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$CaCO_3$ MICROPARTICLE CONTAINING IBANDRONATE–ALGINATE BEADS FOR IMPROVED ADHERENCE TO BISPHOSPHONATE ORAL THERAPY: FORMULATION AND IN-VITRO RELEASE

Jaya Shukla[1,*], BR Mittal[1], A Sood[1], GP Bandopadhaya[2]

[1]Department of Nuclear Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, INDIA
[2]Department of Nuclear Medicine, All India Institute of Medical Sciences, New Delhi-110029, INDIA

***Corresponding author e-mail:** shuklajaya@gmail.com

ABSTRACT

Bisphosphonates are established as supportive therapy to reduce the frequency and severity of cancer-related skeletal complications. Oral formulations are preferred over intravenous if the patients are not hospitalized. The maximum absorption of oral bisphosphonates takes place in stomach. The adverse events with oral dosing are seen in buccal mucosa and gastrointestinal tract**,** which lead to poor adherence to bisphosphonates therapy. In the present study different ibandronate-alginate beads were formulated and characterized for physiochemical parameters like shape, effect of ibandronate and alginate content, encapsulation of drug and drug release. $CaCO_3$ microparticles were incorporated in ibandronate-alginate formulations and studied for increased ibandronate release in simulated gastric fluid (SGF). The ibandronate encapsulation in all formulations was high and was independent on the amount of drug encapsulated. The release of ibandronate from ibandronate-alginate beads was dependent on alginate concentration and not on the amount of drug encapsulated. Additionally, the drug release was more in simulated intestinal fluid (SIF) than in SGF. However, the incorporation of $CaCO_3$ microparticles in ibandronate–alginate beads increased the release of drug in SGF. The scanning electron microscope studies of $CaCO_3$ microparticles containing ibandronate–alginate beads, after incubation in SGF, demonstrated the presence of tiny pores on the surface as well as within the beads. These beads also exhibited increased and sustained ibandronate release in SGF.

KEY WORDS: Alginate, DSC, Ibandronate, Microparticle, SEM, $CaCO_3$ microparticles

INTRODUCTION

Ibandronate is potent N containing third generation bisphosphonate. It has a core of P-C-P structure with side chains having a hydroxyl group at one side and a tertiary nitrogen group on other side [1]. Hydroxyl group of ibandronate enhances the strength of skeletal binding and prevention of hydroxyapatite crystal growth [2].The tertiary nitrogen group binds to bone mineral surface and inhibits osteoclast-mediated bone resorption. Ibandronate inhibits the enzymes responsible for bone resorption, adhesion of tumor cells within the bone and safe for human use. Ibandronate is many times more potent than other bisphosphonates due to these structural attributes [3,4]. It had been demonstrated that bisphosphonates has high selective localization and retention in bones, it enhances bone mineral density, decreases bone fracture rates and is widely used for the prevention of postmenopausal osteoporosis and the treatment of bone pain accompanying bone metastasis [5,6]. Bisphosphonates have shown antitumor activity with decrease in the progression of bone lesions or prevention of bone metastasis [7,8]. The oral bioavailability of bisphosphonate is very low and variable. Therefore it has to be taken early in the

morning, after an overnight fasting, with plenty of water followed by post-dosage fasting of around 60 minutes in upright position. The absorption of bisphosphonates takes place in the stomach and upper part of small intestine [9]. Similar to other bisphosphonate, the oral ibandronate is poorly absorbed by gastrointestinal tract with estimated bioavailability in human is around 0.63% [10,11]. About 40%–60% of absorbed dose is tightly bound to the bone surface and remaining absorbed ibandronate is excreted unchanged through the kidneys. The unabsorbed ibandronate is eliminated unchanged in the feces [12].

Oral formulations are always preferable than intravenous route by the patients suffering from bone metastasis and other bone diseases. Bisphosphonate tablet causes oropharyngeal and gastrointestinal tract ulceration [13]. These difficulties have thwarted the efforts to achieve an efficient formulation [14]. Patients on ibandronate need to follow stringent dosing requirements that may lead to discontinuation of treatment, poor compliance and reduce clinical efficacy [15-19]. Low bioavailability and adverse effects in gastrointestinal tract has limited the efficacy of oral preparation. Several steps have been taken in the development of weekly and monthly regimens, with improved therapeutic adherence. But the overall rate of patients staying on these treatments is still low, indicating the need for another ways to facilitate the increased bioavailability [15,16,20]. To improve oral bioavailability, microencapsulation demonstrates a promising concept [21].

