Carbohydrate Polymers 89 (2012) 667–675

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

pH-sensitive sodium alginate hydrogels for riboflavin controlled release

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ARTICLE INFO

Article history:
Received 25 February 2012
Received in revised form 15 March 2012
Accepted 25 March 2012
Available online 3 April 2012

Keywords:
PGMA-*g*-SA hydrogels
Calcium alginate
Characterization
Drug delivery systems
Riboflavin
Controlled release

ABSTRACT

Sodium alginate (SA) grafted with polyglycidyl methacrylate hydrogels (PGMA-*g*-SA) was prepared as pH sensitive drug delivery matrices for riboflavin (RF). The hydrogel copolymer matrices were compared with calcium alginate (CA) beads for swelling, degradation, entrapment efficiency and in vitro release of RF. The structure, surface morphology of the CA beads and the prepared hydrogels as well as the chemical stability of the encapsulated drug were characterized by FT-IR and SEM, respectively. The results demonstrate that the optimal formulation was achieved with PGMA-*g*-SA proportion of (0.75 mol/1 g) and loaded RF 0.03 g. It has been observed that the in vitro release study of RF from this formulation was superior to the other ones and was able to maintain the release for ~3 and 4 days for the simulated intestinal fluid (SIF) and simulated gastric fluid (SGF), respectively. In general, it has been shown that, GMA grafted onto SA enhanced drug entrapment efficiency, decreased swelling and degradation behaviors of the carrier. In addition, it slowed and controlled the release of RF from the PGMA-*g*-SA hydrogel compared with pure SA beads crosslinked with Ca^{2+} ions alone, which thereby provides a facile and effective method to improve the drug delivery systems.

1. Introduction

Utilization of natural polymeric hydrogels for the design of drug delivery system has long been the subject of great interest during the past decades (Lü, Liu, & Ni, 2010; Saboktakin, Maharramov, & Ramazanov, 2009; Vieira, Ferreira, Coelho, & Gil, 2008; Winzenburg, Schmidt, Fuchs, & Kissel, 2004). It is well known that hydrogel can respond to surrounding conditions such as pH, ionic strength, temperature, and electric current. The pH-sensitivity of hydrogel is an important factor in designing polymers for controlled drug release in the gastrointestinal tract, which has a variation of pH from the stomach to the intestine (Piyakulawat, Praphairaksit, Chantarasiri, & Muangsin, 2007). Among the most commonly used natural polymeric hydrogels alginates which are naturally occurring polysaccharides obtained mainly from marine brown algae belonging to the *Phaeophyceae* (Almeida & Almeida, 2004; Tønnesen & Karlsen, 2002).

Sodium alginate is a polyanionic linear copolymer of 1,4-linked-α-L-guluronic acid and β-D-mannuronic acid residues which considered as a unique biocompatible, biodegradable, and non-toxic material for drug delivery system (George & Abraham, 2006). SA is soluble in water and can be formed as an insoluble gel beads by dropping the SA aqueous solution into a divalent or polyvalent cations solution. Although this is a simple and fast way of obtaining drug carriers, the method presents a major limitation consisting of drug loss during bead preparation (Liu & Krishnan, 1999; Torre et al., 1998). In addition, the alginate beads formed are usually very permeable and little or no drug release can actually be controlled in the case of soluble drugs (Rastogi et al., 2007; Wang, Zhang, & Wang, 2009). Hence, a preferential use for alginate gel beads in the delivery of low solubility or macromolecular drugs has been suggested (Imai, Kawasaki, Nishiyama, & Otagiri, 2000; Pillay, Dangor, Govender, Moopanar, & Hurbans, 1998), while some researchers were able to circumvent this problem by mixing alginate with other polymers such as pectin (Jaya, Durance, & Wang, 2009; Yu et al., 2009), chitosan (Finotelli et al., 2010; Xu, Zhan, Fan, Wang, & Zheng, 2007), hydroxyethyl cellulose (Rao et al., 2006), hydroxypropyl methylcellulose (Patel, Baria, & Pandya, 2009; Shishu & Nidhi, 2007) and gelatin (Xiao, Zhu, Liu, Zeng, & Xu, 2009). Successful attempts involving the grafting copolymerization by synthetic polymers such as poly(N-isopropylacrylamide) (Shi, Alves, & Mano, 2006), poly(lactic-co-glycolic acid) (Zhenga, Gaoa, Zhang, & Liang, 2004), polyacryl-amide (Kulkarni & Sa, 2009), and poly(acrylic acid) (Lin, Ou, Lin, & Ling, 2010) have also been reported.

In the present study, an improved and simplified emulsification method for the preparation of polyglycidyl methacrylate grafted sodium alginate (PGMA-*g*-SA) hydrogels has been described. The structure and morphology of the developed hydrogels were characterized and the encapsulation efficiency, swelling properties and

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http://dx.doi.org/10.1016/j.carbpol.2012.03.074

drug release behaviors were also systematically evaluated using RF as the model drug. The behaviors of PGMA-*g*-SA hydrogels in all studies were compared with those of CA beads.

2. Experimental

2.1. Materials

Sodium alginate (SA, Fluka) with dual function as polymer and emulsifier, glycidyl methacrylate (GMA, Aldrich) of high purity, calcium chloride (Merck), ammonium peroxy disulfate (APS, Merck) and riboflavin (RF, BASF) were used as received. Double distilled water (DDW) was used in all experiments. Other chemicals and reagents employed were of analytical grade and were used without any further purification.

2.2. Methods

2.2.1. Preparation of calcium alginate (CA) beads

Calcium chloride solution of 500 mmol and sodium alginate solution of 2% (w/v) were prepared by dissolving the appropriate amounts of calcium chloride and sodium alginate, respectively, in DDW. The CA beads were prepared by dropwise addition of 10 ml of alginate solution into 20 ml of calcium chloride solution through a fine stainless steel needle. The distance between the edge of the needle and the surface of the calcium solution was 15 cm. The beads were left in the gelling medium for 15 min, then separated from the solution through a stainless steel grid and left at room temperature for 15 min before using for further investigations. In the case of drug loaded CA beads the appropriate amount of RF was added to the alginate solution (Pasparakis & Bouropoulos, 2006). The mixture was dissolved under magnetic stirring and the formation of the beads was performed by ionic gelation as previously described.

