Research Article

Diltiazem loaded floating microspheres of Ethylcellulose and Eudragit for gastric delivery: *in vitro* evaluation

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Abstract

Objective: A novel multiparticulate system for the gastric delivery of Diltiazem (DTZ) was developed for effective treatment of hypertension. **Materials and methods**: Floating microspheres of ethylcellulose and Eudragit loaded with DTZ were successfully developed and evaluated for their gastro-retentive and controlled-release properties. The effect of various process variables on the particle morphology, micromeritic properties, *in vitro* floating behavior, percentage drug entrapment, and *in vitro* drug release were studied. **Results and discussion**: Microspheres were found to be porous with spherical shape. The entrapment efficiency of optimized formulation was found to be maximum (69.24%) as compared to other formulations. The buoyancy of microspheres was observed in the range from 44.48 to 67.68% in simulated gastric fluid. The release pattern of DTZ in simulated gastric fluid from floating microspheres followed Higuchi matrix model and Peppas-Korsmeyer model. **Conclusion**: Preliminary results from the study suggested that these floating microspheres could used to incorporate other anti-hypertensive drugs. Such developed formulation will be subjected to *in vivo* studies in future in order to prove their efficacy against hypertension.

Keywords: Diltiazem; floating microspheres; ethyl cellulose; Eudragit; pharmacokinetic study

Introduction

A number of anti-arrhythmic agents have been used in various regimens to treat the arrhythmia. The properties of different medications may have some impact on the therapy result. Clinical trials are undertaken to search for simpler but equally effective (or more effective) regimen. The calcium channel blockers are in the focus of attention from that point of view mostly in case of long term and combinational therapy (Toyomi et al., 1968).

Diltiazem (DTZ) is an orally administered, nondihydropyridine calcium-channel blocker that is used for the treatment of hypertension and atrial fibrillation (Henry 1980). According to the biopharmaceutics classification system (BCS), DTZ is a class I substance, meaning that it is highly soluble and highly permeable. The drug is completely absorbed throughout the intestinal tract, but it is extensively metabolized in the liver by deacetylation resulting in incomplete bioavailability (about 35–40%).

Due to its very short half-life (3-4.5 h), repeated administration of immediate drug releasing formulation (up to four times per day) is desired, especially in the case of hypertension and angina pectoris that require continuous and constant care (Sweetman et al., 2000; Hermann et al., 1983). Immediate-release dosage forms are also associated with fluctuations in plasma concentrations. To optimize therapy and patient compliance, several extended release (ER), once or twice a day formulations have been developed. Three brands, which are single or multiple-unit coated tablets or capsules, are available in the Lebanese market. Generics offer a cost-effective alternative to brand-name products. However, the main concern with modified-release formulations is product substitution. Because the rate or extent of release could differ from one product to another, the patient may be placed at unnecessary risk. Prolonging the gastric residence of a dosage form may be of therapeutic value. Amongst the methods available to achieve this, floating dosage forms show considerable

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promise (Whitehead et al., 1998).

Further, the extended gastro retentive dosage forms (GRDFs) are also required if either drug action is required at stomach or if the drug is not absorbed through the small intestine. In such cases, the dosage form that can spend much time in stomach such as micro beads, floating tablets etc., are well employed. Floating systems have the property of retaining the dosage units in the stomach for prolonged time and are useful for drugs acting locally in the gastro intestinal (GI) tract (drugs that are poorly soluble and unstable in intestinal fluids). Our research group successfully developed floating microspheres of repaglinide and orlistat using low-density calcium silicate (CS) as a porous carrier (Jain et al., 2005; Jain et al., 2006; Jain et al., 2008). The objective of the present investigation was to prepare and evaluate floating microspheres loaded with DTZ, which is capable of floating on gastric fluid and delivering the therapeutic agent over an extended time.

Materials and methods

Diltiazem (DTZ) was received from M/s Cipla Ltd., Mumbai, (India) as a gift sample. Ethyl cellulose (EC) was obtained from S. D. Fine Chemicals Ltd., Mumbai (India). Eudragit® RS100 (EU) was purchased from Evonik Degussa Pvt. Ltd, Mumbai (India). Ethanol was obtained from Merck Pvt. Ltd. (India). Dichloromethane (DCM) and Tween 20 were procured from CDH Pvt. Ltd. (India). All other chemicals used were of analytical grade.

