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pH sensitive alginate–guar gum hydrogel for the controlled delivery of protein drugs

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Abstract

Design of a pH sensitive alginate–guar gum hydrogel crosslinked with glutaraldehyde was done for the controlled delivery of protein drugs. Alginate is a non-toxic polysaccharide with favorable pH sensitive properties for intestinal delivery of protein drugs. Drug leaching during hydrogel preparation and rapid dissolution of alginate at higher pH are major limitations, as it results in very low entrapment efficiency and burst release of entrapped protein drug, once it enters the intestine. To overcome these limitations, another natural polysaccharide, guargum was included in the alginate matrix along with a cross linking agent to ensure maximum encapsulation efficiency and controlled drug release. The crosslinked alginate–guar gum matrix is novel and the drug loading process used in the study was mild and performed in aqueous environment. The release profiles of a model protein drug (BSA) from test hydrogels were studied under simulated gastric and intestinal media. The beads having an alginate to guar gum percentage combination of 3:1 showed desirable characters like better encapsulation efficiency and bead forming properties in the preliminary studies. The glutaraldehyde concentration giving maximum (100%) encapsulation efficiency and the most appropriate swelling characteristics was found to be 0.5% (w/v). Freeze-dried samples showed swelling ratios most suitable for drug release in simulated intestinal media (∼8.5). Protein release from test hydrogels was minimal at pH 1.2 (∼20%), and it was found to be significantly higher (∼90%) at pH 7.4. Presence of guar gum and glutaraldehyde crosslinking increases entrapment efficiency and prevents the rapid dissolution of alginate in higher pH of the intestine, ensuring a controlled release of the entrapped drug.

Keywords: pH sensitive; Alginate; Guargum; Hydrogel; Protein drug; Oral delivery

1. Introduction

The most challenging task in the development of protein pharmaceuticals is to deal with physical and chemical instabilities of proteins. Protein instability is one of the major reasons by which protein pharmaceuticals are administered traditionally through injection rather than taken orally like most small chemical drugs (Wang, 1999). Peptide and protein drugs are readily degraded by the low pH of the gastric medium in the stomach. Problems such as acid catalyzed degradation in the stomach, proteolytic breakdown in the GI tract, poor permeability across the gastrointestinal mucosa and first-pass metabolism during transfer across the absorption barrier and in the liver must be overcome for the efficient delivery of drugs into the blood stream (Xing et al., 2003). In order to achieve the successful oral delivery of protein drugs, they need to be protected from the harsh environment in the stomach. For designing oral dosage forms, the formulator must consider that the natural pH environment of GI tract varies from acidic (pH ∼1.2) in the stomach to slightly alkaline in the intestine (pH ∼7.4) (Shargel and Yu, 1999).

In the design of oral delivery of peptide or protein drugs, pH sensitive hydrogels have attracted increasing attention. Swelling of such hydrogels in the stomach is minimal and thus the drug release is also minimal. Due to increase in pH, the extent of swelling increases as the hydrogels pass down the intestinal tract. A variety of synthetic or natural polymers with acidic or basic pendant groups have been employed to fabricate pH sensitive hydrogels (Kimura, 1993), for getting the desired controlled release of protein drugs. Most of the synthetic polymers are immunogenic and the incorporation of proteins in to these polymers require harsh environment which may denature and inactivate the desired protein. The use of natural polymers is getting the favor of scientists, will circumvent some of these problems.

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Alginate is a water soluble linear polysaccharide extracted from brown sea weed and is composed of alternating blocks of 1–4 linked α-L-guluronic and β-D-mannuronic acid residues. Alginate can be ionically crosslinked by the addition of divalent cations in aqueous solution. It was reported that alginate is non-toxic and biodegradable when given orally (Mumper et al., 1994). In theory, alginate shrinks at low pH (gastric environment) and the encapsulated drugs are not released (Chen et al., 2004). It was reported that the biological activity of drugs can be retained in the calcium-crosslinked alginate encapsulation process (Gray and Dowsett, 1988). The entrapment of several proteins from alginate has been reported including IgG (Gray and Dowsett, 1988; Chevalier et al., 1987), fibrinogen (Chevalier et al., 1987) and insulin (Kim and Lee, 1992), melatonin (Lee and Min, 1996), heparin (Edelman et al., 2000), hemoglobin (Ribeiro et al., 2005), vaccines (Kim et al., 2002; Romalde et al., 2004), etc.

