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Preparation and in vitro characterization of porous carrier-based floating microspheres of model drug for gastric delivery

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ABSTRACT

Floating microspheres have been utilized to obtain prolonged and uniform release of drug in the stomach for development of once-daily formulations. A controlled-release system designed to increase residence time in the stomach without contact with the mucosa was achieved through the preparation of floating microspheres by the emulsion solvent diffusion technique, using (i) calcium silicate (CS) as porous carrier; (ii) Atenolol, an oral antihypertensive agent; and (iii) Eudragit® S as polymer. The effects of various formulations and process variables on the internal and external particle morphology, micromeritic properties, in vitro floating behavior, drug loading, and in vitro drug release were studied. The microspheres were found to be regular in shape and highly porous. The prepared microspheres exhibited prolonged drug release and remained buoyant for >10 h. The mean particle size increased and the drug release rate decreased at higher polymer concentrations. No significant effect of the stirring rate during preparation on drug release was observed. In vitro studies demonstrated diffusion-controlled drug release from the microspheres. Microsphere formulation F4, containing 1:4 drug: calcium silicate, showed the best floating ability (85% buoyancy) in simulated gastric fluid. The release pattern of Atenolol in 0.1 N HCl from all floating microspheres followed the Higuchi matrix model and.

Keywords: Calcium silicate, floating drug delivery system, Atenolol, Microspheres, Solvent diffusion method.

INTRODUCTION

To develop oral drug-delivery systems, it is necessary to optimize both the residence time of the system within the gastrointestinal tract and the release rate of the drug from the system. Various

attempts have been made to prolong the residence time of the dosage forms within the stomach. [1],[2] Prolongation of the gastric residence time (GRT) of delivery devices could be achieved by promoting adhesion to the mucous membranes,[3] which acted by preventing passage of the microspheres through the pylorus[4] or by maintaining them in a buoyant fashion in gastric juice.[5],[6],[7] With regard to the floating devices, Innucelli et al. ,[8],[9],[10] reported that an air-contained multiple-unit compartment system showed excellent buoyancy in vitro and prolonged GRT relative to the controls in vivo in the fed state. However, in the fasted state, the intragastric buoyancy of the devices did not influence GRT. Yuasa et al, [11] attempted to prepare an intragastric floating and sustained-release preparation, which derived its buoyancy from the air trapped in the pores of calcium silicate when these particles were covered with polymer. Murata et al, [12] prepared calcium-induced alginate gel beads that, upon oral administration, were capable of floating on gastric juice.

Atenolol is a cardioselective beta-1 adrenoceptor blocker devoid of intrinsic sympathomimetic and Membrane- stabilizing activity. It is poorly absorbed from the lower GIT. The oral bioavailability of atenolol has been reported to be 50% reported by Melander et al. [13] Amidon et al., [14] reported that the human jejunal permeability and extent of absorption is also low. Thus, it seems that an increase in GRT may increase the extent of absorption and bioavailability of the drug. Based on this, an attempt was made through this Investigation to formulate floating microspheres of atenolol using low density carrier. The prepared microspheres were evaluated for various parameters.

MATERIALS AND METHODS

Atenolol was supplied as a gift sample by Cipla ltd, (Mumbai, India); CS (Florite RE) was supplied as gift sample from Tomita pharma. (Japan); and ES (Eudragit^[sup][R] S) was received as a gift sample from Cipla ltd, (Mumbai, India). Ethanol, dichloromethane (DCM), and the other solvents were purchased from SD Fine Chemicals (Mumbai, India). All chemicals were of analytical-reagent grade and were used as received.

Preparation of Atenolol-absorbed CS

CS was dispersed in 10 mL ethanolic solution of Atenolol to prepare slurry. The slurry was ultrasonicated for 30 minutes in an ice bath using a bath sonicator (S.W 4.5 TOSHCON, India) to entrap the drug solution inside the pores of porous carrier. The excess ethanolic solution was removed by filtration and then dried at room temperature for 24 hr, which resulted in Atenolol absorbed CS powder. [15]

Preparation of floating microspheres

Microspheres were prepared by the emulsion solvent diffusion method established by Kawashima et al. [15] as follows: The Atenolol-absorbed CS was added into the polymer solution of ES (1 g) in ethanol and DCM (2:1) and sonicated using the bath sonicator (TOSHCON). The resulting suspension was poured into a 200 mL aqueous solution of polyvinylpyrrolidone (0.75% w/v) in a 500 mL beaker at 40°C. The emulsion/suspension was stirred at 500 rpm employing a 2-bladed propeller-type agitator (Remi, Mumbai, India) for 3 h. The microspheres were separated by filtration using Whatman filter paper, washed with water,

and dried at room temperature in a desiccator for 24 h. The microspheres of Atenolol without CS (F1) were also prepared using the same method for comparative study.

