# FLOATING ALGINATE BEADS: STUDIES ON FORMULATION FACTORS FOR IMPROVED DRUG ENTRAPMENT EFFICIENCY AND *IN VITRO* RELEASE

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**Abstract**

Floating calcium alginate beads based on gas-generation technique often suffer from poor drug encapsulation and rapid drug release in the acidic environment of the stomach. To overcome the above mentioned demerits, in the present study we have evaluated the influence of formulation factors such as incorporation of mixtures of gas-generating agents ($NaHCO_3$ and $CaCO_3$), incorporation of gelatin type B and xanthan gum into the calcium alginate beads prepared by ionotropic gelation in acidic $CaCl_2$ solution. Further, feasibility of floating polyelectrolyte complex (PEC) beads based on sodium alginate (SA) and low molecular weight chitosan (LMWC) to overcome the demerits of floating calcium alginate beads was also investigated. In all experiments ketoprofen was used as model drug. The data from excipient-excipient interaction studies carried out by using FTIR and DSC techniques revealed the PEC formation between SA and LMCH and between SA and gelatin type B, whereas, the data from drug-excipient interaction studies revealed the interaction between ketoprofen and the cations. It was found that all the studied formulation factors markedly affect the entrapment efficiency and drug release in 0.1 M HCl (pH 1.2).

**Rezumat**

În prezentul studiu a fost evaluată influenţa factorilor de formulare a peletelor flotante cu alginat de calciu: încorporarea amestecurilor de agenţi efervescenţi, a gelatinei tip B şi a gumei xantan. Au fost de asemenea evaluate avantajele formării complexului flotant polielectrolitic constituit din alginat de sodiu şi chitosan cu masă moleculară mică.

În toate determinările a fost folosit ketoprofenul ca substanţă activă.

Rezultatele obţinute în urma studiilor de interacţiune realizate prin spectroscopie în infraroşu cu transformată Fourier şi calorimetrie diferenţială au demonstrat formarea complexului polielectrolitic şi interacţiunea dintre substanţa activă şi cationi.

În concluzie, toţi factorii de formulare studiaţi prezintă o importanţă deosebită în procesul de încorporare a substanţei active şi de eliberare a sa în mediu de HCl 0,1M.

**Keywords:** calcium alginate beads, PEC, floating beads, encapsulation efficiency, drug release.

## Introduction

During the last few years, considerable attention has been paid to the development of floating drug delivery systems for the stomach specific sustained/controlled delivery of drugs. Among these, the use of floating hydrogel beads is the simplest approach. These beads are generally fabricated using modified ionotropic gelation technique. In this approach, an aqueous dispersion of negatively charged polymer together with a gas-generating agent ($CaCO_3$ or $NaHCO_3$) was added drop-wise into acidic gelation medium consisting of divalent cations such as $Ca^{2+}$. As the droplet dips into the acidic gelation medium, gas-generating agent effervesces, releasing $CO_2$ which then is entrapped in the gel matrix making it lighter than stomach fluids and simultaneously $Ca^{2+}$ present in gelation medium interacts with anionic groups on polymer molecule causing immediate formation of strong aggregation of pairs of helices, leading to strong bead shaped gel structures[1]. Although these beads shaped drug delivery systems are very popular, they often suffer from poor drug encapsulation due to curing in acidic gelation medium and rapid drug release in acidic environment of the stomach [2-4]. In past, a number of efforts have been made to improve the physico-chemical characteristics of these beads, which include incorporation of oils/waxes to produce emulsion gel beads; combination of hydrocolloids to retard drug release, however, these modifications often lead to the systems which are difficult to reproduce and scale up [5-7]. In an effort to search the possible remedies for the disadvantages associated with conventional floating calcium alginate beads, we have carried out extensive experiments in our laboratory. These include incorporation of mixtures of gas-generating agents ($NaHCO_3$ and $CaCO_3$) instead of using single gas-generating agents incorporation of polymers like gelatin type B or xanthan gum to the calcium alginate beads and the feasibility of polyelectrolyte complex formation between sodium alginate (SA) and low molecular weight chitosan (LMCH) (SA-LMCH core-shell PEC beads) in the form of floating beads as an alternative to calcium alginate beads. In all the experiments ketoprofen was used as model drug. The reason for using mixtures of gas-generating agents (core crosslinking with released $Ca^{++}$) was to improve the mechanical strength (with respect to drug entrapment efficiency and release in acidic medium, 0.1M HCl) of the calcium alginate beads due to core crosslinking with released $Ca^{++}$. The second modification involved incorporation of gelatin type B or xanthan gum to the calcium alginate beads prepared with $NaHCO_3$ alone as gas-generating agent. The reason for choosing gelatin type B is the fact that, gelatin is a polyampholyte able to complex with both negatively and

positively-charged polyelectrolytes, below and above its isoelectric point [8] and, therefore, it is expected to form plyelectrolyte complex (PEC) with SA (PEC formation in the core of the bead) thereby giving strength to the bead structure. On the other hand xanthan gum is an anionic polymer and it will not form PEC with SA, however, it is expected to confer increased viscosity of resulting SA solution which may produce alginate beads with improved mechanical strength. The third modification, that is, core-shell PEC beads between SA and LMCH (SA core and SA-LMCH polyion complex shell) was also studied as PECs are reported to exhibit a very high degree of ordering and crystal like properties and have quite compact structures. In these PEC beads (SA-LMCH) buoyancy was induced using $NaHCO_3$ or mixtures of $NaHCO_3$ and $CaCO_3$. Considering the above mentioned properties of PECs, it is expected that, if these complexes in the form of beads employed as carrier for drug delivery, it might be possible to overcome the demerits associated with calcium alginate beads, as they offer improved/altered drug entrapment efficiency, drug stability and dissolution [9]. The reason for selecting ketoprofen as model drug was to develop a sustained release oral dosage form, beneficial for optimal therapy with non steroidal anti inflammatory drugs (NSAID's), with respect to the efficacy, safety and patient compliance, more specifically for geriatric population.

