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Porous Carrier Based Floating Granular Delivery System of Repaglinide

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ABSTRACT A floating granular delivery system consisting of calcium silicate (CS) as porous carrier; repaglinide (Rg), an oral hypoglycemic agent; and hydroxypropyl methylcellulose K4M (HPMC K4M), ethyl cellulose (EC) and carbopol 940 (CP940) as matrix forming polymers was prepared and evaluated for its gastro-retentive and controlled release properties. The effect of various formulation and process variables on the particle morphology, micromeritic properties, in vitro floating behavior, drug content (%) and in vitro drug release was studied. The transit of floating granules of optimized formulation in the gastrointestinal (GI) tract was monitored by gamma scintigraphy in albino rabbits. The optimized formulation was compared in vivo with lactose granules (RgSCLG) prepared from identical polymers with their optimized composition ratio. Repaglinide-loaded optimized formulation was orally administered to albino rabbits and blood samples collected were used to determine pharmacokinetic parameters of Rg from floating granular formulation. Results were compared with pharmacokinetic parameters of marketed tablet formulation of Rg. The optimized formulation ($RgSCG_4$) demonstrated favorable in vitro floating and release characteristics. Prolonged gastric residence time (GRT) of over 6 hr was achieved in all subjects for calcium silicate based floating granules of Rg. The relative bioavailability of Rg-loaded floating granules increased 3.8-fold in comparison to that of its marketed capsule. The designed system, combining excellent buoyant ability and suitable drug release pattern, offered clear advantages in terms of increased bioavailability of repaglinide.

KEYWORDS Repaglinide, Calcium silicate, Floating granules, Gamma scintigraphy, Pharmacokinetic parameters

INTRODUCTION

Floating drug delivery is of particular interest for drugs that act locally in the stomach; are primarily absorbed in the stomach and poorly soluble at an alkaline pH; have a narrow window of absorption; and are unstable in the intestinal or colonic environment (Singh & Kim, 2000). Floating has been achieved with the preparation of low density solid systems by inclusion of sponges or highly porous systems (Muller & Anders, 1989; Nakamichi et al., 1996) or with systems, which decrease in density upon contact with gastric fluids

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based on the expansion of swelling agents (Bolton et al., 1989) or CO_2 generation (Ichikawa et al., 1987). Dennis et al. (1992) proposed a loose powder filled capsule that is buoyant so that it will float on gastric juices and thereby improve drug availability. Streubel et al. (2002) used polypropylene foam powder as porous carrier for the development of verapamil HCl-loaded floating microparticles. They also prepared low density foam-based, floating microparticles of chlorpheniramine maleate, diltiazem HCl, theophylline and verapamil HCl using Eudragit RS and polymethyl methacrylate as polymers (Streubel et al., 2003a). Jain et al. (2005) reported preparation and in vitro characterization of calcium silicate based floating microspheres of repaglinide. These microspheres were also evaluated for their gastro-retentive behavior using gamma scintigraphy study and pharmacokinetic parameters (Jain et al., 2006a). Recently, Jain et al. (2006b) developed calcium silicate based floating granules of ranitidine hydrochloride using hydroxypropyl methylcellulose and ethylcellulose.

The objective of the present investigation was to develop and evaluate a floating granular delivery system consisting of highly porous carrier material like calcium silicate (CS); repaglinide (Rg), an oral hypoglycemic agent; and matrix forming polymers such as hydroxypropyl methylcellulose K4M (HPMC K4M), ethyl cellulose (EC) and carbopol 940 (CP940), that are capable of floating on gastric fluid. Thus, the proper composition of these polymers is proposed to deliver the therapeutic agent over an extended period. CS, which has a characteristically porous structure with many pores and a large pore volume, has floating ability due to the air trapped within its pores when they are covered with a polymer and also has sustained release property (Yuasa et al., 1996). Rg, a fast and short acting meglitinide analog has a very short half-life (1 hr), low bioavailability (56%) and is completely absorbed from GI tract (Davis & Granner, 2001). All these limitations can be overcome if a floating controlled release system with increased GRT is developed for Rg.

MATERIALS AND METHODS

Materials

Repaglinide was generously supplied as a gift sample by Torrent Pharmaceuticals, Ahmedabad, India. Calcium silicate and stannous chloride were purchased from Sigma Aldrich Laborchemikalien GmBH, Germany. Different grades of hydroxypropyl methylcellulose, i.e., HPMC K4M, HPMC E4M, HPMC E5LV, and HPMC K100LV were purchased from G. S. Chemical Testing Laboratories, New Delhi, India. Ethyl cellulose and carbopol 940 were purchased from HiMedia Laboratories Ltd, Mumbai, India. Technetium-99m (as pertechnetate) ($^{99m}TcO_4^-$) was obtained from 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. The in vivo study was performed in accordance with the protocol approved by the Institutional Animals Ethical Committee of Dr. Hari Singh Gour University, Sagar, India following 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. In vivo study was performed at Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, India.

Preparation of Polymer Coating Solution

Polymer solutions (5% w/v) of HPMC K4M and EC (as primary coating solution) and of EC, CP940, HPMC K4M (as secondary coating solution) in different ratios were prepared by dissolving them in ethanol with mild heating. Their viscosity was measured using a Synchro-Lactric Viscometer (Brookfield Engineering Laboratories, Stoughton).