Table 1: Formulation of Atenolol Floating Microspheres

Formulation code	Drug:(FLR)	EUDRAGIT S	DCM: Ethanol
F1	1:0	1	1:2
F2	1:1	1	1:2
F3	1:2	1	1:2
F4	1:3	1	1:2
F5	1:4	1	1:2
F6	1:5	1	1:2

Process variables

Amount of polymer: 500, 1000, and 1500 mg; stirring rate: 250, 500, and 1000 rpm; Temperature of the preparation: 20, 30, 40, and 50°C; volume of aqueous phase: 200, 300, 400, and 500 mL; solvent ratio (ethanol: DCM): 1:1, 2:1, and 3:1; amount of carrier: 50, 100, 150, 200, and 250 mg.

Characterization of microspheres

Micromeritic properties

The prepared microspheres are characterized by their micromeritic properties, such as microsphere size, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose. [18]

Bulk Density:

The bulk density is defined as the mass of powder divided by bulk volume. The bulk density was calculated by dividing the weight of the samples in grams by the final volume in cm. [18]

$$\text{Bulk Density} = \frac{\text{Mass of microspheres}}{\text{Bulk Volume of microspheres}} \quad [1]$$

Tapped Density:

Tapped density is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume. [18]

$$\text{Tapped Density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}} \quad [2]$$

Carr's Compressibility Index:

This is an important property in maintaining uniform weight. It is calculated using following equation,

$$\% \text{Compressibility Index} = \left[1 - \frac{\text{Bulk Density}}{\text{Tapped Density}} \right] \times 100 \quad [3]$$

Lower the compressibility values indicate better flow. [18]

Hausner ratio:

A similar index like percentage compressibility index has been defined by Hausner. Values less than 1.25 indicate good flow, where as greater than 1.25 indicates poor flow. Added glidant normally improve flow of the material under study. Hausner's ratio can be calculated by formula,

$$\text{Housner ratio} = \left[\frac{\text{Tapped Density}}{\text{Bulk Density}} \right] \times 100 \quad [4]$$

Angle of Repose (θ):

Good flow properties are critical for the development of any pharmaceutical tablet, capsules or powder formulation. It is essential that an accurate assessment of flow properties be made as early in the development process as possible so that an optimum formulation can be quickly identified. Interparticle forces between particles as well as flow characteristics of powders are evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane.

The angle of repose of each powder blend was determined by glass funnel method. Powders were weighed accurately and passed freely through the funnel so as to form a heap. The height of funnel was so adjusted that the tip of the funnel just touched the apex of the heap. The diameter of the powder cone so formed was measured and the angle of repose was calculated using the following equation,

$$\tan\theta = \frac{h}{r} \quad [5]$$

Where, θ = angle of repose

h = height of the pile and,

r = radius of the powder cone respectively.

Angle of repose affects particle size distribution, as larger the particle size, it will flow freely and vice-versa. It is a helpful parameter to monitor quality of powdered or granular pharmaceutical formulations. For good flowing materials then, angle of repose should be less than 30°. [18]

Morphology

The morphological study was carried out by Scanning Electron Microscope (SEM). Microspheres were scanned and examined under Electron Microscope HITACHI SU 1500, Japan connected with Fine coat, JEOL JFC-1100E Ion sputter. The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold.