## Materials and Methods

*Materials*

Ketoprofen (KT) was a gift from Pfizer Laboratories Kotdwar, India. Sodium alginate (SA) from brown algae, Gelatin type B [Gel strength = 225 g Bloom], Low molecular weight chitosan (LMCH) [Brookfield Viscosity 25 cps, 1 % solution in 1 % acetic acid at 26 °C] and Xanthan gum (XG) were purchased from Sigma-Aldrich (St. Louis, USA). Water used in the formulations was of HPLC grade (Merck) and all other chemicals used were of analytical grade.

## Methods

*Preparation of SA, SA-gelatin or SA-XG and LMCH solutions*

SA (1.25 %w/v) was dispersed in deionized water and then heated to 90 $^0$C with agitation until total dissolution. The SA-gelatin or SA-XG solutions were prepared by dissolving known amounts of SA containing either type B gelatin or XG in hot (60$^0$C) deionized water with agitation until complete dissolution. Chitosan solution (0.6%w/v) was prepared by dissolving it by stirring for 4 hours in 1% acetic acid in deionized water. The solution was kept overnight at room temperature (around 28-29$^0$C) for

complete hydration. The pH of the solution was adjusted to 4.5 one hour before the experiment.

*Preparation of floating calcium alginate beads*

The floating calcium alginate beads, whose composition (M1-M9) is shown in Table I, were prepared by ionotropic gelation technique as described by Choi et al with some modifications [1]. Briefly, a solution of SA or a solution of SA and type B gelatin or XG containing drug and gas-generating agent (s) was added drop wise into gelation medium consisting of varying concentrations of $Ca^{++}$ as $CaCl_2$ in 10%v/v acetic acid using 25mL hypodermic syringe through a 20G needle. This medium was continuously stirred during bead formation to enhance the mechanical strength of the beads and also to prevent their aggregation. The beads were cured for 10 min, separated by filtration, washed thrice with deionized and dried at 40 °C in a hot air oven (DR 101, Universal, India).

*Preparation of floating SA-LMCH polyelectrolyte complex (PEC) beads*

Floating core-shell SA-LMCH beads (SA core and SA-LMCH polyion shell) with or without drug were prepared by extruding a solution of SA containing gas-generating agents or their mixtures with the help of a 25 mL hypodermic syringe fitted with 20G needle into LMCH solution (0.6%w/v in 1% acetic acid, pH 4.5) at room temperature (28 $^0$C). The beads were cured for 10 min, separated by filtration, washed for three times with deionized water and dried at 40 °C in a hot air oven (DR 101, Universal, India).

**Table I**

Formulation composition of floating gel beads containing KT

| Formulation code | SA (% w/v) | KT (mg) | Gelatin Type B (% w/v) | XG (% w/v) | $NaHCO_3$ (% w/v) | $CaCO_3$ (% w/v) | LMCH (% w/v) | $CaCl_2$ (% w/v) |
|---|---|---|---|---|---|---|---|---|
| M1 | 1.25 | 100 | | | 0.6 | | | 3 |
| M2 | 1.25 | 100 | | | 0.6 | 0.1 | | 3 |
| M3 | 1.25 | 100 | | | 0.6 | 0.2 | | 3 |
| M4 | 1.25 | 200 | | | 0.6 | | | 3 |
| M5 | 1.25 | 200 | | | 0.6 | 0.1 | | 3 |
| M6 | 1.25 | 200 | | | 0.6 | 0.2 | | 3 |
| M7 | 1.25 | 100 | 0.3 | | 0.6 | | | 3 |
| M8 | 1.25 | 100 | | 0.15 | 0.6 | | | 3 |
| M9 | 1.25 | 100 | | 0.3 | 0.6 | | | 3 |
| M10 | 1.25 | 100 | | | 0.6 | | 0.6 | |
| M11 | 1.25 | 100 | | | 0.6 | 0.1 | 0.6 | |
| M12 | 1.25 | 100 | | | 0.6 | 0.2 | 0.6 | |

*** Total volume of each formulation was 10 ml**

*Excipient-excipient and drug-excipient and interactions*

Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) were employed to study excipient-excipient and drug-excipient interactions. Approximately 5mg samples were placed on a standard aluminium pan and heated to 100 to 200 $^0$C (for pure drugs at a rate of 2$^0$C/min.) and upto 300 $^0$C (at a rate of 10$^0$C/min.) for polymers and polymer beads. Fourier Transform Infrared Spectroscopy (FTIR) was employed. Spectra were recorded for pure polymers, polyelectrolyte complex, drug and drug-loaded beads using a FT-IR facility (Shimadzu, model 8400S). The samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400-4000 cm$^{-1}$ and the resolution was 2 cm$^{-1}$.

*Assessment of in vitro buoyancy of the beads*

*In vitro* study of bead buoyancy was performed using a USP XXXI dissolution apparatus type II (paddle type, Electrolab, Mumbai, India). The beads were dispersed in 500mL of 0.1M HCl (pH 1.2) at 37±1$^0$C with continuous agitation at 50 rpm for 24 h. The floating beads were separated from submerged beads and their proportion was determined (%) as described earlier.

*Morphology and bead size analysis*

The size of prepared beads was determined with an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. Twenty dried beads were measured to determine the mean diameter of the beads. All measurements were in triplicate. The shape, surface morphology and internal structure of the dried beads were assessed with a scanning electron microscope (Leo 435VP, variable pressure, Oxford, U.K.) at various magnifications.

In vitro drug release studies

*In vitro* release of KT from the beads was evaluated with a USP XXXI dissolution apparatus type II (paddle type, Electrolab, Mumbai, India) at 50rpm in 500mL 0.1M HCl (pH 1.2) at 37±0.5$^0$C. At predetermined intervals, a 1mL aliquot was withdrawn and replenished with an equal volume of fresh dissolution medium. The withdrawn samples were analyzed by UV spectrophotometry.

