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Evaluation of Porous Carrier-based Floating Orlistat Microspheres for Gastric Delivery

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ABSTRACT

The purpose of this research was to prepare floating microspheres consisting of (1) calcium silicate as porous carrier; (2) orlistat, an oral anti-obesity agent; and (3) Eudragit S as polymer, by solvent evaporation method and to evaluate their gastro-retentive and controlled-release properties. The effect of various formulation and process variables on the particle morphology, micromeritic properties, in vitro floating behavior, percentage drug entrapment, and in vitro drug release was studied. The gamma scintigraphy of the optimized formulation was performed in albino rabbits to monitor the transit of floating microspheres in the gastrointestinal tract. The orlistat-loaded optimized formulation was orally administered to albino rabbits, and blood samples collected were used to determine pharmacokinetic parameters of orlistat from floating microspheres. The microspheres were found to be regular in shape and highly porous. Microsphere formulation CS4, containing 200 mg calcium silicate, showed the best floating ability (88% ± 4% buoyancy) in simulated gastric fluid as compared with other formulations. Release pattern of orlistat in simulated gastric fluid from all floating microspheres followed Higuchi matrix model and Peppas-Korsmeyer model. Prolonged gastric residence time of over 6 hours was achieved in all rabbits for calcium silicate–based floating microspheres of orlistat. The enhanced elimination half-life observed after pharmacokinetic investigations in the present study is due to the floating nature of the designed formulations.

KEYWORDS: Orlistat, calcium silicate, floating drug delivery, microspheres, gamma scintigraphy, pharmacokinetic study.

INTRODUCTION

Floating drug delivery is of particular interest for drugs that (1) act locally in the stomach, (2) are primarily absorbed in the stomach, (3) are poorly soluble at an alkaline pH, (4) have a narrow window of absorption, and (5) are unstable in the intestinal or colonic environment.[1] To provide good floating behavior in the stomach, the density of the device should be less than that of the gastric contents (≈1.004 g/cm^3). Srivastava et al[2] reported cimetidine-loaded floating microspheres of hydroxypropyl methylcellulose and ethyl cellulose. The prepared microspheres exhibited prolonged drug release (~8 hours) and remained buoyant for >10 hours. Sato et al[3] developed hollow microspheres or microballoons (MB) of riboflavin, aspirin, salicylic acid, ethoxybenzamide, and indomethacin using Eudragit S100 as enteric polymer. Sato et al[4] also reported gamma scintigraphy of riboflavin-containing MB to establish its gastro-retention in human volunteers. Simultaneously, pharmacokinetic examination of riboflavin release from MB was conducted in fasted and fed human subjects. Streubel et al[5] used polypropylene foam powder as porous carrier for the development of verapamil HCl-loaded floating microparticles. We have developed and reported in vitro characterization of calcium silicate (CS)-based floating microspheres of repaglinide.[6] The developed formulation was also evaluated for the microspheres' gastro-retentive behavior and pharmacokinetic parameters by gamma scintigraphy and blood plasma studies, respectively.[7] Recently, Jain et al[8] reported a CS-based floating granular delivery system of ranitidine hydrochloride using hydroxypropyl methylcellulose and ethylcellulose. CS was first used as a floating and sustained-release carrier for the development of floating microspheres by our research group.[6,7]

Orlistat (OT) is a lipase inhibitor for obesity management that acts by inhibiting the absorption of dietary fats. It has short half-life (<2 hours) and requires administration 3 times a day.[9] The main sites of action of OT are the stomach and pancreas.[9] Because of its short half-life, dosing frequency, and gastric side effects at high concentration, OT was considered to be a potential candidate for floating controlled-release formulations. CS, which has a characteristically porous structure with many pores and a large pore volume, has a sustained-release property. It has floating ability due to the air trapped within its pores when covered with a polymer.[10] The objective of the present investigation was to prepare and evaluate floating microspheres consisting of (1) CS as porous carrier; (2) OT, an oral anti-obesity drug; and (3) Eudragit S (ES) as polymer, which is capable of

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floating on gastric fluid and delivering the therapeutic agent over an extended period of time.