Drug content

The drug content of Eudragit® S microspheres was determined by dispersing 50 mg formulation (accurately weighed) in 10 mL ethanol, followed by agitation with a magnetic stirrer for 12 h to dissolve the polymer and to extract the drug. After filtration through a whatman filter, the drug concentration in the ethanol phase was determined spectrophotometrically at 224.6 nm (Shimadzu 1700, UV-spectrophotometer) by making desired dilution with 0.1N HCl. Eudragit® S and the CS powder did not interfere under these conditions. Each determination was made in triplicate. The percentage drug entrapment and yield were calculated as follows: [18]

$$\% \text{ Drug loading} = \frac{\text{Actual drug content}}{\text{Weight of microspheres}} \times 100 \quad [6]$$

$$\% \text{ Incorporation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad [7]$$

Floating behavior

Fifty milligrams of the floating microparticles were placed in 0.1 N HCl (100 mL) containing 0.02% w/v Tween 80. The mixture was stirred at 100 rpm in a magnetic stirrer. After 10 h, the layer of buoyant microparticles was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until a constant weight was obtained. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles. [19]

$$\% \text{ Buoyancy} = \left(\frac{W_f}{W_f + W_s} \right) \times 100 \quad [8]$$

Where, W_f and W_s are the weight of the floating and settled microspheres respectively.

Differential scanning calorimetry

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation stage during the development of solid dosage form. Differential Scanning Calorimeter (DSC TA-60WS) allows the fast Evaluation of possible incompatibilities, because it shows changes in the appearance, Shift of melting endotherms and exotherms, and/or variations in the corresponding enthalpies of reaction. The DSC thermograms of pure drug and optimized final formulation were recorded. The thermal analysis was performed in a nitrogen atmosphere at a heating rate of 10°C/min over a temperature range of 40°C to 300°C.

In vitro release studies

The release rate of Atenolol from floating microspheres was determined in a United States Pharmacopeia (USP) II type dissolution apparatus. A weighed amount of floating microspheres equivalent to 50 mg drug was filled into a hard gelatine capsule (No. 0) and placed in the basket of the dissolution rate apparatus. Five hundred millilitres of the 0.1 N HCl containing 0.02% w/v of Tween 80 was used as the dissolution medium. The dissolution fluid was maintained at 37±1°C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release study. 5 millilitre samples were withdrawn at different time intervals, filtered through whatman filter, and analyzed spectrophotometrically at 224.6 nm to determine the concentration of Atenolol present in the dissolution medium. The initial volume of the dissolution fluid was maintained by adding 5 mL of fresh dissolution fluid after each withdrawal. All experiments were run in triplicate. [20]

Drug release pattern from microspheres

Five kinetic models including the zero order (Eq 9), first order (Eq 10), Higuchi matrix (Eq 11), Peppas- korsmeyer (Eq 12) and Hixon-rowell (Eq 13) release equations were applied to process the in vitro release data to find the equation with the best fit using PCP Disso v3 software.[21],[22]

$$R = k_1 t \quad [9]$$

$$\log UR = \frac{k_2 t}{2.303} \quad [10]$$

$$R = k_3 t^{0.5} \quad [11]$$

$$R = k_4 t^n \text{ or } \log R = \log k_4 + n \log t \quad [12]$$

$$(UR)^{1/3} = k_5 t \quad [13]$$

RESULTS AND DISCUSSION**Formation of microspheres**

The floating microspheres were prepared by the emulsion solvent diffusion technique. A solution or suspension of Eudragit® S and Atenolol with CS in ethanol and dichloromethane were poured into an agitated aqueous solution of polyvinyl alcohol. The ethanol rapidly partitioned into the external aqueous phase and the polymer precipitated around dichloromethane droplets. The subsequent evaporation of the entrapped dichloromethane led to the formation of internal cavities within the microspheres. The incorporation of drug-adsorbed CS into the formulation may produce a porous structure within the microspheres. The ultrasonication produced drug-adsorbed CS in a fine state of subdivision.

