IN-SITU FORMATION OF POLY(ACRYLAMIDE) WITHIN CALCIUM ALGINATE BEADS FOR IMPROVED STABILITY

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In the present work, the stability of calcium alginate beads has been remarkably improved by a novel strategy consisting of *in-situ* formation of poly(acrylamide) within the calcium ions crosslinked sodium alginate beads. The resulting beads have been found to be stable for more than 48 h, in physiological fluid (PF) of pH 7.4, while the plain alginate beads disintegrated within a couple of hours. The water uptake behavior of the beads was investigated under various composition parameters, such as amount of alginate, concentration of ionic crosslinker Ca^{++} ions, monomer acrylamide (AAm) contents and degree of crosslinking. The beads also exhibited fair stability in media of varying pH. Finally, the release of the model drug riboflavin from the beads was also investigated.

Keywords: alginate, *in-situ* polymerization, water uptake, riboflavin release

INTRODUCTION

Alginate is one of the most abundant biodegradable and sustainable natural resources in the world and is widely used in the textiles, food, and chemical industries as a thickening and gelling agent. Alginate is a polysaccharide that is extracted from brown algae and consists of 1,4-linked β-D-mannuronic (M) and α-L-guluronic acid (G) residues arranged randomly along the chains.[1-2] It undergoes ionic gelation in the presence of di-valent ions, such as $Ca^{++}$and acquires a bead-like structure of crosslinked chains, usually explained on the basis of the 'egg-box' model.[3-4] These beads have served as a potential for the entrapment of a variety of bioactive ingredients. The major advantages of loading these bioactive materials into calcium alginate are that the beads are produced under normal experimental conditions, such as the use of aqueous medium, maintenance at room temperature, non-toxicity of crosslinking Ca^{++} ions, control over the size of beads, cost-effectiveness of the entrapment process etc.[5-6] These beads have been used in a variety of biomedical applications such as enzyme immobilization, oral drug delivery, cell-encapsulation, bone tissue engineering, as scaffolds etc.

Being a biopolymer with no toxicity, calcium alginate has been widely exploited for oral drug delivery applications. However, the use of Ca^{++} ions-crosslinked alginate beads as drug carrier has some limitations. These carriers do not exhibit fair stability in the physiological fluid or phosphate buffer of pH 7.4.[7-8] The presence of counter ions in the swelling medium induces ion-exchange between crosslinking Ca^{++} ions that are present within the so called 'egg-box' cavities, which finally results in the degradation of alginate beads within a few hours. In addition, in order to mimic transition of drug-loaded beads from the stomach to the large intestine, they are usually placed in simulating gastric fluid (SGF) of pH 1.0 for 2-3 h, followed by their transfer into simulating intestinal fluid (SIF) of pH 7.4. In this case, the beads are degraded at a faster rate when they are transferred in SIF.[9-10] This ultimately reduces the scope for usage in oral drug delivery, where the formulation is expected to release the entrapped drug for a longer time period to provide the best therapeutic level.

The present work is an attempt to enhance the stability of the calcium alginate beads, intended to be used for oral drug delivery. The vinyl monomer acrylamide (AAm) has been

polymerized *in-situ* within the bead matrix so as to provide fair stability to the resulting beads. These calcium alginate/poly(AAm) composite drops have been found to be stable not only in the SIF of pH 7.4, but also in media of varying pH to mimic transition from mouth to colon.

EXPERIMENTAL

Materials

Sodium alginate (SA, molecular weight 218.2 kD and viscosity of 1% aqueous solution was 236cPs at 27 °C) was purchased from Hi Media Chemicals, Mumbai, India. The vinyl monomer acrylamide (AAm), crosslinker N,N' methylene bisacrylamide (MB), initiator potassium persulphate (KPS), ionic crosslinker $CaCl_2$ and model drug riboflavin were received from Merck Chemical Industries, Mumbai, India, and were of analytical grade. Double distilled water was used throughout the investigations.

