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European Journal of Pharmaceutics and Biopharmaceutics 65 (2007) 85–93
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Development of hollow/porous calcium pectinate beads for floating-pulsatile drug delivery

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Received 26 August 2005; accepted in revised form 13 July 2006
Available online 21 July 2006

Abstract

The purpose of this work was to develop hollow calcium pectinate beads for floating-pulsatile release of diclofenac sodium intended for chronopharmacotherapy. Floating pulsatile concept was applied to increase the gastric residence of the dosage form having lag phase followed by a burst release. To overcome limitations of various approaches for imparting buoyancy, hollow/porous beads were prepared by simple process of acid-base reaction during ionotropic crosslinking. The floating beads obtained were porous (34% porosity), hollow with bulk density <1 and had $F_{t50\%}$ of 14–24 h. In vivo studies by gamma scintigraphy determined on rabbits showed gastroretention of beads up to 5 h. The floating beads provided expected two-phase release pattern with initial lag time during floating in acidic medium followed by rapid pulse release in phosphate buffer. This approach suggested the use of hollow calcium pectinate microparticles as promising floating-pulsatile drug delivery system for site- and time-specific release of drugs acting as per chronotherapy of diseases.

Keywords: Floating-pulsatile drug delivery; Calcium pectinate beads; Diclofenac sodium; Hollow beads; Gamma scintigraphy; Chronotherapy

1. Introduction

Natural biodegradable polysaccharides like pectin, guar gum, chitosan, carrageenans, sodium alginate and gellan gum have been used in controlled drug delivery [1–5]. Multiparticulate systems obtained by ionotropic crosslinking of these polymers have been used to develop floating drug delivery. Various approaches to induce buoyancy in crosslinked beads, some of which include freeze-drying, entrapment of gas or gas forming agents, use of volatile oils or fixed oils, have been used [6–8]. These approaches are complicated, as they require specific equipment and handling techniques with limited acceptance. The oil containing beads have limitations of coalescence of oil droplets yielding beads of wider particle size distribution, volatilization or leaching of oil [9]. Comparatively, the floating dosage forms containing sodium bicarbonate as buoyancy imparting agent are simple to produce which have been already attempted [10,11]. Their floating property is based on the evolution of carbon dioxide when in contact with acidic environment followed by the ability of polymer gel to entrap it which decreases their density below one. On the other hand, violent gas generation, disintegration of dosage form, burst release, dose dumping and alkaline microenvironment [12] are limitations of these dosage forms. Choi et al. [13] have developed porous alginate beads containing riboflavin where the carbon dioxide gas was allowed to generate during crosslinking only, followed by freeze-drying to improve porosity. Talukder and Fassihi [14] developed a floatable multiparticulate system by crosslinking low methoxylated pectin and sodium alginate. The beads obtained by freeze-drying remained buoyant over 12 h, whereas the air-dried beads remained submerged. The study revealed the presence of air-filled hollow spaces

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doi:10.1016/j.ejpb.2006.07.010

inside the freeze-dried beads, which was responsible for the flotation property of the beads. Sriamornsak et al. [15] developed floating calcium pectinate beads by emulsion-gelation method. Such technique can be considered as alternative to overcome limitations of sodium bicarbonate containing floating drug delivery systems. Chronopharmacotherapy, the drug regime based on circadian rhythm, is recently gaining much attention worldwide. Various diseases like asthma, hypertension, acidity, and arthritis show circadian variation, that demands time-scheduled drug release for effective drug action, e.g., inflammations associated with morning body stiffness, asthma, and heart attack in early hours of the day [16]. To follow this principle one must have to design the dosage form such that it can be given at the convenient time, e.g., bed time for the above-mentioned diseases with the drug release in the morning. Drug pharmacokinetics too show circadian variation for various anti-inflammatory drugs like indomethacin, ketoprofen and diclofenac sodium which have greater absorption in morning as compared to evening [17], and site-specific absorption from small intestine [18,19]. Therefore, to develop dosage form for chronopharmacotherapy the desired drug release should be time-specific as well as site-specific also.

The purpose of the present study was to produce hollow/porous-floating beads of pectin by a process of evolution of carbon dioxide during crosslinking in acidic environment. Diclofenac sodium, an acid-insoluble NSAID, was used as model drug. The obtained beads were evaluated for drug content, size analysis, porosity, mechanical strength, in vitro and in vivo floating properties and in vitro drug release.