*Determination of mechanism of drug release*

The i*n vitro* data were fitted to Higuchi's square root model [10] to analyze the kinetics of drug release from the beads (see Equation 1).

$$Q = K\sqrt{t} \quad (1)$$

where Q is the amount of drug released in time t, K is the release constant of the equation. The data were also subjected to Korsmeyer-Peppas power law [11] as in Equation 2:

$$Mt/M\infty = Kt^n \quad (2)$$

where $Mt/M\infty$ is the fraction of drug released in time t, K is the structural and geometric constant, and n is the release exponent.

*Statistical analysis*

All data were analyzed by Student's t test and one-way ANOVA to determine the statistical differences in the results. A probability value $p < 0.05$ was considered statistically significant. The software used was SigmaPlot® 11 (Systat Software Inc).

## Results and Discussion

Floating calcium alginate beads were prepared by ionotropic gelation of SA with $Ca^{++}$ in acidic medium. The most important property of alginates is their ability to form gels by reaction with divalent cations such as $Ca^{++}$. Monovalent cations and $Mg^{++}$ do not induce gelation of alginates [12]. The gelation and crosslinking of SA are mainly achieved by the exchange of $Na^+$ from the guluronic acids with $Ca^{++}$, and stacking of these guluronic groups to form the characteristic egg- box structure. The $Ca^{++}$ binds to the α-L-guluronic acid blocks in a highly cooperative manner and the size of the cooperative unit is more than 20 monomers [13]. Each alginate chain dimerizes to form junctions with many other chains and as a result gel networks are formed [14]. The $Ca^{++}$ reactivity to alginates is the result of $Ca^{++}$ induced dimeric association of G-block regions. Core-shell PEC beads were prepared by drop-wise addition of SA solution containing $CaCO_3$ to LMCH solution in acetic acid. SA is a polyanion with $COO^-$ groups, whereas, chitosan is a well-known natural cationic polyelectrolyte that possesses primary amine groups ($NH_2$) that become protonated in acidic environments to become $NH_3^+$. When anionic SA solution was added drop-wise to the acidic LMCH solution (pH 4.5), core-shell beads were formed as soon as $COO^-$ group on SA network reacted with $NH_3^+$ group on LMCH. Prepared beads comprised of SA core and a LMCH-polyanion shell. The reason for selecting the low pH is the fact that the ionization degree of

chitosan ($pK_a$ 6.2) increases as pH decreases [9], which means that amino groups become highly charged. As a result, a higher number of ionic linkages occurred between SA and LMCH, resulting in structures of higher mechanical strength.

*Drug-polymer and polymer-polymer interactions*

*FTIR spectroscopic characterization*

IR spectrum of SA (figure 1) is attributed to its saccharide structure. The bands at 1617 and 1420$cm^{-1}$ are assigned to asymmetric and symmetric stretching bands of carboxylate groups. The band at 3435$cm^{-1}$ assigned to O-H stretching. The FTIR spectra of KT showed bands due to carbonyl group at 1697$cm^{-1}$ and 1654$cm^{-1}$, and have previously been assigned to dimeric carboxylic acid-carbonyl group and ketonic carbonyl group stretching vibrations, respectively [15, 16]. The band at 1593$cm^{-1}$ is attributed to symmetric stretching vibrations of –COOH group.

The FTIR spectra of blank floating calcium alginate beads (blank M1) exhibited characteristic bands at 1555$cm^{-1}$ and 1460$cm^{-1}$ indicating the involvement of $COO^-$ group in the coordination process with $Ca^{++}$; and 3413$cm^{-1}$ indicating the involvement of hydroxyl group with $Ca^{++}$. The FTIR spectra of blank floating calcium alginate beads (blank M2) also exhibited bands at1555$cm^{-1}$ and 3410$cm^{-1}$ respectively, however the band at 1460$cm^{-1}$ in the spectrum of blank M1 was further shifted to 1469$cm^{-1}$ (M2). In the FTIR spectrum of KT loaded calcium alginate beads (both M1and M2) the band due to ketonic carbonyl group stretching vibrations became broader and the band due to dimeric carboxylic acid-carbonyl group stretching vibrations became much smaller than the corresponding bands in the FTIR spectrum of KT. Further, the band at 1593$cm^{-1}$ was completely missing. The corresponding changes in the FTIR spectrum of KT loaded calcium alginate beads could be attributed to the interaction of carboxylic acid functional groups with cations. The FTIR spectra of LMCH showed bands at 3424.28 and 2879.36$cm^{-1}$ for O-H stretching in alcohols and symmetric stretching of-$CH_2$ group. The band at 1654.01$cm^{-1}$ is due to amide N-H bending, whereas, band at 1380.2$cm^{-1}$ attributed to amide III.

The FTIR spectrum of blank floating SA-LMCH polyelectrolyte complex (Blank M10) beads (Figure 1) showed shifting of O-H stretching vibration to 3435$cm^{-1}$. This could be attributed to the free –OH groups of both polymers existing in the PEC structure. The band due to asymmetric and symmetric stretching of $COO^-$ group in SA shifted to 1608 and 1444$cm^{-1}$ indicating the involvement of $COO^-$ in the coordination process. In the FTIR spectrum of KT loaded SA-LMCH PEC beads (M10), the band

due to ketonic carbonyl group stretching vibrations became more intense and the band due to dimeric carboxylic acid-carbonyl group stretching vibrations became much smaller than the corresponding bands in the FTIR spectrum of KT. However, the band due to symmetric stretching vibrations of –COOH group also appeared in the spectrum.