Preparation of drug loaded floating microspheres

Microspheres with an internal hollow structure were prepared by an emulsion solvent diffusion method with minor modification (Kawashima et al., 1992). The drug DTZ and a combination of EU and EC (1:1) were co-dissolved at room temperature in an Ethanol: Dichloromethane mixture (1:1). The disperse phase was poured into a stirred medium containing light liquid paraffin containing 1.5 % w/v Span 60. The poured solution was finely dispersed into discrete droplets, forming oil in water (o/w) emulsion. The dispersion system was stirred at a variable speeds (fixed for each batch) for 3 h until evaporation of solvents was complete. The floating microspheres were separated by filtration, washed with n-hexane, dried in an air oven at 50°C for 12 h, and stored in desiccators. Excess liquid paraffin was washed off using n-Hexane and dried to get hollow microspheres.

Optimization of formulation

Various process variables, which could affect the preparation and properties of floating microsphere were identified and studied. The method of preparation was accordingly optimized and validated. Preparation of floating microsphere involves various process variables out of which drug polymer ratio, stirring speed and organic solvent ratio were selected for the optimization of formulation (Table 1). The procedure adapted for the control variables is as follows.

Drug polymer ratio: To optimize drug polymer ratio, amount of drug was kept constant and the polymer ratio was varied to give a final drug: polymer ratio, i. e. 1:1, 1:2, 1:3, and 1:4.

Stirring rate: Stirring rate was selected by preparing the microsphere at different stirring rate viz. 300, 500 rpm and keeping other variables constant as described in the general procedure for preparing solvent diffusion method.

Table 1. The composition, formulation code, and variables used in the preparation of microspheres

S. No.	Formulation code	Drug: Polymer ratio	Stirring rate (rpm)
1.	A1	1:1	300
2.	A2	1:1	500
3.	B1	1:2	300
4.	B2	1:2	500
5.	C1	1:3	300
6.	C2	1:3	500
7.	D1	1:4	300
8.	D2	1:4	500

Organic solvent ratio: Variations in organic solvent ratio were employed to establish any significant relationship between the solvent system, drug loading, and release properties (Table 2).

Table 2. Formulation code and variable organic solvent ratios to study effect in optimized formulation

Formulation code	Optimized Drug: Polymer ratio	DCM	Ethanol
S1	1:3	1	1
S2		2	1
S3		1	2

Shape and surface morphology

The external and internal morphology of the floating microsphere were studied by scanning electron microscopy (SEM). The sample for SEM was prepared by sticking the microspheres on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 °A using a sputter coater. Then the samples were kept inside the vacuum chamber, scanned and photomicrographs were taken.

Micromeritic properties

The microspheres were characterized for their micromeritic properties, such as particle size, true density, tapped density, compressibility index and flow properties. The size

was measured using an optical microscope, and the mean particle size was calculated by measuring 600 particles with the help of a calibrated ocular micrometer. The tapping method was used to determine the tapped density and percent compressibility index as follows:

Tapped density = Mass of microspheres / Volume of microspheres after tapping

$$\% \text{ Compressibility index} = [1 - V/Vo] \times 100$$

where V and Vo are the volumes of the sample after and before the standard tappings, respectively. True density was determined using benzene displacement method. Porosity (ε) was calculated using the equation:

$$\varepsilon = (1 - P_p / P_t) \times 100$$

where P_t and P_p are the true density and tapped density, respectively. Angle of repose of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method.

Percentage yield and drug entrapment efficiency (DEE)

The prepared microspheres with a different size range were collected and weighed. The measured weight was divided by the total amount of all non-volatile components, which were used for the preparation of the microspheres.

The drug content of floating microspheres was carried out by dissolving the microspheres in a small amount of ethanol in a separating funnel and extracting the drug into SGF, pH 2.0 by evaporating ethanol (Pietras et al., 2004). Determination of drug loading was carried out at 237 nm spectrophotometrically. All experiments were performed in triplicate.

***In vitro* buoyancy studies**

The microspheres (300 mg) were spread over the surface of six-basket dissolution apparatus (Dissolution rate test apparatus USP/IP/BP STD, Jyoti Scientific Laboratories, Gwalior). Vessel was filled with 900 ml of simulated gastric fluid (SGF, pH 2.0), containing 0.02% of tween 80. The dispersion was stirred using paddle at 100 rpm for 8 h. Tween 80 served to mimic the effect of natural surfactants in the stomach. The floating and the settled portions of the floating microspheres were recovered separately, dried, and weighed. All experiments were performed in triplicate. Buoyancy was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres (Gupta and Garg 2010).

$$\text{Buoyancy } (\%) = Q_f / (Q_f + Q_s) \times 100$$

where Q_f and Q_s are the weights of the floating and the settled microspheres, respectively.