2.2.2. Preparation of PGMA-g-SA hydrogels

For the synthesis of the PGMA-*g*-SA hydrogels, an emulsion polymerization technique was employed using APS as an initiator. The polymerization was carried out in a three necked flask (100 ml) fitted with a condenser and a thermometer. The system also had a nitrogen inlet and was stirred with a magnetic stirrer. SA (1 g) and DDW (50 ml) were added into the reaction vessel and heated to 65 °C while flushing nitrogen through the solution. Then specific amount of GMA and APS (0.15 g) were added and the reaction ingredients were stirred vigorously at 65 °C for 4 h. The prepared stable emulsion was transferred to Petri dish till gel formation. The PGMA-*g*-SA hydrogels were cut into pieces with cubic style having dimensions of 5 mm. The PGMA-*g*-SA hydrogels was rinsed with dichloromethane to remove any residual of the unreacted monomer and PGMA homopolymer. The hydrogels pieces were immersed in ethanol for 7 days for complete removal of unreacted monomer. Finally, the hydrogel pieces were collected and stored at room temperature till further uses.

2.2.3. Preparation of PGMA-g-SA hydrogels loaded with RF drug

The same procedure as described in Section 2.2.2 was applied except adding specific amounts of RF drug before finishing the polymerization process. For the formation of drug delivery devices, the hydrogels pieces loaded with RF were left to dry for 2 days by keeping it in desiccators under vacuum at room temperature.

2.3. Testing

2.3.1. Grafting percentage of GMA

Dried PGMA-*g*-SA (50 mg) was dissolved in 10 ml distilled water containing $Na_2S_2O_3{\cdot}5H_2O$ (2 mmol) and acetic acid (1 mmol) and 10 ml acetone was added. The solution was incubated at 40 °C for 20 min, cooled and titrated with 0.1 N NaOH. Grafted PGMA was determined with standard curve against GMA standard solution (Kubota & Ujita, 1995). In addition, the grafting percentage of GMA was calculated gravimetrically according to Eq. (1):

$$\text{Grafting percentage}\,(G)\,(\%) = \frac{W_a - W_b}{W_a} \times 100 \quad (1)$$

where W_a and W_b are the sodium alginate mass before and after the grafting process, respectively.

2.3.2. Swelling behavior of CA beads and PGMA-g-SA hydrogels

The swelling studies of the beads and hydrogels were measured in three aqueous media: DDW, SGF and SIF at 37 °C. Preweighed wet and dry samples ranging from 2.5 to 3 g were immersed in 25 ml of the previous media at 50 rpm until they swelled to equilibrium. After excess surface water was removed gently with filter paper, the fully swollen samples were weighed. All experiments were done in triplicate. The dynamic weight change of the beads and hydrogels with respect to time was calculated by Eq. (2):

$$\text{Weight change}\,(\%) = \frac{W_s - W_i}{W_i} \times 100 \quad (2)$$

where W_s and W_i are the weight of the beads and hydrogels in the swollen state and the initial weight of the beads and hydrogels, respectively.

It is noteworthy to mention that the simulated gastric fluid (SGF) consisted of 2.0 g of sodium chloride, 7.0 mL of 37% hydrochloric acid and 1000 mL of DDW. The final pH was 1.2 (Maltais, Remondetto, & Subirade, 2010). In addition, the simulated intestinal fluid (SIF) consisted of 6.8 g of monobasic potassium phosphate dissolved in 250 mL of DDW and added to 190 mL of 0.2 N sodium hydroxide and 400 mL of DDW. The pH was adjusted to 7.5 using 0.2 N sodium hydroxide and the final volume was brought to 1000 mL with double-distilled water (Piyakulawat et al., 2007).

2.3.3. Degradation behavior of CA beads and GMA-g-SA hydrogels

In order to study the degradability of the beads and hydrogels, in vitro degradation tests were carried out in phosphate buffer saline (PBS) pH 7.4 at 37 °C. The degradation process was monitored by measuring the dry weight loss. Each bead and hydrogel was placed in a vessel containing 50 ml of PBS. At predetermined time intervals, beads and hydrogels were moved from the PBS solution and then dried under vacuum at room temperature to constant weight. All experiments were done in triplicate. The weight loss ratio (WLR) was calculated by Eq. (3):

$$\text{WLR}\,(\%) = \frac{W_o - W_t}{W_o} \times 100 \quad (3)$$

where W_o and W_t are the weights of beads and hydrogels before and after degradation, respectively.

2.3.4. Determination of the drug encapsulation efficiency

The encapsulation efficiency (EE) was measured after extracting RF drug from the prepared beads and hydrogels (Hu, Ma, Li, Yang, & Wang, 2010). 100 mg of drug-loaded beads and hydrogels were dispersed in 100 mL PBS (pH 7.4) and stirred for 30 min to ensure the complete extraction of RF. The mixture was stirred magnetically at 1000 rpm for 4 h. The mixture solutions increased to 250 mL volumes with PBS (pH 7.4). After centrifuging at 4000 rpm for 30 min, these solutions were diluted and analyzed by a UV–vis spectrophotometer at 445 nm. All experiments were done in triplicate in Amber vessels to prevent photodecomposition of RF. The EE of drug is expressed according to Eq. (4):

$$\text{Encapsulation efficiency}\,(\%) = \frac{\text{practical drug loading}}{\text{theoretical drug loading}} \times 100 \quad (4)$$