Preparation of Drug Absorbed CS

The CS (1 g) was dispersed into 10 mL ethanolic solution of Rg (50 mg) to make slurry. The slurry was ultrasonicated for 10 min in an ice bath at 40% voltage frequency using a probe sonicator (Soniweld, Imeco Ultrasonics, India) to entrap the drug solution inside the pores of the porous carrier. The excess ethanolic solution was removed by filtration and drying in vacuum, which resulted in drug absorbed porous carrier.

Preparation of Floating Granules

Rg absorbed CS powder was placed in a sigma blade mixer and sufficient HPMC K4M/EC solution

(5% w/v in ethanol) was added drop-wise with constant mixing at 100 rpm to form a wet mass of desirable consistency. Mixing was allowed for 30 min. The wet mass was passed through a screen (# 22), spread on drying trays and dried in a forced air oven at 50°C for 3 hr. These granules were designated as RgPCG's (primary-coated granules of Rg) (Table 1). Among these primary-coated granules, $RgPCG_5$ (HPMC K4M:EC ratio 25:75) were selected. Thus, these $RgPCG_5$ were mixed and coated with polymer solution of HPMC, EC and CP940 (5% w/v in ethanol) following the same procedure. The coated granules were dried at 50°C for 3 hr and thus the secondary-coated granules containing Rg (RgSCG) were obtained (Table 2) (Bandelin, 1999).

Micromeritic Properties

The granules were characterized for their micromeritic properties, such as flowability, true density, bulk density, and porosity (Gorden et al., 1999). The flowability of CS and granular formulations were determined using angle of repose method. True density of CS and different floating formulations was determined using helium densitometer. Bulk density was determined using tapping method. Porosity was calculated by the following formula:

$$\% \text{ Porosity} = [1 - \text{bulk density/true density}] \times 100$$

TABLE 1 **Formulation Code and Ratio of Polymers Used in Primary Coating of Different Formulations**

S. no.	Formulation code	Ratio of HPMC K4M: EC
1	$RgPCG_1$	05:95
2	$RgPCG_2$	10:90
3	$RgPCG_3$	15:85
4	$RgPCG_4$	20:80
5	$RgPCG_5$	25:75

Rg–Repaglinide, PCG_{1-5}–Primary-coated granules.

TABLE 2 **Formulation Code and Ratio of Polymers Used in Secondary Coating of Different Formulations**

S. no.	Formulation code	Ratio of EC:CP934: HPMC K4M
1	$RgSCG_1$	90:10:0
2	$RgSCG_2$	80V:10:10
3	$RgSCG_3$	70:10: 20
4	$RgSCG_4$	60:10:30
5	$RgSCG_5$	50:10:40
6	$RgSCG_6$	90:0:10
7	$RgSCG_7$	70:20:10
8	$RgSCG_8$	60:30:10
9	$RgSCG_9$	50:40:10

Rg–Repaglinide, SCG_{1-9}–Secondary-coated granules.

Morphology

The morphology of the granules and CS were studied by scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling the powder on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 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 in a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan).

Floating Behavior

The floating test of each formulation was carried out using eight dissolution rate (DR) test apparatus USP XXIII method II. The granules weighing 300 mg were immersed in 900 mL simulated gastric fluid (SGF, pH 2.0) containing Tween 20 (0.02% w/v), maintained at 37°C, which was agitated by a paddle rotated at 100 rpm. The paddle blades were positioned at the surface of dissolution medium. The granules floating on the surface of SGF were recovered with a sieve (# 60) at 1 hr time interval for 8 hr from these dissolution apparatus, such that first sample of floating granules was collected at 1 hr from first DR apparatus, second sample after 2 hr from second apparatus and so on. The granules so collected were dried and weighed. The buoyancy of the granules was calculated by the following equation:

$$\text{Buoyancy } (\%) = W_f/(W_f + W_s) \times 100$$

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

Drug Content

An accurately weighed quantity (100 mg) of granular formulation of Rg was digested with 10 mL of ethanol

(95%) for 1 hr. The digested heterogenate granular mass was centrifuged (Remi, India) at 3000 rpm for 3 min. The supernatant was decanted off and the residue left in the centrifuge was redigested with another 10 mL of ethanol for 15 min and again centrifuged for 3 min. The supernatant was decanted off and mixed with the first extract and volume made up to the mark in a 10 mL volumetric flask and filtered through Whatmann filter paper (No. 41). After appropriate dilution with distilled water, it was analyzed for Rg content spectrophotometrically at 243.3 nm using GBC Cintra-10 UV/Vis spectrophotometer.

In Vitro Release Studies

The release behavior of Rg from various floating granular formulations was determined in simulated GI fluid using USP XXIII basket type dissolution apparatus. A weighed amount of floating granules equivalent to 50 mg Rg was filled into a capsule (# 3) and placed in the basket of dissolution rate apparatus. The dissolution medium, SGF (pH 2.0) (500 mL) containing 0.02% w/v of Tween 20 was maintained at 37 ± 1°C at 100 rpm. Five mL sample was withdrawn at each 30 min interval, and passed through a 5 μm membrane filter (Millipore) followed by analysis using GBC Cintra-10 UV/Vis spectrophotometer at 243.3 nm for Rg to determine the concentration of drug dissolved in the dissolution medium. The initial volume of the dissolution fluid was maintained by adding 5 mL of fresh dissolution fluid after each withdrawal. The dissolution study was repeated using simulated intestinal fluid (SIF, pH 7.4). Perfect sink condition prevailed during the drug release study. The in vitro drug release in SGF and SIF from marketed product of Rg, i.e., Rapilin tablet was also carried out following the same procedure. All experiments were conducted in triplicate.