Alginate has been used as oral or nasal delivery system for the encapsulation of wide varieties of bioactive materials, proteins, enzymes and antibodies etc. [22]. The physical properties of alginate depend on the sequence of mannuronic acid (M) and glucuronic acid (G) residues as well as on the average molecular weights and the molecular weight distribution of the polymer [23]. In the presence of divalent (e.g. Ca^{2+}, Ba^{2+}) or trivalent (e.g. Al^{3+}) cations, alginate spontaneously forms gel in a single-step process [24,25]. The gel is insoluble at low pH and soluble at neutral or higher pH. The alginates with higher percentage of glucuronic acid show more ability to form gel [24]. Moreover, alginate demonstrates low toxicity and low immunogenicity. It is cost effective and readily available [26].

The aim of the study was to formulate a delivery system which could release ibandronate in the stomach and upper part of intestine for increased drug absorption. We tried to formulate pH responsive beads by incorporation of $CaCO_3$ microparticles. The in-vitro release profiles of encapsulated ibandronate in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were also studied.

MATERIALS AND METHODS

Materials: The ibandronate was a kind gift from NATCO, India; sodium alginate, anhydrous calcium chloride, copper sulphate and calcium carbonate were procured from Sigma-Aldrich. All other reagents used were of analytical grade.

Preparation of Ibandronate-Alginate Beads: Ibandronate-alginate beads were prepared by the method described by Pandey et al with some modifications [27]. Briefly**,** 0.5 ml ibandronate of different strength (10 mg, 15 mg, and 20 mg) was added in 10 ml of 2% and 5% alginate solution in milli Q water, separately. Solution was mixed and allowed to stand for 20-30 min to make the solution bubble free. The resulting solution was allowed to fall drop wise into 50 ml of 0.1M calcium chloride solution with the help of 5 ml syringe. Beads were instantly formed. Beads without ibandronate were also prepared. Beads were recovered by filtration after 30 minutes and washed twice with deionized water and dried at room temperature.

Preparation of pH responsive Ibandronate-Alginate Beads: Calcium carbonate microparticles were incorporated to formulate pH sensitive ibandronate-alginate beads by the modified method of Han et al [28]. Briefly, 10 ml alginate solution (2% and 5%) solution was prepared and 100 mg ibandronate was added. The solution was allowed to stand for 20-30 min to make the solution bubble free. Calcium carbonate (0.25%) was homogenized for 3 min with 10 ml poly vinyl alcohol (PVA-0.15%). The resultant microparticles were added in ibandronate-alginate solution, mixed properly and allowed to fall drop wise onto 50 ml of 0.1M calcium chloride solution with the help of a syringe. Beads were instantly formed, recovered by filtration after 30 min, washed twice with deionized water and dried at room temperature.

Physical Characterization: The beads were viewed under scanning electron microscope (LEO 435 VP, Cambridge, UK) for surface characterization. The beads were also monitored after incubation of prepared beads in simulated gastric fluid (SGF**,** pH-1.2) and simulated intestinal fluid (SGF**,** pH-6.8), separately. Beads were coated with gold after mounting on metal stub with adhesive tape, directly or after cutting the beads with the help of blade.

Thermal Characterization: Thermal characterisation was done to assess the drug inside the beads by differential scanning calorimetry (DSC) using TA 3100 thermal analyzer having *910 DSC module*. 3-4 mg ibandronate-alginate beads were placed in hermetically sealed cell and the measurements were taken over a temperature range 30^0-250^0C at a heating rate of 10^0C/min under static air atmosphere.

Water Entrapment Property: Water entrapment property was recorded to study the swelling behavior of the beads. Wet and dry weight of formulated beads was recorded and % water loading was calculated by the formula:

$$\frac{Ws - Wd}{Ws} \times 100$$

Ws-weight of swollen beads, Wd- weight of dry beads

Alginate beads (5 mg) were suspended in 2 ml phosphate buffer (pH-7.4) by stirring in capped bottle at 37°C on shaker. Supernatant, containing bisphosphonate, was collected after centrifugation for quantification of released ibandronate by the method used by Koba et al [29]. Ibandronate-Cu^{2+} complex was prepared by adding ibandronate in 1.5 mmol/L $CuSO_4$ and 1.5 mmol/L HNO_3 (pH 2.8). The λ_{max} for ibandronate was determined by spectrophotometer (UV-1 Evolution100 Thermo Electron Co.). Then the standard curve was obtained for direct extrapolation of encapsulated and released ibandronate.