Guar gum is a non-ionic polysaccharide consisting of a (1-4)-linked β-D-mannopyranose backbone with branch points from their 6-positions linked to α-D-galactose (i.e. 1-6-linked-α-D-galactopyranose). That is, it consists of linear chains of (1 → 4)-β-D-mannopyranosyl units with α-D-galactopyranosyl units attached by (1 → 6) linkages. It is derived from the seeds of *Cyamopsis tetragonolobus*, of the Leguminosae family. There are between 1.5 and 2 mannose residues for every galactose residue. Guargum is used as a thickener in cosmetics, sauces, salad dressings and as an agent in ice cream that prevents formation of ice crystals. In pharmaceutical formulations, guargum is used as a binder and disintegrant in solid dosage forms and as a suspending, thickening and stabilizing agent in liquid formulations. There are many reports of the use of guargum for oral delivery of drugs. Guargum has been used in colon-specific drug delivery as matrix forming material and as a compression coat (Wong et al., 1997; RamaPrasad et al., 1998; Krishnaiah et al., 1999). Guar gum is a potential hydrophilic matrix carrier for oral controlled delivery of drugs with varying solubility (Krishnaiah et al., 2002). Guar gum on exposure to dissolution fluids gets hydrated and forms a viscous gel layer that slows down further seeping-in of dissolution fluids towards the core of the matrix tablet (RamaPrasad et al., 1998; Krishnaiah et al., 1998a,b, 1999). The present paper describes the design of a controlled delivery matrix for the intestinal delivery of protein drugs.

2. Materials and methods

2.1. Materials

Sodium alginate (viscosity: 395 cps for a 5% solution) and guar gum were obtained from Central Drug House, India. Bovine serum albumin (BSA) and glutaraldehyde was purchased from Sigma (USA). All other reagents used were of analytical grade.

2.2. Preparation of alginate–guagum hydrogels and drug loading

The hydrogel beads used for the study were prepared by extrusion of the mixture through a syringe having a diameter of 0.1 mm. Alginate–guagum hydrogels with distinct alginate to guar gum percentage weight ratios were prepared (4:0, 3.75:0.25, 3.5:0.5, 3.25:0.75, 3:1, 2.75:1.25). The guar gum solution of the required concentration is prepared first and then the required amount of alginate is added and stirred well to form a uniform mixture. To this mixture, glutaraldehyde was added to a final concentration of 0.25, 0.3, 0.4 and 0.5% (w/v), followed by BSA to a final concentration of 0.2% (w/v), and blended well. The final solution is kept undisturbed for sometime to remove the trapped air bubbles. Beads are made in 0.5 M $CaCl_2$ solution, cured for 1 h in the same solution and removed by filtration and washed with deionized water to remove excess glutaraldehyde and $CaCl_2$. The beads were then either lyophilized or air-dried at room temperature and then stored in the refrigerator. To find the entrapment efficiency, a known weight of the beads (0.1 g) was dissolved in 10 mL of 0.1 N NaOH, centrifuged and the protein content of supernatant was found out at 280 nm.

$$\text{entrapment efficiency} = \frac{\text{actual protein concentration}}{\text{theoretical protein concentration}}$$

2.3. Scanning electron microscopy

The surface morphology of freeze- and air-dried hydrogels was determined using a scanning electron microscope (JEOL JSM-5600 LV, Japan). Hydrogel samples were sputtered with gold and scanned at an accelerating voltage of 15 kV.

2.4. Swelling characteristics of alginate–guagum hydrogels

The swelling characteristics of the test alginate–guagum hydrogels were determined by a method described previously (Chen et al., 2004). The dried test samples were immersed in 5 mL of a solution of pH 1.2 (HCl–KCl buffer) or 7.4 (phosphate buffer) for 6 h. At specific time intervals, the samples were taken out and were blotted with a paper towel to absorb excess water on the surface. The swelling ratios (Q_s) of test samples were calculated from the equation:

$$Q_s = \frac{W_s - W_d}{W_d}$$

where W_s is the weight of the swollen sample and W_d is the weight of the dried test sample. The sample which had the best swelling characteristics suitable for the protein drug delivery among the studied groups was selected for further BSA release profile studies.

