fixed to a glass Petri dish with the cynoacrylate glue. c) The Petri dish systems were attached to USP disintegration test apparatus d) while the test running, at predetermined time intervals the number of beads detached from the mucosa were observed visually and percent mucoadhesion calculated.**

Fig. 2. The workflow of the novel *in vitro* mucoadhesion study of Alf-loaded floatingmucoadhesive beads in USP disintegration test apparatus

postulated that the majority of particles will remain above stomach contents for an extended time period, therefore remain in the gastric region for several hours, and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment (38,39). Since the present study aimed to enhance the gastric residence time of Alf *via* two mechanisms, floating and mucoadhesion, it is therefore reasonable to believe that this will consequently result in reducing the dosing frequency and fluctuated blood levels, prolonging its duration of action and enhancing its bioavailability.

Calcium silicate is known to posses highly porous structure with lots of pores and a large individual pore volume; it is suggested that when the air trapped in CaSi enormous pores is covered and sealed with polymer such as HPMC, the multiparticulate system floats. In addition, its hydrophilic nature improves dispersal of drug-loaded suspension in aqueous sodium alginate solution and its fine particle size allows extrusion of powder from needle bore (12,23,26,40).

Floating systems require fluids in the stomach in order to float, this might be the case in fed state; while in fasted state, there will be little fluids and a liquid given at the time of administration will empty rapidly resulting in unexpectedly shorter floating time with consequent compromised bioavailability (41). Therefore, we aimed to overcome this shortage by augmenting the floating behavior with the mucoadhesion using HPMC as mucoadhesion-enhancing material. Mucoadhesion could prolong the intimate contact time of the beads on the gastric mucosa by adhering to the surface of mucus layer; in addition, HPMC acted as viscosity adjusting and drug dispersing agent, minimizing drug loss during formulation, and also it was reported to enhance the sustained release of alginate by providing a denser inner matrix for the beads (17,30). Additionally, the hydrophobic nature of MgSt was reported to significantly improve the buoyancy of the beads and extended drug release (13,19). The decision of using the simultaneous gelation method was based on the presence of two Ca^{+2}-donating agents; CaSi and $CaCl_2$. The dropwise addition of the anionic water-soluble natural polysaccharide, sodium alginate, to the aqueous solution containing calcium ions is expected to lead to ionic interaction and intramolecular bonding between the divalent metal ions (*e.g.*, Ca^{2+}) and carboxylic acid group located on the alginic acid; such interaction is stronger than that with monovalent sodium ions. This phenomenon is extensively utilized in the development of alginate beads for sustained release of drugs (17,27).

Preliminary studies have been performed in order to optimize the concentrations of each of the selected excipients. Such preliminary studies to obtain beads were carried out using various concentrations of Na-Alg, HPMC, MgSt, and $CaCl_2$ as cross-linking medium. It was observed that increasing Na-Alg concentration above 2% or increasing HPMC above 0.5% resulted in vast increase in the viscosity of the drug-loaded Na-Alg dispersion to the extent that could block the needle and hinder the formation of drops during extrusion. Therefore, the concentrations of *Na-Alg* and *HPMC* were restricted to ≤2% and 0.3%, respectively. Also, preliminary studies showed that increasing $CaCl_2$ concentration negatively influenced the floating behavior of the beads, therefore, $CaCl_2$ concentration was maintained at 2% for the entire formulations.

Characterization of Alf-Loaded Mucoadhesive-Floating Beads

Beads' Yield and DEE

The percentage yield of Alf-loaded CaSi/Ca-Alg beads were found in the range of 57.03–78.51% and the DEE ranged from 49.78% to 83.26% as presented in Table 1. Statistical analysis of the beads yield using analysis of variance (ANOVA) proved that increasing the concentration of either CaSi or HPMC significantly increased the beads yield ($p<0.01$); however, the concentration of HPMC showed higher effect than CaSi concentration as indicated by the coefficients of X_2 and X_1, respectively (Fig. 3). Also, the interaction between the concentrations of the two excipients was insignificant. The polynomial equation, as determined by ANOVA, was found to be statistically significant with R^2 value of 0.9844:

$$\text{Beads yield}(\%) = 65.16 + 4.27X_1 + 7.68X_2 + 0.68X_1^2 + 1.91X_2^2 + 0.84X_1X_2$$

As X_1 is CaSi concentration and X_2 is HPMC concentration. The significant increase in yield with increasing CaSi concentration might be due to increased bonding and encapsulation of particles in beads. In the formulation of CaSi-based Ca-Alg beads, the beads were fabricated by simultaneous internal and external gelation resulting from the ionic interaction between Ca^{+2} ions and Na-Alg. The increased concentration of the dispersed CaSi in the alginate solution in addition to the $CaCl_2$ in the coagulation fluid provided sources for free divalent cation (Ca^{2+}) leading to increased interaction and intramolecular bonding with the carboxylic acid group located on the alginic acid and respectively caused gelation externally and internally. In addition, the hydrophilic nature of CaSi causes greater contact between solid particles and sodium alginate solution leading to higher yield percent (26,27). In addition, one of the major goals in using HPMC in the present study was to act as viscosity-enhancing agent; as the principle of gelation of Na-Alg with $CaCl_2$ is based on the formation of a tight junction between the guluronic acid residues and Ca^{2+} ion during formulation; increasing HPMC concentration will prolong the contact time between interacting moieties leading to increase in the number of the apparent cross-linking points, with consequent increase in percentage yield.