All experiments were done in triplicates and the mean values were taken. The percentage entrapment was calculated as:

% Drug Entrapment=

$$\frac{\text{Mass of drug present in beads}}{\text{Mass of drug used in the formulation}} \times 100$$

The in-vitro drug release from beads was monitored by suspending ibandronate beads (5mg/ml) in SGF (pH-1.2) and SIF (pH-6.8). The samples were stirred at 400 rpm at 37°C. The supernatant was taken out after centrifugation at 30 min and then after every hour up to 6 hours. The volume, in respective vials, was maintained by replacing same amount of SGF or SIF. The amount of ibandronate released was determined by spectrophotometer after complexation with $CuSO_4$ in dilute HNO_3.

RESULTS

Preparation of Ibandronate-Alginate-Beads: Beads were formulated using 2% and 5% alginate concentration with ibandronate strengths of 10 mg, 15 mg and 20 mg. Another set of beads using 100 mg ibandronate in 2% and 5% alginate formulation was prepared by incorporating $CaCO_3$ microparticles. This resulted in eight different formulations; six formulations were without $CaCO_3$ microparticles and two with $CaCO_3$ microparticles. All the beads were spherical in shape. The size of beads prepared with 2% alginate was smaller (500 μm) than the beads prepared with 5% alginate (1.0 mm) and with 5% alginate.

Water Entrapment Property: All formulations demonstrated high water entrapment property. The percent water uptake was approximately 95% with marginally high uptake in beads formulated with 5% alginate concentration. $CaCO_3$ microparticles had no effect on water uptake property of alginate beads.

Surface characterization: The scanning electron microscope (SEM) revealed rough surface and irregular shape of ibandronate-alginate beads when prepared with 2% alginate whereas, ibandronate-alginate beads with 5% alginate were relatively smooth and spherical in shape irrespective of presence of $CaCO_3$ microparticles and amount of ibandronic acid used for encapsulation (Figure 1b,1c). The SEM of beads of 2% and 5% alginate formulation with $CaCO_3$ microparticle incubated in SGF demonstrated pores on the surface and cross-section of beads (Figure 2a-c). However, pores were not present on the bead surface prepared without $CaCO_3$ microparticles (Figure-2d). All the bead formulations led to degradation and dissolution when kept in SIF (Figure 3a-c).

Thermal Characterization: DSC profiles of ibandronate, alginate and ibandronate-alginate are shown in (Figure 4). Two melting endothermic transitions were observed at 127.84°C and 189.2°C in DSC thermogram of ibandronate. In the thermogram of alginate, an endothermic peak around 200°C was assigned to alginate. But only one endothermic peak around 200°C was observed in thermogram of ibandronate-alginate beads. $CaCO_3$, due to thermo resistance (m.p.>800), had no effect on DSC.

Drug entrapment and *in vitro* ibandronate release study: The maximum absorbance was seen at 238 nm after complexation with acidic copper sulphate ($CuSO_4$ in dil HNO_3). Further encapsulation and release studies were done at 238 nm. The percentage of entrapped ibandronate, in all formulations of alginate beads, was calculated and represented in table-1. The results demonstrated ≥ 85% drug encapsulation with all formulations either prepared with or without $CaCO_3$ microparticles or with different alginate (2% or 5%) concentrations. The in-vitro ibandronate release from alginate beads was

monitored in SGF (pH-1.2) and SIF (pH-6.8). All the formulation demonstrated high water entrapment (~ 95%) however; it was observed that the swelling of alginate ibandronate beads was more in SIF (pH 6.8) than in SGF (1.2). The cumulative release of ibandronate from ibandronate-alginate beads, formulated with 5% alginate, in SGF was 3.9%, 5.2%, 8.2% and 10.3% at 1h, 2h, 3h and 6h, respectively (Figure 5a). The release was independent of amount of drug encapsulation (10, 15, 20 mg). The ibandronate release was increased when 2% alginate formulation were used. The cumulative release of 35.2% was observed in 6 hr and 9.5%, 15.8%, 21.8% at 1, 2 and 3 hr, respectively (Figure 5b). The dissolution of hydrogel was observed in SIF if the beads remained in SIF further (Figure 5).