In Vivo Scintigraphic Study

The optimized formulation ($RgSCG_4$) and lactose granules (RgSCLG) of 500 mg each loaded with $SnCl_2$ and Rg were placed in test tube and soaked in 10 mL of normal saline (0.9% NaCl) for 15 min. A small amount of sodium pertechnetate solution equivalent to radioactivity of 40 mBq in a sterile vial obtained from a technetium generator was added to the tube. The suspension was mixed intermittently for 15 min using a vortex shaker (Superfit, India) and granules were allowed to settle. The supernatant was removed and the labeled granules were recovered by filtration through a Whatmann filter paper (No. 41) followed by washing thoroughly with deionized water. The floating granules were then allowed to dry in air for 15 min. The in vivo transit behavior of the ^{99m}Tc tagged $RgSCG_4$ and RgSCLG was monitored using 12 1-year-old male albino rabbits. These rabbits were divided into two groups, i.e., group I and group II. None of them had symptoms or a past history of GI disease, or were administered any medication. In order to standardize the conditions of GI motility, the rabbits were fasted for 12 hr prior to the commencement of each experiment. $RgSCG_4$ (100 mg) was orally administered in suspension form to animals of group I and RgSCLG to group II followed by sufficient volume of drinking water. All four 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 gamma camera. 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. Anterior images and radioactive counts after definite time interval (5 min) were recorded using E-Cam Single Head Gamma Camera (Siemen's, Germany). The images were recorded using an online computer system (Macscnsetch, Germany), and stored on magnetic disk for analysis. In between the gamma scanning, the animals were freed and allowed to walk and carry out normal activities but they were not allowed to take any food or drink until the formulation had emptied the stomach completely.

High-Performance Liquid Chromatography (HPLC) Analysis in Blood Samples

The HPLC system used for the analysis of Rg in biological fluids consisted of a LC-10AT pump with SCL-10AT*vp* system controller (Shimadzu, Japan) equipped with an autoinjector (Model, SIL-10 AD*vp*) and a UV/VIS detector (Shimadzu, Japan). Chromatographic separations were achieved on Spheri-5 RP-18 column (5 μm, 100 × 4.6 mm i.d., Pierce Chemical Company, Rockford) preceded with guard column packed with the same material. The eluent was monitored at absorption wavelength of 243 nm and

chromatograms were integrated using CLASS-VP (version 6.12 SP5) software (Shimadzu, Japan). The mobile phase was prepared by mixing acetonitrile and ammonium acetate buffer, 10 mM (pH 4.0) in the ratio of 50:50 by volume and was then degassed for 20 min in an ultrasonic bath (Bransonic Cleaning Equipment Company, Danbury, Connecticut, USA) prior to use. The chromatography was performed at ambient temperature.

Pharmacokinetic Studies

The in vivo studies were conducted in healthy male albino rabbits weighing 2.2–2.5 kg. Rabbits were kept for one week in animal house to acclimatize them and provided fixed standard diet. Twelve rabbits were divided into two groups of six each and were fasted for 24 hr. One group was fed with Rapilin tablet (marketed product) equivalent to 1 mg of Rg, while $RgSCG_4$ was fed to second group of animals equivalent to 1 mg of Rg. Water was allowed *ad libitum* during fasting and throughout the experiment. The rabbits were not anaesthetized during or prior to the treatment and were administered formulation with an oral cannula. They 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 hr 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% w/v sodium citrate solution. The samples were centrifuged at 2500 rpm for 5 min. 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 (Milipore). The repaglinide concentration in blood plasma samples was analyzed using HPLC method as described earlier.

RESULTS AND DISCUSSION

Floating granular delivery system of Rg using CS as a floating carrier was prepared for improved retention in the stomach. Method adopted for preparation of floating granules was based on the absorption of drug inside the pores of CS particles that were coated with polymer solutions, which had floating ability due to the presence of air pockets in the pores thereby increasing GRT. Rg was absorbed in the pores of CS by ultrasonication. The drug absorbed CS powder was coated with 5% w/v ethanolic solution of HPMC K4M and EC in different ratios (Table 1). The vacuum dried and sieved granules, designated as primary-coated granules (RgPCG) were again coated with 5% w/v ethanolic solution of EC, CP934 and HPMC K4M in different ratios, which were designated as secondary-coated granules (RgSCG) (Table 2).

Morphology and Micromeritic Properties

SEM photomicrographs of the surfaces of the CS particles, RgPCG, and RgSCG are shown in Fig. 1 From these photomicrographs, it is clear that in case of primary-coated granules, the adsorption of polymer is observed on CS particle, whereas with secondary-coated granules the area of pores of CS covered with a polymer was found to be increased.

The mean diameter of primary and secondary-coated granular formulations was in the range of 620 to 705 μm and 790 to 940 μm, respectively (Table 3). The angle of repose of $RgPCG_5$ was found to be 40.8 ± 1.6°. The angle of repose of secondary-coated granules of each composition was found to be below 40°, which is considered satisfactory in flow during various processes, i.e., capsule filling and tablet punching. The true density of PCG was found to be in the range of 1.33–1.39 g/cm^3. The true density of RgSCG's was found to be increased from 1.35 ± 0.27 g/cm^3 to 1.67 ± 0.06 g/cm^3. The bulk density of PCG and SCG was found to be less than 1 g/cm^3, which is essential for their floating in SGF (pH 2.0). The bulk density of secondary-coated granules was higher than that of primary-coated granules due to increasing weight and amount of polymer solution adsorbed by CS (Table 3). The percentage porosity was also considerably decreased in SCG as compared to PCG. The percentage porosity of CS and granular formulations suggested that decrease in porosity of SCG granules may be due to increasing number of pores covered with polymer in secondary coating of formulations (Table 3).