2.5. Protein release studies

To study the protein release from the test hydrogels, the dried, drug loaded beads were immersed in solutions with pH value 1.2 or 7.4 (Chen et al., 2004). 20 mL of the solutions were taken in a conical flask and were placed in a rotary water bath shaker at 100 rpm. At predetermined time points, 100 μL of this solution was taken out and analysed by a UV spectrophotometer (UV2100, Shimadzu, Japan) at 280 nm for released BSA, while the dissolution medium is replaced with the same amount of

fresh buffer. The percentage of cumulative amount of released BSA was calculated and plotted against time.

3. Results

3.1. Preparation of hydrogels

From the different combinations of alginate and guar gum tested, the 3:1 combination was found to be the most suitable combination as it could be made into beads with ease and also it was found to have comparatively better entrapment efficiency in the preliminary studies (Table 1). The swelling ratio studies (Fig. 1) also supported the 3:1 combination, as it showed the most suitable swelling ratios in gastric and intestinal media. All other groups of beads exhibited rapid swelling in the intesti-

Table 1
Percentage entrapment efficiency of beads with different alginate–guargum ratios and glutaraldehyde concentrations

Alginate (%)	Guargum (%)	Glutaraldehyde concentration (%)	% Entrapment efficiency
4	0	–	39.0
3.75	0.25	–	42.0
3.5	0.5	–	49.3
3.25	0.75	–	58.5
3.0	1.0	0	64.8
3.0	1.0	0.25	92.2
3.0	1.0	0.3	94.2
3.0	1.0	0.4	98.5
3.0	1.0	0.5	100.0
2.75	1.25	–	65.9