Materials and Methods

Materials

OT was generously supplied as a gift sample by M/s F. Hoffmann-La Roche Ltd (Basel, Switzerland). CS and stannous chloride ($SnCl_2$) were purchased from Sigma-Aldrich GmBH (Munich, Germany). ES was received as a gift sample from M/s Rohm Chemische GmBH (Fabrik, Germany). Polyvinyl alcohol (PVA) was purchased from Sigma Chemical Co (St Louis, MO). Ethanol, dichloromethane (DCM), and other solvents were purchased from HiMedia Laboratories Ltd (Mumbai, India). Technetium-99m (as pertechnetate) ($^{99m}TcO_4$) was obtained from the Nuclear Medicine Department, Jawaharlal Nehru Cancer Hospital and Research Centre (Bhopal, India). All other chemicals were of analytical reagent grade and were used as received.

Preparation of Orlistat-absorbed CS

CS (1.0 g) was dispersed in 10 mL ethanolic solution of OT (50 mg) to prepare slurry. The slurry was ultrasonicated for 10 minutes in an ice bath at 40% voltage frequency using a probe sonicator (Soniweld, Imeco Ultrasonics, Mumbai, India) to entrap the drug solution inside the pores of porous carrier. The excess ethanolic solution was removed by filtration and then by drying in vacuum, which resulted in OT-absorbed CS powder.

Preparation of Floating Microspheres

Microspheres were prepared using a modified emulsion solvent diffusion technique.[11] The OT-absorbed CS was added into the polymer solution of ES (1 g) in ethanol and DCM (2:1) and sonicated using probe sonicator (Soniweld). The resulting suspension was poured into a 200-mL aqueous solution of PVA (0.75% wt/vol) in 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 hours. The microspheres were separated by filtration using Whatman filter paper (No. 41, Whatman, Brentford, UK), washed with water, and dried at room temperature in a desiccator for 24 hours. The microspheres of OT without CS (WC) were also prepared using the same method for comparative study.

Preparation of Nonfloating Microspheres

Nonfloating microspheres (NFM) were prepared using the procedure reported by Choi et al.[12] ES (1.0 g) and OT (50 mg) were dissolved in 10 mL of DCM/ethanol mixture (2:1), followed by addition of 1 mL of aqueous phase containing 0.25% wt/vol of Tween 80. The initial water/oil (w/o) emulsion was prepared by stirring the mixture for 20 seconds. The w/o emulsion was slowly added into 500 mL of corn oil, the second oil phase containing 0.02% wt/vol of Span 80 as a surfactant, with stirring at 250 rpm at 25°C. The mixture was stirred for 1 hour and the hardened microspheres were collected by filtration. The collected microspheres were washed with n-hexane 3 times and soaked in fresh hexane with gentle shaking for 24 hours. The microspheres were separated and then dried in an oven overnight at 50°C.

Micromeritic Properties

Microspheres were characterized for micromeritic properties, such as particle size, true density, tapped density, compressibility index, and flow properties.[13] The size was measured using an optical microscope, and the mean particle size was calculated by measuring 200 to 300 particles with the help of a calibrated ocular micrometer. The tapping method was used to determine the tapped density and percentage compressibility index as follows:

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}} \quad (1)$$

$$\%\ \text{Compressibility index} = \left[1 - \frac{V}{V_o}\right] \times 100, \quad (2)$$

where V and V_o are the volumes of the sample after and before the standard tappings, respectively.

True density was determined using a Helium densitometer (No. 1305, Shimadzu, Kyoto, Japan). Porosity (ε) was calculated using the following equation:

$$\varepsilon = \left[1 - \frac{P_p}{P_t}\right] \times 100, \quad (3)$$

where P_p and P_t are the tapped density and true density, respectively.

Angle of repose (θ) of the microspheres was determined by the fixed funnel method.

Morphology

The morphology of the microspheres and CS were studied by scanning electron microscopy (SEM). The samples for

SEM were prepared by lightly sprinkling the powder on a double-sided adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with an SEM (Jeol JSM-1600, Tokyo, Japan).