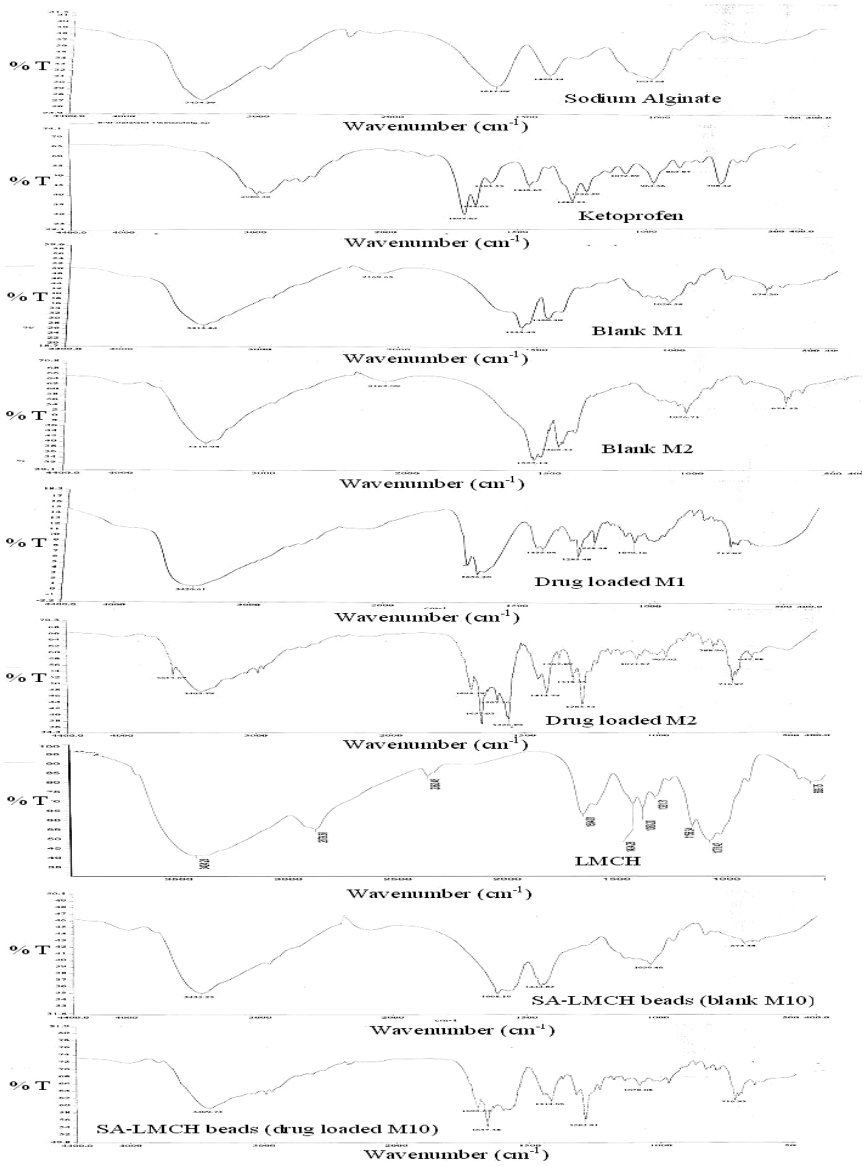


**Figure 1**

FTIR spectra of SA, KT, blank M1and M2, drug loaded M1 and M2, LMCH, blank SA-LMCH PEC beads and drug loaded SA-LMCH PEC beads.

*Thermal characterization*

The DSC thermogram of SA exhibited an exotherm at $307^0$C, corresponding to the decomposition of SA (Figure 2). The DSC thermogram of pure KT (Figure 2) showed a sharp endothermic peak at $95.38^0$C corresponding to the melting point of KT.

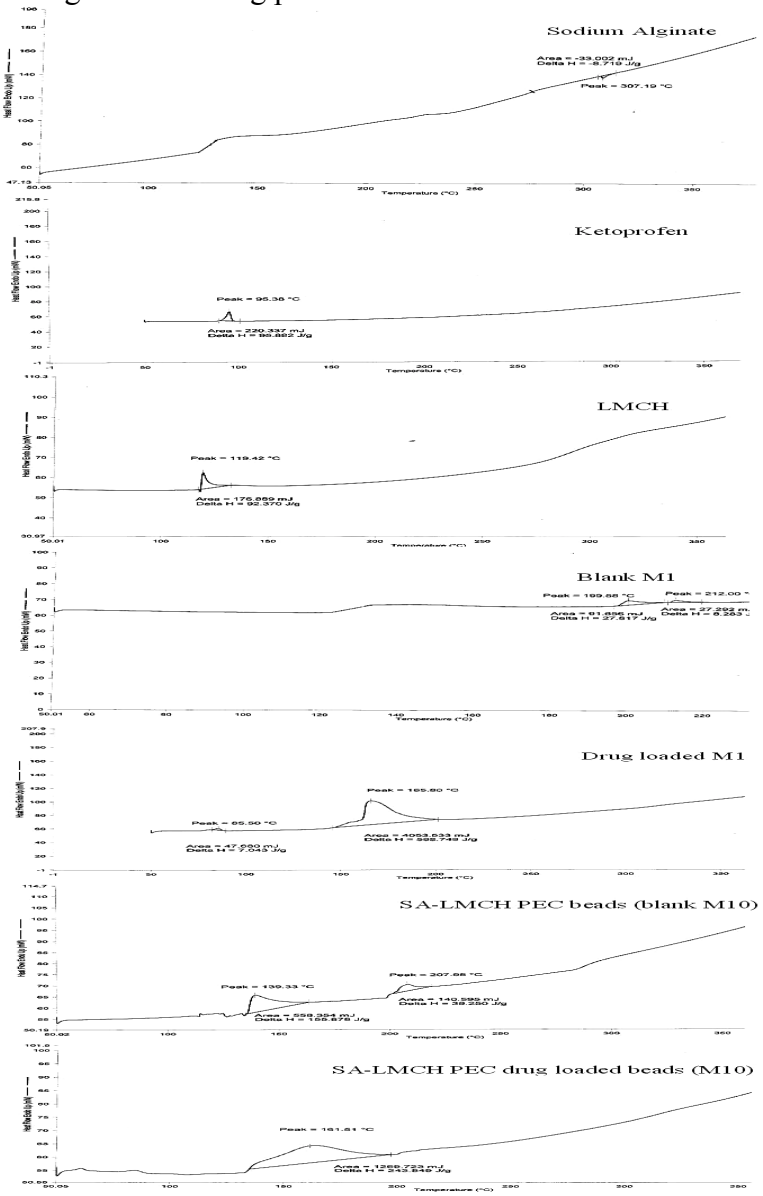


**Figure 2**

DSC thermograms of SA, LMCH, SA-LMCH PEC bead, blank calcium alginate beads, ketoprofen, drug loaded calcium alginate and PEC beads.