A potential advantage of using large volumes of the external aqueous phase are the reduction of the required stirring times. The solubility of dichloromethane in water is 1% w/v. With larger volumes (400-500 mL), the diffusion of dichloromethane into the aqueous phase, and hence solidification of particles, occurred faster as compared to that with 200 mL. Thus, particles could be separated after shorter stirring times. It was found that a saturated solution of polymer produced smooth and high-yield microspheres. The undissolved polymer produced irregular and rod-shaped particles. Preparation at 20°C or 30°C provided porous microspheres having higher porosity, with a surface so rough as to crumble upon touching. At 40°C, polymer and the drug were co-precipitated and the shell was formed by the diffusion of ethanol into the aqueous solution and simultaneous evaporation of dichloromethane. In contrast, microspheres prepared at 50°C demonstrated a single large depression at the surface, which was a consequence of rapid evaporation of dichloromethane. A portion of the polymer solution aggregated into a fiber-like structure as it solidified prior to forming droplets or, alternatively, the transient droplets were broken before the solidification was complete. As ethanol quickly diffused out of the organic

phase (polymer solution) into the aqueous phase, Eudragit® S dissolved in ethanol solidified in fiber-like aggregates. It is documented that when the diffusion rate of solvent out of emulsion droplet is too slow, microspheres coalesce together. Conversely, when the diffusion rate of solvent is too fast, the solvent may diffuse into the aqueous phase before stable emulsion droplets are developed, causing the aggregation of embryonic microsphere droplets. The ratio of dichloromethane with ethanol also affected the morphology of the microspheres and the best result with a spherical shape was obtained when the ratio of ethanol to dichloromethane was 2:1. However, the average particle size increased and the wall thickness also increased as the amount of Eudragit® S increased. When the amount of Eudragit® S was 1.5 g in 15 mL of organic phase, it started to form aggregates. When the amount of Eudragit® S was less than 0.5 g in 15 mL of organic phase, it started to form irregular microspheres with some pores. It is obvious that the rotation speed of the propeller affects the yield and size distribution of microspheres. As the rotation speed of the propeller increased from 250 rpm to 1000 rpm, the average particle size decreased, while maintaining its morphology. The optimum rotation speed for this experimental system was 500 rpm, as judged from the results of particle size and size distribution and the drug content.

Micromeritic properties

The mean particle sizes were 141 μm for FLR powder and 385, 412, 476, 544 and 665 μm for formulation containing FLR in the range of 50–250 mg. The particle size of formulation F1 was found to be 184 μm . The tapped density values ranged from 0.41 to 0.64 g/cm³, while their true densities ranged between 1.62 to 1.92 g/cm³ of all the formulations, which may be due to the presence of low density FLR particles in the microspheres. The porosity of all the formulations was found to be in the range of 60–80%. The compressibility index ranged between 25.0% to 34.6%. All formulations showed excellent flow ability as expressed in terms of angle of repose ($>40^\circ$) except for F6, probably due to higher content of FLR. The better flow property indicates that the floating microspheres produced are non-aggregated.

Table 2: Micromeritic properties of different floating microspheres

Formulation code	Drug:FLR	Mean particle size (μm)	True density (g/cm ³)	Tapped density (g/cm ³)	Compressibility index (%)	Porosity (%)	Angle of repose
FLR	-	141 $\pm$ 19	1.32 $\pm$ 0.25	0.19 $\pm$ 0.01	23.3 $\pm$ 1.2	81.0 $\pm$ 4	48.0 $\pm$ 5
F1	1:0	184 $\pm$ 21	1.59 $\pm$ 0.14	0.61 $\pm$ 0.03	24.9 $\pm$ 1.9	62.5 $\pm$ 3	50.4 $\pm$ 4
F2	1:1	325 $\pm$ 15	1.78 $\pm$ 0.09	0.42 $\pm$ 0.04	25.6 $\pm$ 0.9	74.6 $\pm$ 6	46.8 $\pm$ 4
F3	1:2	382 $\pm$ 12	1.74 $\pm$ 0.12	0.41 $\pm$ 0.03	27.1 $\pm$ 0.8	77.9 $\pm$ 4	41.4 $\pm$ 8
F4	1:3	423 $\pm$ 15	1.81 $\pm$ 0.16	0.51 $\pm$ 0.05	28.8 $\pm$ 0.8	71.3 $\pm$ 8	45.4 $\pm$ 6
F5	1:4	478 $\pm$ 25	1.85 $\pm$ 0.10	0.54 $\pm$ 0.09	32.2 $\pm$ 0.6	70.5 $\pm$ 6	40.6 $\pm$ 4
F6	1:5	512 $\pm$ 38	1.88 $\pm$ 0.21	0.57 $\pm$ 0.06	34.1 $\pm$ 1.6	72.5 $\pm$ 4	37.4 $\pm$ 3

Morphology

FLR based Eudragit® S microspheres were predominantly spherical in appearance; however some were found to be elongated. The porous nature of the FLR and spherical shape of the microspheres are evident from their SEM photomicrographs. Some of FLR also found to be uncoated.

