Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca$^{2+}$ and Ba$^{2+}$ ions

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Abstract

Spherical beads have been prepared by ionotropic gelation of sodium alginate in the presence of CaCl$_2$ and BaCl$_2$ solutions and their swelling behavior has been studied. The barium ion-crosslinked beads exhibit almost minimum swelling of 40 ± 3% in PBS at pH 7.4 but possess greater stability while calcium alginate beads exhibit nearly 160% of water uptake and subsequently dissolve. The beads appear to swell through ion-exchange process which was confirmed by monitoring the Ca$^{2+}$ release from the calcium alginate beads. The release was found to be diffusion controlled. On treatment with 0.1 M HCl, the calcium alginate beads demonstrated a decrease in water uptake in PBS at pH 7.4 with faster degradation while for acid treated barium alginate beads, the water uptake was found to increase on treatment with HCl. When the two beads samples were put in media of continuous varying pH (to mimic the passage of beads from mouth to colon), barium alginate beads possessed greater stability, thus showing potential to be used for colon-targeted oral delivery.

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1. Introduction

Continued progress in many fields of biotechnology has produced numerous, kinds of engineered peptides and proteins as novel therapeutic drugs for drug delivery applications. The oral administration of these drugs seems to be the effective, safe and convenient approach and is preferred over parental medication which suffers from the drawback that it results in rapid increase and subsequent rapid decrease of the blood serum concentration level [1]. However, oral delivery of protein and peptide drugs is not so easy to achieve possibly because of their sensitivity to gastric acid and their vulnerability to gastrointestinal enzymes [2].

The site specific oral drug delivery to the target receptor site has the potential to reduce the side effects and to increase pharmacological response. One of the interesting areas to target the drug orally for the systemic delivery is the colon, the proximal part of large intestine. Due to unique

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physiological features of large intestine, colonic delivery can be achieved in many ways. One such approach is to use the enzymatically degradable polymeric systems [3–7] which can release the encapsulated protein/peptide drug at the colon through their enzymatic degradation by their respective enzymes which are produced in the colon by intestinal flora [8]. Another approach is based on the fact that the luminal pH of the healthy distal colon is slightly higher than that of the proximal small intestine and this has led to the development of oral dosage forms [9–12] which release the drug at the colonic pH via the mechanism involving pH-dependent swelling.

However, the above mentioned approaches involve using materials and conditions that are not compatible with very sensitive protein drugs. Such drugs may lose their activity when exposed to harsh conditions like elevated temperature, organic solvents and extremes of pH [13]. Moreover, synthetic polymers used in pH-sensitive drug delivery and the degradation products of enzymatically degradable hydrogels (e.g. aromatic amines in azo-containing polymers) have also been reported to be highly toxic [14]. These drawbacks may be eliminated by encapsulating protein drugs into natural polymers like alginate, carbomethyl cellulose, guar gum etc. via their ionotropic gelation with multivalent metal ions in the aqueous medium at room temperature [15].

Alginate is a common term used for a family of unbranched polymers composed of 1,4-linked β-D-mannuronic and α-L-guluronic acid residues in varying proportions, sequence and molecular weight. Alginate gelation takes place when divalent cations (usually Ca²⁺), interact ionically with blocks of guluronic acid residues, resulting in formation of three-dimensional network which is usually described by ‘egg-box’ model [16]. As the encapsulation method is mild, and done at room temperature in aqueous medium, several sensitive drugs [17], proteins [18], living cells [19], enzymes [20], spermatozoa [21] etc. have been successfully released through alginate beads.

As mentioned above, the alginates have been used widely for the delivery of bioactive materials. However, we realize that in order to use alginate beads particularly for colon-targeted oral delivery special attention should be paid on the various parameters involved in architecture of the beads and the mechanistic aspects of the swelling and subsequent degradation of the beads in the simulated gastric and intestinal fluids. For example, the beads should reside for nearly 1–4 h in stomach prior to their entry to the small intestine and then to colon. The stay of beads in the highly acidic environment of stomach may result in the hydrolysis of alginate into more soluble low molecular weight alginic acid. This causes reduction in their mechanical strength and degree of crosslinking hence the beads may degrade at faster rate after arriving at colon. Thus, instead of residing for 8–12 h in the colonic fluid, they may degrade in very short time which ultimately fails to solve the purpose of colon-targeting. So, it becomes necessary to impart proper mechanical strength to the beads, so that after passing through stomach and small intestine, they can reside for a sufficient longer time period in colon. Thus, the present work describes a detailed investigation of swelling/degradation behavior of alginate beads crosslinked with different metal ions. The major objective of the present in vitro study is to get some idea about the possible behavior of beads in gastrointestinal tract with respect to their stability so that they may be used in vivo for oral administration of protein drugs. A thorough survey of the literature reveals that no such type of investigation has been carried out to compare the stability and swellibility of beads crosslinked with different metal ions.