The drug entrapment efficiency increased by increasing CaSi concentration from 1% to 2%, but further increase to 4% resulted in consequent reduction in DEE. This could be explained on the bases that increased CaSi concentration to 4% resulted in enormous number of pores that facilitated drug leakage out of Alf-adsorbed CaSi particles during formulation of the beads. In addition, increasing CaSi concentration with the resultant increase in Ca^{2+} ions might lead to more cross linking and tight junctions in the beads between the guluronic acid residues and Ca^{2+} ion during the formulation that does not allow the incorporation of higher drug content. However, ANOVA test of the DEE showed that the neither the effects of CaSi nor HPMC concentrations were significant at $p<0.05$.

Particle Size Characterization, Morphological Analysis, and Surface Topography

The mean particle sizes of the beads varied for different formulations between 1.35±0.18 and 2.11±0.2 mm, and the particle size distribution of each formulation was within a narrow range as indicated by low standard deviations values in Table I. Such variation in particle sizes of the beads might be on account of many formulation factors such as concentration of the porous carrier and bead-forming polymers, viscosity of the extruded phase and dispersion medium, stirring speed, needle size, *etc.* Therefore, it is possible to prepare beads of the desired size by adjusting such parameters (17).

As indicated by the average beads' diamters and ANOVA of the results (Table I and Fig. 4), it was obvious that the beads size significantly ($p<0.0001$) increased with increasing CaSi concentration; this might be due to the fluffy low density CaSi entrapped in the beads, the more CaSi entrapped, the significantly larger will be the beads. Similar results were previously

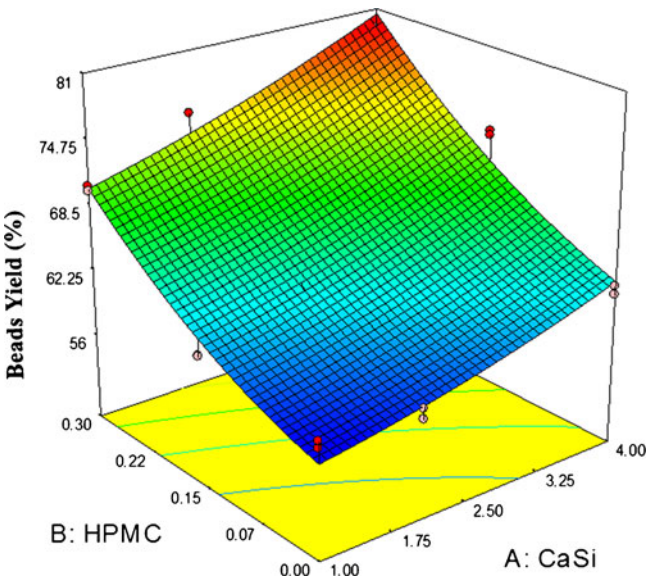


Fig. 3. Response surface plot showing the significant positive effects of both CaSi and HPMC concentrations on the beads yield

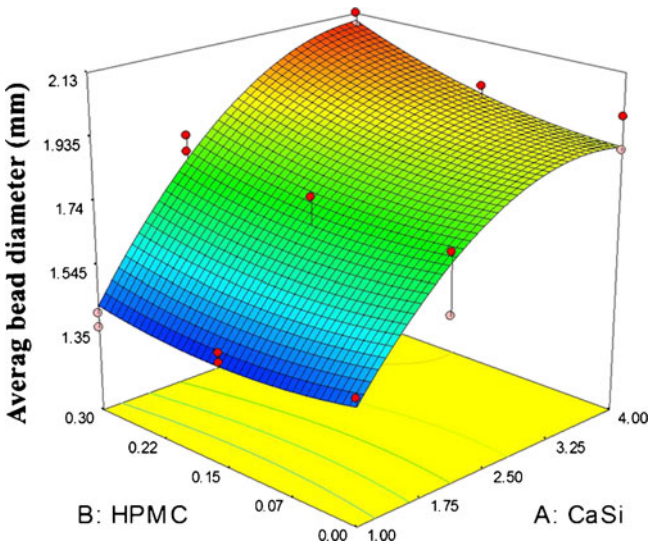


Fig. 4. 3D response surface plot declaring the significant influence of CaSi concentration on the beads diameters

observed with CaSi-based beads studies by Javadzadeh *et al.* who observed that the size of the silicate-based beads is directly related to the amount of calcium silicate (17). In addition, for high CaSi concentrations, increasing HPMC concentration was accompanied by increase in particle size, which could be attributed to an increase in the relative viscosity of the polymer solution at higher HPMC concentration leading to the formation of larger droplets during addition of the polymer solution to the cross-linking agent. However, the influence of HPMC concentration on the beads sizes was insignificant (P=0.0525).

The digital photographs of the image analysis (Fig. 5) showed that the beads were discrete and almost spherical in shape with rough surfaces. Similar results were shown in previous studies (17,26,29–31). However, formulations having higher HPMC concentration (A3 and A9) showed beads with more oblong and irregular shapes and were very sticky during filtration and drying phase. Ca-Alg beads might obtain their spherical shapes through the drop-wise addition of aqueous alginate solution to the aqueous solution containing calcium ions *via* ionic interaction and intramolecular bonding; therefore, increasing HPMC concentration to 0.3% might increased the viscosity of the coagulation fluid to an extent that drop formation was strongly impaired and the produced beads were very sticky and hardly separated from each other. Sahasathian *et al.* obtained similar results with chitosan-coated beads (29).