The DSC thermogram of LMCH showed an endotherm at $119^0$C corresponding to the dehydration of water. The DSC thermogram of blank calcium alginate beads (blank M1) also showed two endothermic peaks at 199.58 and $212^0$C may be due to change in rotational energy of the molecular chains due to the formation of salt complexes. The DSC thermogram also indicated disappearance of exothermal peak of SA; this may attributed to enhanced chain stiffness due to crosslinking with $Ca^{++}$. In the DSC thermogram of KT loaded $Ca^{++}$ crosslinked SA beads (drug loaded M1), the endothermic peak of drug shifted to lower temperature, that is, 85.5 $^0$C. This could be attributed to the formation of a dimer between carboxylic acid groups of KT and $Ca^{++}$. Due to the formation of the dimer, the melting endotherm of KT was shifted to lower temperature. On the other hand, the DSC thermogram of blank SA-LMCH PEC beads (blank M10) showed two new endothermic peaks at 139 and $\approx 207^0$C. This is suggestive of an indication of the PEC formation at the surface of SA droplet. The DSC thermogram of drug loaded SA-LMCH PEC bead showed (M10) a broad endotherm at $161.5^0$C, which suggests that interaction of KT with cations shifted the endothermic peak at a higher temperature with the disappearance of the second endothermic peak at $\approx 207^0$C present in the spectra of blank PEC beads (blank M 10).

*Microscopic and Scanning Electron Microscopic (SEM) characterization*

The mean diameters of emulsion gel beads are given in Table II. The mean diameter of floating calcium alginate beads ranged from 1.3 – 2.1 mm, whereas, the diameter of SA-LMCH PEC ranged from 1.7-1.8 mm respectively. The scanning electron micrographs (SEM) of various KT loaded floating beads are shown in figure 3 (A-E). Floating calcium alginate beads prepared with a mixture of $NaHCO_3$ and $CaCO_3$ as gas-generating agent were spherical and exhibited rough outer surface with numerous internal pores. On the other hand beads prepared with $NaHCO_3$ alone as gas-generating agent were relatively irregular with large internal pores (data not shown) which are consistent with the previous work [1]. SA-LMCH PEC beads were also spherical with interior almost as porous as floating calcium alginate beads. The surface of SA-LMCH PEC beads clearly showed the formation of PEC membrane (Figure 3E).