2. Experimental

2.1. Materials

Sodium alginate (SA, average molecular weight 60,000 ratio of mannuronic acid to guluronic acid residues (M/G) (1.75 ± 0.12), medium viscosity 200 cP for 1% aqueous solution at 20 °C) was obtained from Research Lab, Bombay, India. The crosslinkers aluminium chloride, barium chloride and calcium chloride were obtained from Research Lab, India. The double distilled water was used throughout the investigations.
2.2. Preparation of beads

Sodium alginate was dissolved in distilled water at a concentration of 4% (w/v) unless otherwise noted. The polymer solution was then added drop-wise into gelation media of 250 ml CaCl₂ solution of definite concentration (w/v) using a 25 ml hypodermic syringe through a needle #21 under constant stirring at room temperature. The beads, thus formed, were cured in the gelation medium for 15 min and then taken out, followed by washing with distilled water and then allowed to dry at 30 °C in a dust-free chamber. Similarly, beads crosslinked with BaCl₂ and AlCl₃ were prepared. Here it is worth mentioning that the mode of drying process affects the stability of the beads. The partial drying of the beads reduces the porosity of the beads. The complete dehydration of the beads may result in surface cracking which can facilitate the surface erosion of the beads upon rehydration. This will affect the swelling/degradation behavior of the beads.

One more point to be noted here is that in hydrogel, water exists in two states namely bound water and free water. Hence it may be probable that on drying of the above beads at 30 °C, the free water has come out of the beads while bound water still remains within the beads. Therefore, the crosslinking ions may be present in hydrated state in the beads.

Experimental conditions such as distance between the syringe and gelation media, number of drops of polymer solution falling into gelation media per minute and the temperature were maintained uniform. The beads shall be denoted by CA(X), BA(X) and AA(X) where CA, BA and AA represent calcium alginate, barium alginate and aluminum alginate, respectively, and X denotes percent concentration of crosslinker solution.

2.3. Beads size measurement

Five samples of the completely dried beads from each formulation were selected and their size was measured with the help of a micrometer screw gauge (Kayco, India) with an accuracy of ±0.01 mm.

2.4. Swelling study of beads

The pre-weighed dry beads were immersed in 0.1 M HCl (pH 1.0), and in the phosphate buffer (0.1 M, pH 7.4) at 30 °C and their ‘weight change’ was monitored at different time intervals till the beads showed complete dissolution. The fractional weight change was transformed to a percentage using the following empirical relationship

\[
\text{Dynamic weight change (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

The measurements were made in triplicate and average data was used for calculations. In the various curves plotted, the complete dissolution of beads has been indicated by -100% weight change.

3. Results and discussion

3.1. Beads formation and crosslinking

In the case of beads prepared from pure sodium alginate, the formation of beads takes place due to ionotropic gelation of spherical drops by M⁺⁺ ions. The polyguluronate units in the alginate molecules form a chelated structure with metal ions, called an ‘egg-box’ junction with interstices in which the cations may pack and be coordinated [22]. The junction between the chains formed in this way are kinetically stable towards dissociation [23] while the polymannurionate units show the normal polyelectrolyte characteristics of cations binding. The two interactions as depicted in the Fig. 1 thus result in formation of spherical beads.