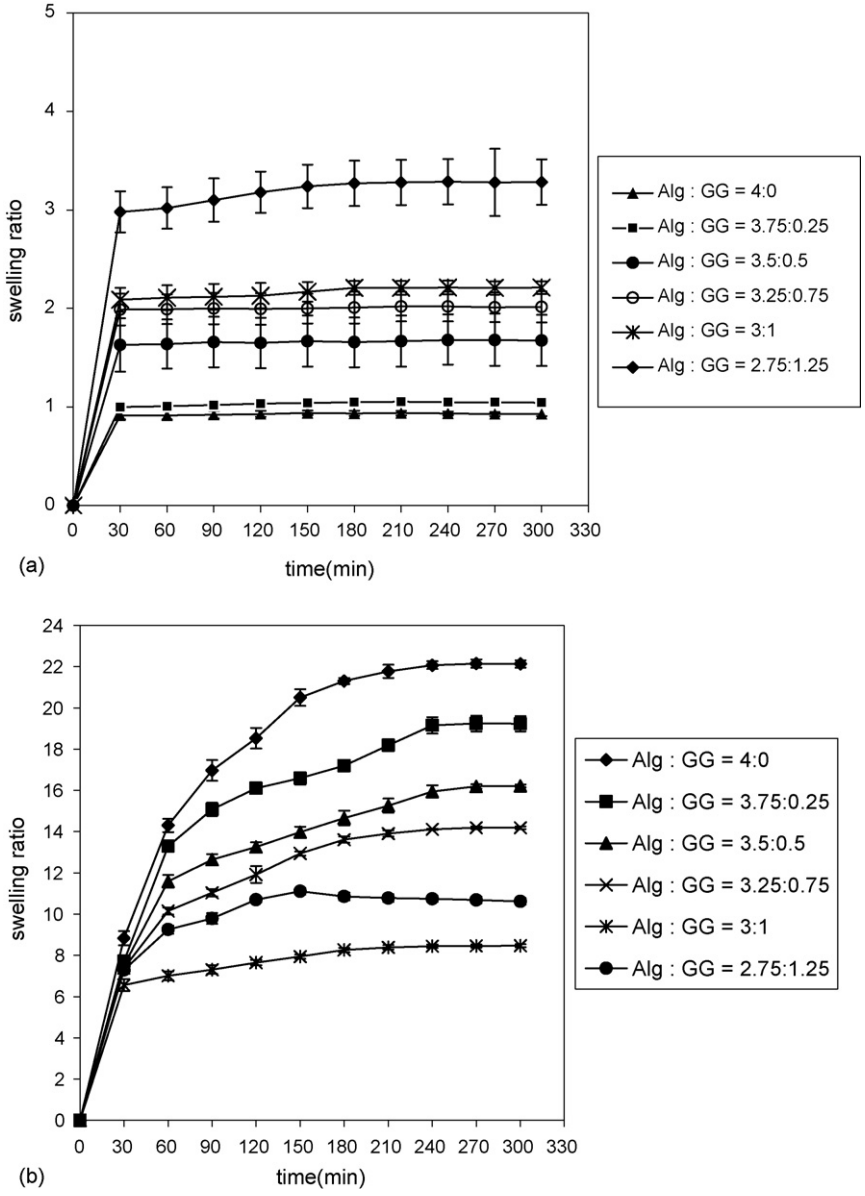

Fig. 1. Swelling characteristics of the alginate/guar gum hydrogels with different weight ratios (a) at pH 1.2 and (b) at pH 7.4 [data presented as mean ± S.E. (standard error)].