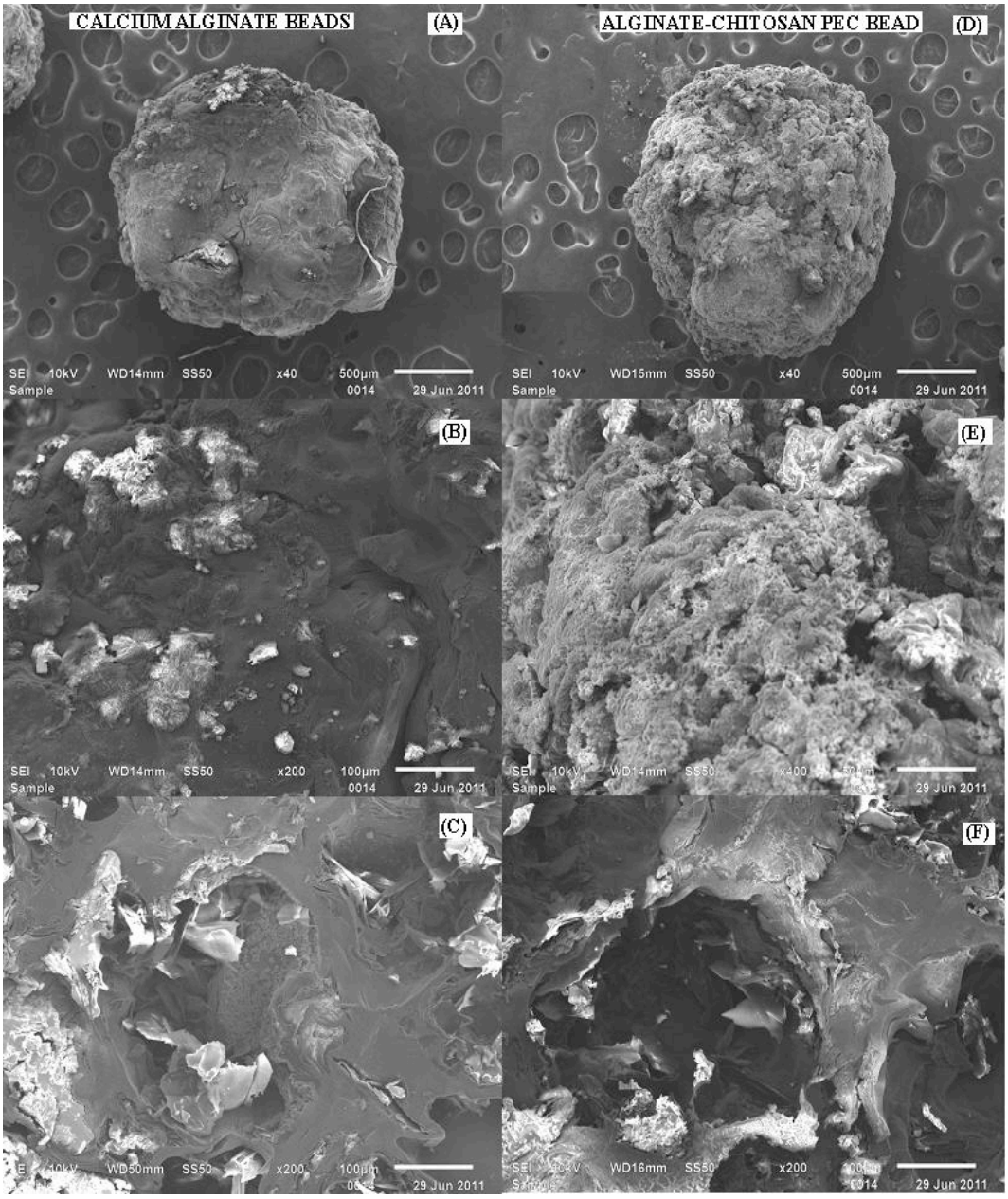


**Figure 3**

Scanning electron microscopic images of various formulations of drug loaded floating beads: (A),(B) and (C) showing shape, surface and transverse section of calcium alginate and; (D), (E) and (F) showing shape, surface morphology and transverse section of SA-LMCH PEC beads respectively

*Determination of encapsulation efficiency*

*Floating calcium alginate beads*

KT is a hydrophobic drug (aqueous solubility = 10 mg/L) [17], therefore, a high entrapment efficiency was expected. KT entrapment in floating bead formulations ranged from 58% to 92% (Table II). KT entrapment in formulation M1 was found to be 58%, which was below our expectations. This could be explained as KT is a weak acid with pKa value ~4.6 and reported to exhibit higher solubility at acidic pH (aqueous solubility at pH 2.0 = 205 mg/L; pH 4.0 = 280 mg/L and at pH 4.6 = 490 mg/L) [17]. The gelation medium used for the preparation of beads was acidic (pH = 4.0), so it seems that owing to higher solubility at lower pH values, some of the KT leached out of the beads during the processing leading to entrapment efficiency which was below our expectations (M1).

Incorporation of $CaCO_3$ into the bead formulations (M2 and M3) resulted in a significant improvement in entrapment efficiency ($p<0.05$, M1 compared to M2 and M3). The observed difference in drug incorporation efficiency could be attributed to the interaction of liberated $Ca^{++}$ with $COO^-$ groups on SA. SA consists of two uronic acids, β-D-mannuronic acid and α-L-guluronic acid. In the presence of divalent cations, SA forms stable gels as $Ca^{++}$ interacts ionically with blocks of uronic acid residues, to form a chelated structure with $Ca^{++}$, called an "egg-box" junction with interstices in which the $Ca^{++}$ may pack and be coordinated [12-14]. This has resulted in efficient core crosslinking of beads by liberated $Ca^{++}$ followed by surface crosslinking with $Ca^{++}$ present in gelation medium for better drug entrapment. On the other hand, $Na^+$, eliberated from reaction of $NaHCO_3$ with acidic gelation medium does not have the capacity to induce gelation of SA, thus, conferring only buoyancy and not the core crosslinking.

An increase in KT contents (M4-M6) in the beads resulted in a significant improvement ($p<0.05$, M1 compared to M4) in the entrapment efficiency. However, incorporation of $CaCO_3$ did not result in further improvement in the entrapment efficiency.

Type B gelatin was added to the formulation (M7) with a view that this would form polyelectrolyte complex with SA as it is reported that the presence of both basic and acidic groups along the backbone which makes gelatin a poly-ampholyte which can complex with both negatively and positively-charged polyelectrolytes at below and above its isoelectric point, respectively. Apart from this, the simultaneous presence of hydrophilic and hydrophobic regions further enhances the multi-functionality of gelatin, and is an important feature for generating strong packaging with hydrogels [8].

As expected, addition of type B gelatin to formulation M7 significantly enhanced the drug encapsulation efficiency ($p< 0.05$, M7 compared with M1 and M4). This could be attributed to the formation of polyelectrolyte complex between gelatin and SA together with insoluble nature of drug. In order to confirm the PEC formation between gelatin type B and SA, we have carried out DSC studies on blank SA-gelatin type B beads (Figure 4). The DSC thermogram of gelatin type B (Figure 4) showed an endothermic peak at around 60°C corresponding to the glass transition temperature and multiple peaks between 226-300°C (that is, at 226.84 °C and at 269.85°C). These endothermic peaks were considerably shifted to 197.8 and 282.51$^0$C in the DSC thermogram of SA-gelatin type B beads, suggesting formation of PEC between SA and gelatin type B in the core of the beads. Further, there was an additional exothermic peak at 299.47 $^0$C attributed to the thermal breakdown of PEC.

Addition of XG to the bead formulations (M8 and M9) resulted in significant improvement in the entrapment efficiency ($p<0.05$, M7 compared with M8 and M9 compared to M1). However, there was no significant difference in the entrapment efficiency between formulation M8 and M9. The increase in the entrapment efficiency of beads could be attributed to increase in micro-viscosity of formulations arises from incorporation of XG.