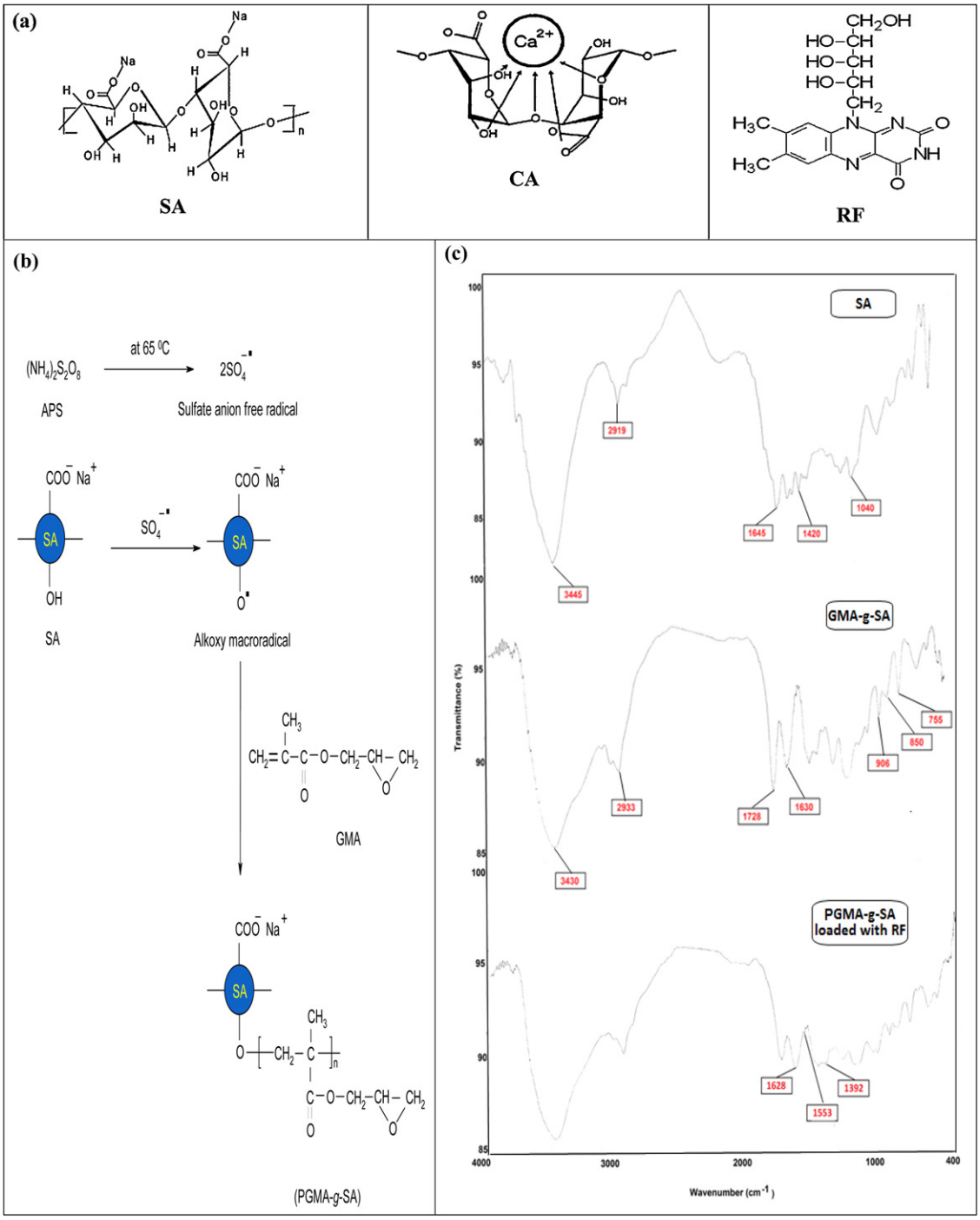

Fig. 1. (a) The chemical structures of SA, CA and RF and (b) the grafting reaction mechanism of the GMA onto SA, and (c) FT-IR of SA, PGMA-*g*-SA and PGMA-*g*-SA loaded with RF.

2.3.5. Determination of in vitro RF release

RF released into the fluids was determined from the measurement of absorbance at 445 nm and a standard curve (in DDW, SGF and SIF media) and expressed as follows:

$$\text{RF release}\,(\%) = \frac{\text{released RF}}{\text{total RF}} \times 100 \quad (5)$$

where, released RF was calculated from the RF concentration (mol/L) measured in the total solution volume and total RF was the amount loaded in each bead or hydrogel. All experiments were done in triplicate in Amber vessels to prevent photodecomposition of RF.

2.4. Characterization

FT-IR spectra were recorded on a FT-IR spectrophotometer (Nicolet 670, range from 4000 to 400 cm^{-1}, USA) using KBr pellets. Surface morphology was visualized by scanning electron microscopy (JXA-840A Electron probe microanalyzer, JEOL, Japan) using an accelerating voltage of 30 kV after coating with gold film using S150A Sputter Coater (Edwards, England). The drug loading and release studies were measured using double-beam Spectrometer (Shimadzu UV-2401 PC, Japan). Digital caliper (0–150 mm, Japan) used to measure the mean diameters of formed beads and hydrogels.