2. Materials and methods

2.1. Materials

Low methoxy pectin, GENU®LM-104As, was the generous gift of C P Kelco (Denmark). Diclofenac sodium was received from Emcure Pharmaceuticals, Pune (India). Other materials used in the study were calcium chloride dihydrate (Sisco Research Lab. Pvt. Ltd., Mumbai, India), sodium bicarbonate (Loba Chemie, Mumbai, India), acetic acid, glacial (100%) (E Merck, Mumbai, India), stannous chloride (E Merck, Mumbai, India), and technicium −99m (as pertechnate) $TC^{99}O_4^-$. All chemical reagents used were of analytical grade.

2.2. Preparation of beads

Three hundred milligrams of pectin was dissolved in 10 ml of deionized water, 150 mg diclofenac sodium and various amounts of sodium bicarbonate were uniformly mixed, as shown in Table 1. The dispersion was sonicated for 30 min. (Ultrasonicator, Toshcon, Ajmer, India) to remove any air bubbles. The resultant dispersion was dropped via a 23-gauge syringe needle (0.65 mm internal diameter) into 80 ml of 2% w/v calcium chloride ($CaCl_2$) solution containing 10% acetic acid. The content was stirred at 100 rpm using magnetic stirrer for 15 min. The beads were then filtered, washed three times with distilled water and subsequently oven-dried at 50 °C for 4 h.

2.3. Drug content

20 mg beads of each batch were placed in100 ml phosphate buffer, pH 7.4, and mechanically agitated on shaker (Steelmet Industries, Pune, India) at 200 rpm for 24 h. The resultant dispersions were filtered and analyzed at 277 nm using UV spectrophotometer (JASCO-V500, Kyoto, Japan). The encapsulation efficiency was determined by the following formula:

$$\text{Encapsulation efficiency } (\%) = \text{AQ}/\text{TQ} \times 100,$$

where AQ is the actual drug content of beads and TQ is the theoretical quantity of drug present in beads.

2.4. Bead characterization

2.4.1. Infrared spectroscopy

The infrared spectra of diclofenac sodium, calcium pectinate beads (without drug, sodium bicarbonate and acetic acid) and drug-loaded porous calcium pectinate beads were recorded on FTIR (JASCO-FTIR 5300). The samples were prepared on KBr press (Spectra Lab, Mumbai, India).

2.4.2. Size analysis

Randomly selected 20 beads were observed under a stereomicroscope (Carl Zeiss, Germany) attached with a digital camera (Watec, WAT-202, Japan). Biovis image plus

Table 1
Composition, percent yield and encapsulation efficiency profiles of calcium pectinate beads

Batch No.	A1	A2	A3	A4	A5
Amount of pectin (mg)	300	300	300	300	300
Amount of drug (mg)	150	150	150	150	150
SBC (mg)[a]	–	–	0.075	0.150	0.225
Amount of $CaCl_2$ (g)	1.6	1.6	1.6	1.6	1.6
Acetic acid 10% (v/v) (ml)	–	8	8	8	8
% encapsulation efficiency	63.78 ± 1.92	77.86 ± 2.29	71.48 ± 1.10	76.66 ± 2.66	80.53 ± 1.81
% yield	92.86 ± 1.07	93.55 ± 1.05	90.03 ± 1.03	92.83 ± 1.40	88.55 ± 1.23

[a] SBC: Sodium bicarbonate.

software (Expert Tech Vision, India) was used to analyze the images of beads and were then expressed in terms of different parameters such as diameter, roundness and circulatory factor.

2.4.3. Bead porosity and bulk density

The bead porosity was assessed using mercury porosimetry (Autoscan 60 Porosimeter, Quantachrome software, USA) [11]. The pressure was applied from 0 to 6000 psi. The mercury intrusion data were recorded and plotted against pressure. Standard values for the contact angle and surface tension of mercury were used for calculations. The bulk densities of the beads were also measured using same mercury porosimeter.

2.4.4. Scanning electron microscopy (SEM)

Beads and their cross-sections were coated with a thin gold–palladium layer by sputter coater unit (VG- Microtech, UK). The surface topography was analyzed with a scanning electron microscope (Cambridge Stereoscan S120, Cambridge, UK) operated at an acceleration voltage of 10 kV.

2.4.5. Crushing strength

Crushing strength of 20 randomly selected beads of all the batches was determined using Jaroz and Parrot's mercury load cell method [20].