Methods

Preparation of calcium alginate/poly (AAm) composite beads

The method consists of *in-situ* polymerization of monomer AAm within the freshly prepared calcium alginate beads. In brief, a pre-calculated quantity of sodium alginate (SA) was dissolved in distilled water under gentle stirring to ensure complete dissolution. To this, definite amounts of monomer AAm, crosslinker MB and initiator KPS were added and the resulting solution was kept at 15 °C so as to prevent any polymerization reaction in the solution. This solution was added dropwise, using a syringe with an internal diameter of 0.5 mm, into $CaCl_2$ solution of a definite concentration, pre-maintained at 15 °C. The alginate solution was stirred gently to prevent agglomeration of the beads. The crosslinking time was 5 min. The crosslinked beads were filtered, washed superficially with distilled water and then placed in an electric oven (Temp star, India) at 60 °C for a period of 2 h. After the *in-situ* polymerization within the alginate beads was over, the beads were taken out and allowed to dry in a vacuum chamber till they attained constant weight. In all, eight bead samples were prepared. The compositions of various samples, plain as well as composite beads, are given in Table 1.

Preparation of drug-loaded beads

The model drug riboflavin loaded CA/poly(AAm) beads were prepared by the same method as described above. A pre-determined quantity of drug was added into the sodium alginate solution before the dropwise addition into $CaCl_2$ solution.

Characterization of beads

The FTIR spectra of the synthesized beads were recorded on an FTIR spectrophotometer (Shimadzu, 8400S) using KBr. For this, dry beads were ground and mixed with KBr. The scans recorded were the average of 100 scans and the spectral range selected was 400 to 4000 cm^{-1}.

The thermogravimetric analysis was performed using a thermogravimetric analyzer (Mettler, Teledo TGA/SDTA 851, Switzerland). A definite quantity of ground sample was placed in a ceramic crucible and analyzed over the temperature range of 30 to 800 °C, at the heating rate of 10 °C min^{-1} under constant flow of N_2 at the rate of 30 ml min^{-1}. The X-ray diffraction patterns were recorded with an X-PERT-PRO diffractometer equipped with a PW 3050/60 channel control goniometer and proportional counter. Radiation was generated from a copper anode tube (Cu Kα 1.54060 Ao) using an X-ray generator operated at 40 kV and 30mA. UV-Visible Spectroscopy was carried out using a JASCO-UV-VIS-NIR spectrophotometer with a bare glass slide as reference.

In order to investigate the surface morphology of calcium alginate and calcium alginate/ poly(acrylamide) composite beads, SEM images were recorded with a Hitachi S-4700 (New Jersey, USA) operating at an acceleration voltage of 15kV. All samples were dried in vacuum at room temperature and coated with gold before scanning. Surface morphologies were imaged at different magnifications.

Table 1
Composition of various beads prepared

Sample code	Alginate (%)	Acrylamide (g)	MB (g)	KPS (g)	$CaCl_2$ (%)	H_2O (mL)
A	4.0	2.0	0.2	0.2	5.0	25
B	4.0	2.0	0.1	0.2	5.0	25
C	6.0	2.0	0.2	0.2	5.0	25
D	4.0	1.0	0.2	0.2	5.0	25
E	4.0	2.0	0.2	0.2	4.0	25
F	6.0	-	-	-	5.0	25
G	4.0	-	-	-	5.0	25
H	4.0	-	-	-	4.0	25

Swelling measurements of beads

The water absorption behavior of CA/poly(AAm) beads was studied gravimetrically.[11] The pre-weighed beads were placed in phosphate buffer solution of pH 7.4 under sink conditions at 37 °C. The beads were taken out at regular time intervals, wiped superficially to remove loosely bound surface water, weighed accurately and then put again in the swelling medium. The mass measurement process was continued until the swollen beads could be weighed properly. The percent swelling (PS) was determined using the following expression:

$$PS = (M_t - M_0) \times 100/M_0 \quad (1)$$

where M_0 and M_t are the initial dry mass and mass at time 't' respectively. All the experiments were carried out in triplicate and average values are reported in the data.

Drug release study

The pre-weighed drug loaded CA/poly(AAm) beads were placed in 200 mL of release medium (*i.e.* physiological fluid PF of pH 7.4) at 37 °C. After regular time intervals, 2 mL of the aliquot was taken out and its absorbance was measured spectrophotometrically at 560 nm. The quantity of drug released was calculated using Lambert-Beer's plot obtained for drug solutions of known concentrations. The release experiments were continued till the two successive measurements yielded almost the same value of absorbance.