For more thorough assessment of the shape and surface topography of the beads, careful examination of the surface and cross-sectional SEM pictures revealed more detailed information regarding their external and internal morphological features. As demonstrated in Fig. 6, the outer surface of the beads was rough, textured with characteristic wrinkles and micropores, while the internal structure clearly showed pores, cavities, and hollow zones of various sizes. The number of observed pores appeared to be directly related to the CaSi concentration in the beads, formula A7 structure (higher CaSi concentration) showed more pores and cavities than those observed with formulae A1 and A3 due to the highly porous nature of CaSi, similar results were previously observed by Javadzadeh *et al.* (17). However, this was not clear in formula A9 due to high HPMC concentration that almost blocked the pores. Regarding HPMC concentration, comparing beads of formula A1 with formula A3 and comparing those of formula A7 with formula A9, it was observed that the increase in HPMC concentration resulted in denser and more tightly packed cavities (as observed in Fig. 6) due to the higher HPMC level permeated into the beads.

DSC and FT-IR

One of the most classic applications of DSC analysis is in the determination of the possible interactions between a drug entity and the excipients in its formulation; it is very important to establish the existence of any incompatibilities during the preformulation stage to ensure the success of the subsequent studies (42). Figure 7 presents the thermal behaviors of the pure components (Alf, CaSi, Na-Alg, and HPMC) in addition to the thermal behavior of the PM and the final Alf-loaded mucoadhesive floating beads. In the beads formulations, Alf was either loaded on CaSi (formula A3) or free Alf unadsorbed on CaSi (control). Pure Alf demonstrated sharp characteristic endothermic peak at 235.68°C corresponding to its melting temperature (43); such sharp endothermic peak signifies that Alf used was in pure crystalline state. There was also a small inflection at 298.77°C.

DSC thermogram of pure CaSi shows no peaks up to 320°C while that of Na-Alg showed characteristic exothermic peak at 242.55°C; this peak was observed but very slight in both beads formulations A3 and control at 238.59°C and 238.26°C, respectively. DSC of HPMC showed broad endotherm at 87.51°C which might be due to the volatilization of adsorbed water; such broad endotherms were also observed in both beads formulations A3 and control at 82.91°C and 79.23°C, respectively.

However, there were no apparent differences between the DSC thermograms of the beads of formula A3 and the control formula having free Alf (unadsorbed on CaSi); they both verified complete disappearance of both characteristic peaks of Alf. Usually, the complete disappearance of the drug-melting peak in DSC might indicate the drug conversion to an amorphous solid solution; *i.e.*, Alf was solubilized in acetone or water during beads formulation and then molecularly dispersed and adsorbed into the CaSi in the solubilized form (a mechanism similar to that of solid solution or liquisolid techniques). Such disappearance of the drug peaks upon formulation was in agreement with McCauley and Brittain (42) who declared that the complete suppression of all drug thermal features, undoubtedly indicate the formation of an amorphous solid solution. Moreover, Mura *et al.* (44) proved that the total disappearance of the drug melting peak indicates that drug amorphization was obtained.

FTIR studies were performed to determine interactions and structural changes in the drug and carrier used. The FTIR spectra of Alfuzosin HCl, porous carrier (CaSi), and Alf-loaded mucoadhesive beads (formulations A4 and A6) are presented in Fig. 8 with the characteristic peaks of distinction marked for both Alf

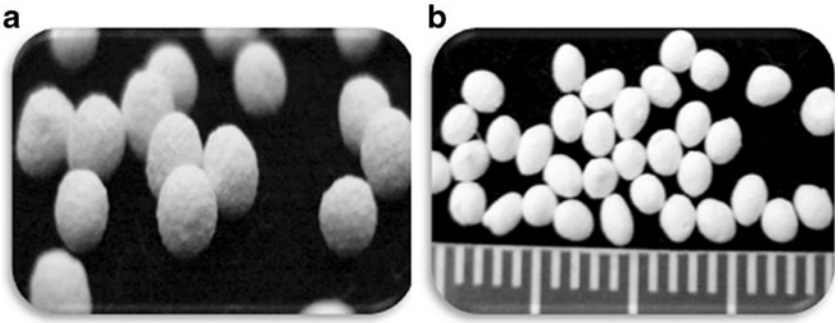


Fig. 5. Representative digital photographs used for morphological examination and particle size analysis of the beads: **a**) surface morphology of formula A7, and **b**) size analysis for formula A5 (*scale* in millimeter)

