

Chitosan Beads as a New Gastroretentive System of Verapamil

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Abstract

The main objective of this project is to design a new extended release gastroretentive multiparticulate delivery system for verapamil (VP) by incorporation into hydrogel beads made of chitosan. The beads were formed by dropping solutions of VP and chitosan in a solution of tripolyphosphate using a syringe pump with adjustable constant rate. The formed beads were then further crosslinked using glutaraldehyde and the excess glutaraldehyde were then washed. The physical properties of the prepared beads such as beads sizes, shapes, friabilities and loading efficiencies were determined. The floating characteristics and the release profiles were also studied. The produced beads from all batches showed a very good spherical geometry with mean diameter in the range of 1.3 – 2.0 mm. The drug loading efficiency was around 42% for all batches. The % friabilities were less than 1% indicating that the beads surfaces are highly resistant to attrition. Batches B₃ and B₄ (prepared using medium molecular weight chitosan) showed both the slowest release rate among all the prepared batches and showed good floating characteristics comprising short onset (around 5 minutes) and the long duration of buoyancy (more than 6 hours). All the batches exhibited a kinetic model of combined mechanism of diffusion partially through a swollen matrix and partially through water-filled pores. The preparation of chitosan beads using this technique would provide a simple and commercially viable method of preparation of chitosan beads for controlling the release of some drugs.

Keywords

Verapamil, floating, chitosan, gastro-retentive, controlled-release.

Introduction

Verapamil hydrochloride, the first calcium channel blocker to be approved by the FDA in 1981, is currently the major Ca-antagonist in the market. It is effective in managing and treating supraventricular tachycardias including paroxysmal supraventricular tachycardia, atrial fibrillation and/or flutter, and re-entrant tachycardias involving the AV node [1,2]. In addition, it is effective in the treatment of hypertension and chronic stable, variant, and unstable angina. In atrial fibrillation, VP is more effective than digoxin for controlling ventricular rate [3]. Verapamil can be effective in decreasing left ventricular hypertrophy, presumably due to afterload-reducing effects, and has been shown to decrease reinfarction in patients with uncompromised left ventricular function [4]. The pharmacokinetic parameters of VP showed a very high variability primarily due to its extensive first pass metabolism [5]. The incorporation of VP in an extended-release oral dosage form would have many advantages such as improving the patient compliance by reducing dosing frequency, since the drug is indicated in chronic diseases. In addition, some reports showed that side effects and therapeutic responses were beneficially modified when sustained release forms were used [6,7].

Gastroretentive drug delivery systems are defined as systems that increase the retention of a per-oral dosage form in the stomach offering numerous advantages for drugs exhibiting an absorption window in the GI tract, drugs that are poorly soluble in the alkaline medium, and drugs that are intended for local action on the gastro-duodenal wall [8,9]. Over the last three decades, various approaches have been pursued to design gastroretentive delivery systems including floating systems [10], modified shape systems [11], swelling and expanding systems [12], bioadhesive systems [13,14], and high density systems [15].

Hydrogels have been widely used in controlled release systems [16]. Among all the hydrogels, chitosan has attracted a lot of attention in the pharmaceutical and

medical fields because it has favorable biological properties such as biodegradability [17] and biocompatibility [18,19]. Chitosan, a deacetylated derivative of chitin, is a naturally occurring polysaccharide found abundantly in marine crustaceans, insects and fungi.

In recent years, biodegradable and biocompatible polymeric micro/nanoparticles have attracted a considerable attention as potential carriers for the controlled and site-specific delivery of drugs [20,21]. The multiparticulate dosage forms have many advantages over single unit preparations, including more uniform dispersion in the GI tract, more uniform drug absorption, less inter- and intra-individual variability, and more flexible formulation process [22].

Chitosan microspheres have high potential for developing a successful gastroretentive drug delivery system since they combine both bioadhesion and floating capabilities [23], especially for drugs such as VP that are poorly soluble in intestinal medium and readily soluble acidic medium. The chitosan beads will serve as depot reservoir that will allow the continuous gradual release of small amounts of VP in solution form to the upper part of the small intestine (the main site of absorption) leading to higher and more uniform blood levels of the drug. Thus reduced adverse effects are highly expected.

The multiparticulate dosage forms have many advantages over single unit preparations, including more uniform dispersion in the GI tract, more uniform drug absorption, less inter- and intra-individual variability, and more flexible formulation processes. Chitosan combines both floating and bioadhesion characters in addition to its high biocompatibility and safety and this makes it highly favorable to the formation of hydrogel beads.