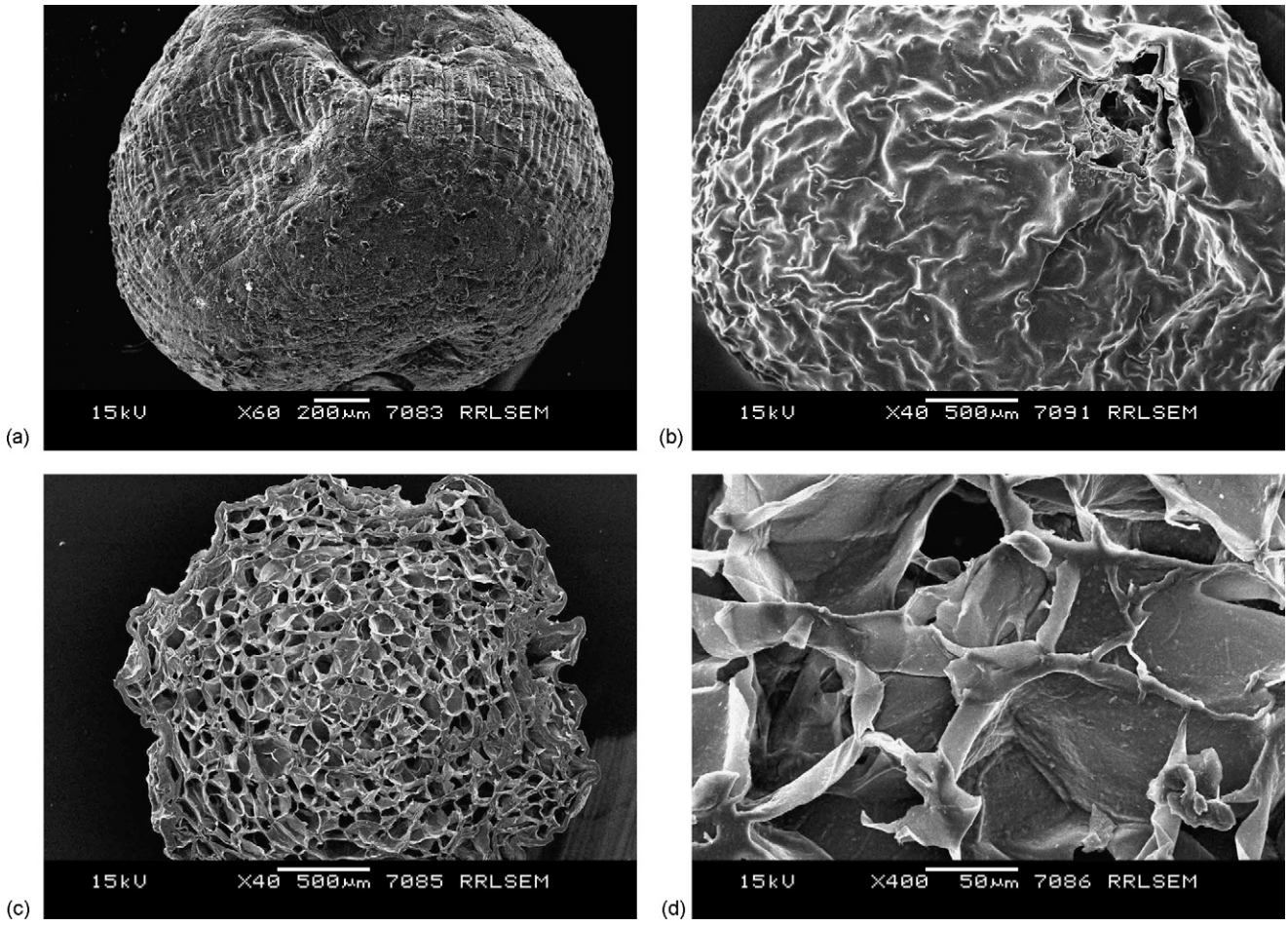

Fig. 2. Scanning electron micrographs of: (a) Surface morphology of air-dried beads, (b) surface morphology freeze-dried beads, (c) and (d) cross section of freeze-dried beads.

nal pH which is not desirable, as it may cause rapid release of the entrapped protein in the intestine. From the different glutaraldehyde concentrations studied, a concentration of 0.5% (v/v), gave 100% entrapment efficiency (Table 1). Therefore, we selected the beads with a glutaraldehyde concentration upto 0.5% for the swelling ratio studies, to find which combination shows the most desirable swelling ratios in the different pH studied.

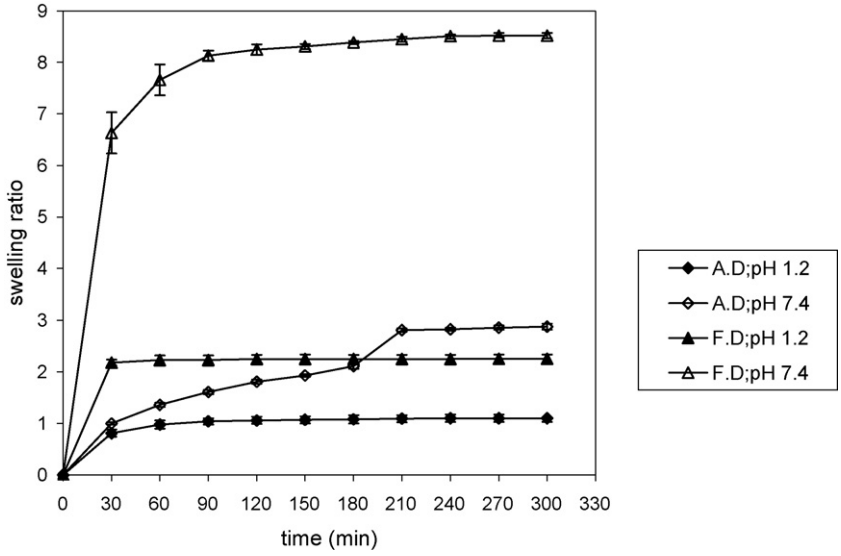

Fig. 3. Swelling ratios of air-dried and freeze-dried hydrogels in pH 1.2 and 7.4 (data presented as mean ± S.E.).

3.2. SEM

The SEM pictures of freeze-dried beads revealed a highly porous nature (Fig. 2); whereas, the air-dried beads were found to be non porous. The cross sectional view of freeze-dried beads exhibited numerous open channel like structures and network like structure was also seen in the cross section.

3.3. Swelling characteristics

The swelling patterns of air-dried and freeze-dried test hydrogels are shown in Fig. 3. Freeze-dried beads showed swelling behaviour which is appropriate for intestinal delivery (∼2 at pH 1.2 and ∼8.5 at pH 7.4); whereas the swelling ratios of air-dried beads were very low even at the higher pH of 7.4 (1.1 at pH 1.2 and 2.8 at pH 7.4). From the swelling ratio studies of different alginate–guagum combinations (Fig. 1), the 3:1 combination was found to be better with a swelling ratio of ∼2.2 at pH 1.2 and ∼8.5 at pH 7.4 and it was selected as the test hydrogel for further studies. Among the different glutaraldehyde concentrations studied (0.25, 0.3, 0.4, and 0.5%), the one giving the most suitable swelling characteristics in gastric and intestinal media was found to be 0.5% (Fig. 4). It showed very low swelling at pH 1.2 and swelling at pH 7.4; while others showed high swelling at both pH 1.2 and 7.4. The rapid swelling exhibited by other groups of beads will tend to limit their efficiency for controlled drug release at intestinal pH. This is because of the increased chance of entrapped protein drugs for a rapid release in the intestinal pH and subsequent denaturation by proteolytic enzymes. Thus the freeze-dried beads with an alginate to guar gum ratio of 3:1 and crosslinked with 0.5% glutaraldehyde was selected for protein release studies.