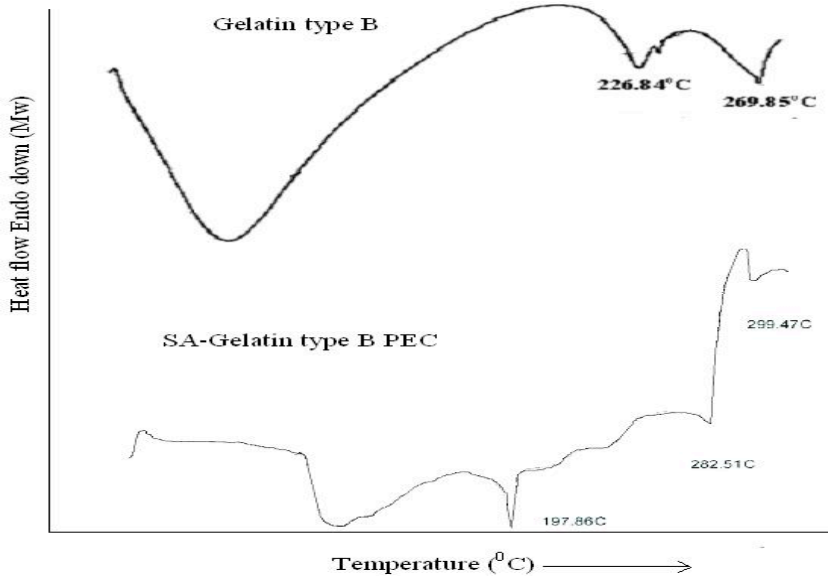


**Figure 4**
DSC thermograms of gelatin type B and SA-gelatin beads

*Floating SA-LMCH PEC beads*

The encapsulation efficiency of floating SA-LMCH PEC beads (M10, M11 and M12) ranged from 63-91%. Although there was an increase in KT entrapment, but it was not significantly different from calcium alginate beads ($p>0.05$, M10 compared with M1). Like calcium alginate beads, incorporation of $CaCO_3$ into the PEC bead formulations resulted in significant improvement in entrapment efficiency ($p<0.05$, M10 compared to M11 and M12). This could be attributed to the interaction of liberated $Ca^{++}$ with $COO^-$ groups on SA in the core of the bead together with the formation of SA-LMCH PEC membrane on the bead surface which effectively encapsulate the drug into the bead network.

**Table II**

Physiochemical characteristics of various floating bead formulations

| Formulation code | % EE* | Bead Size* (mm) | % Buoyancy | Duration of floating (hrs) |
|---|---|---|---|---|
| M1 | 58.8±2.35 | 1.3±0.01 | 100 | 12 |
| M2 | 88.6±4.51 | 1.5±0.01 | 100 | 12 |
| M3 | 92.4±4.11 | 2.1±0.02 | 100 | 12 |
| M4 | 72.7±3.24 | 1.3±0.01 | 100 | 8 |
| M5 | 76.5±2.56 | 1.5±0.02 | 83 | 10 |
| M6 | 72.7±2.36 | 1.9±0.03 | 100 | 10 |
| M7 | 86.7±2.78 | 1.8±0.02 | 100 | 8 |
| M8 | 74.6±2.33 | 1.9±0.03 | 100 | 9 |
| M9 | 68.9±1.78 | 1.7±0.01 | 100 | 8 |
| M10 | 63.8±3.21 | 1.7±0.02 | 97 | 8 |
| M11 | 91.4±3.77 | 1.8±0.02 | 100 | 10 |
| M12 | 90.5±3.54 | 1.8±0.01 | 100 | 10 |

*Determined in triplicate, ±SD.

*In vitro drug release studies*

*Floating calcium alginate beads*

KT release from floating calcium alginate beads in 0.1M HCl (pH 1.2) is shown figure 4a. In the case of formulation M1, 25, 51, 74 and 99% drug was released at the end of 1st, 3rd, 4th and 5th hour. Incorporation of $CaCO_3$ into the bead formulation resulted in a significant retardation of drug release with 13, 52, 70 and 99% drug was released at the end of 1st, 3rd, 6th and 8th hour from formulation M2.

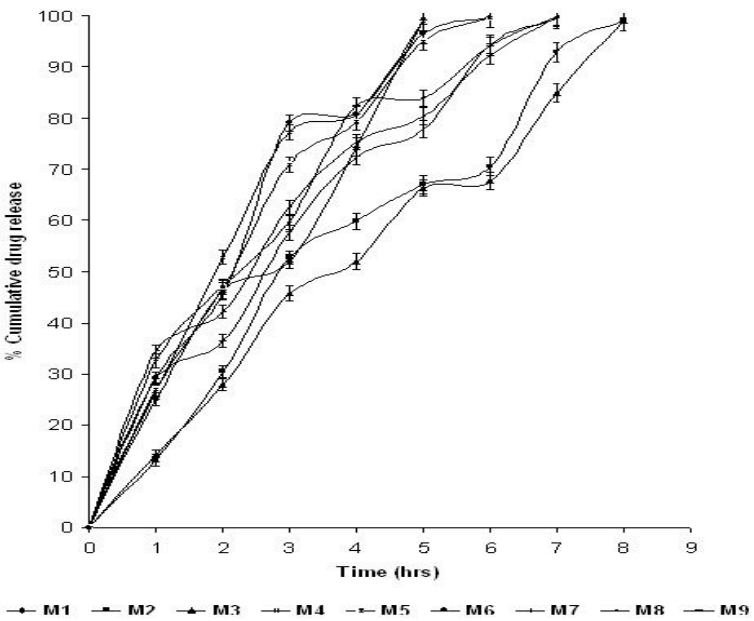


**Figure 4a**

Percent of cumulative KT release from floating calcium alginate beads

Further increase in $CaCO_3$ concentration resulted in almost no change in KT release profile with 14, 45, 68 and 98% drug was released at the end of 1st, 3rd, 6th and 8th hour from formulation M3. The retardation of KT release from formulation M2 and M3 could be attributed to use of $CaCO_3$, which has resulted in more efficient core crosslinking of SA beads due to librated $Ca^{++}$ compared to $Na^{+}$. Increasing the KT contents in the floating beads resulted in fast drug release ($p<0.05$, M4 compared with M1) with 26, 53, 77 and 98% drug was released at the end of 1st, 2nd, 3rd and 5th hour. Here also incorporation of $CaCO_3$ significantly ($p<0.05$, M5and M6 compared with M4) retarded the KT release from the beads. Incorporation of type B gelatin (M7) or XG (M8 and M9) into floating beads although extended the drug release up to 7-8 hours but KT release profile were not significantly different from formulation M1. This could be attributed to PEC formation in the core of the beads (M7) and increase in micro-viscosity of formulations arises from incorporation of XG (M8 and M9) respectively.