In the present study, VP was incorporated in a multiparticulate system consisting of hydrogel beads. These beads were formed by chitosan and pentasodium tripolyphosphate (TPP) and were investigated for their in vitro drug release and floating characteristics.

Experimental

Materials

Verapamil (Adwic Chemical Co. Cairo, Egypt), high and medium molecular weight chitosan, (Aldrich Chemical Co., Milwaukee, WI, USA), low molecular weight chitosan, (Fluka Biochemika, Switzerland), TPP (Sigma Chemical Co., St. Louis, MO, USA) and aqueous glutraldehyde solution 50% (BDH, Poole, BH15 ITD, England) were purchased and used as received.

Methods

Preparation of VP loaded beads

Drug-loaded hydrogel beads were produced by a modified literature technique [24,25] using TPP as the gelling counterion. Chitosan solutions were prepared by dissolving a known amount of chitosan in a 1% (v/v) acetic acid and stirring at ambient temperature over night. The desired quantity of the drug was dissolved in water. The two solutions were mixed for about 20 minutes. Then 1% glycine was added to the mixture and stirred for one hour. The beads were formed by dropping the bubble-free mixture through a disposable plastic syringe with a 22-gauge needle, using a KD Scientific Model 100 push-pull syringe pump (Boston, MA, USA) at a certain speed of 70 ml/hour into 20 ml of a gently agitated 0.136 M solution of the gelling counterion, TPP, prepared in pH 7.2, 0.05 M Tris HCl buffer. Different amounts of 50% aqueous glutraldehyde solution were added. The formed beads were kept at 60°C for 75 minutes and then washed twice using few milliliters of hot and cold water to remove excess glutraldehyde. The resulting beads were dried under vacuum at ambient temperature. Similar procedures were used to prepare placebo beads, which have no entrapped drug. All batches were prepared in triplicate. Table (1) contains the exact contents and specifications of each of the prepared batch.

	Batch							
	B1	B2	B3	B4	B5	B6	B7	B8
Chitosan Mol. Wt.	Low	Low	Med.	Med.	High	High	Med.	Med.
Drug:Polymer ratio	1:3	1:5	1:3	1:5	1:3	1:5	1:3	1:3
%Glutraldehyde (w/v)	5.0	5.0	5.0	5.0	5.0	5.0	2.5	3.75

Table 1: Composition of each batch of the prepared chitosan beads

Characterization of the prepared beads

- a. Determination of the average bead size for each batch using sieve analysis method.
- b. Friability test: by calculating the percent loss in weight of the beads after they are subjected to 100 revolutions at 25 rpm using a friability tester.
- c. Beads morphology: The surface and cross-sectional morphologies of the dried beads were studied using electron microscopy [26].
- d. Entrapment efficiency: a portion of dried beads from each batch were weighed and powdered in a glass mortar. Verapamil was extracted by sonication in distilled water and assayed spectrophotometrically at $\lambda = 278$.
- e. Thermal analysis for the prepared beads: A differential scanning calorimeter, DSC, (912, Du Pont Instruments, UK), was used to determine the crystalline state of the formulation components before and after formation of microspheres. Samples were scanned from $-20\text{ }^{\circ}\text{C}$ to $225\text{ }^{\circ}\text{C}$ at a heating rate $5\text{ }^{\circ}\text{C}/\text{min}$. The thermograms of the prepared microspheres were compared with thermograms of placebo chitosan beads (containing no drug), pure medium M. wt. chitosan and pure VP.

Floatation characteristics

A portion of each batch was placed in a 250 ml beaker, containing 200 ml of simulated gastric fluid with pepsin (according to USP24 NF19) maintained at $37^{\circ}\text{C} \pm 0.5$ in a water bath. Their physical state was observed for 12 hours. Both the lag time and duration of buoyancy were determined visually.

Dissolution profiles

The dissolution of VP from different formulae was monitored using standard USP apparatus No.2 (rotating paddle method). The dissolution medium was 900 ml 0.1 N HCl and the stirring rate was 50 ± 1 rpm. Samples absorbance was measured spectrophotometrically at 278 nm. The release kinetics was determined.

Statistical analysis

Data were expressed as the mean of three experiments \pm the standard deviation and were analyzed using one way analysis of variance (ANOVA), followed by Tukey's post test. Statistical differences yielding $p < 0.05$ were considered significant.

Results

Beads morphology

The produced beads from all batches showed very good spherical geometry as shown in Fig. 1 (B and C). The mean diameter was in the range of 1.3 – 2.0 mm and the drug loading efficiency was around 42% for each batch of the prepared beads. The % friabilities were less than 1% indicating that the beads surfaces are highly resistant to attrition.