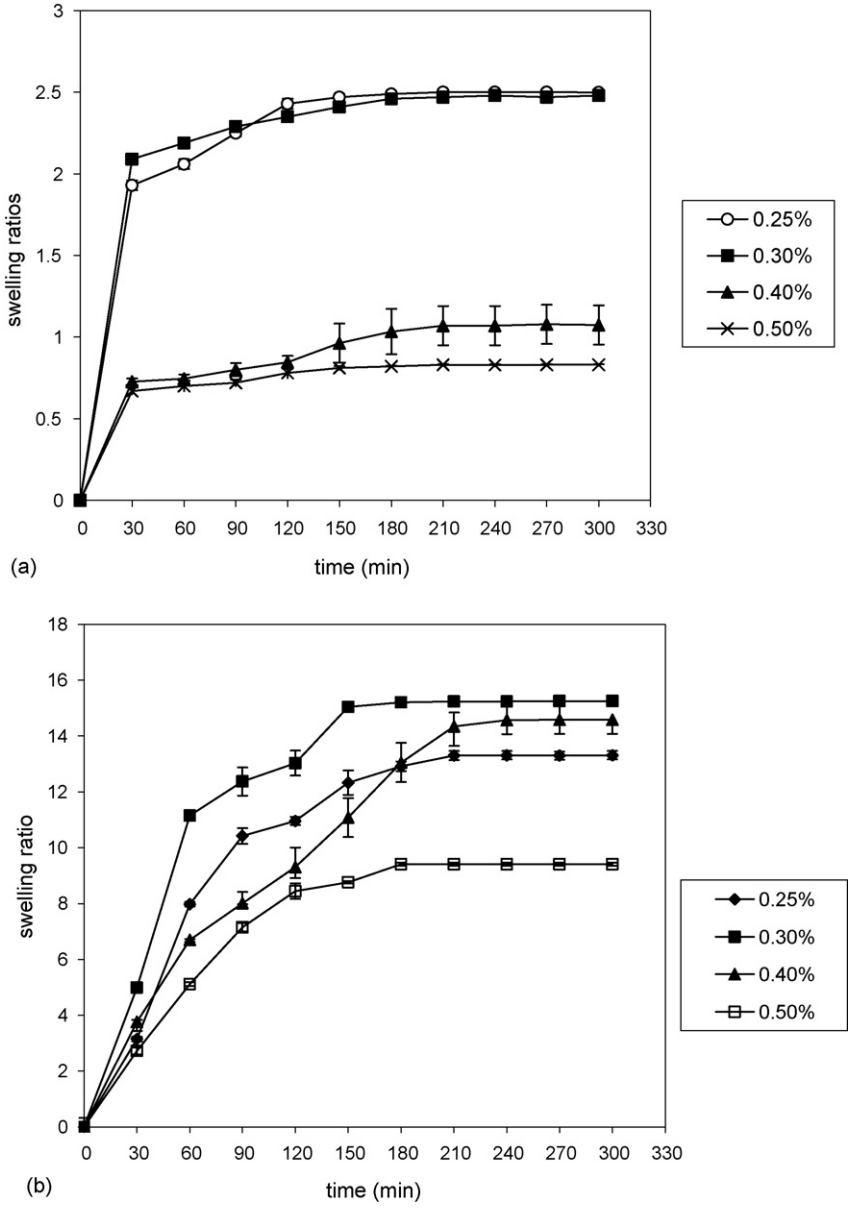

Fig. 4. Swelling characteristics of hydrogels crosslinked with different glutaraldehyde concentrations at pH 1.2 and 7.4 (data presented as mean ± S.E.).