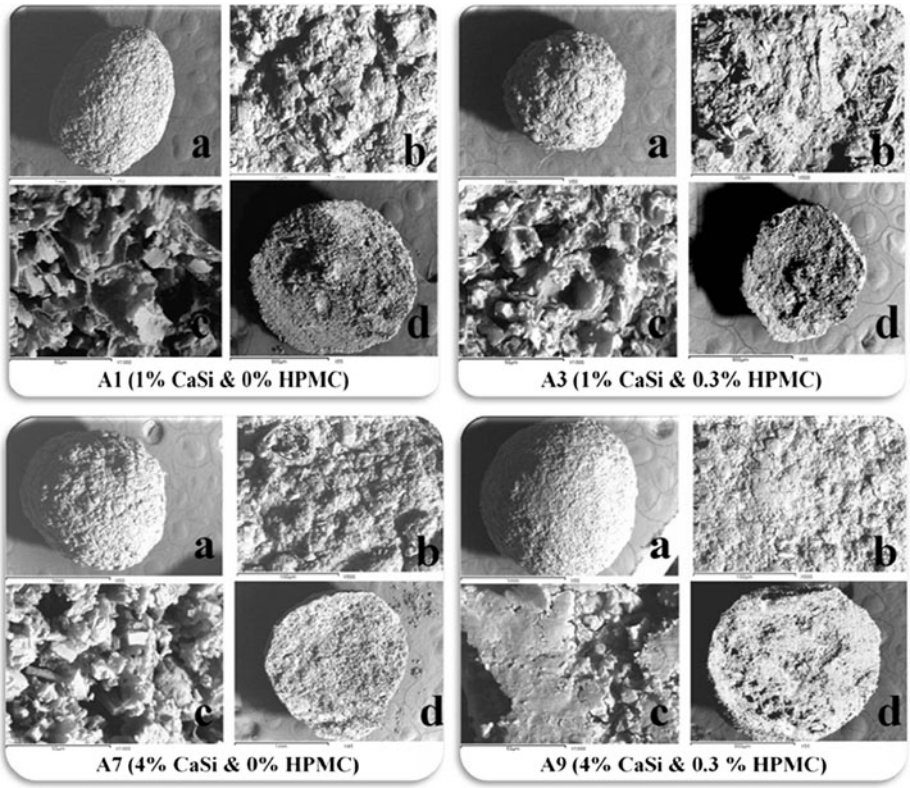


Fig. 6. Scanning electron micrographs of Alf-loaded floating-mucoadhesive beads (*A1*, *A3*, *A7*, and *A9*), for each of them: **a**) intact appearance of beads (magnification, ×50), **b**) surface of the beads (magnification, ×500), **c**) internal structure of the beads (magnification, ×1,000), and **d**) cross-section of the beads (magnification, ×55)

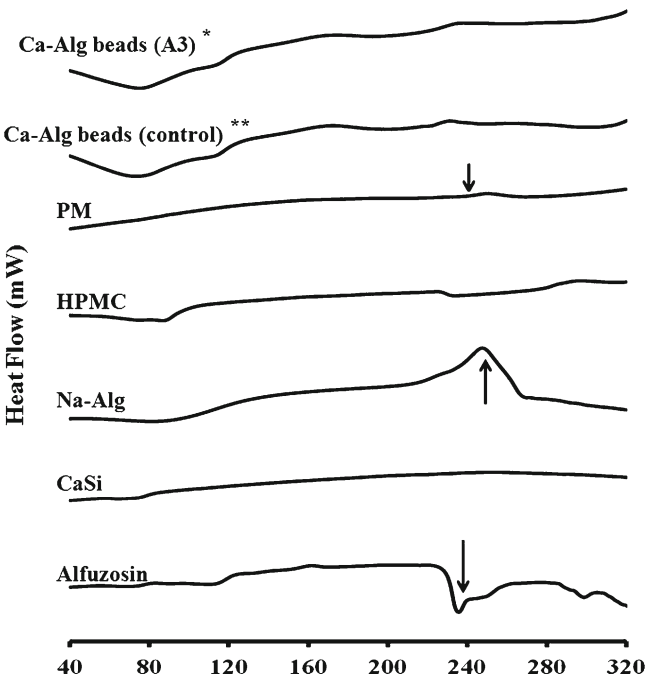


Fig. 7. DSC thermograms of pure Alf, CaSi, Na-Alg, HPMC, PM, and the floating mucoadhesive beads containing Alf either adsorbed on CaSi (*A3) or free (unadsorbed on CaSi; **control). The *curves* have been displaced vertically for better visualization

and CaSi. FTIR spectrum of pure alfuzosin HCl (Fig. 8) showed principle characteristic peaks at 3,366.13 cm^{-1} (secondary –NH), 1,590.98 cm^{-1}, and 1,499.38 cm^{-1} (C=O stretching), 2,938.03 cm^{-1} (aromatic C–H stretch vibration or aliphatic (saturated) C–H stretching bands), also some prominent bands appears in the fingerprint region ranging from 1,000 to 600 cm^{-1} such as 875.52 and 728.8 cm^{-1}. Similar characteristic peaks for Alf were previously reported (43,45). CaSi showed distinct characteristic peak at 3,446.16 cm^{-1} (Si-OH), also, such hydrogen bonding has a significant influence on the peak shape and intensity, generally causing peak broadening and shifts in absorption to lower frequencies. Another distinct peaks at 1,506.13 and 1,620.87 cm^{-1} (Si=O stretching), and at 670 and 821 cm^{-1} (Si-O and Si-O-Si) (46,47). Most of the characteristic peaks of both the drug and CaSi were also present in FT-IR spectrum of the beads (both A4 and A6 formulations) with some broadening and reduction in intensity, such results indicates no strong interaction between the drug and the polymers, also, it is suggested that the interaction between them had physical nature rather than chemical one for the adsorption and formulation processes studied.