3.2. IR spectra

The alginate IR spectrum (Fig. 2) shows the characteristics peaks at 3383 cm⁻¹ (OH stretching), 1607 cm⁻¹ (COOH stretching) and 1036 cm⁻¹ (C–O–C stretching).
3.3. Swelling behaviour of alginate beads

Fig. 3 depicts the dynamic weight change for the alginate beads, crosslinked in the 2%, 3% and 4% CaCl₂ solutions, in the phosphate buffer medium of pH 7.4. It is clear from the figure that the beads CA(2), CA(3) and CA(4) exhibit maximum water uptake of 318 ± 6%, 252 ± 8% and 160 ± 6% in the PBS at 30 °C. After attaining maximum water uptake, the beads begin to lose weight and finally dissolve. The observed behavior may be explained as follows:

When CA beads are placed in the phosphate buffer saline (PBS) of pH 7.4, the Na⁺ ions present in the external solution undergo ion-exchange process with Ca²⁺ ions which are binding with COO⁻ groups mainly in the polymannuronate sequences. As a result the electrostatic repulsion among negatively charged –COO⁻ groups increases which ultimately causes the chain relaxation and enhances the gel swelling. Thus it can be said that in the initial phase of the swelling process the Ca²⁺ ions present in polymannuronate units are exchanged with Na⁺ ions, thus causing the beads to swell along with uptake of water. This argument is further supported by the observation that some turbidity appears in the system due to formation of calcium phosphate. Therefore, phosphate buffer is mainly responsible for the swelling of the beads. The Na⁺ ions undergo ion-exchange with Ca²⁺ ions thus making the bead structure loose, and phosphate ions interact with calcium ions to form calcium phosphate. In this way the swelling and water uptake of the beads seem to be due to the presence of sodium phosphate buffer. We also confirmed this by allowing the beads to swell in the Tris–HCl buffer of pH 7.4. However, the beads demonstrated almost no swelling thus confirming our hypothesis that water uptake of beads is due to ion exchange between Ca²⁺ ions and Na⁺ ions from the sodium phosphate buffer. In the later stage of swelling process, the Ca²⁺ ions which are binding with –COO⁻ group of the polyguluronate units and thus form the tight ‘egg-box’ structure also start to exchange with Na⁺ ions of the buffer medium. This consideration is plausible because polyguluronate sequences have a strong auto-cooperative binding of Ca²⁺ ions [23] and serve as a stable crosslinking structure within the gel. Finally, the alginate beads begin to disintegrate when Ca²⁺ ions in the egg-box buckled structure diffuse out into the medium. Therefore, the beads start to lose their weight and finally dissolve. It is also clear that as the extent of crosslinking increases, the maximum water uptake decreases.
A critical analysis of the swelling process reveals two underlying molecular processes: penetration of the solvent molecules to the void space in the network and subsequent relaxation of the network segments. The fundamental equation \[ M_t / M_\infty = k t^n, \]
where \( M_t \) and \( M_\infty \) are the percentage of water uptake at time ‘\( t \)’ and at equilibrium, respectively, \( k \) is gel characteristics constant and \( n \) is swelling exponent, defines three situations:
1. For a perfectly Fickian process where the rate of solvent penetration is the slowest and hence is the rate determining step, the value of \( n = 0.5 \).
2. When the penetrant velocity is far greater then the chain stretching rate then solvent uptake is proportional to the time, i.e., \( n = 1.0 \) (case II transport).
3. When the two rates are comparable, the swelling exponent ‘\( n \)’ falls between 0.5 and 1.0, thus indicating a non-Fickian type swelling mechanism.

Using \( n \) and ‘\( k \)’, the diffusion coefficient \( D \) of solvent in the matrix was calculated using the following equation \[ D^n = K / 4(\pi r^2)^n, \]
where \( r \) is radius of the beads.

Table 1 describes the above swelling parameters for the beads crosslinked with 2\%, 3\% and 4\% calcium chloride solutions. It is clear from the values of ‘\( n \)’ given in the Table 1 that the swelling of the beads follows case II mechanism for the beads crosslinked with 4\% CaCl\(_2\) whereas other beads samples (i.e. crosslinked with 2\% and 3\% calcium chloride solutions) demonstrate super case II mechanism. The higher values of ‘\( n \)’, obtained for all bead samples may be attributed to the fact that the swelling process is mainly governed by the relaxation of polymeric segments induced by the ion-exchange process taking place between Na\(^+\) ions present in the outer solution and Ca\(^{2+}\) ions present inside the beads. As the ionic strength of the phosphate buffer used is sufficiently high (i.e. 0.1 M), the ion-exchange process is also prominent. The higher values of diffusion coefficients also support the above experimental findings.

