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Formulation and *In Vitro* Evaluation of Sunflower Oil Entrapped within Buoyant Beads of Furosemide

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Abstract

The purpose of the present study was to develop buoyant beads for the intragastric delivery of furosemide in order to evaluate the effect of incorporated sunflower oil on physiochemical properties of alginate beads. Sunflower oil entrapped buoyant alginate beads of furosemide were prepared by the emulsion-gelation technique. During the preparation of various batches of beads, the ratio of sunflower oil to water (v/v), the ratio of drug to polymer (w/w), were kept as variables at two levels; either high or low. Smooth, spherical beads with nominal weight variation were obtained. All batches of beads floated for 24 hours with a lag time of 5–10 min. The release of drug followed for 5 hours. Higuchi and first order kinetic modeling indicated a diffusion-controlled release of drug from the beads. The study also demonstrated the influence of sunflower oil on drug entrapment (81–95%) and in vitro release. A higher level of oil increased drug entrapment efficiency but retarded drug release rate as compared to a lower level of oil containing beads.

Keywords

Furosemide • Alginate beads • Sunflower oil • Buoyant system

Introduction

Furosemide is the prototype high ceiling (Loop) diuretic which acts by inhibiting Na^+-K^+-$2Cl^-$ cotransport at the thick ascending limb of the loop of Henle. Since furosemide has a narrow absorption window (i.e. mainly absorbed from stomach and proximal part of small intestine [1]), the design of a gastro retentive dosage form is desirable to retain the dosage form in the stomach. This assists in improving the bioavailability of the drug at the site of absorption. Several approaches are currently used to prolong gastric retention time using floating drug delivery systems [2, 3]. Higher absolute bioavailability in a floating dosage form than from a nonfloating commercial product of furosemide has already been demonstrated in dogs [4]. Improved bioavailability of furosemide from a floating tablet in human volunteers has also been reported [5]. Although single unit dosage forms of furosemide have been extensively investigated, few studies have been reported using multiple unit dosage forms of furosemide [6]. Multiple unit dosage forms may be more advantageous than single-unit systems by avoiding the ‘‘all or none’’ effect by emptying from the stomach during migrating myoelectric complex activity. Among the various floatable multiple unit dosage forms, calcium alginate gel beads have been developed in recent years as a unique vehicle for a multiple-unit drug delivery system. This is due to their excellent biocompatibility, biodegradability, reproducibility, simple method of preparation, abundant sources, low cost and minimal processing requirements. Buoyancy in these systems is imparted by incorporating various types of low density oils such as sunflower oil [7]. Besides aiding in flotation, the oil provides advantages of prolonging the floating duration (≥ 24 hours), decreasing lag time as well as increasing entrapment efficiency and modifying drug release. Currently, there is a scarcity of literature are available concerning influence of oil on drug entrapment efficiency and release from floating beads.

The aim of this study was to develop an oil entrapped multiple-unit floating dosage form of Furosemide and evaluate the effect of incorporated sunflower oil on the physiochemical properties of alginate beads. The alginate gel beads containing drug were prepared by the emulsion-gelation technique using sunflower oil. The influence of the ratio of drug to polymer (w/w), ratio of sunflower oil to water (v/v) on the physicochemical properties, buoyancy and release were evaluated. In vitro release media was selected using phthalate buffer of pH 4.6 since furosemide has increased solubility and absorption in the body at pH around 4. On an empty stomach, the pH is 1.2, however, after a meal it increases to around pH 6. Hence, the dosage form should be taken after a meal with sufficient water.

Experimental

Materials

Sodium alginate (low viscosity grade; 250 cp of 2% solution at 25°C) was purchased from Loba Chemie Pvt. Ltd., Mumbai. Furosemide (drug) was donated by Cipla Ltd., Mumbai. Sunflower oil (relative density 0.94) was purchased from Agro Tech Food Ltd., Secundrabad, India. Both Calcium chloride dihydrate and Hydrochloric acid (35% GR) were purchased from E. Merck India Ltd., Mumbai, India. All materials were used with no further purification. Double distilled water was used throughout the study.

Formulation design of drug containing polymeric beads

In the formulation design of various batches of drug containing alginate beads, the ratio of drug to polymer and oil to water were kept as variable parameters. Each variable parameter was investigated at two levels; (high and low) in order to determine the effect of these formulation variables on bead size, drug entrapment, floating time and the in vitro release profile. The composition of eight batches (F1, F2, F3, F4, F5, F6, F7, and F8) of drug loaded alginate beads were given in Table 1.