Liquid Chromatography-Mass Spectrometry of Orlistat

Chromatographic Conditions

A PerkinElmer Series 200 high-performance liquid chromatography (HPLC) system (PerkinElmer Inc, Wellesley, MA) consisting of flow control valve, vacuum degasser, pump, and auto sampler was used to deliver mobile phase (acetonitrile and ammonium acetate buffer 10 mM, in the ratio 90:10) at a flow rate of 0.65 mL/min. The mobile phase was degassed for 20 minutes in an ultrasonic bath (Branson Ultrasonic Corp, Danbury, CT) before use. Chromatographic separation was achieved on Spheri-5 RP-18 column (5 μm, 100 × 4.6 mm inner diameter [id], Pierce Chemical Co, Rockford, IL) preceded with guard column packed with the same material. The samples (20 μL) were injected through autoinjector onto the liquid chromatography tandem mass spectrometry (LC/MS/MS) system. The column and autoinjector were maintained at ambient temperature.[14,15]

Mass Spectrometric Conditions

API-4000 LC/MS/MS (Applied Biosystems/MDS SCIEX, Toronto, Ontario, Canada) was operated with standard electrospray ionization (ESI) source coupled with a LC separation system. Analyst 1.4.2 software was used for the control of equipment, data acquisition, and analysis. For optimization of MS parameters, approximately equimolar solutions of each analyte were prepared in acetonitrile:ammonium acetate buffer (90:10). Zero air was used as nebulizing gas spectrometry (GS 1, 25 psi) and nitrogen as curtain gas (20 psi). Declustering potential (DP) was optimized, while ion spray voltage and nebulizing and curtain gas conditions were used in default mode. The dwell time and mass width were set at 0.2 seconds and ± 10 atomic mass units (amu), and MS scan was performed in both positive and negative ion modes.[14,15]

Percentage Drug Entrapment

The OT content in ES microspheres was determined by dispersing accurately weighed 50-mg formulation in 10 mL of ethanol followed by agitation with a magnetic stirrer for 12 hours to dissolve the polymer and extract the drug. After filtration through a 0.25-μm membrane filter (Millipore Corp, Billerica, MA), the OT content was determined in filtrate using LC/MS/MS method as described earlier. Each determination was made in triplicate.

Floating Behavior

Fifty milligrams of the floating microspheres were placed in 100 mL of the simulated gastric fluid (SGF, pH 2.0) containing 0.02% wt/vol Tween 20. The mixture was stirred at 100 rpm with a magnetic stirrer. After 8 hours, the layer of buoyant microspheres 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 constant weight was achieved. 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.[6]

$$Buoyancy\ (\%) = \frac{W_f}{(W_f + W_s)} \times 100, \qquad (4)$$

where W_f and W_s are the weights of the floating and settled microparticles, respectively. All the determinations were made in triplicate.

In Vitro Release Studies

The release rate of OT from floating microspheres was determined in a United States Pharmacopeia (USP) XXIII basket type dissolution apparatus. A weighed amount of floating microspheres equivalent to 50 mg drug was filled into a hard gelatin capsule (No. 0) and placed in the basket of dissolution rate apparatus. Five hundred milliliters of the SGF containing 0.02% wt/vol of Tween 20 was used as the dissolution medium. The dissolution fluid was maintained at 37°C ± 1°C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release study. Five-milliliter samples were withdrawn at each 30-minute interval, passed through a 0.25-μm membrane filter (Millipore), and analyzed using LC/MS/MS method to determine the concentration of OT 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.

Data Analysis of Release Studies

Five kinetic models including the zero order (Equation 5), first order (Equation 6), Higuchi matrix (Equation 7), Peppas-Korsmeyer (Equation 8) and Hixon-Crowell (Equation 9) release equations were applied to process the in

vitro release data to find the equation with the best fit using PCP Disso v3 software.[16,17]

$$R = k_1 t \quad (5)$$

$$\log UR = \frac{k_2 t}{2.303} \quad (6)$$

$$R = k_3 t^{0.5} \quad (7)$$

$$R = k_4 t^n \text{ or } \log R = \log k_4 + n \log t \quad (8)$$

$$(UR)^{1/3} = k_5 t, \quad (9)$$

where R and UR are the released and unreleased percentages, respectively, at time (t); k_1, k_2, k_3, k_4, and k_5 are the rate constants of zero-order, first-order, Higuchi matrix, Peppas-Korsmeyer, and Hixon-Crowell model, respectively.

Statistical Treatment of Dissolution Data

Differences in in vitro drug release of OT from CS-based microspheres and microspheres devoid of CS were statistically analyzed by 1-way analysis of variance (ANOVA) with posttest (Dunnett multiple comparison test). Statistically significant differences between in vitro OT release of formulations were defined as $P < .05$. Calculations were performed with the GraphPad-Instat software program (GraphPad Software Inc, San Diego, CA).