2.4.6. Moisture content

The total moisture content was measured using about 50 mg of beads from all the batches by Karl Fisher titration (Veegomatic-D, Mumbai, India).

2.4.7. Buoyancy test

The obtained beads were studied for buoyancy [21] and floating time using USP XXIII type 2 dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). One hundred beads of each batch were placed in 900 ml of 0.1 N HCl (pH 1.2) containing 0.02% w/v Tween 80 and agitated at 100 rpm, temperature was maintained at 37 °C ± 2. Number of sinking beads was observed visually.

2.5. Dissolution studies

The dissolution studies of the beads equivalent to 50 mg of diclofenac sodium were performed using USP XXIII type 1 dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). The drug release study was carried out in 0.1 N HCl for initial 2 or 6 h depending upon floating characteristics of beads, followed by dissolution in phosphate buffer, pH 7.4, each 900 ml, maintained at 37 °C ± 2 and agitated at 100 rpm ($n = 3$). Periodically samples were withdrawn and filtered through Whatman filter paper 41 and concentration of diclofenac sodium was measured spectrophotometrically (UV spectrophotometer, JASCO-V500, Kyoto, Japan) at 273 and 277 nm for acidic and basic media, respectively. Analysis of data was done using 'PCP Disso v2.08' software (Poona College of Pharmacy, Pune, India).

2.6. Gamma scintigraphy studies

2.6.1. Preparation of labeled beads

Radiolabeled beads of pectin (3% w/v) containing $TC^{99}O_4^-$ eluted from the generator (TC^{99}m Generator, Saxion Biotech Pvt. Ltd., Delhi, India) and stannous chloride solution were prepared by ionotropic crosslinking method. The labeling efficiency of the process was determined by comparing the radioactive counts obtained from the separated wet beads with the total radioactive count of initial radiolabeled pectin solution.

The TLC of the radiolabeled pectin solution was run in saline solution so as to assess the radiolabeling. The radiolabeled beads were also evaluated for the stability of the calcium pectinate–radioisotope complex. The dried radiolabeled beads were placed in 100 ml of 0.1 N HCl, pH 1.2, for 6 h. Periodically the beads and medium was sampled and assessed for radiocount (Isotope calibrator RC4714, Electronic appliances of India, Hyderabad, India).

2.6.2. Gamma image collection

The in vivo gastric residence of the beads was studied by gamma scintigraphic images [22,23]. Three adult male New Zealand white strain rabbits weighing approximately 2–2.5 kg were used for the study. After fasting for 24 h rabbits were allowed free access for food pellets (Chakan Oil Mills, Pune, India) and water for 12 h just before starting the study [24]. The protocol according to form B was approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC).

The beads equivalent to 50 mg diclofenac sodium were administered through a gastric tube with the aid of 3–4 ml water, using a syringe to push the beads forward. The gamma scintigraphy, the non-invasive technique, was employed to measure the gastric transit rate with GE gamma camera (Model Millennium MPT, Israel). Posterior images of rabbits were collected using collimator, about 1000 counts per second were collected. The gamma scintigraphic imaging was started just after dosing and was carried out for 6 h at specified time interval under the dynamic planer conditions.

3. Results and discussion

Polysaccharides have been widely used as pharmaceutical excipient for their biocompatible, biodegradable, inexpensive and non-toxic nature. They form multiparticulate system by simple ionotropic gelation, which can be formulated to provide various desired drug release patterns. Pectin, heterogeneous anionic polysaccharides with an ability to produce water-insoluble complexes with drug, has been used in oral novel drug delivery systems. In stomach pectin is not digested by gastric enzymes and has minimum

swelling but undergoes rapid gel relaxation/swelling in alkaline environment [25–27].

3.1. Preparation of beads

In our preliminary study, 0.75:1 w/w ratio of sodium bicarbonate and sodium alginate yielded mechanically weak and irregular hollow beads. Compared to calcium alginate beads the calcium pectinate beads of same concentration showed greater mechanical strength, therefore pectin was selected to obtain hollow floating beads. The hollow/porous beads were produced during ionotropic gelling assisted by in situ reaction between sodium bicarbonate in wet pectin beads with acidified calcium chloride crosslinking solution. To observe the effect of acid and alkali component Batch A1 and Batch A2 were prepared as shown in Table 1. Batches A3–A6 were, respectively, prepared using increased sodium bicarbonate level to pectin in ratio of 0.25:1, 0.5:1 0.75:1 and 1:1. Batch A6 produced beads of poor mechanical strength with no spherical shape, due to the excessive liberation of gas, which made pectin matrix too weak to sustain the shape after drying.