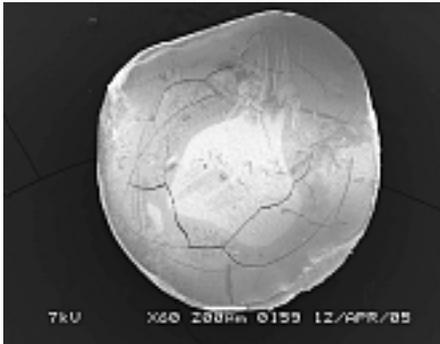
The internal structure of the dried beads showed that VP is incorporated within the core of the beads, which is evident by Fig. 1 (A) where the core containing the drug exhibited lighter color than the coating layer.

Thermal analysis

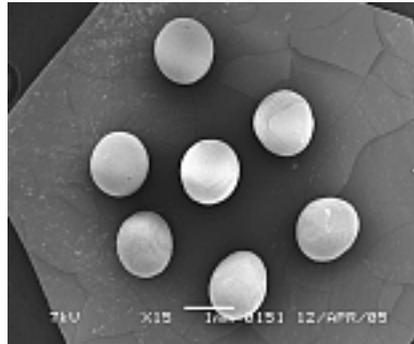
The thermal behavior of the prepared microparticles as well as the placebo chitosan beads in comparison with the thermograms of both pure molecular weight (m. wt.) chitosan and VP is illustrated in Fig. 2. The DSC-thermogram of pure chitosan (trace a) shows sharp exothermic peak at zero °C which is corresponding to the melting of the adsorbed ice and is also present in other three thermograms, in addition to an endothermic peak corresponding to melting of chitosan at 56.6 °C. The thermogram of pure VP (trace b) is characterized by a sharp endothermic peak

at 149.9 °C. The placebo beads thermogram exhibited broadening and shifting in the melting peak of chitosan to higher temperature showing a maximum at 81.4 °C. This may be attributed to the crosslinking of the polymer.

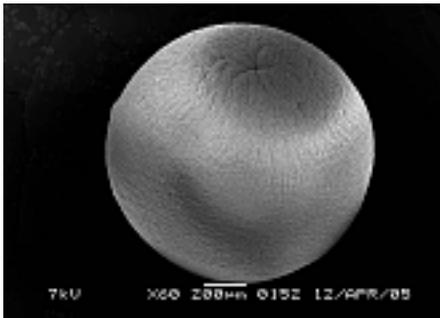
A (Cross-sectional view)



B (Surface view for number of beads)



C (Surface view for single bead)



D (Highly magnified bead surface)

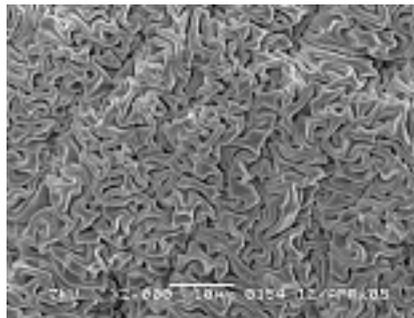


Figure 1: Scanning electron micrograph for number of dried beads from batch B₃.

Floating Characteristics:

The floating characteristic of the prepared batches are presented in Table 2. It is clear that only batches prepared using medium molecular weight chitosan showed good floating characteristics. This is indicated from the short onset (around

5 minutes) and the long duration of buoyancy (more than 6 hours). While other batches ranged from inability to float to floating for periods less than 2 hours.