RESULTS AND DISCUSSION

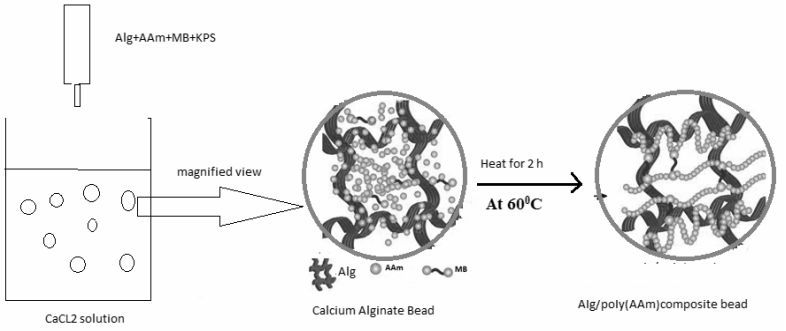
Preparation of CA/poly(AAm) beads

The *in-situ* polymerization of monomer AAm within the calcium alginate beads has proved to be a unique approach to render stability to the alginate beads so that they can withstand the physiological environment against disintegration during their journey from mouth to colon. The overall scheme for the formation of CA/poly(AAm) beads has been illustrated in Scheme 1.

When the sodium alginate solution containing the polymerization mixture is dropped into $CaCl_2$ solution, the Ca (II) ions instantaneously enter the droplet and crosslink alginate chains via 'egg-box' formation. Within these beads, molecules of monomer AAm, crosslinker MB and initiator KPS are present and evenly distributed. These beads are kept in an electric oven at 60 °C. The free radical-induced *in-situ* polymerization of AAm begins within the bead matrices and crosslinked poly(AAm) chains are produced. In this way, the calcium alginate beads contain uniformly distributed polymer network of crosslinked poly(AAm) chains, and these chains are expected to enhance overall stability of beads against dissolution/disintegration in PF. The optical images of plain CA and CA/poly(AAm) beads are shown in Figure 1.

Characterization of beads

The FTIR spectrum of the CA/poly(AAm) composite beads is shown in Figure 2. The spectrum contains all the characteristic peaks of calcium alginate and poly(AAm). In brief, the broad peak at 3206 cm^{-1} is due to symmetrical and asymmetrical stretching of N-H of poly(AAm). A sharp peak at 1680 cm^{-1} corresponds to C=O stretching vibrations of amide (carbonyl group stretching) and the peak corresponding to 1610 cm^{-1} indicates N-H bending vibrations of acrylamide. Finally, C-H stretching appears at 2917 cm^{-1}. The bands around 1028 cm^{-1} (C-O-C stretching) present in the IR spectrum of sodium alginate are attributed to its saccharide structure. The peaks around 3400 and 1600 cm^{-1} correspond to –OH stretching and carbonyl stretching, respectively. In addition, the bands at 1610 and 1419 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups.



Scheme 1: Formation of calcium alginate/poly(AAm) composite beads


(A)

(B)

Figure 1: Optical image of (A) calcium alginate and (B) calcium alginate/poly(AAm) beads

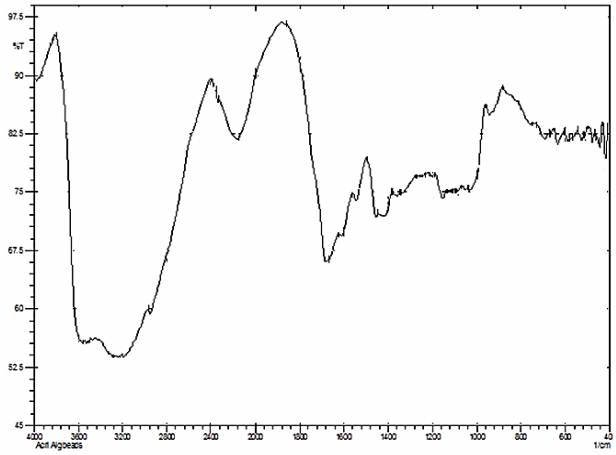


Figure 2: FTIR spectrum of CA/poly(AAm) composite beads

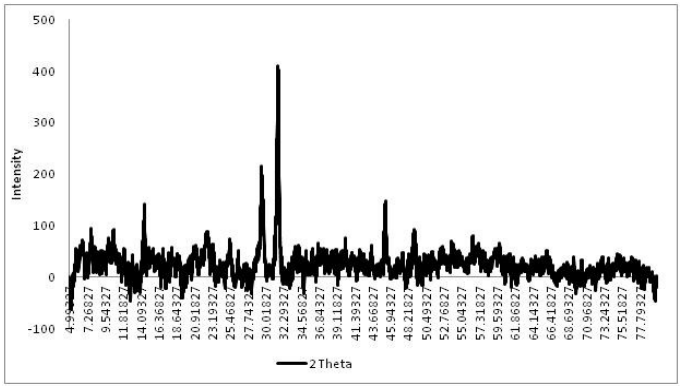


Figure 3: X-ray diffractogram of CA/poly(AAm) beads

The XRD pattern of CA/poly(AAm) is shown in Figure 3. Two typical peaks around 32 and 45° values of 2θ are related to lateral packing among alginate molecular chains and layer spacing along the molecular chain direction.[12] The amorphous poly(AAm) does not make any contribution towards crystallinity of the XRD pattern.