Buoyancy (Floating Behavior) of the Beads

Buoyancy of beads is directly related to the performance of floating drug delivery systems. Most of the prepared beads formulations showed instantaneous *in vitro* floating behavior (100% of the beads floated with zero lag time); this might be

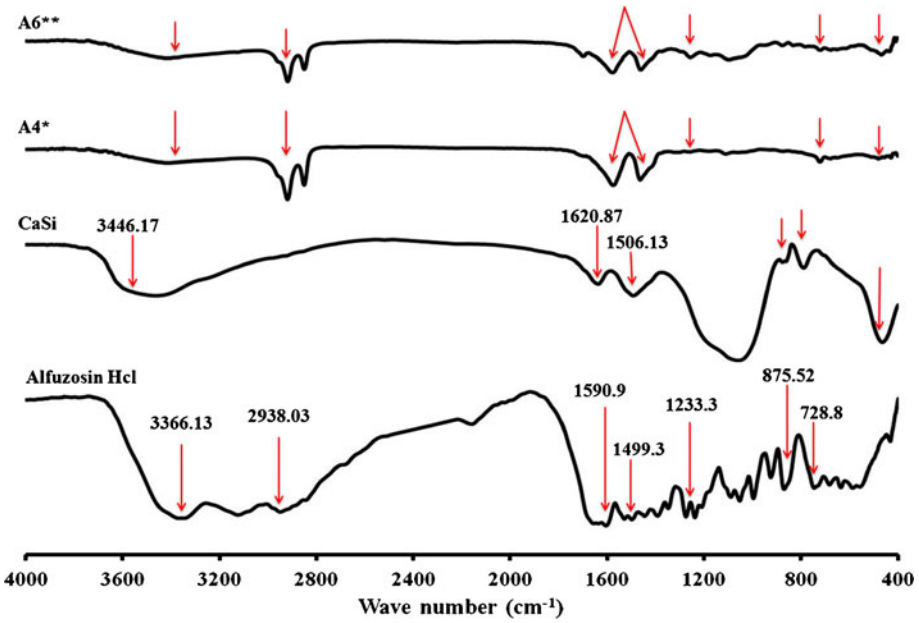


Fig. 8. FTIR spectra of alfuzosin hydrochloride, CaSi, and the floating mucoadhesive beads either HPMC-free (*A4) or containing HPMC (**A6). The curves have been displaced vertically for better visualization

due to low apparent density provided by the porous nature of CaSi. Formula A3 was an exception as it showed only 83.3% of the beads floated in the first 5 min. The floating behavior of different beads formulations over 8 h and the 3D surface plot are presented in Fig. 9a, b, respectively. From both plots, addition of HPMC (0→0.15%) resulted in initial increase in the beads buoyancy, then further increase in HPMC concentration (0.15→0.3%) resulted in corresponding decrease in percentage beads floating over time. Similar results were also obtained with CaSi. For instance, formulation A5 and A6 (containing 2% CaSi) showed the best floating behavior, 100% and 98.33% of the beads remained floating after 8 h, respectively. In order to explain such behavior, the combined effect of both CaSi and HPMC should be considered. Floating is primarily controlled by apparent density of the beads, which in turn is affected by both quantity of calcium silicate and concentration of polymer used (48). Increase CaSi concentration resulted in corresponding increase in the air-trapped porous structure that kept the beads floating. Moreover, adding HPMC caused the air trapped in CaSi enormous pores to be covered and sealed with HPMC leading to better floating behavior.

Yuasa *et al.* (40) suggested that polymer (such as HPMC) forms liquid bridges over the pores present on surface of CaSi and does not intrude completely into the pores resulting in trapped air within the granules causing floating. It might be suggested that, at higher HPMC concentration, the polymer starts to intrude inside the pores displacing the entrapped air, resulting in inferior floating, this was also proved by the fact that increase CaSi concentration resulted in wider and greater number of pores that HPMC could easily penetrate, leading also to poor floating as presented in Fig. 9. As well, swelling of HPMC when it comes in contact with the floating medium might result in further penetration into the CaSi pores o replacing the entrapped air, leading to poor floating; an effect that increase with increasing HPMC concentration. This could explain the fact that worse floating behavior is observed by increase concentration or either CaSi, HPMC, or both.

In Vitro Alf Release and f_2 Analysis

Dissolution test is a frequently used quality control method to evaluate drug release from oral dosage forms (17). To provide an effective therapy using alfuzosin hydrochloride from the mucoadhesive-floating beads, ideally it would be required to release drug rapidly in the initial stages to obtain the desired therapeutic concentration followed by slow release of the drug in order to replace the drug absorbed or destructed in the high pH of intestine. The release patterns of all mucoadhesive bead formulations are presented in Fig. 10.

When the model dependent parameters (*i.e.*, the amount of Alf released after 3 h, Q_3; 6 h, Q_6; and 10 h, Q_{10}) were statistically analyzed using ANOVA to compare the drug release from different formulations, it was found that the effects of both CaSi and HPMC concentrations were significant in the three cases ($p<0.05$). It was observed that CaSi had significant negative effect on Alf release; *i.e.*, increase CaSi concentration significantly delayed Alf release from the beads (for the amount of Alf release after 3 h, p was 0.0001; for the amount of Alf release after 6 h, p was 0.0012; and for the amount of Alf release after 10 h, p was found to be 0.009). This might be due to the highly invaginated network that will be formed at higher CaSi concentration, the drug entrapped inside would require longer time for water to penetrate the network, solubilize the drug inside and take it out, especially, when beads are coated with HPMC, which will further hinder penetration. Therefore, the release data revealed that higher CaSi concentration led to more sustainment of the release patterns compared to formulations containing lower CaSi concentrations.