In Vivo Scintigraphic Study

The optimized formulation (CS4) and nonfloating microspheres (NFM) of 500 mg each, loaded with $SnCl_2$ and OT, were placed in a test tube and soaked in 10 mL of normal saline (0.9% NaCl) for 15 minutes. A small amount of sodium pertechnetate solution equivalent to radioactivity of 40 mBq obtained from a technetium generator and stored in a sterile vial was added to the tube. The suspension was mixed intermittently for 15 minutes using a vortex shaker (Superfit, Mumbai, India). The supernatant was removed and the labeled microspheres were recovered by filtration through a Whatman filter paper (No. 41) followed by washing thoroughly with deionized water. The microspheres were then allowed to dry in air for 15 minutes. Twelve 1-year-old male albino rabbits were used to monitor the in vivo transit behavior of the floating and nonfloating microspheres. These rabbits were divided into 2 groups (group I and group II). None of them had symptoms or a past history of gastrointestinal (GI) disease. In order to standardize the conditions of GI motility, the animals were fasted for 12 hours prior to the commencement of each experiment. CS4 (100 mg) was orally administered in suspension form to animals of group I and NFM to group II followed by sufficient volume of drinking water. All 4 legs of the rabbit were tied over a piece of plywood (20 × 20 inch), and the location of the formulation in the stomach was monitored by keeping the subjects in front of a gamma camera (Siemens, Forcheim,

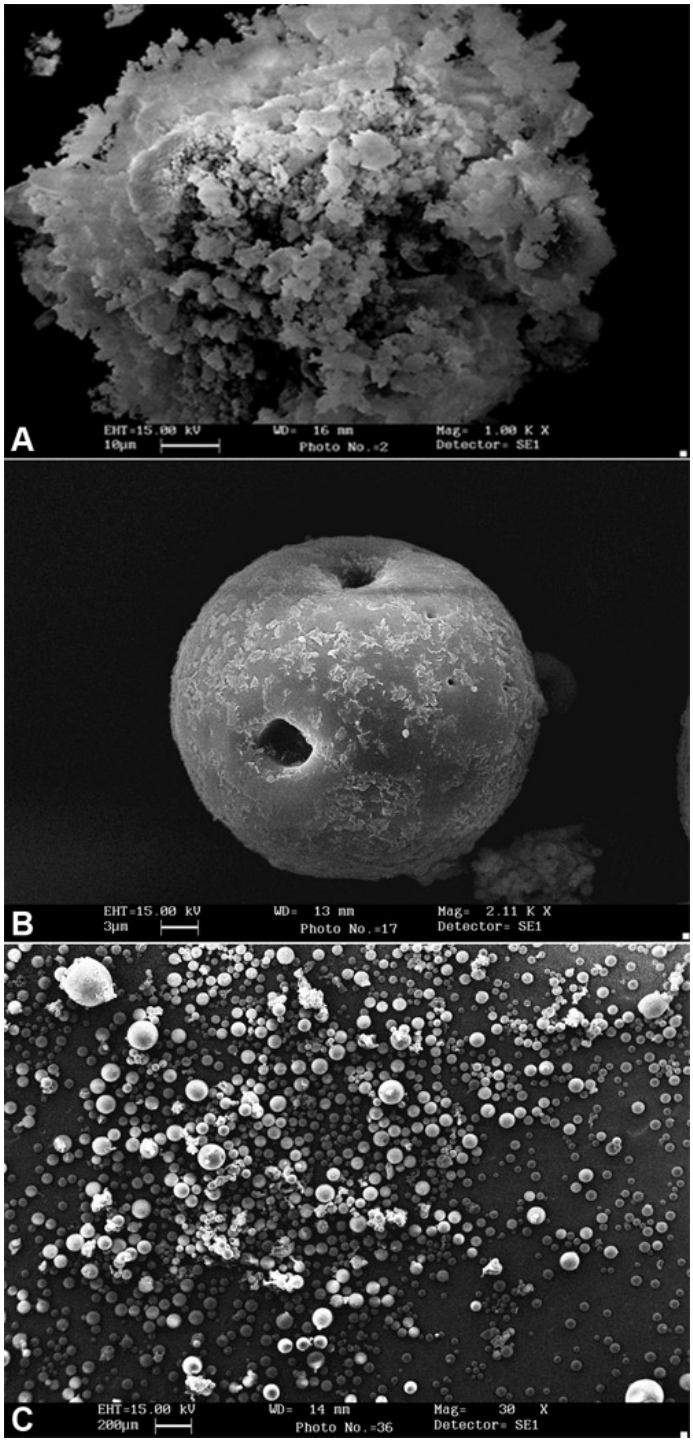

Figure 1. Scanning electron photomicrographs; (A) CS particle, (B) CS based microsphere, and (C) population of microspheres. CS indicates calcium silicate.