3.2. Drug content

Batch A1, prepared in plain crosslinking solution, showed lowest drug encapsulation than other batches; it may be due to decreased drug solubility in acidic crosslinking solution (Table 1). Batch A2 showed high encapsulation than A3 due to the absence of sodium bicarbonate. In Batches A3–A5, encapsulation efficiency increased with the increase in amount of sodium bicarbonate (Table 1). Effect of sodium bicarbonate can be attributed to the formation of alkaline microenvironment inside the bead enhancing drug solubility combined with the effervescent action-giving rise to modifications of bead matrix in situ. In Batch A3, the less amount of sodium bicarbonate acted individually causing scattered micro channels leading to drug loss. This effect can be supported by the fact that the bulk density of this batch is more than 1 (Table 2). For Batches A4 and A5 collective action exerted by the increased amount of sodium bicarbonate leads to the formation of prominent hollow structures due to entrapment of generated gas. This entrapment leads to the coalescence of gas bubbles, which pushed the internal matrix towards periphery forming thick boundaries minimizing drug leaching.

3.3. Bead characterization

3.3.1. Infrared spectroscopy

The IR spectra of calcium pectinate beads showed the characteristic band C=O vibration of COOH group at 1740 cm^{-1} and strong absorption band at 1617 cm^{-1} belonging to the asymmetric stretching of vibration of COO^{-}. The IR spectra of diclofenac sodium showed the strong peak at 1600 cm^{-1} in carbonyl frequency region, peak for NH stretching of aromatic ring at 1450 cm^{-1} and bending at 690 cm^{-1} for meta-di-substituted chlorine on benzene. The IR spectra of drug-loaded calcium pectinate beads of Batch A5 showed all the above-mentioned peaks of calcium pectinate beads and the diclofenac sodium (Fig. 1).

3.3.2. Size analysis

The drug-loaded calcium pectinate beads without sodium bicarbonate were comparatively spherical than other batches (Table 2). The presence of sodium bicarbonate

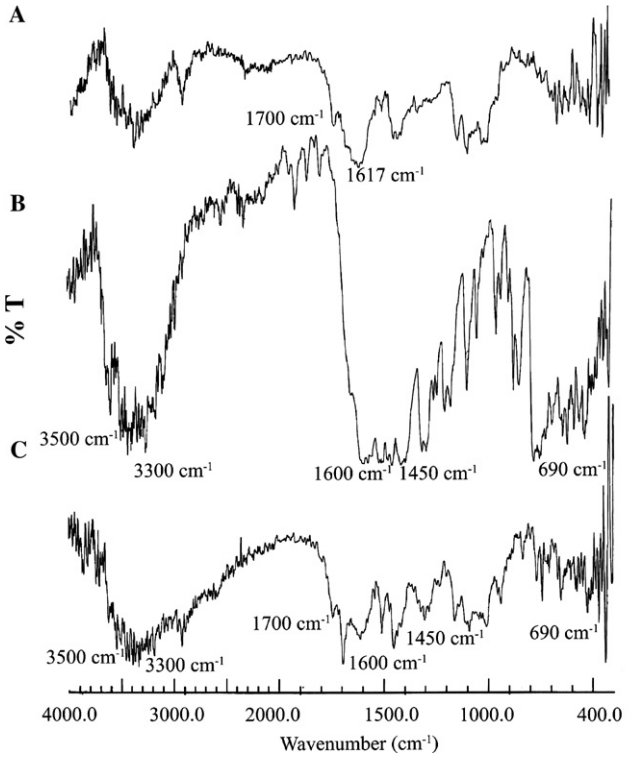

Fig. 1. The FTIR spectra. (A) Plane calcium pectinate bead. (B) Diclofenac sodium. (C) Batch A5.