The thermal stability of pure CA and CA/poly(AAm) beads is shown in Figure 4 (A) and (B), respectively. The initial weight loss up to 200 °C in both the beads is due to moisture loss. Later on, there is continuous weight loss in both samples caused by de-polymerization. Up to 500 °C, the plain CA beads have lost almost 50% of their initial weight, whereas the CA/poly(AAm) beads suffer a weight loss of 33% only. This indicates an improved thermal stability of CA/poly(AAm) beads due to the presence of poly(AAm) segments. Finally, the total weight loss suffered by the plain CA beads up to 700 °C is of 70%, while the CA/poly(AAm) beads lose only 40% of the total weight. In this way, it may

be concluded that CA/poly(AAm) beads are more stable than plain CA beads.

In order to investigate the surface morphology of the plain CA and CA/poly(AAm) beads, their SEM images were recorded. The SEM images of plain and CA/poly(AAm) beads are shown in Figure 5 (A), (B), (C) and Figure 6 (A), (B) and (C), respectively. Figure 5 (A) shows the surface of the CA film obtained at 70X magnification. A dense network of crosslinked and mutually entangled calcium alginate chains can clearly be seen. A more magnified view (*i.e.* 500X magnification) is seen in Figure 5 (B). Here, it is also noticeable that there are voids or spacing within the entangled networks. Finally, Figure 5 (C) shows a 4000X magnified view, which reveals that calcium chloride particles might have been deposited on the alginate chains during the drying of the beads. The surface morphology of the CA/poly(AAm) beads is shown in Figure 6. At a magnification of 250 X, it indicates a drastic change in the surface morphology of the composite beads, as compared to the plain CA beads. Here, the surface looks smoother and the previously observed entangled network of alginate chains disappears completely, thus suggesting that poly(AAm) has been formed throughout the bead surface.

In a 1000 X magnified view (Fig. 6 (B)), there can be observed some cracks that are usually observed when a crosslinked matrix undergoes drying. In addition, some impurities, like $CaCl_2$, and unreacted salts are also visible. Finally, in a 4000X magnified view (Fig. 6 (C)), small pits are also visible throughout the surface, with some depositions as well. Therefore, it may be concluded that there is a drastic change in the surface morphology of the beads due to the formation of poly(AAm) network within the calcium alginate beads.

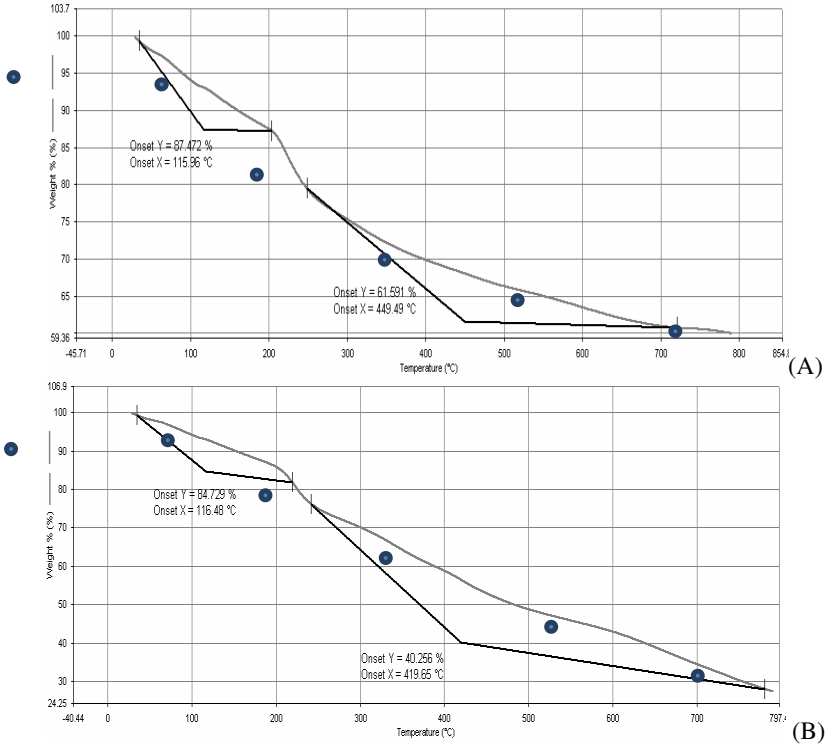


Figure 4: Thermograms of plain CA beads (A) and of CA/poly(AAm) beads (B)