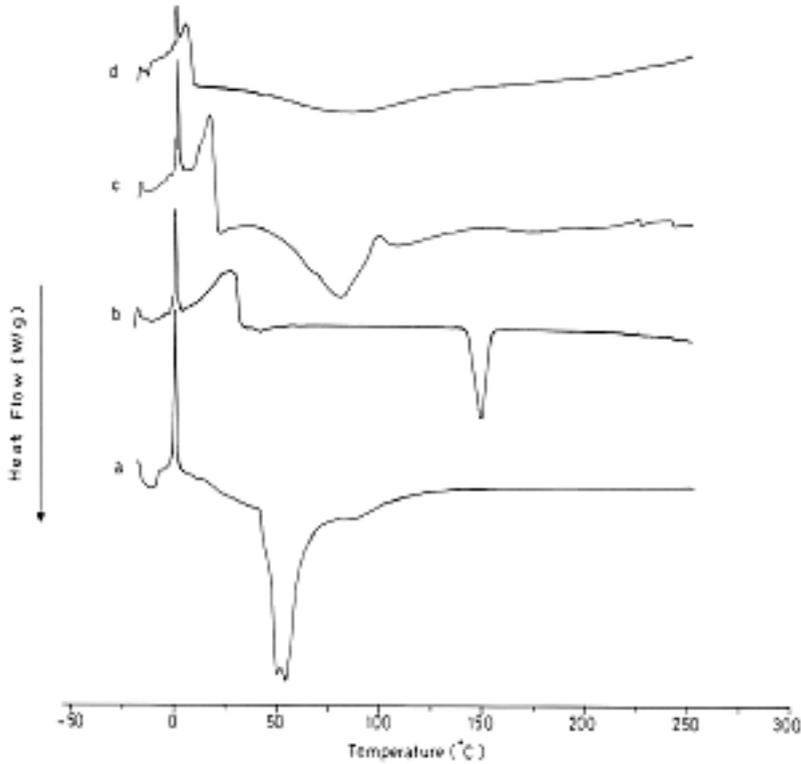


Figure 2: DSC thermograms of:
 a. Pure medium m.wt. chitosan b. Pure VP powder
 c. Placebo chitosan bead d. Chitosan beads B₃

Floating Characteristics	Batch							
	B1	B2	B3	B4	B5	B6	B7	B8
Onset (min)	No	10	5	5	15	9	2	5
Duration (min)	No	85	370	380	115	110	298	310

Table 2: Floating characteristic of each batch of the prepared chitosan beads.

***In vitro* release**

The release profiles of the prepared bead batches (Fig. 3) show that batches B₃ and B₄ (medium m. wt. chitosan) had the slowest release rate among all the prepared batches. Batches B1 and B2 (low m. wt. chitosan) exhibited the fastest release while those prepared using high m. wt. chitosan were unexpectedly intermediate (B5 and B6). It is clear that the release was independent of the drug:chitosan ratio.

The increase in the concentration of the crosslinking agent did not significantly affect the release rate from the beads up to 3.75%. At 5 % concentrations, rate of release was slower in the duration from 2 to 9 hours (Fig. 4).

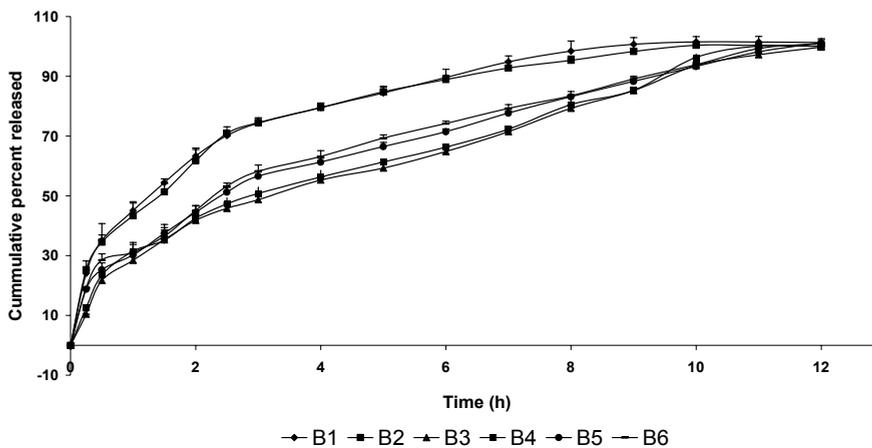


Figure 3: Release profile of verapamil HCl from each batch of the prepared chitosan beads.

Kinetic analysis

In order to determine the release kinetic model and mechanisms, the dissolution data for microspheres, that showed slow release, were analyzed. Three kinetic models including the zero-order release equation (Eq.1), first-order equation

(Eq. 2), and Higuchi equation (Eq. 3) were applied to process the in vitro data to find the equation with the best fit [27,28].

$$Q = k_1 t \quad (1)$$

$$Q = 100 (1 - e^{-k_2 t}) \quad (2)$$

$$Q = k_3 (t)^{0.5} \quad (3)$$

Where Q is the release percentage at time (t). k_1 , k_2 , and k_3 , are the rate constant of zero-order, first-order, and Higuchi, respectively. In order to further investigate the release mechanism, the data were analyzed using the following equation [29]:

$$M_t / M_\infty = K \cdot t^n \quad (4)$$

Where M_t / M_∞ is the fraction released of the drug (using values of M_t / M_∞ within the range 0.10 – 0.60) at time t, K is a constant incorporating structural and geometric characteristics and n is the release exponent characteristic for the drug transport mechanism.