Table 2
Micromeritic properties of calcium pectinate beads

Batch No.	Diameter (mean) (mm)	Roundness	Porosity (%)	Bulk density (g/cm^3)
A1	1.43 ± 0.05	0.77 ± 0.06	–	1.23 ± 0.01
A2	1.47 ± 0.06	0.71 ± 0.08	–	1.85 ± 0.11
A3	1.66 ± 0.06	0.67 ± 0.08	–	1.28 ± 0.19
A4	1.82 ± 0.09	0.75 ± 0.06	28.81	0.89 ± 0.13
A5	1.97 ± 0.10	0.75 ± 0.08	33.64	0.85 ± 0.07

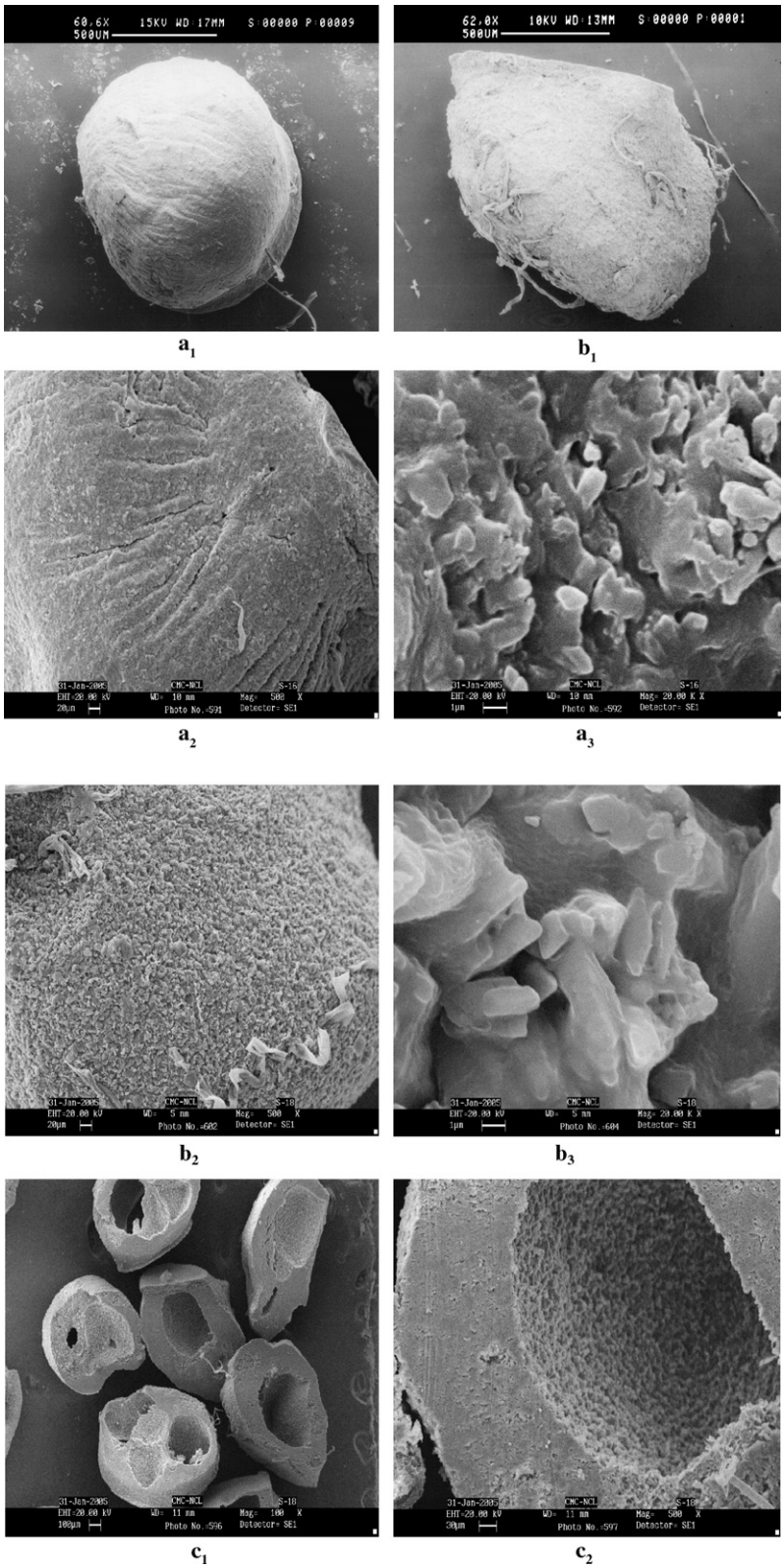

Fig. 2. Scanning electron micrographs of diclofenac sodium-loaded calcium pectinate beads. (a_1) Batch A2 (60.6×). (a_2) Batch A2 surface (500×). (a_3) Batch A2 surface (20K×). (b_1) Batch A5 (62×). (b_2) Batch A5 surface (500×). (b_3) Batch A5 surface (20K×). (c_1) Cross-section of Batch A5 (100×). (c_2) Cross-section of Batch A5 (500×).

amounts (at constant pectin concentration) might be responsible for softening of pectin beads subsequently deformed by the force of agitation. The particle size increases with the increased proportion of sodium bicarbonate in the polymer matrix. This can be attributed to the presence of entrapped gas bubbles. The increase in

porosity was also observed in similar order too (Table 2).