Tab. 1. Composition of drug loaded alginate beads

Batch	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Drug/ Polymer (by weight)	1:3[a]	1:3[a]	1:6[a]	1:6[a]	1:2.33[a]	1:2.33[a]	1:4.66[b]	1:4.66[b]
Oil/Water (by volume)	0.25:1[a]	0.15:1[b]	0.25:1[a]	0.15:1[b]	0.25:1[a]	0.15:1[b]	0.25:1[a]	0.15:1[b]

[a] high level; [b] low level

Preparation of alginate beads

Alginate gel beads were prepared by the ionotropic gelation method. In brief, the solution of sodium alginate was prepared by adding sodium alginate (required % w/v) in 10 ml double distilled water (DDW). Furosemide was added to it with continuous stirring. Sunflower oil was added drop wise to the dispersion by stirring at 6000 rpm continuously for 40 minutes to get a stable o/w emulsion. The emulsion was poured drop wise into 50 ml 5% w/v calcium chloride dihydrate (CCD) solution through an 18-gauge stainless steel needle. The beads were left in contact with CCD solution for 10 minutes. They were separated from the solution using stainless steel grid and air dried for 24 hours.

Determination of bead size, weight, swelling & morphology

The diameter of each air-dried bead and wetted bead (after 8 hr wetting in pH 4.6 sodium phthalate buffer) was measured by digital slide calipers (Digmatic Masschieber, model no-CD-6″ CS, Mitutoyo Corp., Japan). The average size was then calculated by measuring the diameters of three sets of 20 beads (n=3) from each batch. To determine the weight uniformity of the various batches of beads, three sets of 50 beads (n=3) were weighed from each individual batch. From the difference in the average diameter of dried and wetted beads, the percentage swelling was calculated. For the morphological study, beads were mounted on metal grids using double-sided tape coated with gold under a vacuum. Surface morphologies of the beads were investigated with a Scanning Electron Microscope (SEM) (JSM-5310LV Scanning Microscope, Tokyo, Japan) at 15 kv.

Determination of drug entrapment efficiency

A 100 mg portion of dried beads were placed into 100 mL of phosphate buffer (pH 7.4) solution. It was shaken for two hours by mechanical shaker and the resulting solution was filtered. The filtrate was suitably diluted and analyzed at 273 nm using a Beckman DU-64 Spectrophotometer. The entrapment efficiency was calculated according to Eq. 1.

Eq. 1. $$\textit{Entrapment efficiency}\ (\%) = \frac{\textit{Actual Drug content}}{\textit{Theoretical Drug content}} \times 100$$

In vitro buoyancy study

The in vitro buoyancy study for the beads was tested by visual observation in phthalate buffer pH 4.6 [9]. The experiment was carried out using a USP dissolution apparatus II. Twenty beads of each batch were placed into 900 ml of phthalate buffer (pH 4.6) solution maintained at 37±0.5°C and 100 rpm. The time taken for the beads to float at the surface of the medium (known as floating lag time) and duration of floating were noted.

In-vitro drug release study

For the in vitro release of drug from the beads, dissolution apparatus II (Campbell Electronics, Mumbai) was used. An equivalent weight of beads containing 80 mg drug was placed into 900 mL of phthalate buffer (pH 4.6) maintained at 37±0.5°C and stirring at 100 rpm. A 10 mL aliquot was taken out periodically and replaced with fresh dissolution medium. The aliquots were further diluted suitably and analyzed at 332 nm using a Beckman DU-64 spectrophotometer. In order to analyze the furosemide release mechanism, the in vitro release data were fitted into various release equations and the following kinetic models (zero-order, first order [10], Higuchi [11], Korsemeyer and Peppas [12]).

Statistical analysis

The experimental results were expressed as mean ± SD (standard deviation). Student's t-test was applied to determine the level of significance. One-way analysis of variance was also applied to check significant difference in drug release from different formulations. Differences were considered statistically significant when $p<0.05$.

Results and Discussion

The average size of the dried beads was 2.30 (± 0.15) mm considering the spherical shape of the beads (as seen in Fig. 1). The overall size differences among the eight batches of beads with various drug, alginate and oil concentrations were not significant. Beads swell to a lesser extent (range 2.2 to 4.8%) in pH 4.6 phthalate buffer media after 8 hrs. The weight range for three sets of 100 beads from eight batches varied from 374.333 ± 3.5118 mg to 588.666 ± 4.0414 mg (Table 2). The SEM study of alginate beads showed a spherical shape with pores or channels distributed throughout the surface (Fig. 2). Oil entrapped beads had an "orange peel" surface with corrugations.