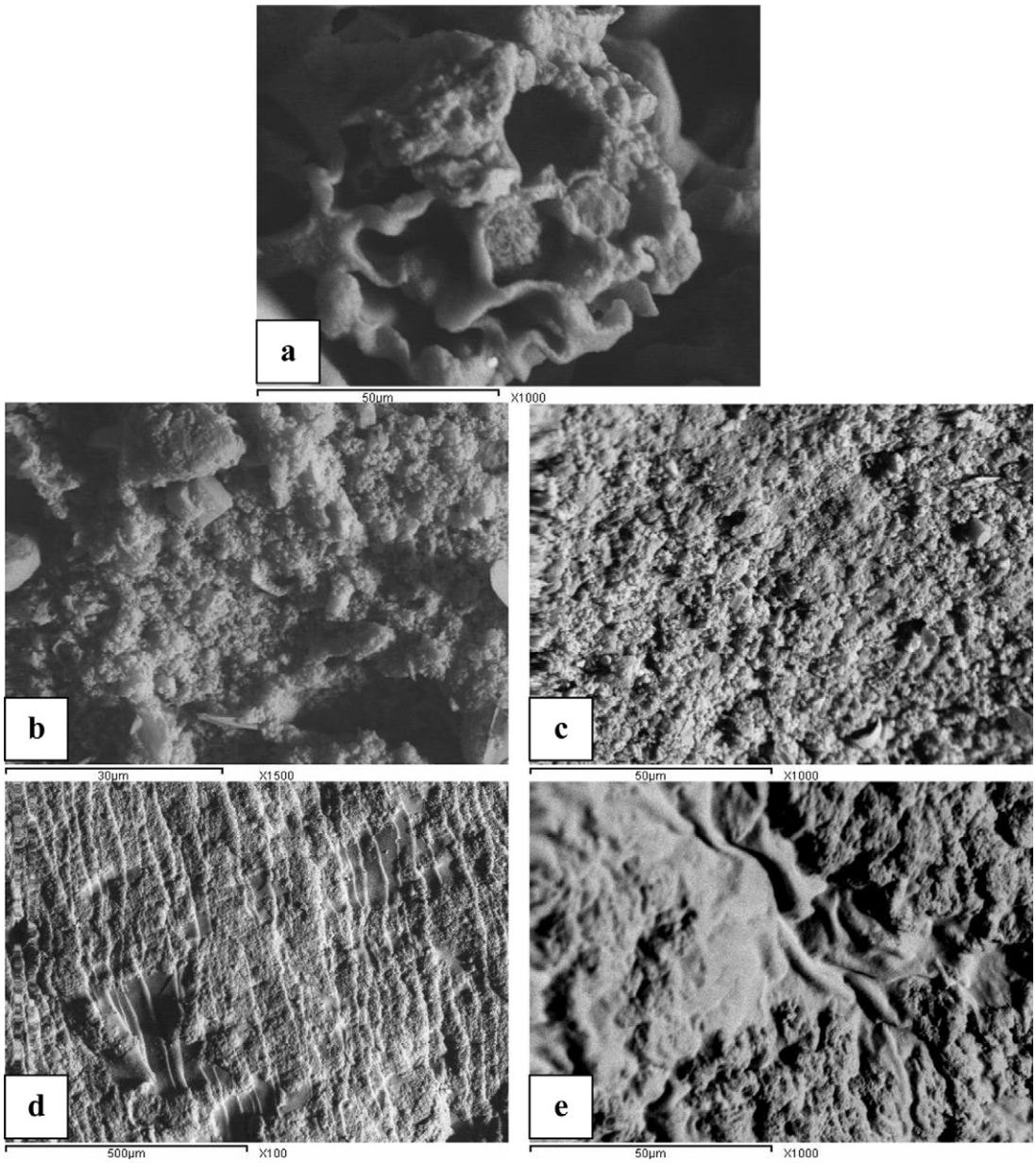

Fig. 2. SEM of (a) CA interior bead, (b) PGMA-*g*-SA hydrogel (0.25 mol GMA), (c) PGMA-*g*-SA hydrogel (0.5 mol GMA), (d) PGMA-*g*-SA hydrogel (0.75 mol GMA), (e) PGMA-*g*-SA hydrogel (1 mol GMA), Photograph of (f) CA beads and (g) CA beads loaded with riboflavin (0.03 g), SEM of (h) CA bead, (i) CA bead loaded with riboflavin (0.03 g), (j) CA interior bead, (k) CA interior bead loaded with riboflavin (0.03 g), (l) PGMA-*g*-SA hydrogel (0.75 mol GMA) and (m) PGMA-*g*-SA hydrogel (0.75 mol GMA) loaded with riboflavin (0.03 g).

3. Results and discussion

SA is a natural, biocompatible, and biodegradable polymer. Hydrogel beads composed of SA crosslinked with Ca^{2+} exhibit low mechanical strength and are, therefore, rapidly degraded after oral administration (Lin et al., 2010). To overcome this limitation, we used GMA to reinforce the structure of the SA and increase its strength by in situ grafting various concentrations of SA (0.5–5 wt%) with a fixed concentration of GMA (0.25 mol). We found that when the SA concentration was higher than 1 wt%, the solution became extremely viscous. Furthermore, we found that the use of a low SA concentration resulted in the production of deformed beads and hydrogels. The optimal SA concentration was identified as 1 wt%. Accordingly a series of hydrogels could be made with this specific SA concentration and various GMA concentrations (0.25–1 mol) without any problem.

Fig. 1a illustrates the structures of the studied SA, CA, and RF. In addition, the grafting reaction takes place according to Fig. 1b.

3.1. Characterization of beads and hydrogels

3.1.1. FT-IR spectroscopy

Fig. 1c shows the FT-IR spectra of SA, PGMA-*g*-SA hydrogels and RF loaded PGMA-*g*-SA hydrogels. The bands around 1040 cm^{-1} (C—O—C stretching) presenting in the IR spectrum of SA are attributed to its saccharide structure. In addition, the bands at 1645 and 1420 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups broad band at 3445 cm^{-1} corresponded to the hydroxyl groups. IR-spectroscopy of the PGMA-*g*-SA is characterized the epoxide group by peaks at 906, 850, 755 cm^{-1}. 1728 cm^{-1} peak is due to stretching vibrations of C—O of ester group. Among the characteristic vibration of the