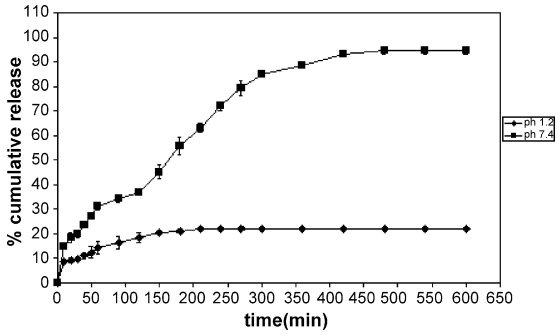

Fig. 5. BSA release profile of test hydrogel (alginate–guargum ratio = 3:1; glutaraldehyde conc. = 0.5%) in pH 1.2 and pH 7.4 (data presented as mean ± S.E.).

3.4. Protein release studies

Figs. 5 and 6 show the BSA release profiles of the test hydrogels at pH 1.2 and 7.4. As shown, the amount of BSA released at pH 1.2 was very low in both one-step and two-step release studies. Only about 20% BSA was released within 10 h. At pH 7.4, the amount of BSA released increased significantly (∼94% within 10 h). In two-step release studies, the protein release at pH 1.2 was similar to that obtained in one-step swelling. But the protein release at pH 7.4 was found to be about 89%.

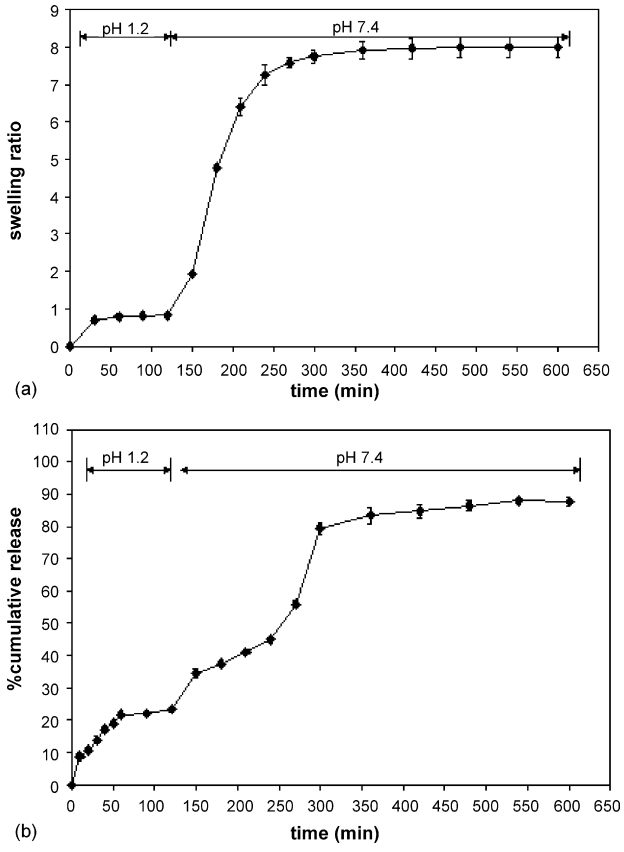

Fig. 6. (a) Swelling characteristics and (b) BSA release profile of test hydrogel (alginate–guargum ratio = 3:1; glutaraldehyde conc. = 0.5%) in pH 1.2 and subsequently in pH 7.4. (data presented as mean ± S.E.).

4. Discussion

The hydrogels with an alginate to guar gum ratio of 3:1 and crosslinked with 0.5% glutaraldehyde was found to posses the most suitable encapsulation efficiency (Table 1) and swelling characteristics. The air-dried hydrogels were found to have lower swelling ratios even in higher pH ranges, whereas the freeze-dried hydrogels were found to posses swelling characteristics, which would help the controlled release of the protein drug in higher pH ranges. The alginate–guar gum hydrogel crosslinked with glutaraldehyde was found to have a better stability and controlled release properties at pH 7.4. In the present study, the freeze drying process generated hydrogels with good porosity. The air-dried hydrogels were found to be non porous and has a higher density (1.2 ± 0.07 g/mm^3). The porous nature of freeze-dried hydogel make it less dense (density = 0.5 ± 0.0 g/mm^3) and provides more surface area. The extensive swelling capacity of the freeze-dried hydrogel compared to that of the air-dried one can be attributed to this highly porous nature (Fig. 2) where the capillary forces help the diffusion of solvent into the hydrogel. In the case of air-dried beads the porosity was very low and therefore the solvent could not penetrate effectively into the matrix (Risbud et al., 2000).