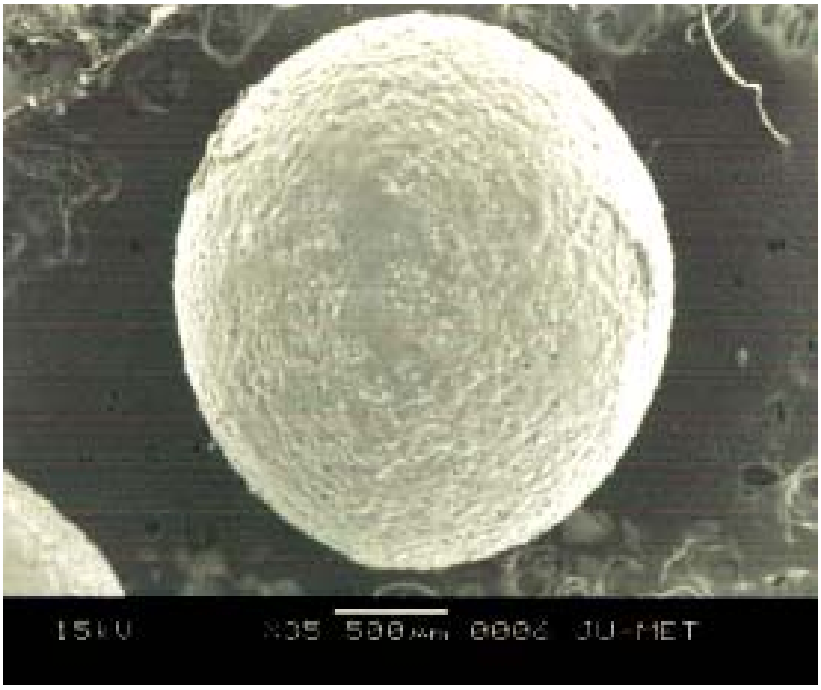

Fig. 1. SEM image of drug & oil incorporated alginate beads

Fig. 2. SEM image of intact beads showing presence of pores on surface

Tab. 2. Physicochemical properties of drug loaded alginate beads

Batch code	Mean dia. of dried beads ±SD (mm)	Mean dia. of wetted beads ±SD (mm)	Mean Swelling (%)	Mean wt. of 100 dried beads ±SD (mg)	Drug entrap. efficiency (%)	Mean Density (gm/cm^3)	FLT (min)	FT (hr)
F1	2.26 ± 0.014	2.34 ± 0.011	3.672	588.66 ± 4.04	90.05 ± 1.2	0.974	9.5 ± 0.5	24
F2	2.32 ± 0.016	2.41 ± 0.014	3.263	462.66 ± 2.51	84.76 ± 3.36	0.7	7.2 ± 0.2	24
F3	2.27 ± 0.011	2.32 ± 0.021	2.107	558 ± 3.61	95.39 ± 1.42	0.902	9.3 ± 0.2	24
F4	2.32 ± 0.025	2.39 ± 0.012	3.008	431.33 ± 3.21	92.67 ± 1.97	0.654	6.0 ± 0.5	24
F5	2.22 ± 0.011	2.32 ± 0.015	4.582	502.334 ± 4.046	86.03 ± 2.15	0.870	8.8 ± 1.0	24
F6	2.26 ± 0.016	2.34 ± 0.02	3.708	403.66 ± 5.13	81.92 ± 1.84	0.664	6.0 ± 1.5	24
F7	2.244 ± 0.024	2.31 ± 0.018	2.941	394.66 ± 3.51	93.89 ± 1.56	0.667	6.2 ± 0.1	24
F8	2.28 ± 0.017	2.39 ± 0.013	4.73	374.33 ± 3.51	86.94 ± 1.31	0.601	5.5 ± 0.3	24

SD…standard deviation; FLT…floating lag time; FT…floating time

Drug entrapment efficiency ranged from 81% to 95% depending on the composition of the eight batches of polymeric beads of furosemide (Table 2). The curing time were kept to 10 minutes since drug is very insoluble in water. There was a correlation observed between proportion of oil and drug entrapment efficiency of the beads. A higher proportion of oil in the formulation of beads increased the drug entrapment efficiency in different batches due to partitioning of the drug in the oil phase. Moreover, it was observed that an increase in the amount of alginate increases drug entrapment efficiency due to increased space for drug molecules to be retained throughout a larger cross linked network of calcium alginate.