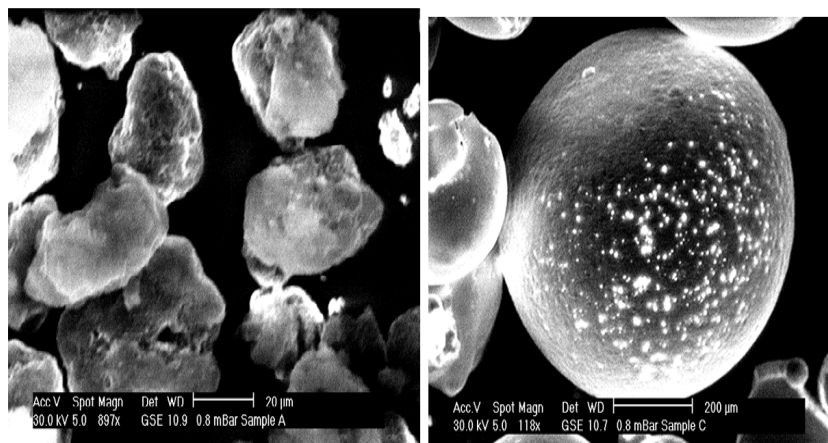


Figure 1: SEM images of (a) CS particle (b) Microsphere

Percentage buoyancy, drug entrapment

The floating test was performed to investigate the floatability of the prepared microspheres. Good in vitro percentage buoyancy was observed for all the microsphere formulations. This characteristic may be attributed to the low tapped density of the microspheres as a result of the entrapment of low density CS within the system. Microsphere formulation F4 showed the best floating ability ($88\% \pm 4\%$ buoyancy) in SGF as compared with other formulations. The floating ability of microspheres for 8 hours may be considered a satisfactory performance of the prepared formulations. The percentage entrapment of Atenolol was found to be good at all loading.

The values of % yield and % entrapment efficiency and %buoyancy are shown in. As the FLR concentration was increased the % drug loading decreased and %entrapment efficiency was increased due to increase in the adsorption of the Drug.

Table 3: Buoyancy and drug entrapment of diff microspheres

Formulations	% Yield	%drug entrapment	% Buoyancy
F1	67 ± 2.0	70 ± 2.5	70 ± 3
F2	73 ± 3.5	73 ± 2.4	77 ± 2
F3	68 ± 2.4	79 ± 3.0	80 ± 4
F4	74 ± 2.5	82 ± 2.8	83 ± 2
F5	76 ± 3.3	85 ± 3.3	88 ± 4
F6	65 ± 3.0	83 ± 2.4	82 ± 5

Differential scanning calorimetry

In order to determine the physical state of drug, i.e., whether amorphous or crystalline, before and after floating microsphere formulation, DSC examination was conducted for the pure drug, the polymer, CS physical mixture. Thermograms of the single component(s) and microspheres are shown in. A sharp melting transition of Atenolol (pure) was observed at 153.89°C (curve A). A DSC thermogram of optimized formulation showed peak at 153.2°C .