Swelling behavior of plain CA beads at pH 7.4

It has been a well-established fact that sodium alginate consists of polymannuronate (M) and polyguluronate (G) residues, both of which contain carboxylate ions. When alginate solution is dropped into calcium chloride solution, the Ca^{++} ions enter the drop and form a buckled structure with G blocks via interacting with the $-COO^-$ ions of G blocks, a so-called 'egg-box' structure, which is mainly responsible for the structural integrity of the CA beads.[13] The carboxylate ions present in the M blocks also

interact with the $–COO^-$ ions via electrostatic attraction forces. The percent swelling (PS) of plain bead samples F, G and H is shown in Figure 7.

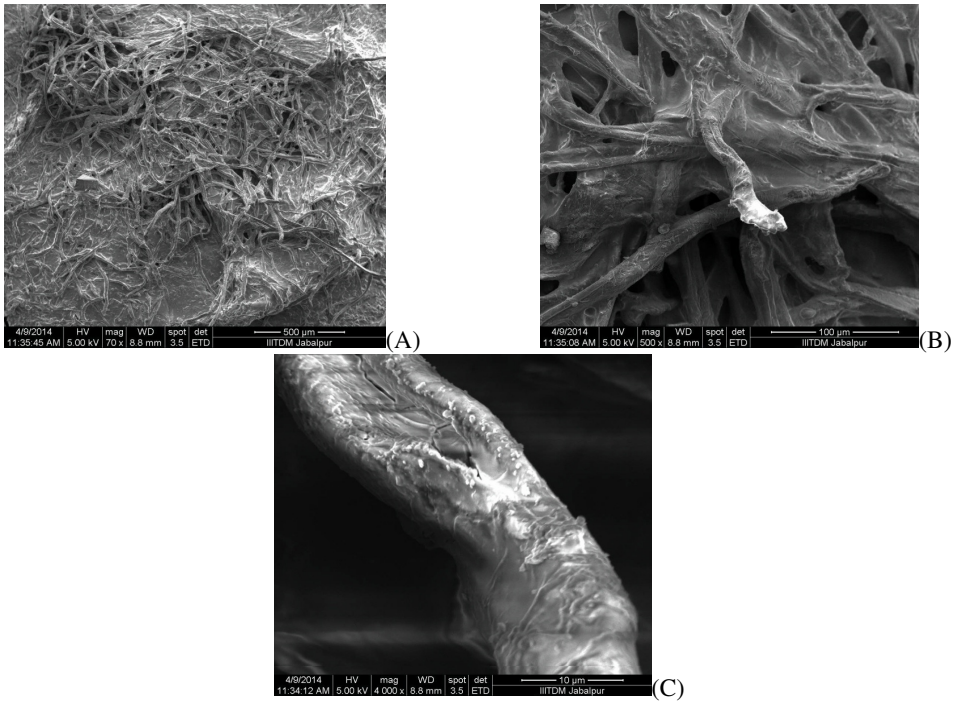


Figure 5: SEM images of CA beads at magnifications of (A) 70X, (B) 500X and (C) 4000X