The incorporation of $CaCO_3$ microparticles in ibandronate–alginate beads has increased the cumulative release of ibandronate from alginate beads. The cumulative release of ibandronate from 5% alginate beads in SIF was increased to 45.2% at 3h, 57% at 4 h and 69.8% at 6h. The release of ibandronate from beads prepared with 2% alginate was 54.2% at 3 h, 66.4% at 4 h and 74.9% at 6h. However, the release of ibandronate from $CaCO_3$ microparticles containing ibandronate–alginate beads with 5% and 2% alginate concentration at 2h, 4h and 6h was 69.4%, 88.4%, 98.6% and 74.4%, 94.4%, 99.1%, respectively (Figure 5 a,b). Alginate beads demonstrated small pores of 4-9 μm in size when incubated in SGF. However in SIF, the beads had tendency to dissolve by surface erosion.

DISCUSSION

The ionic crosslink of alginate was induced immediately after adding alginate (2% or 5%) solution into 0.1M $CaCl_2$ (Ca^{2+}) solution (Figure 1a) [30]. Alginate is an anionic copolymer of 1,4-linked- β –D-mannuronic acid (M) and α -L-glucuronic acid (G) residues. Alginate has open lattice structure that provides gentle environment to encapsulated drugs inside the hydrogel. The reactivity with calcium and formation of water insoluble Ca-alginate gel is a direct function of the average chain length of glucuronic acid. The alginate gel was formed due to the stacking of glucuronic acid (G) blocks with the formation of 'egg-box' calcium-linked junctions. Ca^{2+} ions are located into electronegative cavities like eggs in an egg-box [26]. The electrostatic interactions between carboxylate groups of glucuronate blocks and Ca^{2+} lead to the formation of mechanically stable network of beads [31]. In SGF (pH-1.2), calcium of calcium alginate gel (egg box structure) was totally displaced from polymer network but the beads maintained their structure and mechanical strength like ionically cross-linked beads due to the formation of hydrogen bonds. At this stage beads did not swell but maintained a stable three dimensional network [32].

Calcium carbonate is readily soluble in acidic aqueous medium but sparsely soluble in neutral or alkaline medium. When the beads were in contact with acidic medium (SGF), the calcium carbonate content in the alginate beads was leached out after dissolution in acid and small pores (4-9 μm) were formed within the beads and were visible in electron micrograph (Figure 3). The ibandronate was mixed with polymer matrix for the formulation of beads. Water soluble ibandronate on the surface of beads was lost during gelation and washing. The DSC thermogram indicated that ibandronate in formulated alginate beads exist in either amorphous form or uniformly dispersed at the molecular level in the alginate matrix. Water entrapment property plays an important role in swelling of beads and help in release of encapsulated drug. It was observed in this study that swelling of the alginate matrices was more in SIF (pH 6.8) than in SGF (pH 1.2). The pKa of alginate (the carboxyl groups of uronic acid residues) is ~ 4. At pH 6.8, alginate gets dissolve and form a viscous solution. In acidic environment (in SGF; pH-1.2), the Ca^{2+} ions were displaced but the carboxyl groups were less dissociated and did not allow the polymer matrix to swell and the contents remained intact without change in matrix shape. The alginate chains undergo a process of association and resulting in the formation of a thick network of inter and intra-molecular hydrogen bonds [32,33]. The ionic interactions in egg-box structure between glucuronate and Ca^{2+} form thermostable gel and demonstrated slow release of encapsulated ibandronate. But at high pH or in SIF, carboxylic groups of alginate remained in relaxation state due to ionization. The increased bead porosity resulted into increased swelling and release of encapsulated ibandronate. The solubilization of hydrogel in SIF was due to surface erosion followed by degradation of alginate matrix mesh at higher pH (6.8).