### 3.4. Role of Na\(^+\) ions in the swelling process

Although alginates are hydrophilic and water soluble anionic polysaccharides, but the Ca\(^{2+}\) ions induced crosslinked beads of alginate are sufficiently stable in the aqueous media. It is the ion-exchange process between Na\(^+\) and Ca\(^{2+}\) ions, which is supposed to be responsible for the swelling and subsequent degradation of the beads. In order to confirm this hypothesis, pre-weighed amount of calcium alginate beads CA(4) were put each in 100 ml of distilled water containing 0, 1.5 and 3.0 g NaCl and their swelling behavior was studied at 30 °C. The results, as depicted in the Fig. 4, reveal that beads do not show any tendency to take up water and swell in pure distilled water (not containing NaCl). Moreover they begin to loose their weight slightly i.e. by 40% in 6 h and then do not show any tendency to further loose their weight for next 36 h. However, behavior of beads is totally different in distilled water containing 1.5 and 3.0 g NaCl. The beads show tendency to gain weight and the equilibrium water uptake has been found to be nearly 95±12% and

<table>
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<th>Bead sample</th>
<th>( n )</th>
<th>( k \times 10^2 )</th>
<th>( D \times 10^5 ) cm(^2) min(^{-1} )</th>
</tr>
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<tbody>
<tr>
<td>CA(2)</td>
<td>1.25</td>
<td>6.58</td>
<td>7.34</td>
</tr>
<tr>
<td>CA(3)</td>
<td>1.11</td>
<td>8.00</td>
<td>5.78</td>
</tr>
<tr>
<td>CA(4)</td>
<td>1.00</td>
<td>10.80</td>
<td>5.30</td>
</tr>
</tbody>
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![Fig. 4. Dynamic uptake of water for the beads samples CA(4) in pure distilled water (O), in water containing 0.5 g NaCl (●) and 1.0 g NaCl (△) at 30 °C.](image-url)
360 ± 17% in the two solutions, respectively. It means that as the quantity of NaCl in the medium increase, the value of water uptake of beads also increases. After attaining maximum swelling, the beads begin to show weight loss and finally dissolve completely (data not shown in the figure). A close look at the Fig. 4, also reveals some interesting features. In the distilled water with NaCl, the beads demonstrate a slow uptake of water in the initial phase of swelling process (i.e. nearly 21 ± 3% and 65 ± 7% in 180 min). However, after 3 h, there is drastic increase in water uptake in next 2 h and the beads attain maximum swelling. Later on they begin to show dissolution tendency and finally dissolve. These experimental findings may be explained on the basis of ion-exchange process between Na$^+$ and Ca$^{2+}$ ions and the composition of alginate beads.

The beads dipping in pure distilled water do not show any tendency to take up water because no sodium ions are available in the medium which could undergo ion-exchange process with Ca$^{2+}$ ions percent within the beads. In addition to this, the beads are highly crosslinked (i.e. 4% CaCl$_2$ solution) and hence do not show any tendency to absorb water and swell. This explains why the beads did not swell in pure distilled water. In the later stage, slight weight loss in beads may be due to the little dissolution of polymannuronate blocks and hence may result in weight loss.

The situation is very different with beads which were immersed in the distilled water, containing 1.5 and 3.0 g of NaCl per 100 ml of solvent. Here, there starts ion-exchange process between Na$^+$ ions and Ca$^{2+}$ ions which are in the polymannuronate blocks. Now since the Na$^+$ ions are not able to sufficiently bind the −COO$^-$ ion groups in polymannuronate sequences the extent of crosslinking decreases and hence water uptake increases. However after near by 3 h when most of the Ca$^{2+}$ ions present in the poly(M) blocks have been exchanged with Na$^+$ ions the ion-exchange process between Na$^+$ ions and Ca$^{2+}$ ions present in polyguluronate blocks begins. This results in loosening of egg-box structure and thus permit great amount of water to enter. This may be the probable cause of drastic increase in water uptake by the beads after nearly 3 h. After the attainment of equilibrium water uptake, the highly hydrated egg-box structure begins to disintegrate and hence the beads start to loose their weight.

In this way, we see that the ion-exchange process between Na$^+$ ions binding with carboxylate groups in polyguluronate and polymannuronate blocks is ultimately responsible for the swelling and subsequent degradation of the beads.