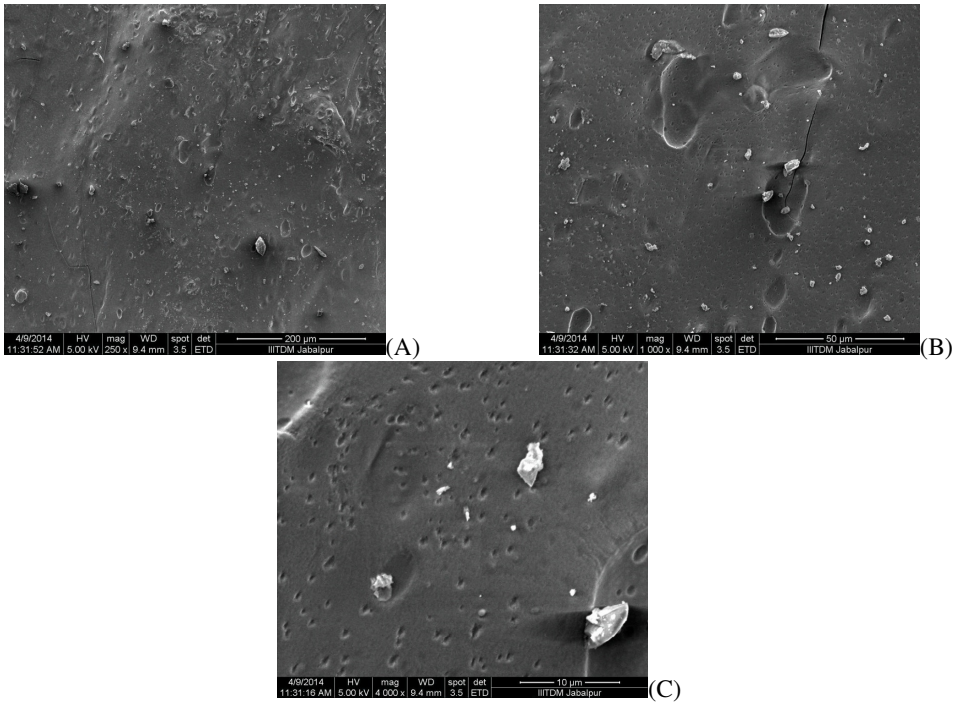


Figure 6: SEM images of CA/poly(AAm) composite beads with (A) 250X, (B) 1000X and (C) 4000X magnifications

It is clear that samples H and G have the same alginate content, but they are crosslinked with 4 and 5% $CaCl_2$ solutions, respectively. This is also reflected in their maximal swelling values, which

are 1111 and 616%, respectively. Obviously, the sample crosslinked with 4% $CaCl_2$ solution demonstrates higher swelling, as compared to the other sample. However, it is interesting to see that both of the fully hydrated samples begin to dissolve/disintegrate after 1 h, and finally dissolve completely after 3 h. The bead sample F, which is prepared with 6% sodium alginate solution and crosslinked with 4% $CaCl_2$, shows different behavior. It exhibits maximum % swelling of 679 (which is comparable with that of sample G), but takes almost 6 h to dissolve completely. Therefore, it can be concluded that the higher is the SA content, the greater shall be the stability of the beads.

The disintegration/dissolution of all the samples, namely F, G and H, may be attributed to the fact that when CA beads are put in PF, Na^+ ions present in the outer solution enter the bead matrix and undergo ion-exchange with Ca^{++} ions, which are attached to _COO- groups of M blocks. This results in relaxation of M chains along with increased swelling. Later on, the external Ca^{++} ions enter the 'egg box' cavities and replace the already existing Ca^{++} ions.[14] This also results in enhanced percent swelling. Now this fully hydrated structure begins to lose its structural integrity due to disruption of 'egg-box' cavities and the alginate chains begin to disintegrate and dissolve.

Swelling behavior of CA/poly(AAm) beads at pH 7.4

The *in-situ* formation of the poly(AAm) network within the CA hydrogel beads is expected to enhance the overall stability of the beads. In order to investigate this, the samples A and B, having same compositions, but 0.2 and 0.1 g of crosslinker MB in the feed mixture, were allowed to swell in PF at 37 °C. The results of their dynamic swelling are shown in Figure 8.

It can be seen that samples A and B obtain maximum % swelling of 157 and 213, respectively. Later on, they begin to lose their weight, probably due to Ca^{++}----Na^+ ion exchange induced dissolution of alginate chains, as has been mentioned earlier. However, the beads continue to undergo a slight weight loss and then attain a constant PS of 95 and 170 at the end of 48 h, respectively. This indicates that the CA/poly(AAm) beads exhibit enhanced stability due to the presence of poly(AAm) chains within the CA bead matrix. The dynamic swelling of beads with different CA contents is shown in Figure 9.