Table 1. Buoyancy and Drug Entrapment of Different Floating Microspheres*

Formulation Code	CS Content (mg)	Buoyancy (%)	Drug Entrapment (%)
WC*	0	70 ± 3	70 ± 2.5
CS1†	50	77 ± 2	78 ± 2.4
CS2†	100	80 ± 4	82 ± 1.4
CS3†	150	83 ± 2	82 ± 3.0
CS4†	200	88 ± 4	84 ± 2.8
CS5†	250	82 ± 5	80 ± 1.4

*CS indicates calcium silicate; WC, floating microspheres of orlistat without carrier; and CS1-5, floating microspheres of orlistat with calcium silicate (n = 3).

Germany). The gamma camera had a field view of 40 cm and was fitted with a medium-energy parallel hole collimator. The 140 keV gamma rays emitted by ^{99m}Tc were imaged. Specific stomach site (anterior) were imaged by E-Cam Single Head gamma camera after definite time intervals and activity counts were recorded for a 5-minute period to calculate the counts per minute (cpm). The gamma images were recorded using an online computer system, stored on magnetic disk, and analyzed to determine the distribution of activity in the oral cavity, stomach, and intestinal region. In between the gamma scanning, the animals were freed and allowed to move and carry out normal activities but were not allowed to take any food or water until the formulation had emptied the stomach completely.[18]

Pharmacokinetic Studies

The in vivo study was performed as per the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The Institutional Animals Ethical Committee of Dr Hari Singh Gour University approved the protocol for the study. The in vivo studies were conducted in healthy male albino rabbits weighing 2.2 to 2.5 kg. Rabbits were kept for 1 week in an animal house to acclimatize them and fed a fixed standard diet. Twelve rabbits were divided into 2 groups of 6 each and were fasted for 24 hours. The first group was fed with Xenical capsule (F. Hoffmann-La Roche Ltd, Basel, Switzerland) equivalent to 12 mg of OT, while CS4 equivalent to 12 mg of OT was fed to the second group of animals. Water was allowed ad libitum during fasting and throughout the experiment. The rabbits were not anesthetized during or prior to the treatment and were administered the formulation with an oral cannula. The rabbits swallowed the formulation without any difficulty. Blood samples (2 mL) were collected from the marginal ear vein into heparinized centrifuge tubes just before dosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours during the study. The blood samples withdrawn as above were transferred to a series of graduated centrifuge tubes containing 0.4 mL of 2.5% wt/vol sodium citrate solution. The samples were centrifuged at 2500 rpm for 5 minutes. The plasma was transferred into another set of sample tubes and frozen until assayed. One undosed plasma sample was kept as blank. The sample was filtered through 0.25-μm membrane filter (Millipore). The OT concentration in blood plasma samples was analyzed using LC/MS/MS method as described earlier.

Results and Discussion

Micromeritic Properties and Morphology

The mean particle size of CS powder was 142 ± 02 μm, while that of microsphere formulations containing CS in the range of 50 to 250 mg measured 550 ± 05 μm, 610 ± 08 μm, 648 ± 12 μm, 720 ± 10 μm, and 828 ± 12 μm. The particle size of microsphere formulation WC was found to be 180 ± 08 μm. The tapped density values ranged from 0.43 ± 0.04 g/cm^3 to 0.68 ± 0.06 g/cm^3, while their true density ranged between 1.66 ± 0.12 g/cm^3 and 1.94 ± 0.10 g/cm^3. This significant difference in the densities may be caused by the presence of low-density CS particles in the microspheres. The porosity of all the microsphere formulations was found to be in the range of 60.6% ± 2.5% to 80.0% ± 4.0%. The compressibility index ranged between 25.0% ± 2.2% and 34.6% ± 3.1%. All formulations showed excellent flowability as expressed in terms of angle of repose (<40º) except formulation CS5, probably because of the higher content of CS. The better flow property of microspheres indicates that the floating microspheres produced are nonaggregated.