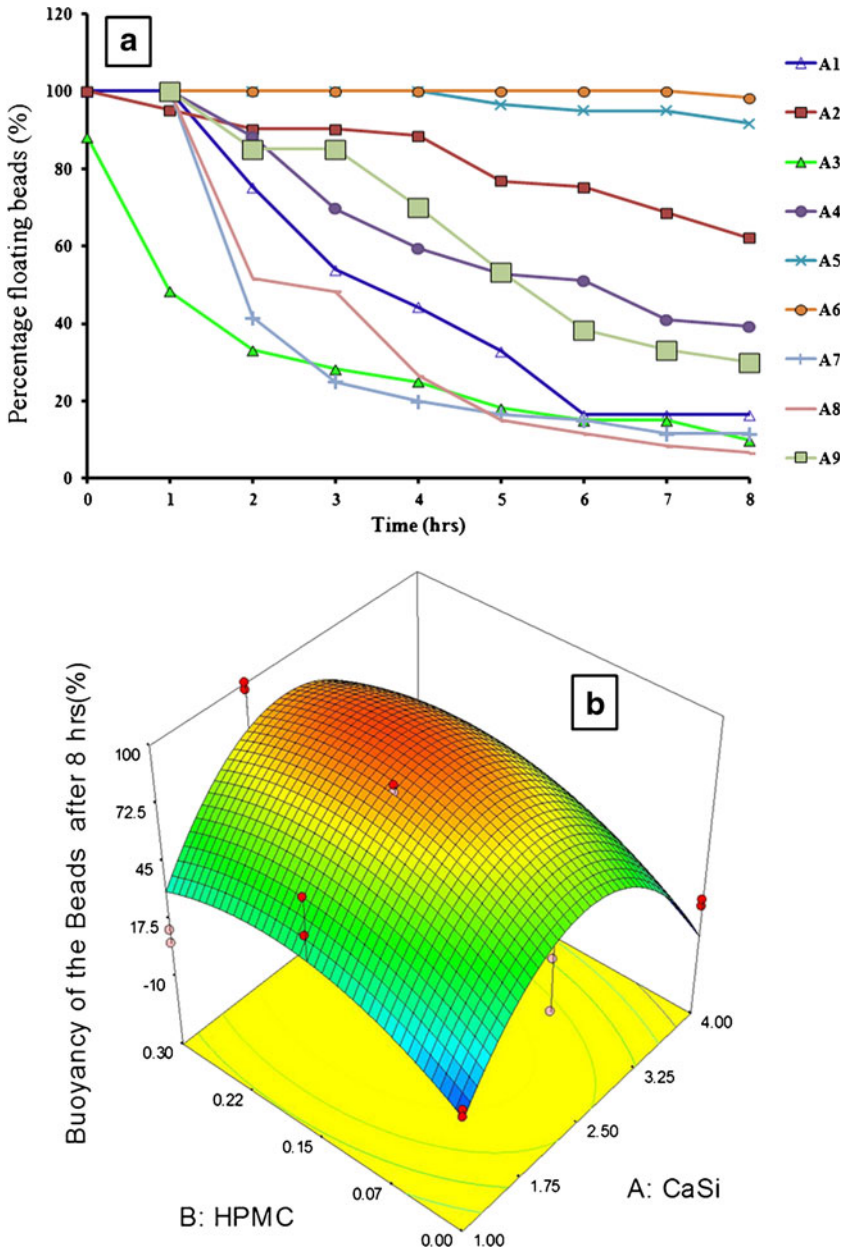


Fig. 9. Buoyancy of the beads **a**) floating behavior of different beads formulations over 8 h represented as percentage buoyancy *versus* time. **b**) 3D response surface plot showing effect of both factors (CaSi and HPMC concentration) on beads buoyancy

On the other hand, HPMC concentration showed significant positive effect of the amount of Alf released after 3, 6, or 10 h ($p<0.05$).

In all cases; Q_3, Q_6, and Q_{10}, the interaction between CaSi and HPMC concentrations was also found to show significant positive effect ($p=0.0064$ after 3 h, 0.0001 after 6 h, and 0.0023 after 10 h).

Moreover, according to the ANOVA results, the sequential model was suggested to be two-factor interaction rather than polynomial types (as quadratic model previously suggested), therefore, when X_1 is CaSi and X_2 is HPMC, the percentage of drug released after 3 h statistical equations was found to be:

$$\text{Alf released after 3 h}(\%) = 78.17 - 9.07X_1 + 4.03X_2 + 6.48X_1X_2$$

For the percentage of Alf released after 6 h, the statistical equation was:

$$\text{Alf released after 6 h}(\%) = 85.36 - 5.96X_1 + 6.84X_2 + 9.94X_1X_2$$

and that for the percentage of Alf released after 10 h was:

$$\text{Alf released after 10 h}(\%) = 90.67 - 5.07X_1 + 5.78X_2 + 7.61X_1X_2$$

Figure 11 represent the 3D surface plot presenting the significant influence of both CaSi and HPMC concentrations on the released Alf after 6 h.

In general, for the purpose of dissolution profiles comparisons, f_2 values higher than 50 (50–100) proves similarity of the dissolution profiles of the compared products (32).