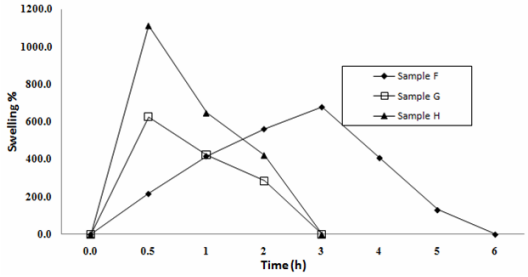


Figure 7: Percent swelling (PS) of plain CA bead samples F, G and H

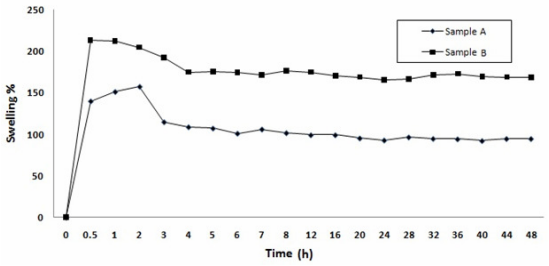


Figure 8: Percent swelling of CA/poly(AAm) bead samples A and B in physiological fluid of pH 7.4 at 37 °C

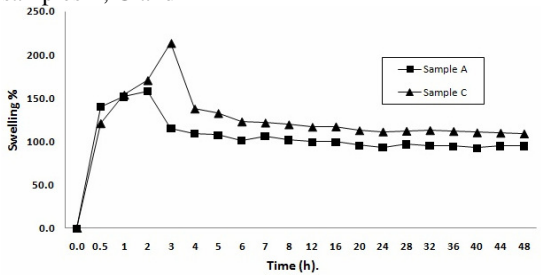


Figure 9: Percent swelling of CA/poly(AAm) bead samples A and C in physiological fluid of pH 7.4 at 37 °C

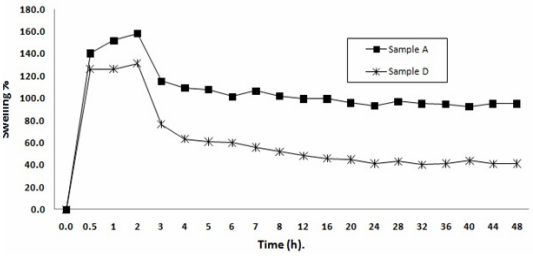


Figure 10: Percent swelling of CA/poly(AAm) bead samples A and D in physiological fluid of pH 7.4 at 37 °C

It is clear that samples A and C, having 4 and 6% of alginate in the feed mixture show almost similar % swelling, but sample A, having 4% alginate content, shows faster weight loss as compared to sample C, which has been prepared with 6% concentration of sodium alginate. This indicates that beads with higher alginate content offer more resistance against dissolution in PF. It is also noticeable that both samples attain almost constant weight after 32 h. This may probably be due to the fact that after 32 h, most of the alginate chains must have dissolved and the stability is now totally governed by the poly(AAm) chains, which are insoluble due to the crosslinked structure.

Finally, the effect of variation in monomer AAm content was studied. The samples A and D, containing 1 and 2 g of monomer AAm in the feed mixture, were investigated for their dynamic water uptake (see Fig. 10).

The results indicate that beads with higher AAm content exhibit less swelling. This may be attributed to the fact that a higher content of monomer AAm results in more entangled macromolecular networks, thus lowering the water invasion into the bead matrix. They attain a maximal swelling of 131 and 153%, respectively. Later on, they begin to lose weight and finally attain % swelling of 95 and 41, respectively. The beads show enhanced stability for more than 48 h.

Swelling in media of varying pH (1.0 and 7.4)

It is well known that when a formulation is taken orally, it passes through the stomach to the large intestine and undergoes exposure to media of varying pH. In the stomach, the pH of the gastric fluid is around 1.0, while in the colon the pH becomes around 7.4. To mimic this transition from stomach to colon, we put the CA/poly(AAm) beads (sample A) in SGF for a period of 2 h, and then transferred them to SIF of pH 7.4 at 37 °C. The plain CA beads (sample G) were used as control to compare the stability of CA/poly(AAm) beads. The results are shown in Figure 11.