Drug Content and Floating Ability

The content of Rg in primary and secondary-coated granules was found to be 80 ± 4% in all the formulations. The floating test was carried out to investigate

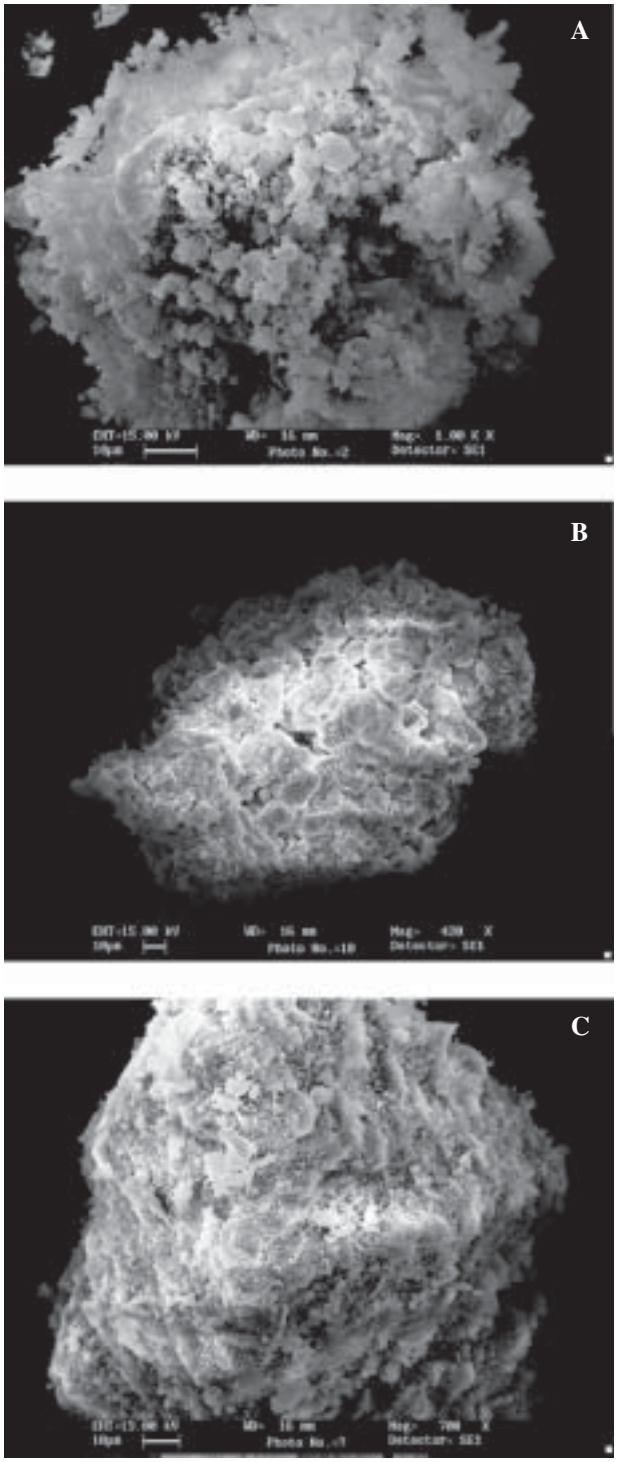

FIGURE 1 **Scanning Electron Photomicrographs of (A) Calcium Silicate Particles at 1000 ↔ (B) Primary-coated Floating Granule at 420 ↔, and (C) Secondary-coated Floating Granule at 700 ↔.**

the floatability of the prepared floating granules. Good in vitro floating behavior was observed for all the prepared formulations. Tween 20 (0.02% w/v), added to SGF, counteracted the downward pulling at the liquid surface by lowering surface tension of SGF and increasing the surface area at the air fluid interface. In contrast to most conventional floating systems (including gas generating ones), these granules floated immediately upon contact with the release medium showing no lag time in floating behavior because the low density was prevailed from the beginning ($t = 0$). The effect of polymer ratio (used for coating of granules) on the floating property of granules was also studied. High viscosity grade (K4M and E4M) HPMC showed slightly better floating properties than low viscosity grade (E5LV) HPMC. The floating behavior of different formulations increased in the rank order $RgPCG_5 > RgPCG_4 > RgPCG_3 > RgPCG_2 > RgPCG_1$ of primary-coated granules. The floating study also suggested that the granules coated with higher proportion of HPMC in polymer solution were more floatable than those with lower proportion of HPMC. The floating capacity or percentage buoyancy of SCG was higher than that of PCG. The floating study of granular formulations revealed that it is possible to cause a high floating property in the secondary-coated granular formulation due to swelling of HPMC in the coating of granules. In order to evaluate the effect of carbopol on the floating property of granules, CP940 was included in floating granules in different ratios with HPMC K4M and EC. It was observed that CP940 played an important role in the drug release and buoyancy of SCG_5, when used in combination with HPMC K4M and EC. In the present formulation carbopol was included in coating solution to control the drug release because carbopol is insoluble in water and artificial gastric fluid. The swelling of HPMC K4M in gastric fluid might have contributed partially to the floating behavior of these granular formulations. The floating behavior of secondary-coated granules in SGF increased in the rank order $RgSCG_5 > RgSCG_4 > RgSCG_2 > RgSCG_3 > RgSCG_6 > RgSCG_7 > RgSCG_8 > RgSCG_9 > RgSCG_1$ (Fig. 2). CP940 appeared to have a negative effect on the floating behavior of granules. Floating ability of granules for 8 hr was considered satisfactory performance. Another observation was that the granules of larger size showed the longer floating time.