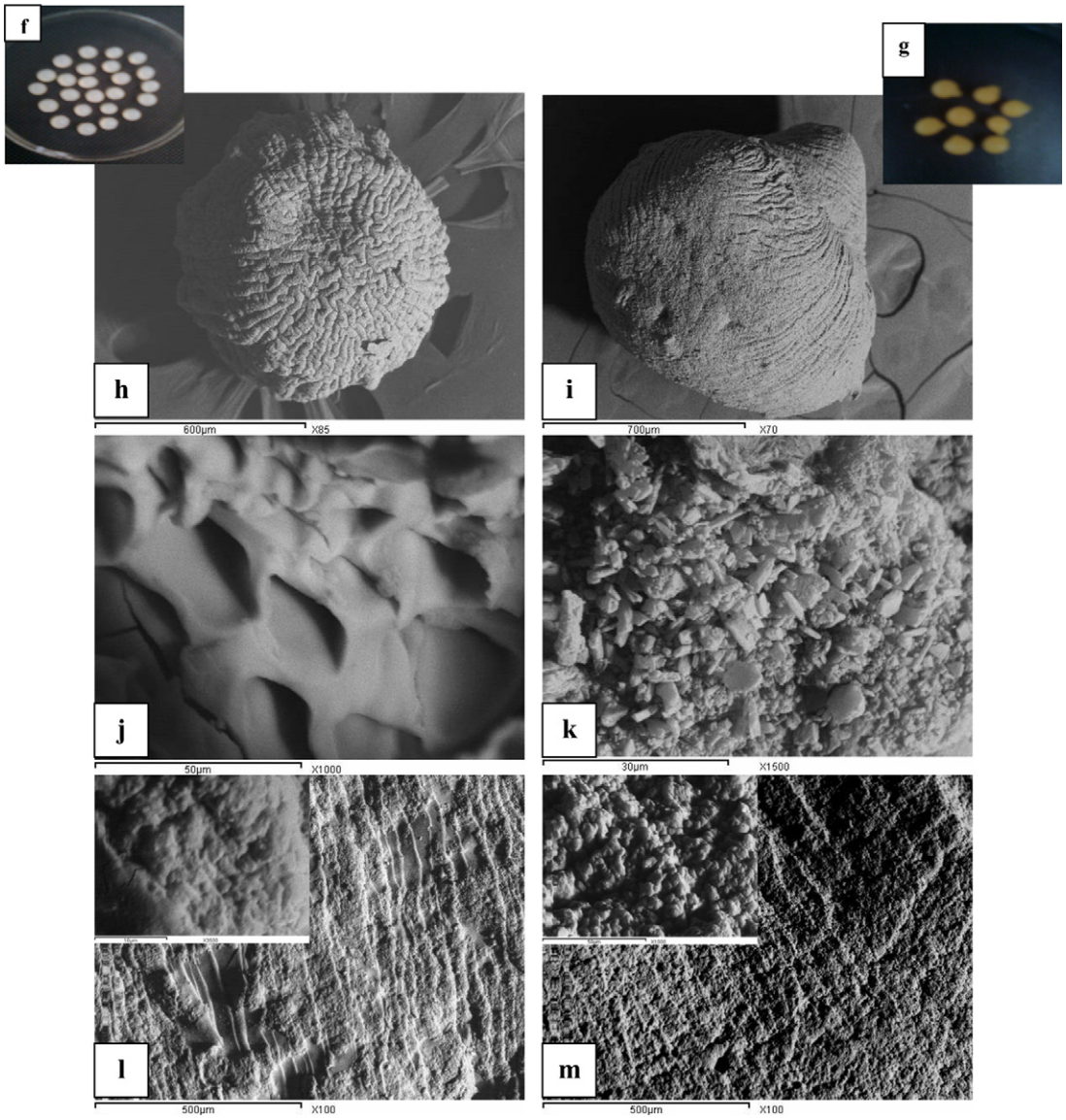

Fig. 2. (*Continued*).

GMA is the methyl vibration at 2924 cm^{-1}. These peaks showed that GMA was grafted to sodium alginate. Furthermore, the IR spectrum of RF loaded GMA-*g*-SA hydrogels exhibits bands of RF at 1628, 1553 and 1392 cm^{-1} that are characteristic stretching modes C=O (amide carbonyl), C=N (conjugated system) and C—N, respectively. These results are in accordance with the previous results (Eckert, Gröbe, & Rothe, 2000; Malele, Ray, & Jones, 2010; Sartori, Finch, & Ralph, 1997; Vaidya et al., 2007).

3.1.2. Beads and hydrogels Morphology

The mean diameter of the CA beads was determined by measuring 20 beads using the digital caliper. In comparison with the size of the wet beads which was measured 3 ± 0.5 mm, dried beads were shrunk at about half value and their diameter were found to be 1.4 ± 0.05 mm. Also, the PGMA-*g*-SA hydrogels pieces dimensions were measured by the digital caliper. The wet hydrogels were of cubic style having dimension in the range of 5 ± 0.3 mm while the dried air samples were also cubic in the range of 2.7 ± 0.5 mm.

SEM images of CA beads and PGMA-*g*-SA hydrogels are shown in Fig. 2. The CA interior beads displayed very porous and spongy morphological structure (Fig. 2a). While, after grafting of GMA on SA and formed PGMA-*g*-SA hydrogels the morphology have very narrow micropores with smooth layers and cracks were observed on the surface (Fig. 2b–d). Furthermore, it was observed that by increasing the ratio of GMA the porosity decreased and the structure of the hydrogel tend to ordinary distribution between the micropores and the smooth layers till 0.75 mol of GMA with high degree of homogeneity (Fig. 2d). The increase of GMA ratio over 0.75 mol led to randomly distribution, decrease in the grafting degree and increase the porosity of the hydrogel (Fig. 2e).

In addition, Fig. 2 illustrates the SEM of pure CA beads and PGMA-*g*-SA hydrogel in the presence and absence of loaded RF. It can be seen from Fig. 2f and g that the pure and loaded CA beads with RF were spherical in shape with smooth surface. The morphology of the exterior surface of CA beads changed from large wrinkles (Fig. 2h) to very tight surface (Fig. 2i) after loading with RF. Also, an examination of the SEM micrographs (Fig. 2j) indicates the presence of many enlarged pores of the interior surface of CA beads which after drug encapsulation were seen to be filled with RF particles (Fig. 2k). Also, the surface morphology in Fig. 2m shows that the micro pores filled with the drug particles which confirm RF loaded PGMA-*g*-SA hydrogels.