CS-based Eudragit microspheres were spherical in shape. The porous nature of the CS and the spherical shape of the

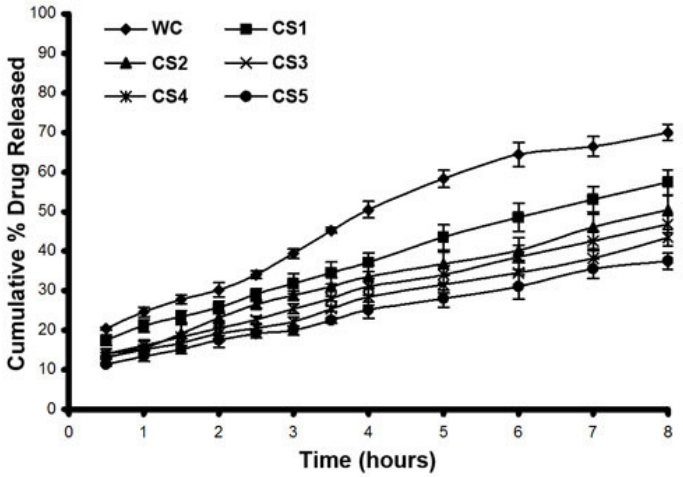

Figure 2. In vitro release of orlistat from various floating microspheres in simulated gastric fluid (pH 2.0) (n = 3). WC indicates floating microspheres of orlistat without carrier; and CS1-5, floating microspheres of orlistat with calcium silicate.

Table 2. The Regression Coefficients and Rate Constants for Release of Orlistat From Floating Microspheres in Simulated Gastric Fluid (pH 2.0)*

	Zero-order Model		First-order Model		H-M Model		P-K Model		H-C Model	
Formulation	r	k_1	r	k_2	r	k_3	r	k_4	r	k_5
WC	0.8762	10.6842	0.9799	–0.1641	0.9902	24.6715	0.9782	24.5398	0.9589	–0.0469
CS1	0.8523	8.4173	0.9564	–0.1140	0.9931	19.4937	0.9807	20.7807	0.9313	–0.0342
CS2	0.8652	7.3073	0.9488	–0.0939	0.9953	16.9078	0.9868	17.0995	0.9270	–0.0287
CS3	0.8674	6.7540	0.9457	–0.0849	0.9924	15.6110	0.9787	16.3321	0.9249	–0.0261
CS4	0.8629	6.1582	0.9362	–0.0755	0.9898	14.2356	0.9736	15.1140	0.9163	–0.0235
CS5	0.8563	5.5036	0.9247	–0.0657	0.9924	12.7386	0.9807	13.4997	0.9053	–0.0206

*H-M, indicates Higuchi matrix; P-K, Peppas-Korsmeyer; H-C, Hixon-Crowell; r, indicates correlation coefficient; k_1-k_5, rate constants of zero-order, first-order; WC, floating microspheres of orlistat without carrier; and CS1-5, floating microspheres of orlistat with calcium silicate.

microspheres are evident from their SEM photomicrographs (Figure 1). A large population of microspheres in the optimized formulation exists in spherical shape, which may be clearly seen in Figure 1.

Percentage Buoyancy and 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 (Table 1). 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.[5,19] Microsphere formulation CS4 containing 200 mg CS showed the best floating ability (88% ± 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 OT was found to be good at all loading. The high entrapment efficiency of OT in microspheres may be attributed to its poor aqueous solubility. The extent of loading influenced the particle size distribution of microspheres.

Table 3. One-way ANOVA (Dunnett multiple comparison test) for In Vitro Release of Orlistat in Simulated Gastric Fluid (pH 2.0)*

Comparison	Mean Difference	q	*P* Value
WC vs CS1	9.033	1.821†	$P > .05$
WC vs CS2	13.833	2.788‡	$P < .05$
WC vs CS3	16.133	3.252§	$P < .01$
WC vs CS4	18.583	3.746§	$P < .01$
WC vs CS5	21.267	4.287§	$P < .01$

*ANOVA indicates analysis of variance; q, parameter obtained with p when performing ANOVA; WC indicates floating microspheres of orlistat without carrier Control-WC; and CS1-5, floating microspheres of orlistat with calcium silicate.
†Nonsignificant.
‡Significant.
§Very significant.

When the loading was high, the proportion of larger particles formed was also high. With 80% drug entrapment, most of the particles were in the size range of 600 to 1200 μm, which is suitable for oral administration. From the experimentally determined yields, it was found that ~35% microspheres did not contain any porous carrier. This may be owing to the difference in particle size of microspheres. Because porous carrier-free microspheres and carrier particles are much smaller in size (100-200 μm) than the microspheres-containing carrier (500-800 μm), these were separated during the sieving step.