***In vitro* drug release studies**

The *in vitro* drug release rate of DTZ from floating microspheres was determined in a United States Pharmacopeia (USP) XXIII basket type dissolution apparatus. A weighed amount of floating microspheres equivalent to 300 mg drug was filled into a hard gelatin capsule (No. 0) and placed in the basket of dissolution rate apparatus. Nine hundred milliliters of the SGF containing 0.02% w/v of Tween 80 was used as the dissolution medium. The dissolution fluid was maintained at 37±0.5°C at a rotation speed of 100 rpm in SGF (pH 2.0). Perfect sink conditions prevailed during the drug release study. Samples (5ml) were withdrawn at each predefined interval, passed through Whatmann filter paper (#41), and analyzed at 237 nm spectrophotometrically after suitable dilution.

Analysis of release data

To analyze the *in vitro* release data various kinetic models were used to describe the release kinetics. The following plots were made: *cumulative % drug release vs. time* (zero order kinetic models); *log cumulative of % drug remaining vs. time* (first order kinetic model); cumulative *% drug release vs. square root of time* (Higuchi model) and *log cumulative % drug release vs. log time* (korsmeyer model).

Results and discussion

Surface morphology and shape

The floating microspheres were predominantly spherical in shape as shown in SEM photographs (figure 1). Their sphericity contributed considerably to their very good flow properties. Distinct pores are evident on the surface of microspheres, which is responsible for the release of drugs. The photomicrographs also showed presence of loose crystals of drug on the surface of few microspheres (Tanwar et al., 2007).

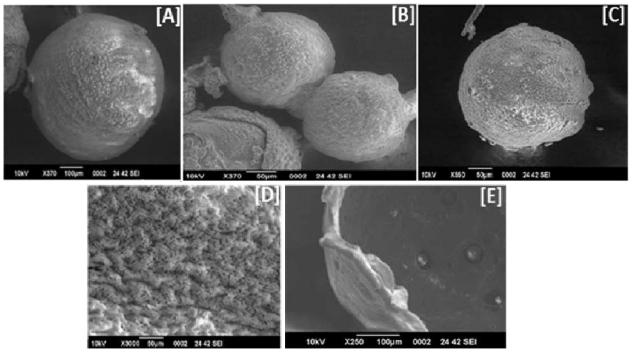

Figure 1. SEM images of [A] Placebo microsphere of EU-EC blend showing pores on surface; [B] Drug loaded intact microspheres; [C] Drug loaded microspheres showing drug crystal on the outer wall; [D] Surface of drug loaded microspheres showing pores and drug crystals; [E] Cross sectional internal morphology of a broken microsphere.

Micromeritic studies

The variation in mean particle size was observed owing to variation in drug-polymer ratio. The viscosity of the medium continuously increased with rise in polymer concentration, which enhanced interfacial tension.

Table 3. Micromeritic studies of the developed formulations

Formulation code	Particle Size (µm)	Bulk density (g/cm^3)	Tapped (g/cm^3)	Compressibility index (%)	Angle of repose (°)
A1	195.37±1.53	0.423	0.526	0.103	27.42
A2	199.14±0.52	0.479	0.595	0.116	31.21
B1	307.65±1.19	0.451	0.623	0.172	35.83
B2	289.015±0.7	0.501	0.593	0.092	32.25
C1	225.44±0.99	0.499	0.645	0.146	30.50
C2	234.31±1.06	0.528	0.69	0.162	26.42
D1	279.63±0.92	0.542	0.646	0.104	28.61
D2	274.27±0.63	0.513	0.623	0.110	30.42
S1	245.49±0.83	0.493	0.601	0.108	28.42
S2	307.65±0.71	0.456	0.592	0.136	32.18
S3	323.59±0.62	0.488	0.630	0.142	31.65

Results are expressed as Mean. (n=3)

Diminished shearing efficiency was also observed at higher viscosities that results in to formation of larger particles (Srivastav *et al.,* 2005). The mean particle size of the microspheres significantly increased with increasing polymer concentration and was found to be in the range of 195.39 ± 1.53 to 323.59 ± 0.62 µm. The formulation S3 showed maximum particle size. Whereas, stirring rate did not show any significant change in particle size of different formulation.

Angle of repose was found in between 26.42 to 35.83°. The good flow property of microspheres indicated their non-aggregating nature. Tapped density was observed in the range between 0.493 to 0.612 g/cm^3. High compressibility index is indicative of the tendency to form bridges. The compressibility index of all formulation was found to be in the range of 12.41 to 17.16 % (Table 3). However, floatation might be influenced owing the low bulk and tapped densities (Jain et al., 2006).