In the present study, the release pattern of encapsulated ibandronate with different concentrations of ibandronate (10,15,20 mg) and alginate (2% and 5%) were demonstrated. It was shown that the release of ibandronate was independent of the amount of ibandronate encapsulated, however slower release was observed with increased alginate (5%) concentration. The pH responsive release was therefore studied with higher ibandronate concentration (100 mg) using 2% and 5% alginate concentration. Our long term aim is to design a formulation that can release its pay load in stomach as the absorption of bisphosphonates is reported to take place in stomach and proximal part

of intestine. We incorporated $CaCO_3$ microparticles in alginate matrix during formulation of beads. The pH-responsive increased release from these beads was observed in *in-vitro* simulated gastric atmosphere (Figure 5). The $CaCO_3$ microparticles were leached out in SGF (containing HCl) and tiny pores of 4-9 µm were created. Micro channels were formed through these pores which facilitated the release of ibandronate. The incorporation of ibandronate in alginate beads may decrease the direct contact of ibandronate to buccal cavity and esophagus. The ibandronate release from $CaCO_3$ microparticles containing beads was sustained in stomach that may also help to decrease the adverse effects of ibandronate when when given in tablet form. Alginate has bioadhesive property and the studies are now needed to further study the in-vivo behavior of ibandronate-alginate beads.

CONCLUSION

Ibandronate-alginate beads were successfully formulated. The above results demonstrated that the beads prepared with 2% alginate; the weight ratio of drug to polymer, 1:2; cross linking time, 30 min; $CaCl_2$ concentration, 0.1M have good formulation and swelling characteristic. The incorporation of $CaCO_3$ microparticles in alginate bead formulations further increased the release of ibandronate in SGF. The formulation may increase the release and absorption of ibandronate in stomach. The encapsulation of ibandronate in the beads may also prevent direct contact of ibandronate with buccal cavity.

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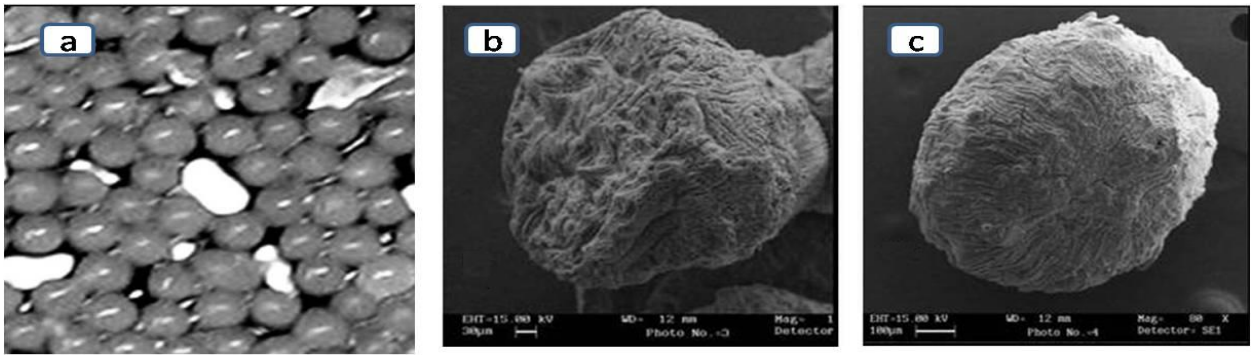

Figure 1: Ibandronate-alginate beads- Instantly formed in 0.1M calcium chloride solution(a), SEM micrograph showing surface morphology of Ibandronate- alginate bead prepared from 2% and 5% alginate (b and c)

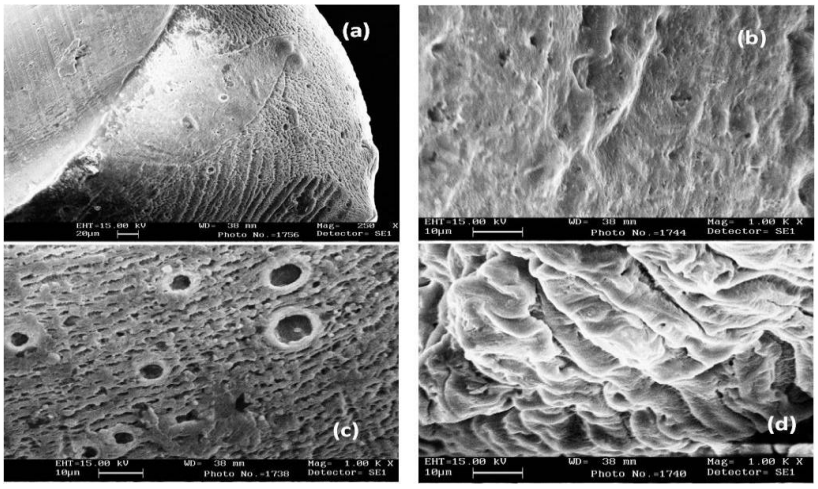

Figure 2: Electron micrograph showing surface morphology of Ibandronate- alginate $CaCO_3$ microprticles containing bead incubated in SGF (a) Surface and cross section at magnification 250 X; (b and c) surface and cross section at magnification 1 K (d) beads without $CaCO_3$ microparticles