3.3.3. Scanning electron microscopy (SEM)

The drug-loaded beads with and without sodium bicarbonate were studied by SEM (Fig. 2). The oven-dried beads of Batch A2 were small and dense with wrinkled circumference due to gradual water loss (Fig. 2a_1, a_2 and a_3). The surface of Batch A5 prepared using highest sodium bicarbonate was very rough and porous (Fig. 2b_1 and b_2). The cross-section of beads from Batch A5 (Fig. 2c_1 and c_2) showed either a hollow core or multiple small hollow pockets in the matrix. The thick matrix boundaries around the hollow core, in the former batch, may be due to the coalescence of the gas bubbles formed in the wet beads. The precipitated drug crystals can be seen embedded in the matrix.

3.3.4. Bead porosity and bulk density

The bulk density of hollow beads (Batches A4 and A5) was less as compared with the beads without sodium bicarbonate (Batch A2). The decrease in bulk density was observed with increase in size and porosity (Table 2).

3.3.5. Crushing strength

Beads of all the batches were mechanically strong to tolerate normal handling. The beads of Batches A1 and A2 showed resistance to crushing when subjected to weight up to 800 g. It indicated the high intermolecular bonding and compactness of beads. Beads of Batches A4 and A5 showed comparatively lower values of crushing strength, 489.68 ± 13.00 and 483.22 ± 17.03 g, respectively. The rapid crushing of porous/hollow beads without deformation or fracture may be due to presence of empty spaces within the matrix.

3.3.6. Buoyancy test

Floating properties of beads were studied by determining buoyancy and time required for sinking all the beads under study. The surfactant was used in medium to simulate surface tension of human gastric juice (35–50 mN/m^2) [21]. Beads of Batches A1 and A2 were completely non-floating and sunk immediately, whereas majority of beads of Batch A3 were non-floating. Batches A4 and A5 produced floating beads without buoyancy lag time (Fig. 3) and remained floating for 7 and 12 h, respectively. F_{t50}, the time required to sink 50% of beads assuming linear approach of sinking, was presumed to be 14 and 24 h, respectively, for Batches A4 and A5. The floating properties of hollow/porous beads may be attributed to the low bulk density and the porosity of the beads; implying that the beads will have the propensity to exhibit an excellent buoyancy effect in vivo.

3.3.7. Gamma scintigraphy and dissolution studies

The in vivo gastric residence time of Batch A5 was studied by gamma-scintigraphic images of radiolabeled beads using rabbit as animal model. The representative gamma images obtained from a rabbit are shown in Fig. 4. It can be interpreted from the images that the beads gave a discrete bright spot at 4 h, after that mass started to diffuse