The protein release from the freeze-dried hydrogel at pH 1.2 was found to be lesser than that in pH 7.4 and this may be due to the pH sensitive behaviour of alginate. In low pH medium (pH 1.2), the beads shrank and the release of BSA was only ∼20%; whereas at pH 7.4 the hydrogel – swelled and the controlled release of protein is effected. Since the glutaraldehyde reinforced the Ca ion crosslinked alginate–guar gum network, the dissolution of alginate did not occur. Also the guar gum in the matrix absorbs water and swells, preventing the further seeping in of dissolution medium and thus helps to control the release of protein.

Alginate is a matrix which has the pH sensitive properties suitable for the intestinal delivery of drugs. But it has a drawback that it gets rapidly dissolved in the higher pH prevailing in the intestine. Guar gum has been proposed as a matrix for the delivery of peptide drugs to the colon. This is because, as the guar gum gets hydrated, it swells and forms a viscous layer that prevents seeping in of dissolution fluids and thus helping to control drug release. In the present work, we have tried a combination which incorporates the pH sensitive property of alginate and drug release retarding property of guar gum. It has been reported that Na–Alg can be crosslinked with glutaraldehyde (GA), the chemical reaction between hydroxyl groups of Na–Alg and GA was confirmed by fourier transform infrared (FTIR) measurements (Yeom and Lee, 1998). The crosslinking effect and the stability provided by the glutaraldehyde to the alginate–guargum matrix also helps in controlling the BSA release for the time period studied (∼10 h).

From the swelling ratios of hydrogels with different alginate to guar gum weight ratios, it was found that the 3:1 ratio beads had a swelling ratio of ∼2 at pH 1.2 even after 5 h of swelling; while it was approximately 8.5 at pH 7.4. Even though beads with some other combinations were found to have lesser swelling at pH 1.2, all of them had very rapid swelling at pH 7.4

(ranging from 10.6 to 22.13). It would not be a desirable feature because, with the rapid swelling, the entrapped protein will also be released rapidly from the hydrogel.

Glutaraldehyde crosslinking of the hydrogel network had a noticeable impact on their entrapment efficiency. 0.5% glutaraldehyde gave the maximum entrapment efficiency (100%). The swelling properties this hydrogel was also found to be suitable for the intestinal delivery applications. Beads with 0.5% glutaraldehyde concentration showed a controlled swelling and release of protein in intestinal pH. This is because the crosslinking by glutaraldehyde made the network more stable and the swelling and drug release is in a controlled manner unlike the other beads. The glutaraldehyde crosslinking prevents the easy dissolution of Ca alginate network in the higher pH ranges of the intestine, thus helping in the controlled release of the drug by the controlled swelling of the hydrogel. Since the concentration of glutaraldehyde used was as minimum as 0.5%, and the excess was washed off after crosslinking, there will be very less amount remaining in the beads.

The BSA release from the beads at pH 1.2 and 7.4, done separately gave a maximum release of 21.77% and 94.41%, respectively in 10 h. In the two-step release testing, a maximum release of 89.32 was observed in 10 h. In this method, when the hydrogel beads are in pH 1.2, alginate shrinks and the pore size is decreased. As the pores get smaller, the release of the protein is also reduced. When it passes into the pH 7.4 medium, the hydrogel swells and the pore size increases and the protein is released gradually. Along with the crosslinking effect provided by glutaraldehyde to the alginate network, guar gum also helps in controlling the release of the drug by preventing the rapid seeping in of the dissolution medium into the hydrogel. Therefore, the protein release from the hydrogel matrix occurs due to the diffusion of the drug though the pores of the swelled matrix in the intestinal pH. The presence of guargum and the crosslinking effect provided by the glutaraldehyde prevents the disintegration of the alginate matrix in this pH.

5. Conclusion

The entrapment efficiency of beads increases with increasing amounts of guargum and with the increasing glutaraldehyde concentration. The crosslinked alginate–guar gum hydrogels, prepared by the freeze drying method generated a matrix with high porosity and highly controlled delivery of the protein drug. The hydrogel released low amounts (∼20%) of BSA at pH 1.2 and higher levels of protein release were observed at pH 7.4 (∼90% in 10 h). The proposed freeze-dried alginate–guar gum hydrogel can be considered as a potent candidate for a protein delivery matrix to the intestine via the oral route.

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