Percentage yield and drug entrapment efficiency (DEE)

The percent yield of floating microspheres was greater than 60% for all the formulations & was in the range of 60.36 to 74.64 (Table 4). At a high stirring speed, the shell was destructed by insufficient diffusion of ethanol into aqueous solution and simultaneous evaporation of dichloromethane.

The drug entrapment of DTZ in all formulation was satisfactory. The high entrapment efficiency of drug is believed to be due to its good aqueous solubility, which facilitates the diffusion of a part of entrapped drug to surrounding medium during preparation of floating microspheres (Bhagwat et al., 2009). However, in case if drug: polymer ratio of 1:4, the drug entrapment falls due to excess pores on the surface. The extent of loading was also influenced by the particle size of microspheres. The encapsulation efficiency of the prepared microspheres was in the range of 42.13 to 68.18%. Encapsulation efficiency of S3 found to be the highest (69.24%) among the formulations.

Table 4. Percentage yield and drug entrapment efficiency of floating microspheres

Formulation code	Yield (%)	Drug entrapment efficiency (%)
A1	62.24	42.13
A2	65.29	43.17
B1	60.36	44.58
B2	64.22	49.65
C1	67.82	68.18
C2	74.64	65.63
D1	64.65	60.72
D2	69.38	58.12
S1	74.16	66.14
S2	67.13	65.66
S3	70.12	69.24

***In vitro* floating behavior**

To assess the floating properties, the microspheres were placed in SGF, pH 2.0 in dissolution apparatus containing surfactant Tween 80 (0.02% v/v), to simulate gastric conditions and the fraction of microspheres settled down as a function of time was quantiated. The microspheres floated for prolonged time over the surface of the dissolution

Table 5. Model fitting of various formulations

Formulation code	Zero order Kinetics	First order kinetics	Higuchi models	Korsmeyer-models Peppas	
	R^2	R^2	R^2	R^2	n
Control	0.708	0.799	0.896	0.923	0.616
A1	0.642	0.706	0.860	0.901	0.613
A2	0.950	0.620	0.809	0.857	0.598
B1	0.771	0.886	0.992	0.929	0.680
B2	0.724	0.834	0.896	0.908	0.651
C1	0.981	0.758	0.981	0.926	0.546
C2	0.695	0.761	0.886	0.916	0.655
D1	0.693	0.785	0.908	0.927	0.598
D2	0.681	0.805	0.989	0.897	0.669
S1	0.612	0.667	0.834	0.882	0.654
S2	0.697	0.978	0.931	0.952	0.478
S3	0.694	0.823	0.915	0.926	0.514

medium without any apparent gelation. Percentage buoyancy of the microspheres was in the range 44.48 to 67.68 % for 8 h study period. It was observed that large size microspheres showed the longer floating time (Figure 2). Floating ability of microspheres might be due to the low bulk and tapped densities of the microspheres.

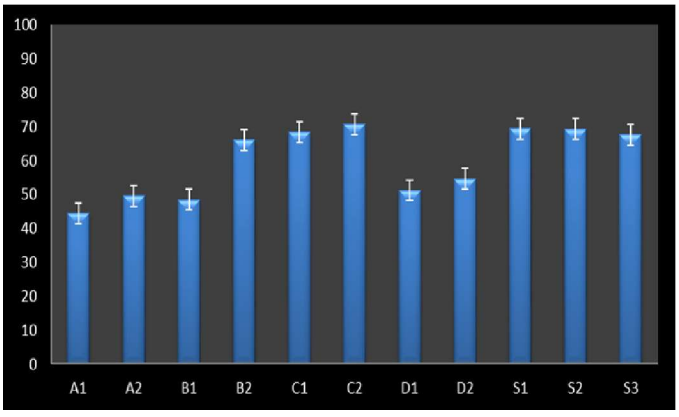

Figure 2. Graphical representation of percent Buoyancy

In vitro drug release

DTZ release from EU-EC microspheres decreased with increasing EC concentration. Increase in the content of EU (which is less permeable than EU) would increase polymer matrix density and thus result in increased diffusion path length, leading to a decrease in drug release from the microsphere. Another factor might be that the smaller microspheres formed at low concentration had a larger surface area exposed to the dissolution medium, thus, giving rise to faster drug release (Figure 3). On the other hand, the greater the content of EU, the higher was the rate of drug release from microspheres. EU is insoluble in acidic medium and exhibits low permeability.