The floating lag time was observed among the various batches (Table 2). The lag time was found to be proportional to the mean density of the beads (range 0.6–0.9 gm/cm^3). It is evident that all bead formulation floated within 10 minutes after being placed into the acidic medium and remained buoyant in the acidic medium throughout the study, irrespective of the proportion of oil in the formulation. Primarily, oil that is entrapped in the bead is responsible for floating (i.e. oil acts as floating aid). This explanation can be corroborated by the observation of failed buoyancy of non-oily beads compared to 24 hr buoyancy for oily beads [8]. Sunflower oil serves as the dispersed phase to prepare uniform emulsion to create multiple tiny pockets in the alginate matrix for better buoyancy. Secondarily during dissolution, drug was released from the matrix of the beads and the swelling reduces density, which also facilitates floating. It had been observed that a higher proportion of the oil (oil/water ratio of 0.25:1.0 by volume) in formulations (F1, F3, F5, F7) decreased the lag time, thereby enabling the beads to float comparatively faster than those formulations (F2,

F4, F6, F8) containing a lower proportion of oil (oil/water ratio of 0.15:1.0 by volume), irrespective of the amount of polymer included in the various batches.

Tab. 3. Results of correlation coefficients for the release data of drug loaded beads using a curve fitting method for zero-order, first-order and Higuchi kinetic model (n=3)

Batch No.	Correlation Coefficients (R^2 ± SD)			Model of Best Fit
	Zero Order	First Order	Higuchi	
F1	0.888 ± 0.023	0.925 ± 0.012	0.995 ± 0.025	Higuchi
F2	0.863 ± 0.090	0.992 ± 0.041	0.979 ± 0.032	First
F3	0.919 ± 0.014	0.991 ± 0.015	0.985 ± 0.045	First
F4	0.883 ± 0.017	0.984 ± 0.020	0.987 ± 0.024	Higuchi
F5	0.823 ± 0.021	0.965 ± 0.013	0.965 ± 0.037	Higuchi
F6	0.702 ± 0.17	0.933 ± 0.090	0.913 ± 0.021	First
F7	0.866 ± 0.012	0.977 ± 0.014	0.974 ± 0.041	Higuchi
F8	0.778 ± 0.11	0.992 ± 0.018	0.951 ± 0.025	First

Tab. 4. Results of correlation coefficients and diffusion exponent for the release data of drug loaded beads using the Korsemeyer–Peppas model (n=3)

Batch No.	Correlation Coefficients (R^2 ± SD)	Diffusion exponent (n ± SD)
F1	0.991 ± 0.078	0.497 ± 0.063
F2	0.973 ± 0.051	0.459 ± 0.014
F3	0.973 ± 0.017	0.657 ± 0.095
F4	0.983 ± 0.091	0.535 ± 0.073
F5	0.951 ± 0.088	0.502 ± 0.028
F6	0.897 ± 0.039	0.354 ± 0.034
F7	0.963 ± 0.077	0.574 ± 0.067
F8	0.926 ± 0.069	0.431 ± 0.046

A curve fitting method according to zero-order, first-order and Higuchi models for the analysis of drug release kinetics are given in Table III. Different batches of the formulation were able to release up to about 100% drug after 5 hours under study. The graphical nature of the cumulative percent released vs. time plot is given (Fig. 3 and 4). From the release profile, it was observed that the drug release kinetics was best fitted with the Higuchi model (F1, F4, F5, F7) and the first order kinetic model (F2, F3, F6, F8). From Table 4, it was observed that the drug release from the polymer matrix system was a diffusion controlled process of the fickian type ($n < 0.5$, in most cases except F3 and F7). At pH 4.6, the swelling of the calcium alginate beads occurs only to the extent of 2–4%. Therefore, a drug molecule is likely to be released by diffusion through insoluble polymer matrix. The initial fast release was attributed to the diffusion of the adsorbed drug particles on or near the outer surface of the beads, while subsequent release was due to slow diffusion of the entrapped drug from the interior core of the alginate matrix. Experimental determination of the saturated solubility of furosemide in sunflower oil (90 ± 1.18 µg/ml as

determined by HPLC method) was found to be about 3 times its solubility in water (30.26 ± 2.69 µg/ml). Interestingly, drug release was slower in formulations with higher oil as demonstrated. Hence, the most reasonable explanation for the slow release is that most of the furosemide remained saturated and dispersed in the oil pockets of the beads to form a drug-oil dispersed matrix. Actually, drug transportation from the beads to the release medium may undergo two steps. Firstly, it diffuses out of oil pockets into alginate matrix. Secondly, it diffuses out of the alginate matrix into the medium. The latter step is very fast compared to the first step.