In Vitro Drug Release

The release of Rg from floating granules prepared using HPMC K4M, CP940 and EC in different ratios

TABLE 3 **Micromeritic Properties of Calcium Silicate and Floating Granular Formulations**

Formulation code	Particle size (μm)	True density (g/cm^3)	Bulk density (g/cm^3)	Porosity (%)	Angle of repose (θ)
CS	142 ± 26	2.27 ± 0.11	0.14 ± 0.04	93.7 ± 1.9	46.7 ± 0.9
$RgPCG_1$	620 ± 19	1.33 ± 0.24	0.50 ± 0.06	60.7 ± 2.4	43.2 ± 2.0
$RgPCG_2$	665 ± 12	1.23 ± 0.26	0.48 ± 0.04	61.8 ± 2.5	43.6 ± 2.1
$RgPCG_3$	705 ± 14	1.35 ± 0.24	0.47 ± 0.08	62.0 ± 2.8	40.6 ± 1.0
$RgPCG_4$	680 ± 16	1.37 ± 0.38	0.42 ± 0.12	66.9 ± 3.5	42.0 ± 1.9
$RgPCG_5$	650 ± 15	1.39 ± 0.32	0.39 ± 0.09	69.4 ± 2.0	40.8 ± 1.6
$RgSCG_1$	790 ± 19	1.35 ± 0.27	0.73 ± 0.12	41.8 ± 3.0	38.6 ± 2.8
$RgSCG_2$	810 ± 18	1.42 ± 0.18	0.72 ± 0.08	43.8 ± 2.5	38.6 ± 2.4
$RgSCG_3$	850 ± 20	1.46 ± 0.12	0.69 ± 0.10	45.0 ± 2.0	37.9 ± 2.1
$RgSCG_4$	840 ± 22	1.51 ± 0.18	0.73 ± 0.05	43.0 ± 1.5	36.4 ± 1.2
$RgSCG_5$	890 ± 19	1.62 ± 0.22	0.79 ± 0.10	39.4 ± 3.0	39.8 ± 1.9
$RgSCG_6$	920 ± 15	1.60 ± 0.20	0.78 ± 0.12	37.4 ± 2.4	37.7 ± 2.3
$RgSCG_7$	940 ± 16	1.64 ± 0.08	0.70 ± 0.08	45.0 ± 0.8	37.9 ± 1.2
$RgSCG_8$	840 ± 12	1.58 ± 0.18	0.73 ± 0.06	43.0 ± 1.8	36.4 ± 0.6
$RgSCG_9$	905 ± 24	1.67 ± 0.06	0.81 ± 0.12	37.4 ± 2.0	37.7 ± 0.9

Rg–Repaglinide, $PCG_{1–5}$–Primary-coated granules, $SCG_{1–9}$–Secondary-coated granules.
Data represent the mean ± SD (n = 3).

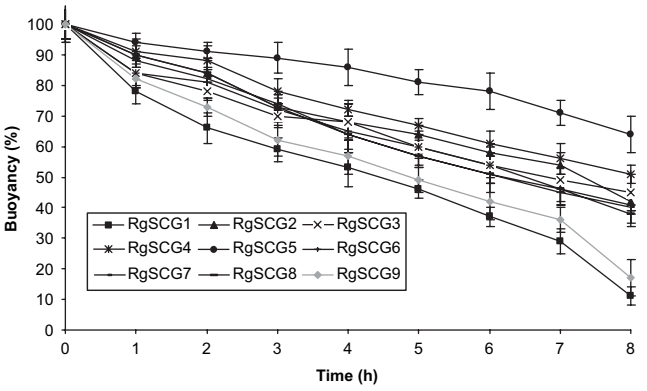

FIGURE 2 **Percent Buoyancy of Different Secondary-coated Granules ($SCG_{1–9}$) in SGF (pH 2.0). Values are Mean ± SD (n = 3).**