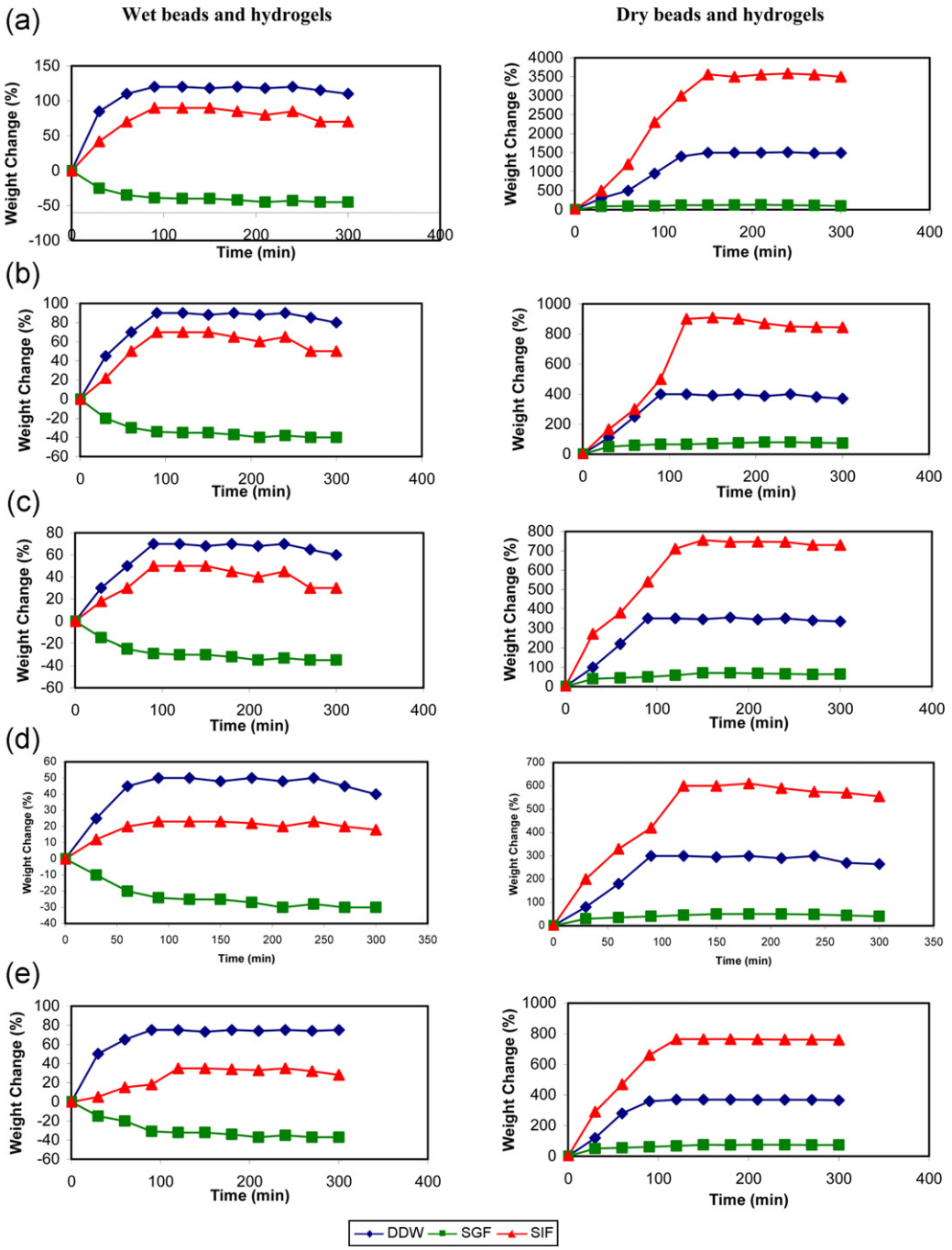

Fig. 3. Swelling profiles of wet and dry beads and hydrogels in DDW, SGF and SIF: (a) CA beads, (b) PGMA-*g*-SA (0.25 mol GMA), (c) PGMA-*g*-SA (0.5 mol GMA), (d) PGMA-*g*-SA (0.75 mol GMA), and (e) PGMA-*g*-SA (1 mol GMA).

3.2. Determination of grafting percentage of GMA in PGMA-g-SA

Grafting percentage of GMA was determined gravimetrically and titrimetrically. The titrimetric method was made according to reaction between epoxy group and sodium thiosulfate. NaOH was produced and reacted with CH_3COOH. Remained CH_3COOH was titrated with 0.1 N NaOH and GMA content was calculated from mole ratios (Kubota & Ujita, 1995). GMA content of PGMA grafted sodium alginate was calculated according to titrimetric method and results were also given in Table 1. As observed from the results, the percentage of grafting increased by increasing the GMA ratio till 0.75 mol then the percentage decreased. The results of titration and gravimetric methods were compared and the results were in accordance.

Table 1
Effect of amount of initial GMA according gravimetric and titrimetric methods.

Amount of initial GMA	Amount of GMA grafted to 100 mg SA[a]	Amount of GMA grafted to 100 mg SA[b]	*G* (%)
177	16.1	16.8	92.9
355	32.5	33.9	93.5
533	51.0	52.4	97.0
710	60.0	61.0	85.2

[a] Gravimetric method.
[b] Titrimetric method.

3.3. Characterization of beads and hydrogels loaded with riboflavin

3.3.1. Effect of pH on swelling behaviors

Swelling behavior is a very important property for drug delivery system because it has a great influence on the drug release behavior (Wang, Xie, Zhang, Zhang, & Wang, 2010). Swelling studies were conducted using both wet and dry beads and hydrogels. The term wet refers to the state of the beads and hydrogels immediately after the preparation and the term dry refers to beads and hydrogels that were left to dry for 24 h at 30 °C in air. In order to simulate the possible effect of pH on drug release rate, a swelling study was conducted in three aqueous media: DDW, SGF and SIF for pure CA beads and PGMA-*g*-SA hydrogels.

3.3.1.1. Wet beads and hydrogels. Pure CA beads exhibit a swelling degree in DDW of about 120% as illustrated in Fig. 3a. The swelling behavior can be well justified due to the fact that wet beads tend to absorb water in order to fill the void regions of the polymer network within the beads that remain dehydrated, until they reach the equilibrium state (Hoffman, 2002). The phenomenon is provoked by the relaxation of the polymer network at the presence of osmotic pressure. Swelling of the wet CA beads in water lasts for about 90 min until the osmotic pressure equals the forces of the crosslinking bonds that maintain the structure of the polymer network stable. When these two forces are equal, no further water absorbing from the beads is observed (Pasparakis & Bouropoulos, 2006). In the acidic environment of SGF, pure CA beads tend to shrink. The observed results may be attributed to the fact that most of carboxylate groups in CA transformed into —COOH groups in the low pH region (pH 1.2) because the pK_a of CA is about 3.2. The hydrogen bonding between carboxylate groups in SA leads to the polymer–polymer interactions and predominate over the polymer–water interactions and hence the electrostatic repulsion among these groups decreases and shrinkage is favored (Hu et al., 2010). As a result, the swelling ratio of the beads is relatively low. While the pH of the outer swelling medium is increased in SIF medium (pH 7.5), the carboxylate groups of SA molecules tend to ionize and give —COO— ions, which facilitate the swelling of beads. The osmotic pressure should increase inside the beads due to the higher concentration of free H^+ and promote water uptake. Furthermore, electrostatic repulsion between carboxylate ions should cause macromolecular chain relaxation and increase swelling (Bajpai & Sonkusley, 2002; Bajpai, Bajpai, & Kalla, 2002; Beaulieu, Savoie, Paquin, & Subirade, 2002). It is evident that the swelling degree of the beads in a buffer of pH 1.2 (SGF) was much lower than that in pH 7.5 (SIF), indicating a pH-sensitive swelling behavior.