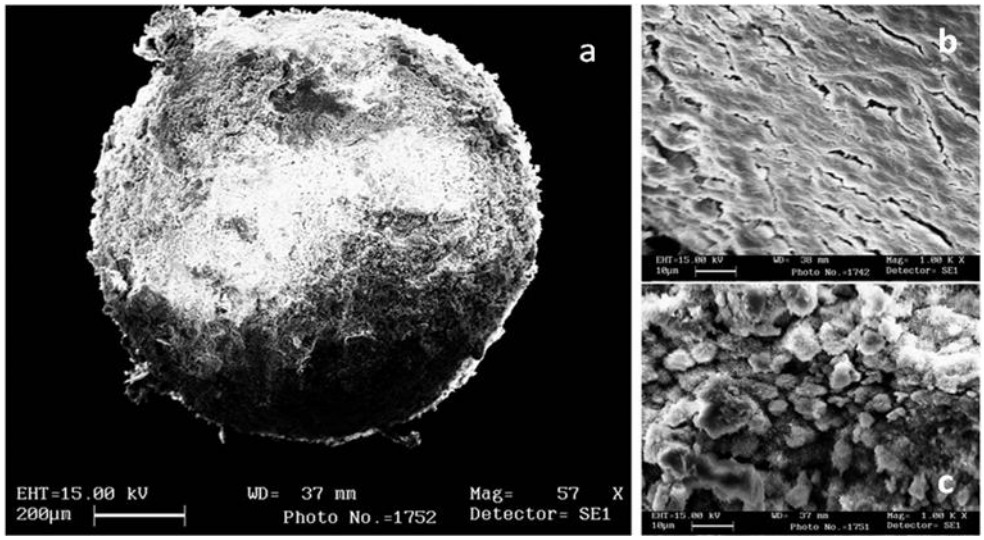

Figure 3: Electron micrograph showing surface morphology of Ibandronate- alginate $CaCO_3$ microprticles containing bead incubated in SIF (a) Surface at magnification 57 X (b) 1 hr incubation and (c) 3 hr incubation

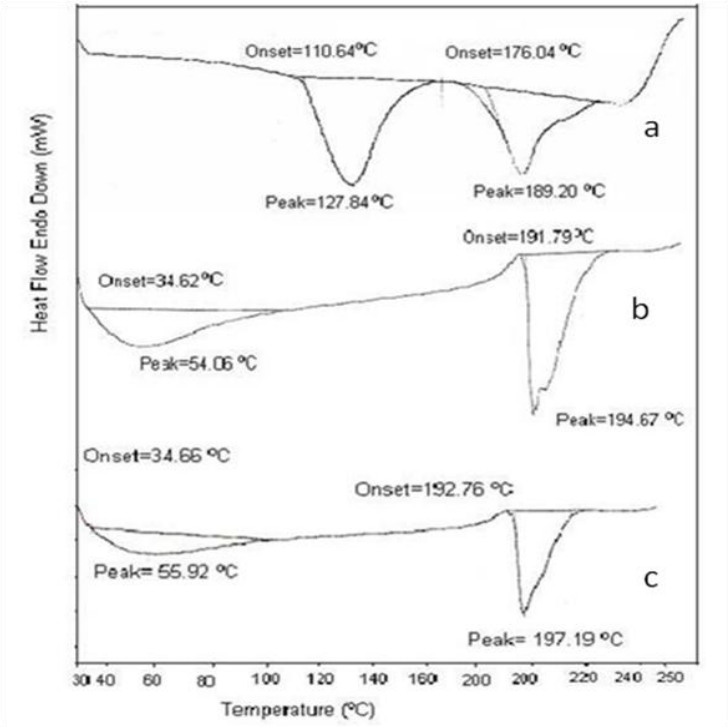

Figure 4: DSC thermogram of (a) Ibandronate, (b) alginate and (c) Ibandronate:alginate beads