### 3.5. Estimation of Ca$^{2+}$ release

Although, ion-exchange induced swelling and subsequent degradation of calcium alginate beads is a well established fact but in order to correlate the ion-exchange process with the swelling profile of the beads we also estimated release of Ca$^{2+}$ ions from the swelling beads by EDTA method [26]. The results, as depicted in the Fig. 5 indicate that amount of Ca$^{2+}$ ions released from the beads increases with the concentration of NaCl in the swelling media. This can be well attributed to the fact that with increase in number of Na$^+$ ions in the external solution, the ion exchange process is also enhanced, thus resulting in faster release of Ca$^{2+}$ ions from the beads. In this way, number of Ca$^{2+}$ diffusing out from the beads depends upon the concentration of NaCl in the external solution. However, on comparing the swelling and Ca$^{2+}$ release profiles of the beads in salt containing media (i.e. Figs. 4 and 5) very interesting results come out. For example, if we compare the swelling and release profiles for the beads immersed in 3 g sodium chloride containing distilled water, it is
interesting to see that beads demonstrate nearly 15% of the total water uptake in first 3 h while 64% of calcium is released from the beads in this time period. This anomaly can be explained with the help of composition of the beads. As stated in the section ‘experimental’, the ratio of mannanuronic acid to guluronic acid residues in nearly 1.72 thus indicating that percent of manunuronic acid residues in the polymer in nearly 61% of the total alginate content. Hence, of the total calcium ions that are bound with carboxyl groups in the beads nearly 62% ions may be supposed to be present within the polymannuronic residues. Therefore, when the beads are put in sodium chloride containing distilled water, in the first 3 h Ca$^{2+}$ ions present in polymannuronate blocks are released. This explains why there is nearly 64% release of calcium ions from the beads in first 3 h. Finally, since polymannuronate residues do not contribute much towards swelling of the beads, the beads swell to nearly 15% of the total swelling in this time interval. Later on, the ion-exchange between Na$^+$ ions and Ca$^{2+}$ ion present in the polyguluronate blocks causes greater water uptake with subsequent degradation.

In order to find out the pattern of calcium release from the beads, ln $M_t/M_\infty$ values were plotted again ln $t$ and the release exponent ‘n’ was evaluated from the slope of the straight lines obtained. The values of release exponent ‘n’ for the beads immersed in the solutions containing 1.5 and 3.0 g NaCl per 100 ml water were found to be 0.22 and 0.25, respectively. These values indicate that the release of Ca$^{2+}$ ions, as induced by the ion-exchange process, follows diffusion controlled or Fickian release pattern. Similar results have also been reported elsewhere [27].

3.6. Crosslinking with other metal ions

The mechanical and swelling properties of alginate beads, produced by ionic crosslinking with cations depend upon a number of factors like valency of ions, size of ions etc. For example, monovalent cations and Mg$^{2+}$ ions do not induce gelation [28] and Ba$^{2+}$ ions produce stronger beads than Ca$^{2+}$ [29]. To investigate this aspect in more detail, we synthesized sodium alginate (4% w/v) beads via crosslinking in 4% solution (w/v) of Ba$^{2+}$, Ca$^{2+}$ and Al$^{3+}$ ions, respectively. The results of their swelling behavior in PBS, pH 7.4 have been well depicted in the Fig. 6. The results obtained display some interesting informations. The equilibrium water uptake values follow the order Ca$^{2+}$ > Al$^{3+}$ > Ba$^{2+}$ and the total ‘lasting time’ before complete dissolution follows the reverse order i.e., Ba$^{2+}$ > Al$^{3+}$ > Ca$^{2+}$.