The swelling behaviors of PGMA-*g*-SA hydrogels in DDW, SGF and SIF media was shown in Fig. 3b–e. It can be seen that they swell and shrink with the same representative pervious behaviors but lesser in weight than observed in the CA beads. This is due to the grafting of SA with GMA caused significant reduction of micro pores with smooth layers and cracks were observed on the surface which in turn lead to decrease the permeability as already shown in the SEM (Fig. 2). The polymer network is denser and exhibits increased resistance to osmotic pressure but the total swelling behavior is dominated by the SA. Furthermore, the PGMA-*g*-SA hydrogel (1 mol GMA) have a swelling behavior higher than the other formulations of PGMA-*g*-SA hydrogels (0.5 mol GMA and 0.75 mol GMA) due to structure morphology which was discussed in Section 3.1.2.

3.3.1.2. Dry beads and hydrogels. The swelling of the dry pure CA beads is mainly attributed to the hydration of the hydrophilic groups of alginate and the water penetration into the inert pores and the cavities in the beads (Huang, Xiao, & Lang, 2012) (Fig. 3a). As already mentioned in Section 3.3.1.1, the grafting of GMA on SA reduces the permeability of the hydrogels. The ability of dry PGMA-*g*-SA hydrogels to swell in DDW is lower than the pure CA beads (Fig. 3b).

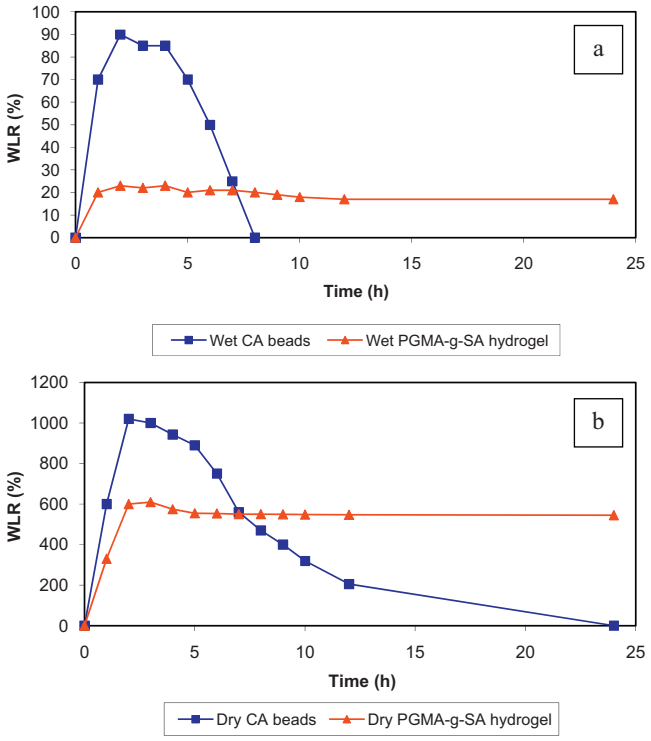

Fig. 4. Degradation profiles of (a) wet CA beads and PGMA-*g*-SA hydrogel (0.75 mol GMA) and (b) dry CA beads and PGMA-*g*-SA hydrogel (0.75 mol GMA), in PBS (pH 7.4) at 37 °C.

In contrast to the wet beads and hydrogels, which shrink in SGF, dry beads and hydrogels exhibit a swelling degree ranging from 80 and 130% for CA beads and PGMA-*g*-SA hydrogels, respectively (Fig. 3). CA dry beads gained extra weight due to the hydration of the hydrophilic groups (Pasparakis & Bouropoulos, 2006). On the other hand, PGMA-*g*-SA has an expansion in the hydrogel networks which lead to the penetration of water (Wang et al., 2010).

Also Fig. 3 shows that all formulations tested exhibited significant swelling rates when exposed to the slightly alkaline environment of SIF. The swelling mechanism in this case is related to the Ca^{2+} and Na^+ exchange in pure CA beads (Bajpai & Sharma, 2004). PGMA-*g*-SA hydrogels still have lower ability to swell than the pure CA beads. Furthermore, the PGMA-*g*-SA hydrogel (1 mol GMA) have a swelling behavior higher than that of PGMA-*g*-SA hydrogel formulations (0.5 mol GMA and 0.75 mol GMA) due to their structure morphology which discussed in Section 3.1.2.

3.3.2. Degradation behaviors of beads and hydrogels

The degradability of the beads and hydrogels was monitored by examining the weight loss of beads and hydrogels with incubation time in PBS (pH 7.4) at 37 °C, as shown in Fig. 4. It was found that in the CA beads case, the SA forms a crosslinked network with Ca^{2+} which is easily broken in PBS and contributes to a large swelling due to the electrostatic interaction and then complete degradation takes place and the spherical form of the beads vanished after 8 h and 24 h for wet and dry beads, respectively. While the presence of GMA in the grafted SA hydrogels leads to reduce the degradation. The hydrogel samples keep their shape throughout the test and

only about 10% and 25% degraded after 24 h of incubation for wet and dry hydrogels, respectively.