in coating solution was studied. Formulation $RgPCG_5$ showed comparatively better controlled release in SGF (pH 2.0), which were coated with polymer solution containing HPMC and EC in ratio of 25:75. Insoluble EC might have played an important role in controlling the Rg release from RgPCG. Formulation $RgPCG_5$ also displayed better buoyancy pattern. Thus, these primary-coated granules with satisfactory buoyancy (formulation $RgPCG_5$) were recoated with polymer solution containing different ratios of EC, CP940 and HPMC K4M and coded as $RgSCG_1$, $RgSCG_2$,.....$RgSCG_9$. Li et al. (2003) reported polymeric system with low viscosity polymer (HPMC K100LV) yielding a faster initial burst effect. Dortunc & Gunal (1997) reported increased viscosity resulting in a corresponding decreased drug release. Similar results were reported by Wan et al. (1995) who demonstrated that HPMC with higher viscosity resulted in thicker gel layer formation. The higher molecular weight of polymer also modifies dissolution behavior. With increasing macromolecular weight the dissolution rate decreases, thus the drug release decreases (Streubel et al., 2003b). CP940 is a cross-linked polymer with high molecular weight (~2×10^6 Da) and viscosity, and, when exposed to water, it becomes viscous and thus tends to bind the mixed polymeric system together, resulting in a reduced erosion of floating granules. Hence formulations without CP940 exhibited faster release due to erosion of HPMC and EC present in the coating. The release rate of Rg increased in the rank order $RgPCG_1 > RgPCG_2 > RgPCG_3 > RgPCG_4 > RgPCG_5$ of primary-coated granules in simulated gastric fluid. In case of secondary-coated granules, drug release in SGF (pH 2.0) followed the order $RgSCG_6 > RgSCG_1 > RgSCG_2 > RgSCG_7 > RgSCG_8 > RgSCG_9 > RgSCG_3 > RgSCG_5 > RgSCG_4$ (Fig. 3). As shown in Fig. 3, formulation $RgSCG_6$ released almost 100% Rg in 6 hr. Rg release in SIF (pH 7.4) followed the order $RgSCG_1 > RgSCG_6 > RgSCG_2 > RgSCG_7 > RgSCG_8 > RgSCG_3 > RgSCG_9 > RgSCG_4 > RgSCG_5$ (Fig. 4). It is clear from comparison of in vitro release

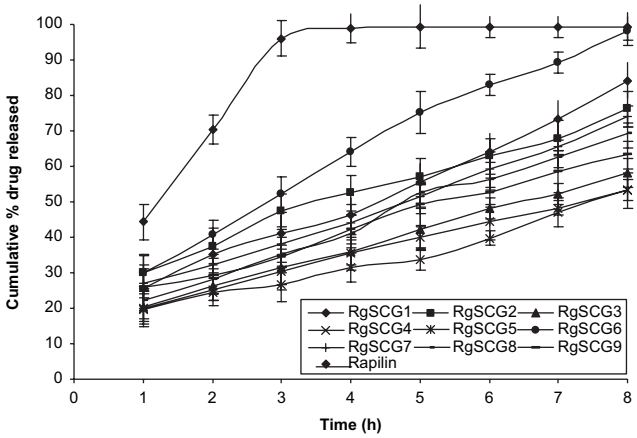

FIGURE 3 **In Vitro Repaglinide Release Profile of Rapilin Tablet and Secondary-coated Floating Granules ($RgSCG_{1-9}$) in SGF (pH 2.0). Values are Mean ± SD (*n* = 3).**

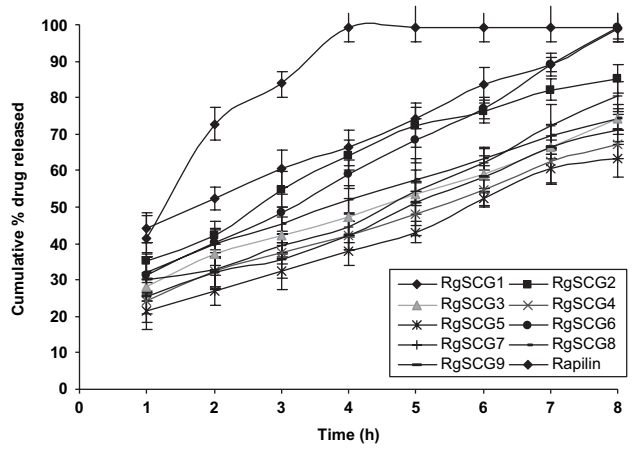

FIGURE 4 **In Vitro Repaglinide Release Profile of Rapilin Tablet and Secondary-coated Floating Granules ($RgSCG_{1-9}$) in SGF (pH 7.4). Values are Mean ± SD (*n* = 3).**

profile of Rg from secondary-coated granules that as the ratio of HPMC decreased, the release was increased. This may be due to the decrease in density of the swollen hydrogel network with reduction in amount of HPMC K4M offering lower hindrance to drug diffusion and hence the drug release. The drug release studies in GI fluids revealed that the release profile depended upon the polymer concentration, amount of adsorbed polymer as well as the ratio of HPMC K4M and CP940 in the polymer solution. The study suggested that the initial high release of Rg from RgPCG and RgSCG might be attributed to the dissolution of Rg from those pores that were not covered with polymer.

Gamma Scintigraphic Studies

Optimized formulation ($RgSCG_4$) had shown good in vitro buoyancy and controlled release behavior and hence was finally selected for in vivo study, i.e., gamma scintigraphy and the results were compared with secondary-coated lactose granules (RgSCLG) prepared using identical polymers and composition ratios. The gamma images of ^{99m}Tc labeled formulations orally administered to albino rabbits were recorded using an online computer system (Macscnsetch, Germany), and stored on magnetic disk for analysis. Stored scintigraphic images were analyzed to determine the distribution of activity in the oral cavity, stomach and intestinal region. Gamma images of the ^{99m}Tc labeled $RgSCG_4$ and RgSCLG are shown in Figs. 5 and 6, respectively. Examination of the sequential