The results obtained can be explained on the basis of the extent of crosslinking in the beads and the size of the cations involved in crosslinking process. Since barium and calcium ions are divalent, their bonding to alginate is expected to occur in a planar two-dimensional manner inside the beads [15]. But, since barium ion has largest radius (1.74 Å) as compared to the other two cations, (i.e. 1.14 Å for Ca$^{2+}$ and 0.68 Å for Al$^{3+}$ ion) it is supposed to fill a large space between the alginate molecules, thus producing a tight arrangement with smaller voids as depicted in Fig. 7(a). Therefore, the exchange of large Ba$^{2+}$ ions in the beads with Na$^+$ ions and also their removal in the form of insoluble barium phosphate is hindered, thus resulting in lowest water uptake and highest stability. The barium alginate beads take almost 18 h to degrade completely. In the case of calcium alginate beads, although there exists two-dimensional crosslinking, but due to relatively smaller size of calcium ions as compared to barium ions, their diffusion from the beads due to ion-exchange process with Na$^{2+}$ ions and subsequent removal as
Calcium phosphate is relatively faster thus leading to greater water uptake and faster degradation. The Al\(^3+\) crosslinked beads display somewhat embarrassing results. The trivalent Al\(^3+\) ions are expected to form a three-dimensional valent bonding structure with the sodium alginate [15]. This three-dimensional bonding results in extended crosslinking through the whole bead. This is because the crosslinking occurs in two different planes at the same time resulting in compacting the alginate molecules (see Fig. 7(b)). Because of the smallest size of Al\(^3+\) ions among the three crosslinking cations (i.e. 0.68 Å) its diffusion from the beads into outer solution is relatively faster as compared to that of Ba\(^2+\) ions. This consequently results in a little more water uptake than by the beads crosslinked with Ba\(^2+\) ions. In other words in spite of three-dimensional crosslinking it exhibits greater water uptake than Ba\(^2+\) ions-crosslinked beads. Moreover, because of the extended three-dimensional crosslinking, the beads exhibit greater stability. Hence it can be concluded that nature of crosslinker cations exerts a great influence on the swelling and degradation behavior of beads. Moreover, the beads, crosslinked with Ba\(^2+\) ions exhibit fair stability with minimum water uptake.

From the above discussion it is clear that Al\(^3+\) and Ba\(^2+\) ions crosslinked alginate beads are sufficiently strong with low water uptake. However, looking to the toxicity of Al\(^3+\) ions, only Ca\(^2+\) and Ba\(^2+\) ions-crosslinked beads were considered for further studies. Here it is worth mentioning that the radio-labelled BaSO\(_4\) is given to the patients for the diagnosis of intestinal disorder. Its non-toxic effect in the body is due to the fact that BaSO\(_4\), being insoluble, is eliminated from the body without being dissolved in the biological fluids. Likewise, the Ba\(^2+\) ions, coming out of the beads (although at extremely slow rate) due to ion-exchange with Na\(^+\) ions, may also get precipitated as barium phosphate in the intestinal fluid (due to present of phosphate buffer) and hence easily eliminated from the body along with waste materials.

3.7. Effect of treatment with 0.1 M HCl

Alginates have been reported to undergo proton-catalyzed hydrolysis which is dependent on time, pH and temperature [30]. A crosslinked alginate matrix, when exposed to low pH, can therefore be converted into alginic acid which may result in lowering of degree of crosslinking and hence faster degradation. Now, prior to entry to the colon, the oral formulation resides for some time in highly acidic environment of stomach. Therefore, in order to use the proposed beads as oral dosage form for colon-targeting, they should also be investigated for their swelling behaviour after their treatment with acid. In order to investigate this, the CA(4) and BA(4) beads were treated with 0.1 M HCl solution for a period of 30 and 120 min and their swelling was studied in the phosphate buffer saline at 30°C. The results obtained for CA(4) beads have been depicted in the Fig. 8. It is clear that the beads, treated with HCl for 0, 30 and 120 min attain a maximum water uptake of 160 ± 6%, 100 ± 9% and 25 ± 4%, respectively. Moreover the time required to attain equilibrium swelling is found to be nearly 180 ± 8, 120 ± 7 and 60 ± 5 min, respectively. Later on, the
gels begin to disintegrate and finally dissolve. The total time taken by the gels to disintegrate and dissolve completely is approximately $360/215$, $300/211$ and $180/219$ min, respectively. Thus, the above data reveals that as the treatment time increases, the time required to attain maximum swelling and the equilibrium water uptake decrease, respectively. Moreover, with the increase in the ‘treatment time’, the gels take less time to disintegrate and dissolve completely. The observed results may be explained as follows:

When CA(4) beads are treated with 0.1 M HCl, alginate gets hydrolyzed to lower molecular weight fraction alginic acid [31]. Due to conversion of COO$^-$ group into unionized carboxylic group, the electrostatic attraction between Ca$^{2+}$ ions and COO$^-$ ions in the ‘egg-box’ junction almost disappears. Moreover, there may occur ion-exchange between H$^+$ ions (presence in the external HCl solution) and free Ca$^{2+}$ ions inside the beads. Thus a reduced Ca$^{2+}$ ions concentration within the beads results in a weaker Ca$^{2+}$ crosslinked beads when put in phosphate buffer at pH 7.4. Therefore, the acid-treated beads are a loosely crosslinked structure containing more soluble alginate as constituent. When such beads are put in the phosphate buffer of pH 7.4, the beads swell at faster rate but do not attain a higher water uptake value due to loosely bound structure of the beads which is unable to retain large amount of water within the matrix. Moreover, there is possibility of ion-exchange between H$^+$ ions (produced due to ionization of carboxylic groups in the buffer of pH 7.4) and Na$^+$ ions present in the buffer, thus resulting in uptake of sodium ions. Moreover the soluble low molecular weight alginate starts to dissolve in the hydrated matrix, thus causing a loss in weight of the beads and finally the beads dissolve completely. Therefore, the beads with longer ‘treatment time’ have lower degree of crosslinking and greater low molecular weight alginic acid content within the beads.

The most surprising part of this study is the behavior of acid treated alginate beads crosslinked with barium ions. The swelling behavior of these beads has been well depicted in the Fig. 9, which clearly indicates that these beads show just opposite behavior. The beads treated for 0, 30 and 120 min in 0.1 M HCl exhibit nearly $50/26\%$, $915/217\%$ and $1060/221\%$ water uptake in PBS at 30 $^\circ$C. It means that the water uptake increases with treatment time which is just contradictory to the results obtained with calcium alginate beads. The observed findings may be explained on the basis of the big size of the Ba$^{2+}$ ions, which imparts greater stability to beads. The acid treatment of the beads causes a reduction in the degree of crosslinking due to the formation of low molecular weight alginic acid as stated earlier. This loosely crosslinked structure continues to take up water and swell in the phosphate buffer of pH 7.4 due to hydrophilic nature of alginate. As the Ba$^{2+}$ ions are of sufficiently large size they undergo very slow ion-exchange process with Na$^+$ ions, thus helping the swelling beads to retain their structure. After
attaining the maximum water uptake, the highly hydrated beads begin to disintegrate due to dissolution of alginic acid content, thus showing a constant weight loss. The beads, treated for 120 min dissolve completely in sixth hour while the beads treated for 30 min took nearly 18 h to dissolve completely. In this way, size of the cross-linking ion plays a key role in governing the swelling behavior of the acid treated beads.

3.8. Swelling in medium of varying pH

When a dosage form is taken orally, first of all it goes to stomach and resides there for a certain period. Then it passes through small intestine and then large intestine and finally reaches the left colon, the distal part of large intestine. The total gastrointestinal transit time for an oral dosage form may vary from 24 to 48 h depending on the physiology of the patient. In the course of this journey along the GI tract, the formulation has to gel exposed to a sharp pH change in the range 1–2 to 7–8 along the whole tract. Thus modeling of beads as oral formulation can be best visualized by exposing them to environment of continuous changing pH.

Satyanarayan et al. [32] carried out gamma scientographic studies in guar gum tablets using $^{99m}$Tc–DTPA as tracer in human volunteers and reported a mean gastric emptying time of $1.08 \pm 0.11$ h and the mean colonic arrival time of $2.83 \pm 0.33$ h. Hence, it means that small intestinal transit time is likely to be $1.75 \pm 0.25$ h, thus suggesting that the formulation should enter the colon between 1.75 and 3.75 h of administration. Relying on this data, we opted to expose the beads for a period of 2 h in the medium of pH 1.0, and 4.0 each and then for the next 6 h in the medium of pH 7.4, thus mimicking the transition of formulation from month of colon.