3.4. Encapsulation and controlled release

3.4.1. Drug encapsulation efficiency of the beads and hydrogels

The percentages of encapsulation efficiency of the RF-loaded beads and hydrogels with different ratios of GMA content are shown in Table 2. The EE % was obtained in the range of 80–100%. Formulation SG7 gave the highest EE % of 99.7% and formulation CA3 gave the lowest EE % of 78.3%. The PGMA-*g*-SA hydrogels showed the EE % to be in the range of 87.4–99.7%, which is higher than that of the pure CA beads. The method of CA beads preparation and its high porosity lead to a small degree of RF loading which is lower than PGMA-*g*-SA hydrogels having dense structures (Fig. 2). In addition, it was observed that the EE is increased with increasing the GMA ratio up to 0.75 mol of GMA after that ratio the EE of the drug decreased. This behavior may be attributed to the high ratios of GMA which lead to a decrease in the grafting degree which reduces the encapsulation efficiency of the loaded RF.

3.4.2. In vitro release studies of riboflavin

To study the release profiles for test beads and hydrogels, dried test samples were immersed in SGF and SIF media at 37 °C. Fig. 5 shows the release profiles of entrapped RF under the same varying pH conditions. The release properties of RF illustrated in Fig. 5 are also closely associated with the swelling and degradation behavior of the beads and hydrogels (Figs. 3 and 4). The release RF reached 100 and 30% after 24 h in SIF for CA beads and PGMA-*g*-SA hydrogels, respectively. In SGF the drug release was 70 and 18% after 24 h for CA beads and PGMA-*g*-SA hydrogels, respectively. The great difference of the release percentages in the two media (SIF and SGF) is attributed to the swelling behaviors of the polymers, as discussed in Section 3.3.1 and also to the RF pH sensitivity. This behavior can be interpreted as follows.

At SIF (pH 7.5), it is noteworthy to mention that RF of pK_{a1} 1.7, pK_{a2} 10.2 (Budavari, 1989) has an amphoteric nature with an isoelectric point at pH 6.0 which is not too far from the pH of SIF. As a result, the drug has small net charge and does not interact with carboxyl groups in PGMA-*g*-SA hydrogels. In addition, carboxyl groups in CA beads are already involved with Ca^{2+} in the formation of cross-links. The weak drug-polymer interactions lead to fast diffusion of RF.

At SGF (pH 1.2), RF is ionized and can engage in ionic interactions with the polymers, which slow down its diffusion into the dissolution medium (Maltais et al., 2010). Table 3 shows the time required for complete release of RF from the polymers at different pH solutions (SIF and SGF). It was noticed that the RF is completely released from CA beads and PGMA-*g*-SA hydrogels after 1 day and 3 days in SIF, respectively. In SGF medium, RF needs 2 days and 4 days for CA beads and PGMA-*g*-SA hydrogels, respectively for complete release.

Table 2
Code, polymers ratio and encapsulation efficiency (EE) for all prepared beads and hydrogels.

Code	Polymer	SA (g)	GMA (mol)	RF (g)	EE (%)
CA1	CA	2	0	0.01	80
CA2	CA	2	0	0.03	86.8
CA3	CA	2	0	0.05	78.3
SG1	PGMA-g-SA	1	0.25	0.01	91.4
SG2	PGMA-g-SA	1	0.5	0.01	98.7
SG3	PGMA-g-SA	1	0.75	0.01	98.5
SG4	PGMA-g-SA	1	1	0.01	88.6
SG5	PGMA-g-SA	1	0.25	0.03	88.3
SG6	PGMA-g-SA	1	0.5	0.03	95.1
SG7	PGMA-g-SA	1	0.75	0.03	99.7
SG8	PGMA-g-SA	1	1	0.03	87.4
SG9	PGMA-g-SA	1	0.25	0.05	89.8
SG10	PGMA-g-SA	1	0.5	0.05	93.6
SG11	PGMA-g-SA	1	0.75	0.05	98.1
SG12	PGMA-g-SA	1	1	0.05	87

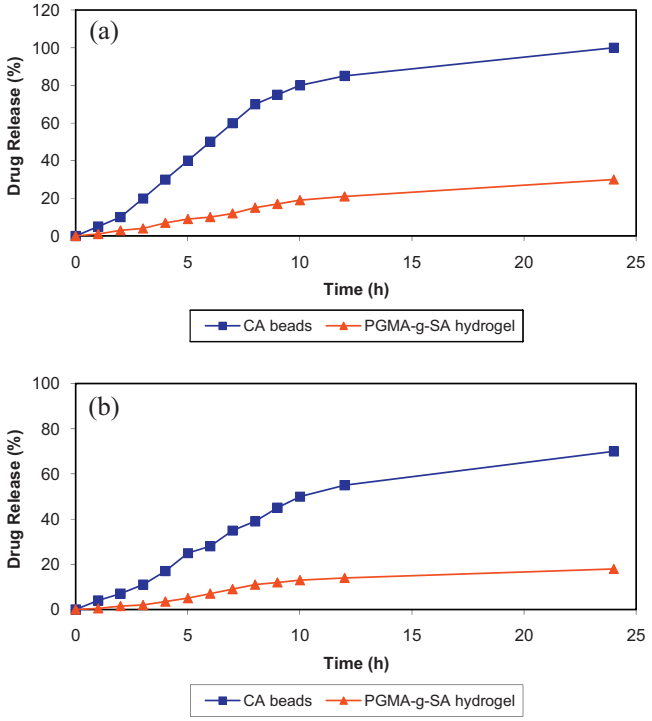

Fig. 5. Riboflavin release from CA beads and PGMA-*g*-SA hydrogels (0.75 mol GMA, 0.03 g RF) in (a) SIF (pH 7.5) and (b) SGF (pH 1.2).

Table 3
Time duration for complete release of riboflavin.

Polymer code	SIF			SGF			
	1 day	2 days	3 days	1 day	2 days	3 days	4 days
CA2	100%	–	–	70%	100%	–	–
SG7	30%	70%	100%	18%	45%	79%	100%

4. Conclusions

RF release studies from the CA beads and hydrogels prepared from PGMA-*g*-SA indicate that PGMA-*g*-SA hydrogels lead to decrease in the release rate of RF and increase the entrapment efficiency due to minimizing the grafted SA porosity. It is also observed that the release of RF is much higher at SGF (pH 7.5) compared to SIF (pH 1.2) and decreases with the increase in PGMA grafting onto SA. The results showed that the design of PGMA-*g*-SA hydrogels is an interesting and pH sensitive device for a controlled release drug delivery system.

Acknowledgments

The authors gratefully acknowledge financial support from National Research Center.

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