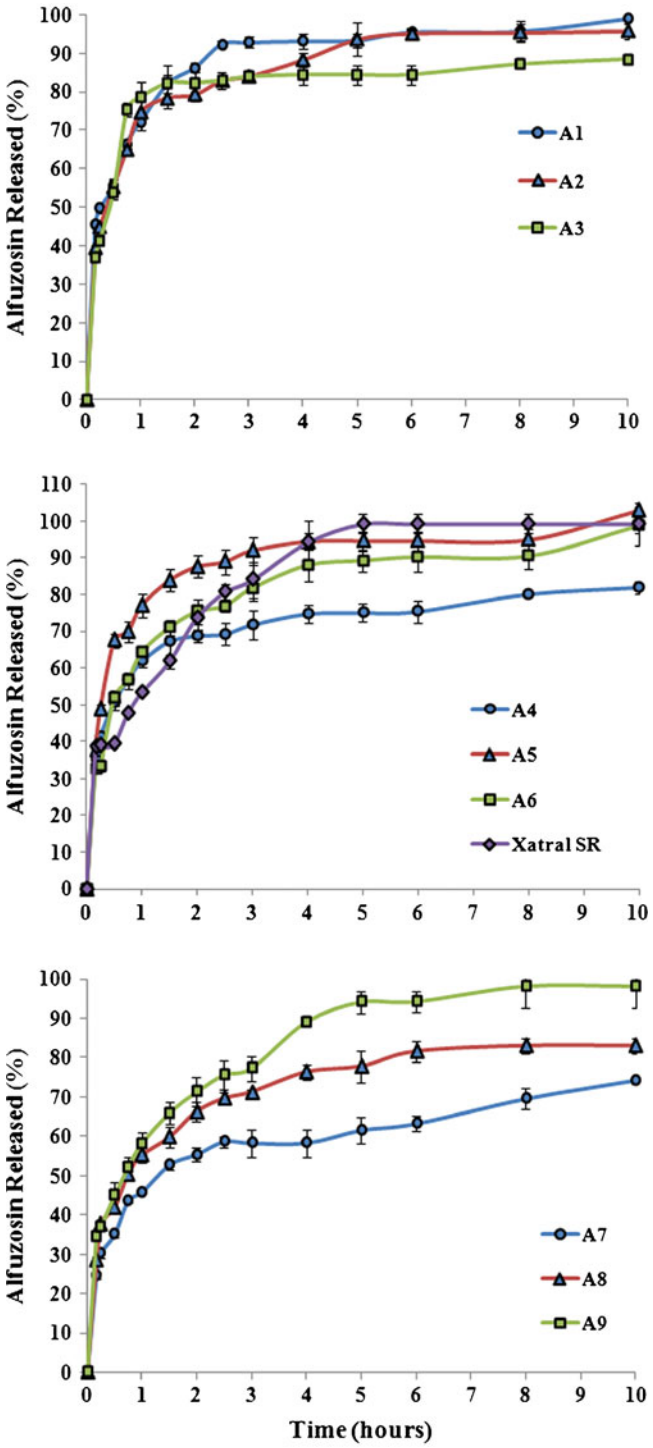


Fig. 10. *In vitro* release of Alf from different mucoadhesive floating beads formulations and the reference market product (Xatral SR)

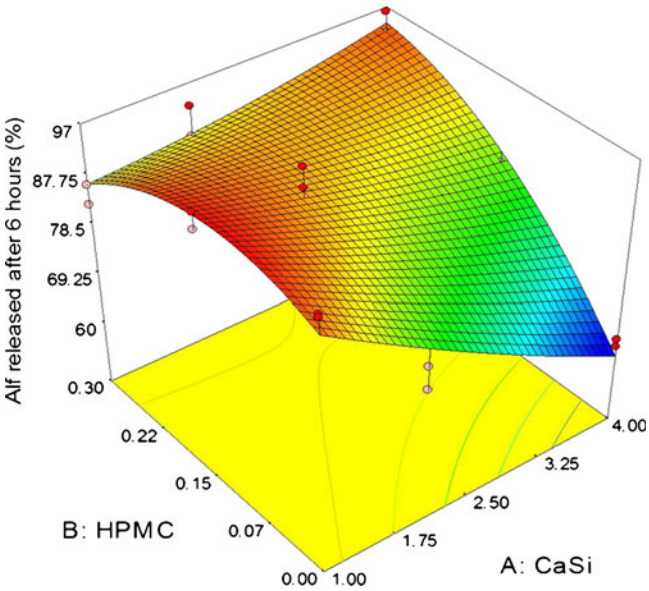


Fig. 11. Response surface plot of the influence of increased CaSi or HPMC concentration on the percentage Alf released following 6 h

The calculated f_2 value obtained in this study is presented in Table III. The results clarify that most of the prepared formulations, although presented sustained release profiles, were not similar to the reference extended release marketed product (Xatral SR). Formulations that showed f_2 values higher than 50 were A6 and A9 indicating that the dissolution profiles of both formulations were similar to the market product and drug release with the optimal formulations were not significantly faster than Xatral SR. However, since the formulation A6 showed better buoyancy behavior compared to A9, it was selected as the optimal formulation.

Novel In Vitro Evaluation of the Mucoadhesiveness of the Beads

Mucoadhesive drug delivery can improve drug effectiveness by targeting them at a specific site for longer period of time (29). Therefore, *in vitro* mucoadhesiveness of the bead formulations was tested in a novel way and the results were calculated as mucoadhesive strength (%) according to Eq. (5) and presented in Table IV. The novel method was established as a combination and modification of the falling liquid film technique (rinsed channel method) using rat or pig stomach mucosa (31,33–35) and the mucoadhesiveness measurement method by Nakamura *et al.* (36) and Tadros (37) using agar plates. They were combined by using freshly excised rat mucosal membrane fixed to the Petri plates; and after hydration of the beads, the beads were dispersed on the mucosal tissue and fixed by applying a light force with finger press for 30 s followed by hydration of the whole system for 20 min. Then,