In Vitro Drug Release Study

Release of OT from CS-based microspheres was evaluated in SGF (pH 2.0). Since the acrylic polymer used is not soluble in acidic pH and starts to dissolve above pH 7, microspheres released the OT only by diffusion in SGF (pH 2.0). The other reason for the slow dissolution rate of drug may be attributed to low solubility of OT at acidic pH. No burst effect was observed from any of these formulations. The release of OT from different formulations followed the order: WC > CS1 > CS2 > CS3 > CS4 > CS5. 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) (Figure 2). The release mechanism of OT 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 OT in SGF were calculated using PCP Disso Version 3 software (Pune, India) and are reported in Table 2. Release pattern of OT in SGF (pH 2.0) from all floating microspheres followed Higuchi matrix model and Peppas-Korsmeyer model. Desai and Bolton[20] and Khattar et al[21] reported that noneffervescent floating systems obeyed the Higuchi model indicating drug release via a diffusion mechanism. When the release data of WC (without CS) was

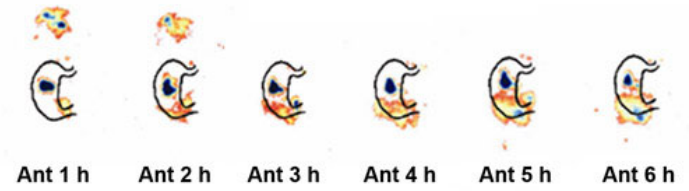

Figure 3. Gamma scintigraphic images of CS4 in albino rabbits. Ant indicates anterior position.

compared with formulations containing CS by 1-way ANOVA (Dunnett multiple comparison test), the difference in in vitro release in SGF from CS2 was found to be significant ($P < .05$) and very significant ($P < .01$) from CS3, CS4, and CS5 (Table 3).

Gamma Scintigraphy

The optimized formulation (CS4) had shown good in vitro buoyancy and controlled release behavior and hence was finally selected for in vivo study (ie, gamma scintigraphy), and the results were compared with NFM prepared using the identical polymers. Gamma images of the ^{99m}Tc-labeled CS4 and NFM are shown in Figures 3 and 4. Examination of the sequential gamma scintigraphic images during the study clearly indicated that the CS4 remained buoyant and uniformly distributed in the gastric contents for the study period of 6 hours. Prolonged gastric retention time (GRT) of more than 6 hours was achieved in all the rabbits for the CS4, which remained buoyant in the stomach for the entire test period. In contrast, NFM showed gastro-retention of less than 2 hours. After swallowing, the floating microspheres adopted a floating position on top of the stomach content. This might be because of the presence of porous low density CS and a hollow cavity inside the microspheres. A measurable number of counts of ^{99m}Tc-tagged CS4 for the 6-hour study period showed very good gastro-retentive propensity as the administered microspheres remained floating and distributed in the stomach contents for the 6-hour study period. In case of NFM, the radioactive counts decreased considerably after 2 hours (Figure 5). Gamma scintigraphy was performed for 6 hours, corresponding to the half-life of ^{99m}Tc.[22]

Pharmacokinetic Studies

In addition to the developed floating formulation that had shown the best in vitro dissolution and floating behavior,

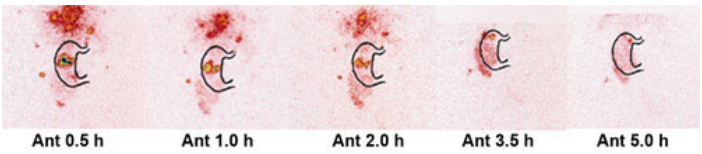

Figure 4. Gamma scintigraphic images of nonfloating microspheres in albino rabbits. Ant indicates anterior position.