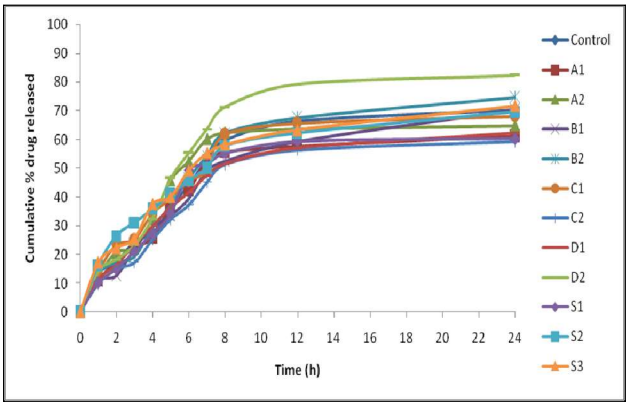

Figure 3. *In vitro* drug release from different microsphere formulations

Analysis of release data through curve fitting

Different kinetics were applied to interpret the release rate of DTZ from floating microspheres i.e. zero order, first order, Higuchi-matrix, Korsmeyer-Peppas. The zero order plots of different formulation were found to be fairly linear, as indicated by their moderate regression values (R^2 = 0.612-0.981) but it seems that drug release from the floating microspheres A2 and C1 followed zero order kinetics. Only one formulation, S2 found to follow first order release. B1, C1, and D2 follow Higuchi matrix model. However, C1

Table 6. Dunnett's multiple comparison data of formulation against control

Comparison	Mean Difference	q- Value	p- Value
Control vs. A1	2.737	0.2859	P>0.05
Control vs. A2	-2.221	0.2319	P<0.05
Control vs. B1	2.995	0.3127	P>0.05
Control vs. B2	-0.8091	0.0844	P>0.05
Control vs. C1	-1.322	0.1380	P<0.05
Control vs. C2	5.796	0.6053	P<0.05
Control vs. D1	3.153	0.3292	P>0.05
Control vs. D2	-6.742	0.7040	P>0.05
Control vs. S1	2.654	0.2771	P<0.05
Control vs. S2	-2.210	0.2308	P<0.05
Control vs. S3	-2.535	0.2648	P>0.05

release mechanism for formulation C1 may be of mixed order as it shows highest regression both in zero order curve and in Higuchi model fit. To confirm the exact mechanism of drug release, the data were fitted according to Korsmeyer-Peppas equation (Korsmeyer et al., 1983). The value of '*n*' gives an indication of the release mechanism; when $n = 1$, the release rate is independent of time (zero-order) (case II transport), $n = 0.43$ for Fickian diffusion and when $0.43 < n < 0.85$, diffusion and non-Fickian transport are implicated. Lastly, when $n > 0.85$ super case II transport is apparent. '*n*' is the slope value of log $M/M\infty$ versus log time curve. Slope values ($0.43<n<0.85$) suggest that the release of DTZ from floating microspheres followed non-Fickian diffusion mechanism i.e. release was governed by both diffusion and swelling of polymer. From the coefficient correlation, it shows that the release rate of C2 (R^2-0.916) show best fit to korsmeyer model (Table 5) (Gattani et al., 2009).

Dunnett's Multiple Comparison test. One way non-paired ANOVA was performed on the release data obtained from formulations and it was found to be significant (Table 6). ANOVA assumed that the sampled population lies within identical standard deviation. This assumption was tested using method of Bartlett. Dunnett's multiple comparison tests were performed to compare the unpaired groups with the control population. The release data of formulation was compared with release profile of control and results confirmed significant variation in SD ($P<0.05$).

In the assumption test of each population, the data was sampled from population that follows Gaussian distribution. This assumption was tested using the method of Kolmogrov and Smirnov (Table 7).

From the assumption test, it is clear that all formulations passed normality test. However, the significance value is up to the limit.

Table 7. Assumption test calculation by Kolmogrov and Smirnov method

Group	KS	p-value	Passed Normality
Control	0.1235	>0.10	Yes
A1	0.1680	>0.10	Yes
A2	0.1743	>0.10	Yes
B1	0.1122	>0.10	Yes
B2	0.1440	>0.10	Yes
C1	0.1256	>0.10	Yes
C2	0.1292	>0.10	Yes
D1	0.1089	>0.10	Yes
D2	0.1343	>0.10	Yes
S1	0.2044	>0.10	Yes
S2	0.8234	>0.10	Yes
S3	0.1193	>0.10	Yes

Conclusion and future perspectives

The blend of EU and EC proves to be a versatile vehicle for drug delivery of DTZ, which is stable in gastric environment. The data indicates that the integrity of drug has been maintained during the formulation process. Drug release can be achieved for a prolonged time for better absorption of drug and avoidance of gastric irritation. The method used for the preparation of microspheres is easy, economic, and easily scalable to industrial level.

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Conflict of Interest

Authors have no conflict of interest.

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