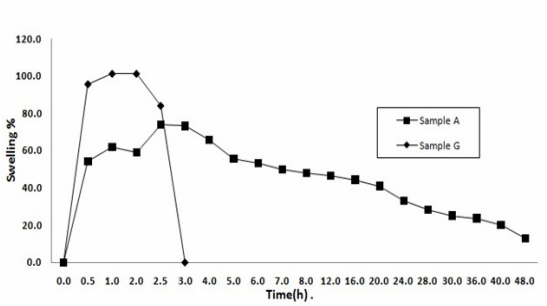


Figure 11: Stability of plain CA sample G and CA/poly(AAm) sample A beads in media of varying pH (*i.e.* pH 1.0 for 2 h, then transferred to pH 7.4 for the rest of the period) at 37 °C

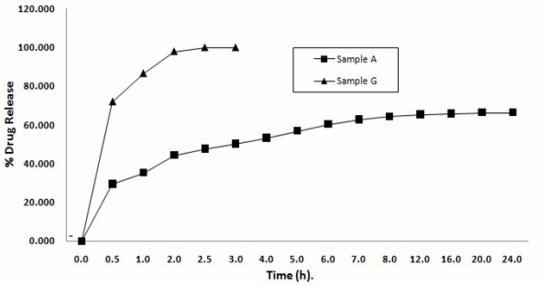


Figure 12: Release of model drug vitamin B_2 from the bead sample A (*i.e.* CA/poly(AAm) sample) and sample G (plain CA beads) in PF at 37 °C

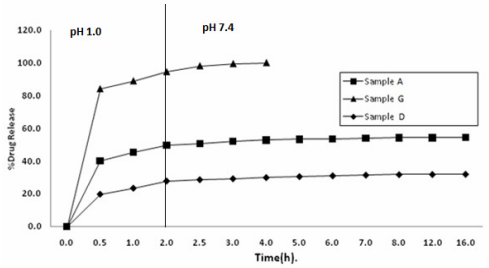


Figure 13: Drug release from plain CA bead sample G and samples A (CA/poly(AAm) and D (CA/poly(AAm)) in media of varying pH (*i.e.* pH 1.0 for 2 h, followed by transfer to pH 7.4 for the rest of the period) at 37 °C

It can be seen that the plain CA beads remain quite stable in the SGF and acquire a percent swelling of 110. However, when they are transferred into SIF, they begin to disintegrate rapidly and within the next 30 min the beads get almost dissolved in the medium. On the other hand, the CA/poly(AAm) beads attain a maximal swelling of 72% in two and a half hours and then

begin to lose their weight slowly. Even after 48 h, they retain the percent water uptake of sample G. This indicates that the CA/poly(AAm) beads are fairly stable in the media of varying pH and therefore they are expected to release the entrapped biologically active ingredient for a longer duration in the colon.

Release of Vitamin B_2 at pH 7.4

From the discussions in the previous sections, it is clear that plain CA beads do not exhibit fair stability in PF and they are supposed to release the entrapped drug in the short time period of 3 h. On the other hand, the CA/poly(AAm) beads undergo a slow disintegration process during an extended period of around 48 h. Therefore, it may be interesting to see their drug release behavior under physiological conditions. The release of model drug vitamin B_2 was investigated from the bead sample B (*i.e.* CA/poly(AAm) sample) and sample G (plain CA beads) in PF at 37 °C. The results of dynamic release of B are shown in Figure 12.

It can be seen that sample G exhibits a total release of 100% during around 3 h and the beads are fully disintegrated. On the other hand, sample B shows a gradual release of the entrapped drug, extended over a period of almost 24 h. The total release from sample A was of around 66%. The rapid release from sample A can be simply the consequence of the fast degradation of plain CA beads, as has been discussed previously. Therefore, it appears that CA/poly(AAm) beads have potential to provide an extended release of the entrapped drug for a longer period.

Release in media of varying pH

Finally, the plain CA bead sample G and samples A (CA/poly(AAm)) and D (CA/poly(AAm)) were placed in media of varying pH (*i.e.* at pH 1.0 for 2 h followed by transfer to pH 7.4 for the rest of the period) at 37 °C. The results, as shown in Figure 13, indicate that these samples exhibit different behavior.

The plain sample G releases around 95 percent of the entrapped drug in the first 2 h in SGF and then the rest of the drug is released in the next hour in SIF. Also, it loses its structural integrity completely and finally dissolves. On the other hand, samples A and D show an extended drug release, which lasts for a period of almost 16 h without losing their integrity. In addition, sample A shows a relatively faster release, as compared to sample D. This may be attributable to the fact that sample A has a greater amount of monomer AAm in the feed mixture (see Table 1) and therefore it exhibits higher swelling compared to sample D. In this way, the release rate can be best controlled by variation in the composition of CA/poly(AAm) beads.

CONCLUSION

It can be concluded from the present study that *in-situ* formation of poly(AAm) within calcium alginate beads results in a drastic increase in the stability of the resulting beads and these beads may be successfully employed for gastrointestinal drug delivery.

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