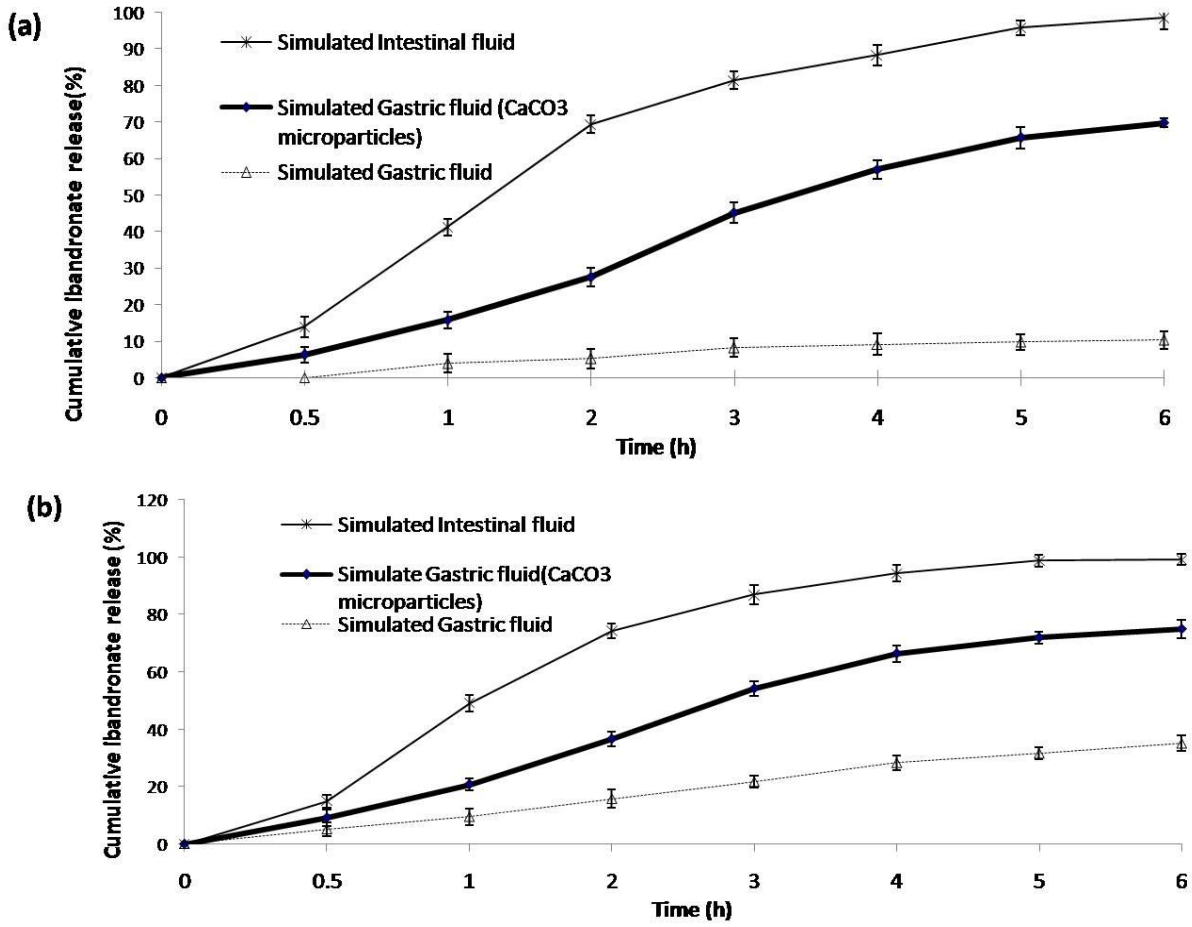

Figure 5: % Release of ibandronate in SGF and SIF with and without $CaCO_3$ microparticles (a) from 5% alginate-ibandronate beads (b) from 2% alginate-ibandronate beads

TABLE-1. Drug encapsulation in different ibandronate-alginate formulations

Sample	Encapsulation (%)
10 mg ibandronate + 5% alginate	87
15 mg ibandronate + 5% alginate	85
20 mg ibandronate + 5% alginate	86
100 mg ibandronate + 2% alginate + 25 mg $CaCO_3$ microparticles	85
100 mg ibandronate + 5% alginate+ 25 mg $CaCO_3$ microparticles	89

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