The swelling behavior of CA(4) and BA(4) beads in the media of varying pH has been depicted in the Fig. 10. It is very clear that the two beads exhibit different behavior. When the calcium alginate beads are immersed in medium of pH 1.0, they exhibit almost minimum swelling (i.e. nearly 25 ± 3%) as expected. Later on when they are transferred into medium of pH 4.0, the water uptake remains almost constant. This may be attributed to the fact that as the sodium citrate buffer has been used for obtaining pH 4.0, the ion-exchange between Na$^+$ ions and Ca$^{2+}$ ions results in loosening of the beads. As a result, beads tend to absorb more water. But at the same time, the lower molecular weight alginic acid (present due to acid catalyzed hydrolysis of alginate in pH 1.0) tends to dissolve. Thus the overall result of the two opposite process is that water uptake remains almost the same for the two hour in the medium of pH 4.0. Finally when the beads are transferred to phosphate buffer medium of pH 7.4, the alginate begins to dissolve, accompanied by fast removal of Ca$^{2+}$ ions from the beads due to enhanced ion-exchange process. Hence the beads get dissolved in duration of next 2 h.

However, the swelling behavior of barium ions crosslinked alginate beads is totally different. In

![Fig. 10. Water uptake as a function of time for the beads CA(4) (○) and BA(4) (●) in the medium of varying pH.](image-url)
the medium of pH 1.0, the beads show almost similar behavior. But, when the beads are transferred into medium of pH 4.0 they demonstrate $300 \pm 14\%$ of water uptake. This is somewhat unexpected result and it may be attributed to the fact that in the medium of pH 4.0 (which is not only the pK$_a$ value of mannnuronic acid but also above the pK$_a$ of guluronic acid), the –COOH group begin to ionize to give –COO$^-$ charged groups and there may start very slow ion-exchange between Ba$^{2+}$ ions present within polymannuronic acid blocks, thus resulting in decrease in degree of crosslinking and greater uptake of water. Moreover, the big sized Ba$^{2+}$ ions present in polyguluronate blocks maintain the integrity of the beads. In this way the water uptake reaches nearly $300 \pm 14\%$. Finally, when the beads are transferred into the phosphate buffer of medium 7.4, the further exchange of Ba$^{2+}$ ions with Na$^+$ ions further relaxes the polyguluronate chains due to increased electrostatics repulsion among –COO$^-$ groups and causes more uptake of water.

In this way the beads demonstrate maximum water uptake of nearly $720 \pm 18\%$. At this stage the alginate chains become unable to retain the highly hydrated structure of beads, thus leading to disintegration of beads. Moreover alginate may also have undergone acid hydrolysis into more soluble low molecular weight alginic acid (previously when the beads were in the medium of pH 1.0) it also begins to dissolve. In this way the beads continue to loose weight and finally dissolve. However, the beads reside for by nearly 10 h in the PBS of pH 7.4. Therefore, from the above discussion it is clear that alginate beads crosslinked with Ba$^{2+}$ ions reside in the medium of pH 7.4 for nearly 10 h which is the average transit time for an oral formulation in the colon. Moreover, the degrading beads may also leads to zero order drug release kinetics (which will be investigated in the next part of this communication) which is most desirable in the field of drug delivery.

4. Conclusions

In the above work, we have compared the swellibility and degradability of alginate beads crosslinked with Ca$^{2+}$ and Ba$^{2+}$ ions which differ appreciably in size. It is found that swelling of calcium alginate beads is governed by the ion-exchange process taking place between Na$^+$ ions (present in the phosphate buffer) and Ca$^{2+}$ ions in the beads. The release of Ca$^{2+}$ ions from the calcium alginate beads was found to be diffusion controlled On acid treatment the two beads exhibit different behavior. The barium crosslinked beads exhibit greater water uptake with increase in acid treatment time while calcium crosslinked beads show opposite tendency. Likewise, beads crosslinked with Ba$^{2+}$ ions, are found to be more stable in medium of varying pH. In this way, the nature of the crosslinking ion exerts great influence on the swelling/degradation. behaviour of the beads. In addition to this, other factors like composition, sequential structure and molecular weight of sodium alginate also affect the physical properties of the beads. It is expected that swelling/degradation of the beads may be affected by varying the composition of sodium alginate solution used in beads preparation. With increase in alginate concentration, the number of apparent crosslinking points should increase, thus resulting in delayed degradation. Moreover, the increased alginate density may also result in decreased mesh size within the gel beads, thus making the ion-exchange process slower. Finally, a minimum concentration of alginate should be required for formation of well shaped beads. Finally, it can be concluded that out of the two bead samples, namely calcium alginate and barium alginate, later exhibits more stability and bears potential for being used as oral drug delivery system for colon-targeting.

References

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