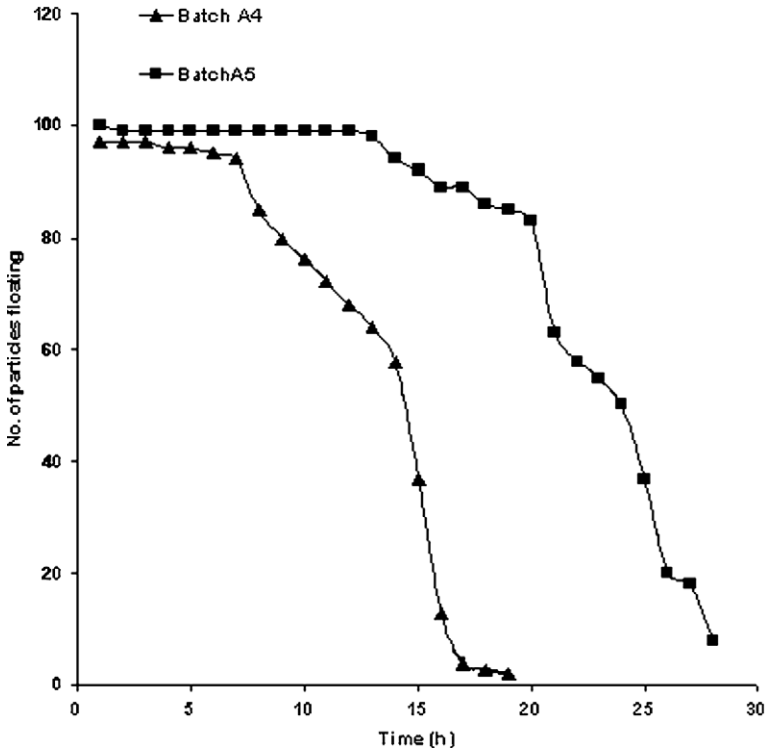

Fig. 3. Floating profile for diclofenac sodium-loaded calcium pectinate beads.

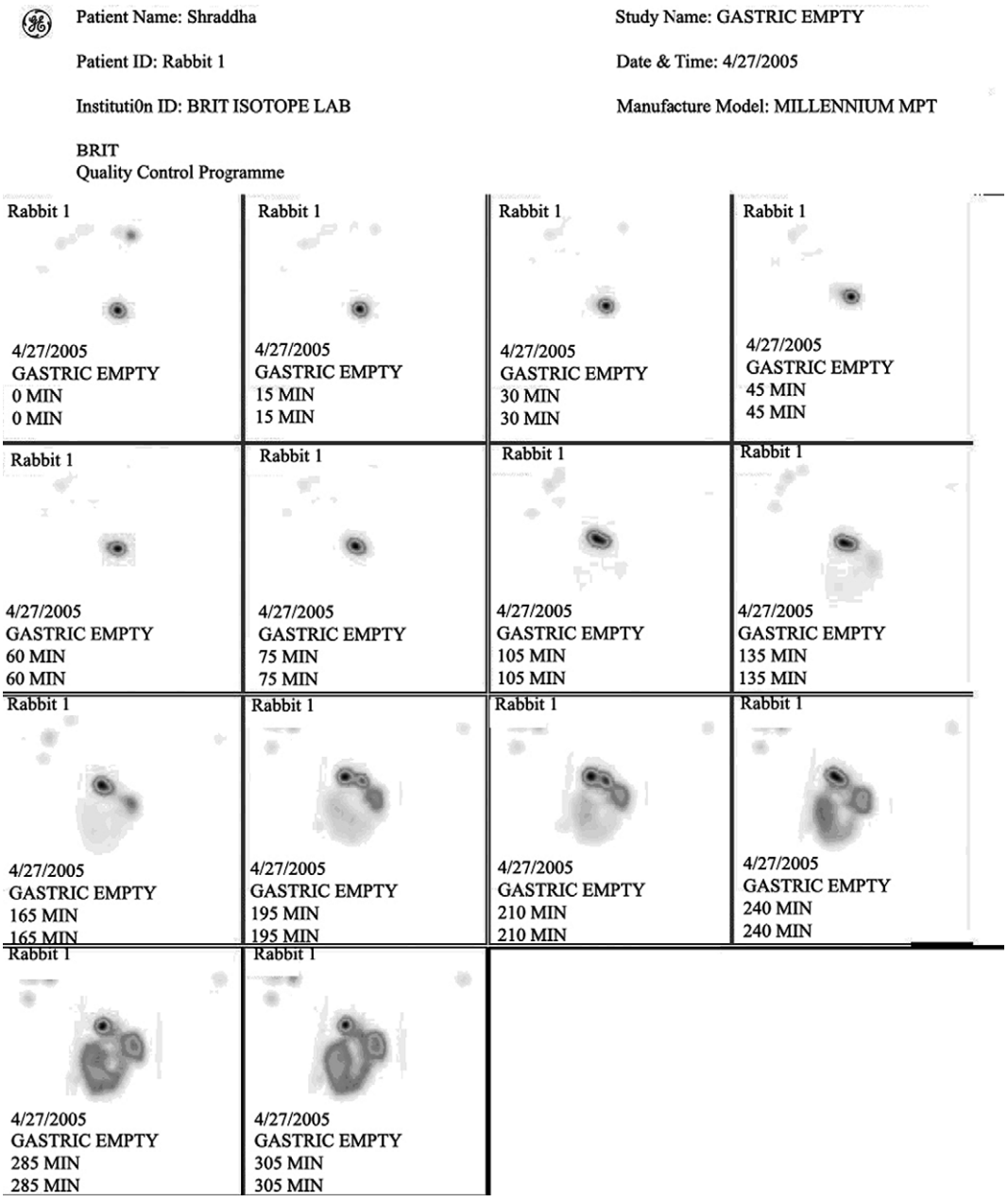

Fig. 4. Gamma scintigraphic images of diclofenac sodium-loaded calcium pectinate beads in rabbits.

to some extent in gastric content up to the end of 5 h, until the end of study.