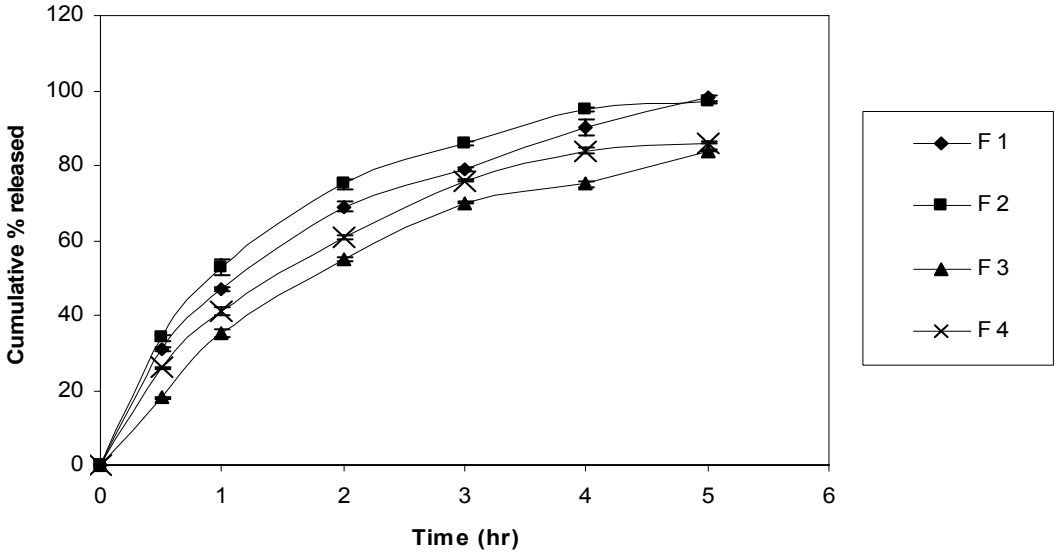

Fig. 3. Furosemide release profiles in phthalate buffer (pH 4.6) of F1 to F4

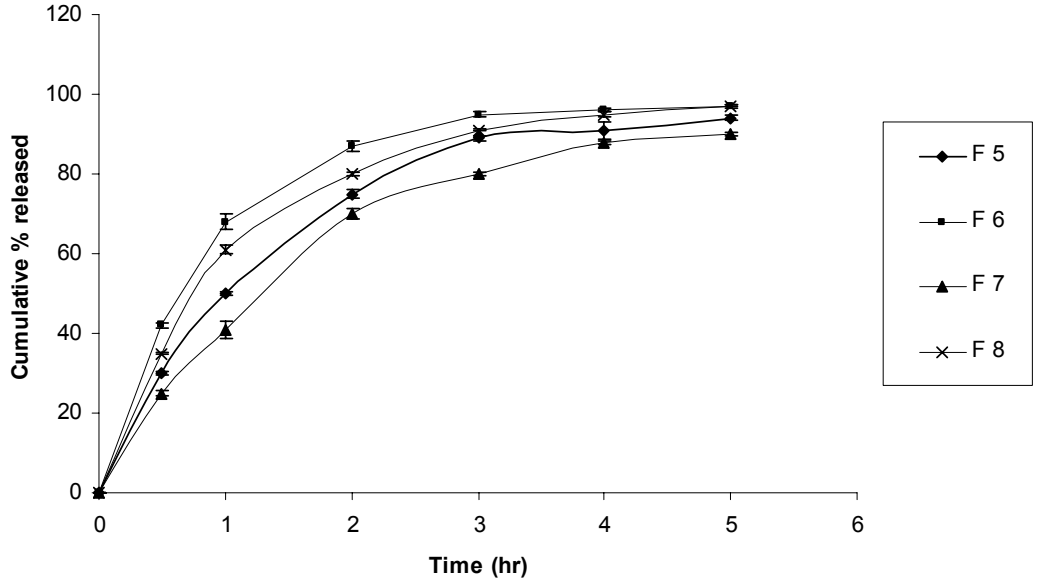

Fig. 4. Furosemide release profiles in phthalate buffer (pH 4.6) of F5 to F8

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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