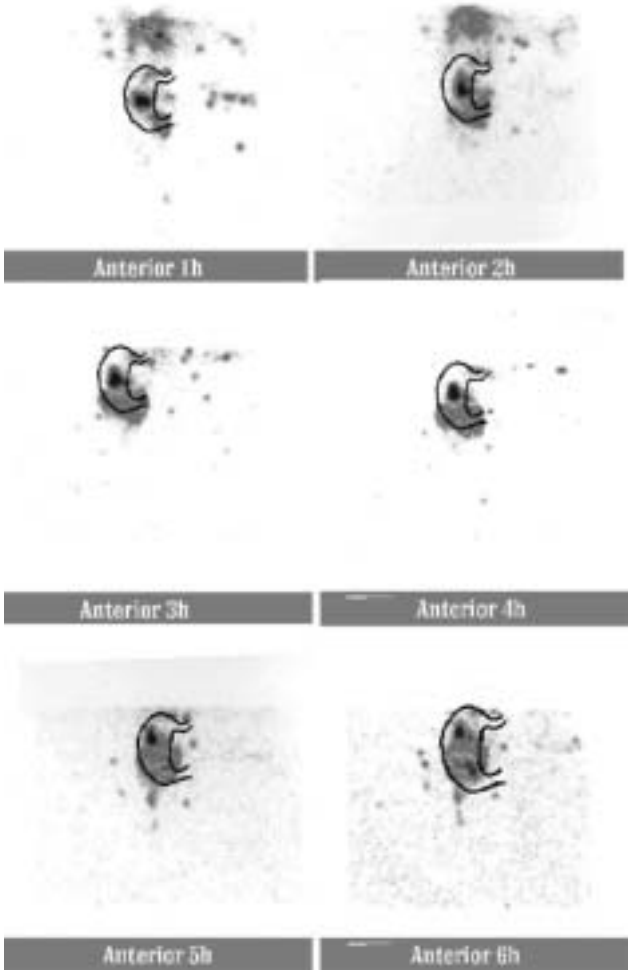

FIGURE 5 **Gamma Scintigraphic Images of $RgSCG_4$ in Albino Rabbit.**

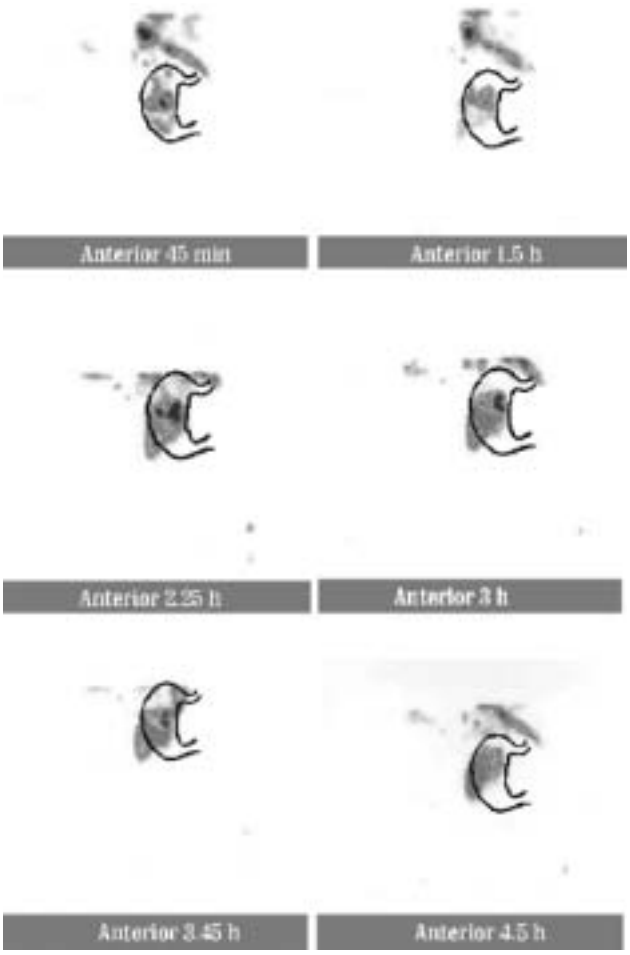

FIGURE 6 **Gamma Scintigraphic Images of RgSCLG in Albino Rabbit.**

gamma scintigraphic images during the study clearly indicated that the optimized $RgSCG_4$ remained buoyant and uniformly distributed in the gastric contents for the study period of 6 hr. Prolonged GRT of over 6 hr was achieved in all the rabbits for the $RgSCG_4$, which remained buoyant in the stomach for the entire test period. In contrast, RgSCLG showed gastroretention of 4 hr, which might be due to the presence of HPMC K4M in both coatings. After swallowing, the floating granules of calcium silicate adopted a floating position on top of the stomach content. This might be due to the presence of both the porous, low density CS and HPMC K4M in the granules. Specific stomach site (anterior) was imaged by E-Cam Single Head gamma camera after definite time intervals and counts were measured for 5 min period. Measurable number of counts of ^{99m}Tc tagged $RgSCG_4$ for 6 hr study period showed very good gastroretentive property of the administered formulation (Fig. 7). The coated granules remained floating and distributed properly in the stomach for study period of 6 hr. In case of RgSCLG, the radioactive counts decreased considerably after 2.25 hr (Fig. 8). This shorter retention time may be due to the absence of calcium silicate (low density porous carrier) in the secondary-coated lactose granules. Gamma scintigraphy was performed for 6 hr, i.e., half-life of ^{99m}Tc.