The beads of Batch A3 were not studied for dissolution rate. The non-floating beads were assumed to remain in stomach for 2 h whereas on the basis of in vivo gastric residence the floating beads were considered to be gastroretentive for 6 h, making basis for in vitro dissolution time in acidic medium. All the beads released 3–4% of the drug in acidic medium irrespective of time. The low drug release in the acidic medium is also advantageous to reduce gastric irritation of NSAIDs. After this lag, it was followed by pulse with complete drug release within 30–45 min in phosphate buffer (Fig. 5). The porous/hollow beads also showed excellent lag in drug release at acidic pH that may be due to insolubility of drug and pectin. At acidic pH, calcium pectinate may get protonated into insoluble form having reduced swelling [28]. The second phase of pulsed release in phosphate buffer, pH 7.4, can be attributed to rapid swelling and gel relaxation of calcium pectinate gel at alkaline pH. Secondly at pH $>$ 6.6 diclofenac sodium is freely soluble that resulted in rapid and complete drug release. Hollowness/porosity of beads did not significantly affect the drug release pattern in both media.

4. Conclusion

Novel hollow calcium pectinate beads containing diclofenac sodium were prepared by simple technique with

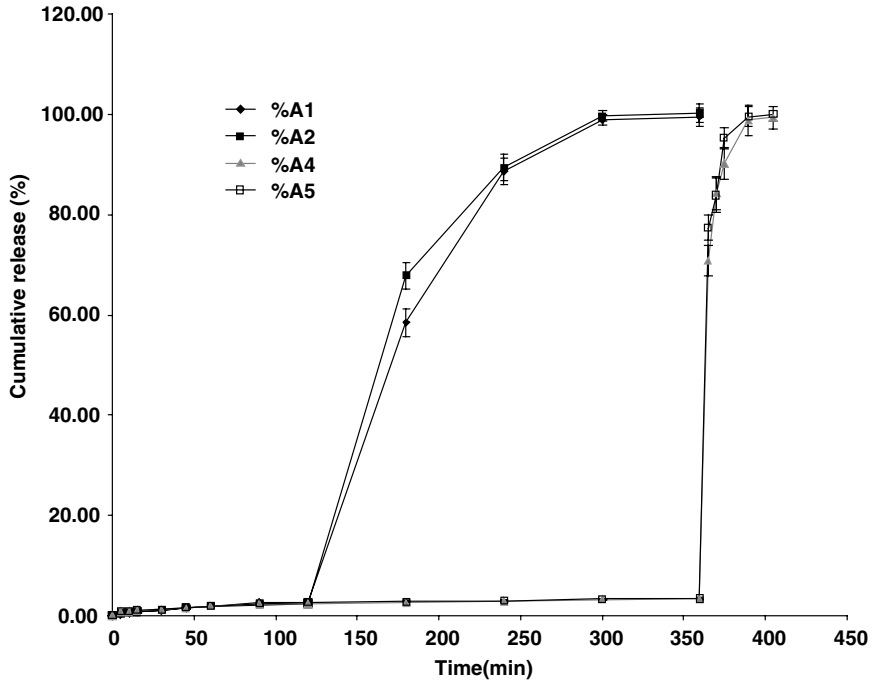

Fig. 5. Cumulative drug release profile.

in situ action of buoyancy imparting agents during formation. Overall, the buoyant beads provided a lag phase while showing gastroretention followed by a pulsatile drug release that would be beneficial for chronotherapy of rheumatoid arthritis and osteoarthritis. This work can be extended for time-scheduled drug release of drugs having low solubility, poor absorption or degradation in lower gastrointestinal tract.

Acknowledgements

Authors are thankful to Dr. S. Ponrathnam of National Chemical Laboratory, Pune, India, for providing the facility of Mercury Porosimetry and SEM and also to D.S. Reddy, C P Kelco, Mumbai, India, for providing the pectin. A.P. Pawar and Praveen Sher are thankful to UGC, India, for funding the major research project.

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