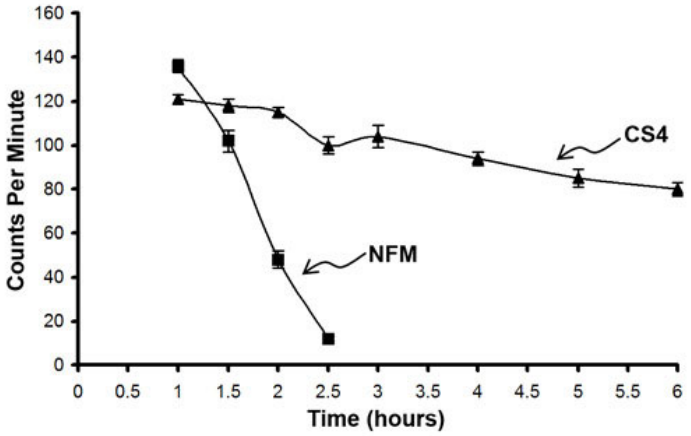

Figure 5. Counts per minute of ^{99m}Tc-tagged optimized floating formulation (CS4) and nonfloating microspheres (NFM) (n = 6).

the optimized formulation CS4 was also evaluated for its pharmacokinetic parameters. For comparison, the marketed preparation of OT (Xenical capsule) was used in the study. In the present study, the peak plasma concentration (C_{max}) remained nearly unchanged, as evident from the data in Table 4, showing 9.2 ng/mL for the marketed preparation (Xenical) and 9.4 ng/mL for floating microspheres (Figure 6). In addition, the time to reach peak plasma concentration (t_{max}) increased from 4 to 8 hours and the area under the curve (AUC) increased from 69.0 ng.h/mL to 113.3 ng.h/mL (nearly 1.6 times) for floating microspheres. Elimination half-life ($t_{1/2}$) was increased by ~1.5 times (4.08 hours) for the CS4 in comparison with the Xenical capsule. The values of C_{max} and t_{max} clearly indicate that the absorption

Table 4. Pharmacokinetic Parameters of Orlistat Formulations After Oral Administration in Rabbits (n = 6)

Serial No.	Pharmacokinetic Parameter	Marketed Preparation (Xenical)	Floating Microspheres (CS4)
1	Peak plasma concentration C_{max} (ng/mL)	9.2 ± 0.42	9.4 ± 0.62
2	Time to reach peak plasma concentration t_{max} (hours)	4	8
3	Area under the curve $AUC_{0–24}$ (ng.h/mL)	69.0	113.3
4	Absorption rate constant K_a (h^{-1})	0.58	0.54
5	Elimination rate constant K_e (h^{-1})	0.26	0.17
6	Elimination half life $t_{1/2}$ (hours)	2.67	4.08
7	Lag time (minutes)	50	45
8	Relative bioavailability	1.00	1.64

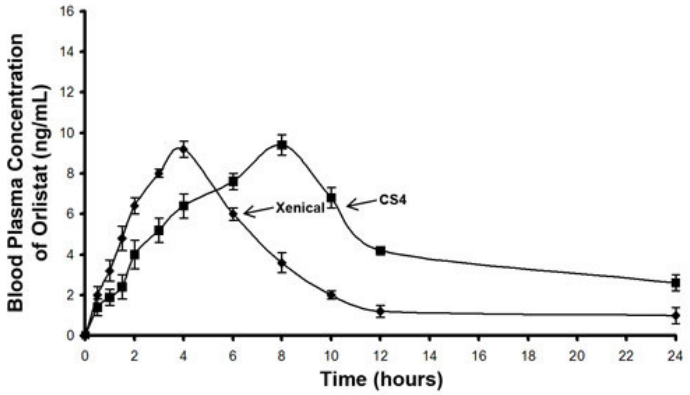

Figure 6. Mean plasma concentration of orlistat following oral administration of its floating formulation and the marketed product (Xenical) (n = 6).

of OT was not increased from GI tract when given in floating formulations. Absorption from the CS4 took 8 hours as compared with 4 hours from Xenical to reach the maximum concentration of OT in blood, indicating delay in absorption and prolonged localized concentration in the stomach and duodenum, the desired site of action of the drug. The site of action of OT is localized in the stomach and duodenum for inhibition of lipase enzyme. The in vivo study of selected floating formulation confirmed its ability to modify the pharmacokinetic behavior of the parent drug in the desired manner. These results clearly indicated the controlled and sustained release of OT from CS based gastro-retentive floating formulations. It may be concluded that the enhanced elimination half-life observed after gamma scintigraphy in the present study is because of the floating nature of the designed formulations.

CONCLUSION

The CS-based floating multiparticulate delivery system radiolabeled with ^{99m}Tc can be successfully visualized by scintigraphy to establish gastro-retentive performance in the rabbit. The results clearly indicated the controlled and sustained release of OT from CS based gastro-retentive floating microspheres. Further, the microspheres could also be compressed into tablets, filled into capsules, or formulated into oral suspension for reconstitution.

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