Table III. Similarity Factor (f_2) for the Dissolution Profile Comparison between the Floating Beads and the Reference Product

Formula number	f_2 Value
A1	46.12
A2	49.37
A3	41.94
A4	42.26
A5	42.31
A6	55.51
A7	31.81
A8	45.50
A9	67.23

Table IV. Mucoadhesion Test Results of Alf-Loaded Mucoadhesive Beads

	Mucoadhesion strength (%)								
Time	A1	A2	A3	A4	A5	A6	A7	A8	A9
15 min	96.7	90	90	100	100	100	60	90	60
30 min	96.7	90	90	93.3	100	100	60	86.7	60
45 min	93.3	90	90	93.3	100	93.3	60	86.7	56.7
1 h	76.7	90	90	86.7	93.3	93.3	53.3	80	43.3
2 h	53.3	86.7	83.3	73.3	80	90	43.3	76.7	43.3
3 h	26.7	30	40	6.67	60	86.7	33.3	33.3	40
4 h	26.7	30	33.3	6.67	53.3	66.7	26.7	33.3	40

A1–A9, Alf-loaded floating-mucoadhesive beads with their corresponding compositions presented in Table I

applying simulated gastric movements by installing the whole plate into USP disintegration test apparatus in pepsin-free SGF at 37±0.5°C (Fig. 2).

A thorough analysis of the data on mucoadhesive test results revealed that the addition of HPMC to the beads facilitated the gastric adherence of the beads and prolonged their adhesion retention period.

When the mucoadhesive strength of the beads formulations after 4 h (%) was statistically analyzed using ANOVA to compare mucoadhessiveness of different formulations, it was found that HPMC concentration had significant positive effect (p=0.0029) on the mucoadhesiveness of the beads, on the other hand, CaSi concentration did not possess such significant effect. Additionally, the model evaluation showed that linear model will prevail over other models (P=0.0103), with the equation:

$$\text{Mucoadhesiveness of beads after 4 h (\%)} = 35.24 + 0.47X_1 + 13.33X_2$$

As X_1 is CaSi concentration and X_2 is HPMC concentration. As Fig. 12 represent the response surface plot of the percentage mucoadhesiveness, it clarify the insignificant influence of CaSi concentration, and on the contrary, it clearly declares the significant positive effect observed on the beads mucoadhesiveness accompanied the increase in HPMC concentration, a result that indicate that HPMC can be efficiently used in these low concentration as mucoadhesion facilitating agent. Furthermore, the results indicated that A5 and A6 represented the highest mucoadhesive strengths of all formulations at all time intervals.

CONCLUSIONS

A novel multiparticulate system combining the advantages of floating and mucoadhesive DDSs have been developed for the sustained gastric delivery of alfuzosin hydrochloride to improve the treatment of benign prostatic hyperplasia. The multiparticulate beads formulation based on low density porous calcium silicate as drug carrier and floating aid, HPMC as mucoadhesive viscolizer, in addition to the cross-linked alginate polymer. The developed formulations showed instantaneous floating for most of the formulations, as well as extended floating time for 8 h for some formulations; 88.3% of the beads remained floating for formula A5 after 8 h and 98.3% for A6. Both formulations also showed the best mucoadhesion properties as 60% and 86.7% of the beads remained adhering after 3 h for A5 and A6, respectively. The study elucidate the combined significant effect of CaSi on the enhanced buoyancy of the beads and HPMC on the enhanced mucoadhesiveness that makes them excellent candidates as mucoadhesive aids for intragastric mucoadhesive drug delivery systems. Furthermore, using ANOVA test, the concentrations of either CaSi or HPMC used were found to significantly affect the properties of the produced beads such as beads yield, beads size, floating behavior, Alf release from the beads and mucoadhesion properties. Therefore, by optimizing the concentrations of these components in formulations, the beads characteristics could be easily modified to provide optimized formulations with controlled simultaneous floating and mucoadhesion characteristics.

In conclusion, these developed mucoadhesive-floating beads formulations could be suggested to provide simple and novel technique for sustained release of drugs in the stomach and the upper part of small intestine.

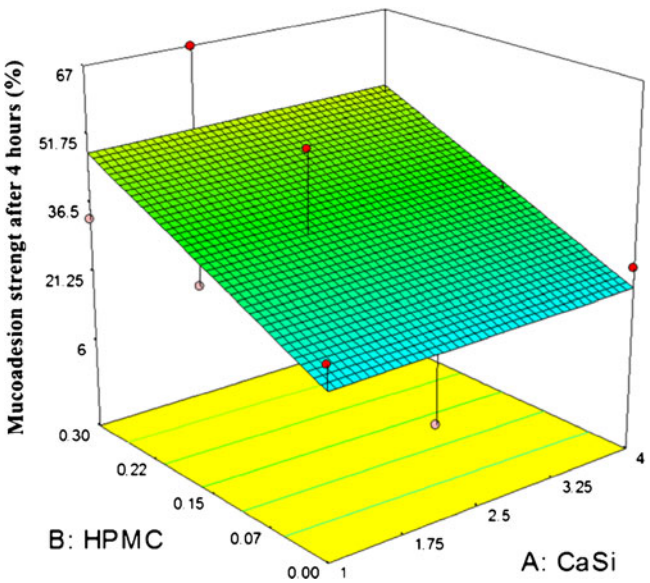


Fig. 12. Response surface plot for the effect of CaSi and HPMC concentrations on the beads mucoadhesiveness

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