Pharmacokinetic Studies

Although the developed floating formulation had shown better in vitro dissolution and floating behavior, the optimized formulation $RgSCG_4$ was also evaluated for its bioavailability in rabbits to ascertain the pharmacokinetic parameters. For comparison marketed tablet preparation of Rg (Rapilin, Torrent Pharma, Ahmedabad, India) was used. In the present study, peak plasma concentration (C_{max}) for Rapilin tablet was found to be 24.4 ng/mL as against 26.2 ng/mL for floating granular formulation ($RgSCG_4$) (Fig. 9). The

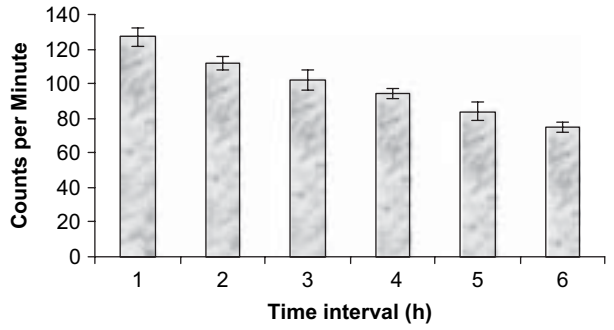

FIGURE 7 **Counts/min of ^{99m}Tc Tagged $RgSCG_4$. Values are Mean ± SD (n = 6).**

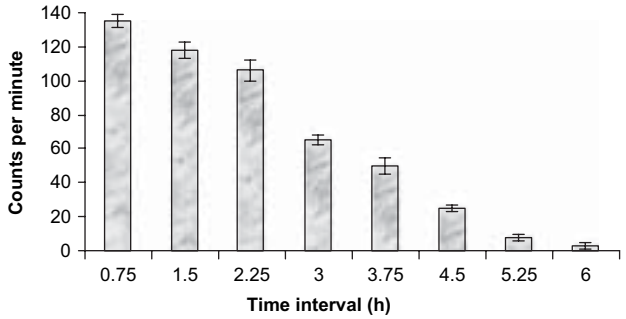

FIGURE 8 **Counts/min of ^{99m}Tc Tagged RgSCLG. Values are Mean ± SD (n = 6).**

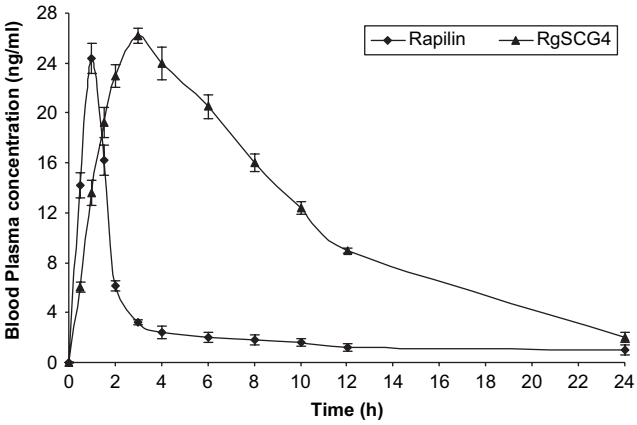

FIGURE 9 **Mean Plasma Concentration of Repaglinide Following Oral Administration of its Floating Granular Formulation and Marketed Product.**

AUC for Rapilin tablet was found to be 72.5 ng.hr/mL, whereas 275.6 ng.hr/mL for $RgSCG_4$. For Rapilin tablet, absorption rate constant (K_a) was 3.0/hr, elimination rate constant (K_e) was 1.36/hr and elimination half-life ($t_{1/2}$) was 0.51 hr, whereas in case of formulation $RgSCG_4$, K_a was 1.57/hr, K_e was 0.13/hr and $t_{1/2}$ was found to be 5.33 hr (Table 4). The comparison of these data clearly indicates that the C_{max} was not much varied and AUC was increased almost four times, in case of floating granular formulation of Rg. Thus, the relative bioavailability of Rg-loaded floating formulation ($RgSCG_4$) was found to be increased about 3.8-fold in comparison to that of its marketed preparation. Elimination half-life ($t_{1/2}$) was increased by about 10 times (5.33 hr) for the granular formulation $RgSCG_4$ in comparison to the marketed tablet formulation (Rapilin).

CONCLUSION

The present study was aimed to prepare floating granular delivery system with an objective to control the release rate of repaglinide. The performance of the formulations was evaluated and the effect of various formulation variables was studied. The floating ability of the granules and the release rate of the drug (repaglinide) from the granules can be controlled by changing the polymer type as well as the composition ratio of HPMC K4M, EC and CP940 in the polymer solution. The study demonstrated that calcium silicate based floating granular delivery system radiolabeled with ^{99m}Tc could be successfully visualized scintigraphically to establish its gastroretentive performance in the rabbit. It is concluded from the present investigation that the novel calcium silicate based floating granules can overcome and alleviate the drawbacks/limitations associated with various SR formulations. The floating granules could be compressed into tablets or filled into capsules.

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TABLE 4 **Pharmacokinetic Parameters of Repaglinide Formulations After Oral Administration in Rabbits**

S. no.	Pharmacokinetic parameter	Marketed preparation (Rapilin)	Floatinggranules ($RgSCG_4$)
1	Peak plasma concentrationC_{max} (ng/mL)	24.4 ± 1.24	26.2 ± 0.50
2	Time to reach peak plasma concentration t_{max} (hr)	1.0	3.0
3	Area under the curveAUC_{0-24} (ng.hr/mL)	72.5	275.6
4	Absorption rate constant K_a/hr	3.00	1.57
5	Elimination rate constant K_e/hr	1.36	0.13
6	Elimination half life $t_{1/2}$ (hr)	0.51	5.33
7	Lag time (min)	0	12
8	Relative bioavailability	1.00	3.80

Data represent the mean ± SD (n = 6).

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