From Table (2), one can see that all of the prepared batches exhibited the highest correlation coefficient (r) when the percentage released was plotted against the square-root of time except B₆ and B₈. Their r values were higher applying the first order formula. However the r values were very close in the case of first order and Higuchi in all the cases. The n values were less than 0.5 for all the batches indicating that the release rates exhibit a combined mechanism of diffusion partially through a swollen matrix and partially through water-filled pores [30].

Discussion

Chitosan microparticles prepared here were highly regular in shape with strong surfaces resistant to attrition. This is very much due to the standardization of needle gauge size, flow rate, dropping distance, and using the same concentration of the counter ion in all the experiments. The low entrapment efficiency is possibly due to diffusion of VP during preparation as it is present in concentrations far below its solubility in the medium.

Many studies have been reported in the literature to evaluate the safety of glutaraldehyde and it has been proven that it is non-carcinogenic and safe [31,32].

The thermal behavior of the prepared beads with comparison to the placebo beads indicated that Vb was stable in the matrices developed without undergoing any chemical changes during particle production. The major thermal change is the disappearance of the sharp characteristic melting peak of VP incorporated into chitosan beads and more broadening of the chitosan peak with shifting toward higher temperature.

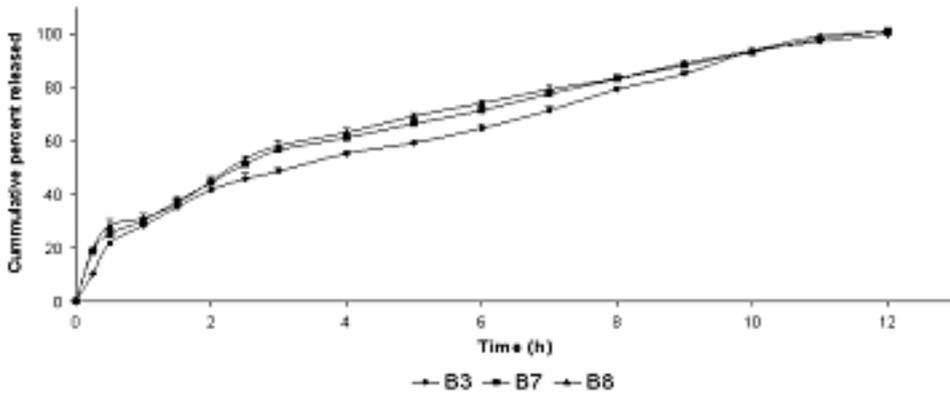


Figure 4: Effect of the glutaraldehyde percent on the release profile of verapamil HCl from chitosan beads prepared using medium m. wt. chitosan.

Model	B ₁	B ₂	B ₃	B ₄	B ₅	B ₆	B ₇	B ₈
Zero order	0.9888	0.9932	0.9525	0.9569	0.9843	0.9816	0.9843	0.9816
First order	0.9972	0.9947	0.9848	0.9845	0.9937	0.9897	0.9937	0.9897
Higuchi	0.9979	0.9951	0.9920	0.9923	0.9938	0.9859	0.9938	0.9859
n	0.4507	0.4097	0.4464	0.4267	0.3872	0.3756	0.3872	0.3756

Table 3: Values of correlation coefficients and release exponents from release data of each batch of the prepared chitosan beads.

This is mainly due to the loss of crystallinity and the solubility of VP in the melted chitosan. Thus, the incorporated VP is mostly in the amorphous state.

The *in vitro* release studies showed no dependence on the chitosan molecular weight or the drug: chitosan ratio. This may be attributed to the possible variation in the degree of deacetylation that can tremendously affect the chitosan properties. It was found that the 5 % of the crosslinker is optimum for both delaying the release and the duration of floating. The proposed release kinetic mechanism was based on the phenomena explained by Peppas [30]. He showed that using equation (4) with porous systems will lead to n values less than 0.5. Thus the crosslinking may increase the porosity of the chitosan microsphere surfaces.

The ability of some beads to maintain *in vitro* floating for more than 6 hours would be valuable for drugs with very poor alkaline solubility and drugs with absorption windows in the upper small intestine.

The preparation of chitosan beads using this technique would provide a simple and commercially viable method of preparation of chitosan beads for controlling the release of some drugs. This method can be easily scaled up for large scale production of a new controlled drug delivery dosage form.

Acknowledgements

The authors would like to thank The Research Center at College of Pharmacy, King Saud University for providing facilities to carry out the study (Grant No. C.P.R.C.143).

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