*Floating SA-LMCH PEC beads*

KT release from floating PEC beads in 0.1M HCl (pH 1.2) is shown in figure 4b. In the case of floating PEC bead formulation M10, about 30, 56, 73 and 99% KT was released at the end of 1st, 3rd, 5th and 8th hour. However, the release profile was not significantly different ($p<0.05$) from

M1. It was also observed that by the incorporation of $CaCO_3$ into the PEC bead formulations, KT release was increased with 30, 60 and 99% KT was released at the end of 1, 2 and 4 hour. Further increase in CaCO3 concentration in the bead PEC formulation did not significantly ($p<0.05$) retarded the KT release from the beads.

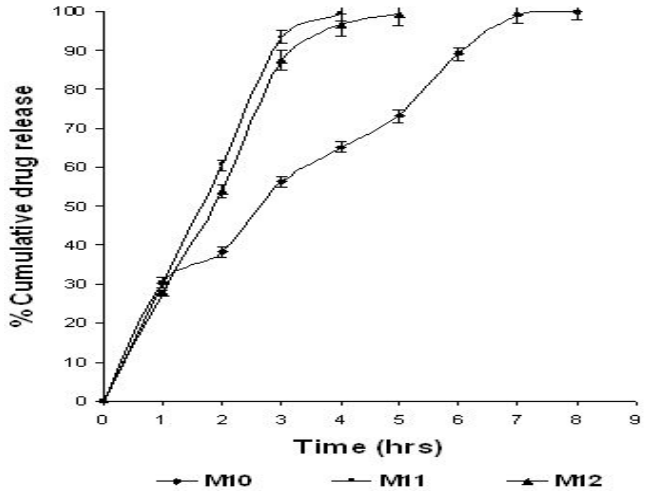


**Figure 4b**
Percent of cumulative KT release from floating SA-LMCH PEC beads

*Mechanism of drug release*

The *in vitro* release pattern of various formulations was analyzed by fitting the dissolution data into various kinetic models (Table III). In the case of floating calcium alginate beads, It was observed the for the formulations M1, M2, and M3, the $r^2$ values were higher when fitted to zero order kinetics, which describes that the drug release rate from the formulations is independent of the concentration of the drug . Whereas, formulations M4-M9 followed the Higuchi release pattern, which describes the release from system, where the solid drug is dispersed in an insoluble matrix and the rate of drug release is related to the rate of drug diffusion. The n values of calcium alginate formulations ranged from 0.57-1.04. Formulations M1, M3, M4, M5, M6, M7, M8 and M9 followed anomalous non-Fickian diffusion, whereas, formulation M2 followed super case II transport. In the case of floating PEC beads, formulations M10 and M12 followed the Higuchi release pattern, whereas formulation M11 followed zero order kinetics. In this case the n value ranged from 0.57-1.04. Formulation M10 followed anomalous non-Fickian diffusion and formulations M11 and M12 followed super case II transport. Anomalous non-Fickian transport suggests that the mechanism and kinetics of drug

release were dependent on the solubility of KT in dissolution medium, with KT being predominantly released by diffusion and anomalous behaviour resulting from the relaxation of macromolecular polymeric chains in gel beads. For true swelling-controlled release systems, the diffusional exponent, *n*, is 1. This type of transport is known as Case II transport and results in zero-order release kinetics. For systems exhibiting Case II transport, the dominant mechanism for drug transport is due to polymer relaxation as the gels swells. If n>1.0, the swelling material is likely to craze and fracture due to the tremendous osmotic pressure differences at the accelerating and advancing front. This type of transport mechanism is known as Super Case II transport. Super case II transport suggests that more than one mechanism, i.e. swelling and erosion may be involved in the release kinetics.

**Table III**

Drug release kinetics of various floating bead formulations

| Formulation code | $r^2$ Value | | | | n value |
|---|---|---|---|---|---|
| | Zero order | First order | Higuchi | Korsmeyer-Peppa's | |
| M1 | 0.9795 | 0.694 | 0.9171 | 0.9681 | 0.74 |
| M2 | 0.9697 | 0.6707 | 0.9409 | 0.9722 | 1.04 |
| M3 | 0.9856 | 0.6834 | 0.9341 | 0.9912 | 0.96 |
| M4 | 0.9592 | 0.6695 | 0.9612 | 0.9998 | 0.97 |
| M5 | 0.957 | 0.6318 | 0.9721 | 0.9955 | 0.87 |
| M6 | 0.9298 | 0.6184 | 0.9622 | 0.9584 | 0.88 |
| M7 | 0.9319 | 0.5658 | 0.9851 | 0.9733 | 0.64 |
| M8 | 0.9437 | 0.5654 | 0.9872 | 0.9343 | 0.57 |
| M9 | 0.967 | 0.6081 | 0.9689 | 0.9304 | 0.67 |
| M10 | 0.9587 | 0.5707 | 0.9782 | 0.953 | 0.57 |
| M11 | 0.9668 | 0.7217 | 0.9424 | 0.9995 | 1.01 |
| M12 | 0.9329 | 0.6712 | 0.9455 | 0.9967 | 1.04 |

## Conclusions

In the present investigation we have compared the drug encapsulation efficiency and release properties (in 0.1M HCl) of floating calcium alginate beads prepared with $NaHCO_3$ alone or a mixture of

$NaHCO_3$ and $CaCO_3$ as gas-generating agent(s) with floating polyelectrolyte complex (between SA and LMCH; SA and gelatin type B) beads prepared with $NaHCO_3$ or a mixture of $NaHCO_3$ and $CaCO_3$ as buoyancy imparting agent. Ketoprofen was found to interact with cations used for the preparation of floating beads. But, overall, our results suggest that by using a mixture of gas-generating agents instead of $NaHCO_3$ alone, it is possible to improve the drug encapsulation efficiency and the modulation of drug release from floating alginate based formulations. These results enable these floating beads to be used as oral stomach specific sustain/control delivery of drugs with absorption window in the upper gastro-intestinal tract.

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