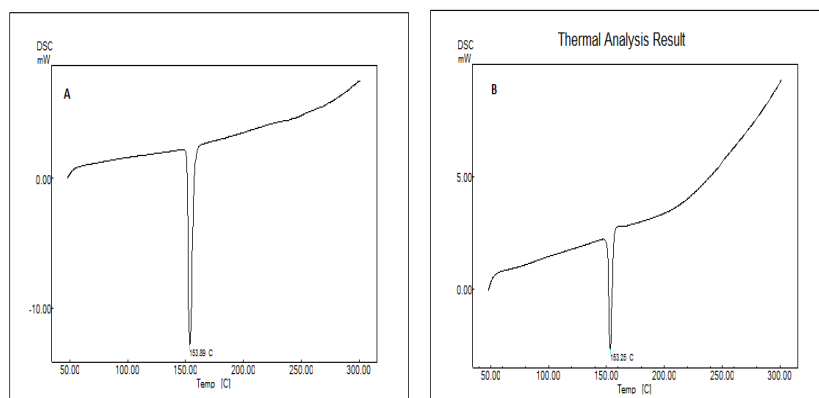


Figure 2: DSC Thermogram of (a) pure drug (b) mixture of drug and polymers

In vitro drug release study

Dissolution studies on all the six formulations of Atenolol floating microspheres were carried out using a USP dissolution apparatus Type I. 0.1N HCl (pH 1.2) was used as the dissolution medium And all the calculations was done by PCP Disso Version 2.8 software (Pune, India) and are reported. Since the acrylic polymer used is not soluble in acidic pH and starts to dissolve above pH 7, microspheres released the Atenolol only by diffusion in 0.1 N HCl. No burst effect was observed from any of these formulations. The release of Atenolol from different formulations followed the order: F1> F2>F3>F4>F5>F6. The pattern also provides an idea about the effect of CS content on drug release from the microspheres (ie, the higher the CS content in microspheres, the lower the drug release). The release mechanism of Atenolol from these floating microspheres was also evaluated on the basis of theoretical dissolution equations including zero-order, first-order, Higuchi matrix, Peppas-Korsmeyer, and Hixon-Crowell kinetic models. The regression coefficients and rate constants from in vitro release profiles of Atenolol in 0.1 N HCl were calculated. Release pattern of Atenolol in 0.1 N HCL from all floating microspheres followed Higuchi matrix model.\

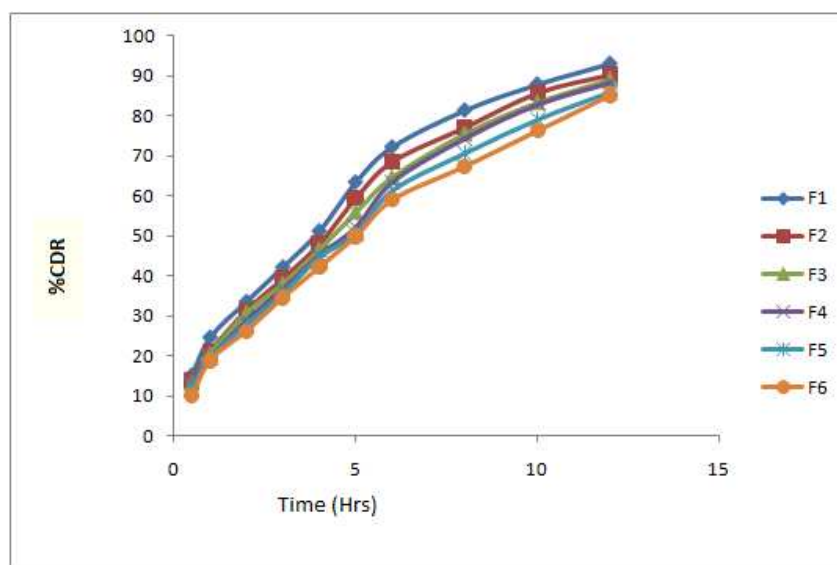


Figure 3: Release plot of all the formulations

CONCLUSION

The present formulation study of Atenolol was performed in an attempt to prepare a floating drug delivery system consisting of a floating multiple-unit system. Incorporation of CS in the microspheres proved to be an effective method to achieve the desired release behavior and buoyancy. The performance of these formulations was evaluated and the effect of various formulation variables was studied. The designed system, combining excellent buoyant ability and suitable drug release pattern, could possibly be advantageous in terms of increased bioavailability of Atenolol. The major advantages of the system include: (i) ease of preparation, (ii) good buoyancy, (iii) high encapsulation efficiency, and (iv) sustained drug release over several hours. Certain other drugs can be formulated for the same formulation e.g. Famotidine, verapamil Hcl

The developed formulation overcomes and alleviates the drawbacks and limitations of sustained-release preparations in the drug-delivery art through the introduction of CS-based floating microspheres suitable for controlled release of drug after oral administration. The microspheres could be compressed into tablets, filled into capsules, or formulated into oral suspensions for reconstitution.

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