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ORIGINAL ARTICLE

L-Arginine grafted alginate hydrogel beads: A novel pH-sensitive system for specific protein delivery

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Abstract Novel pH-sensitive hydrogels based on L-arginine grafted alginate (Arg-g-Alg) hydrogel beads were synthesized and utilized as a new carrier for protein delivery (BSA) in specific pH media. L-arginine was grafted onto the polysaccharide backbone of virgin alginate via amine functions. Evidences of grafting of alginate were extracted from FT-IR and thermal analysis, while the morphological structure of Arg-g-Alg hydrogel beads was investigated by SEM photographs. Factors affecting on the grafting process e.g. L-arginine concentration, reaction time, reaction temperature, reaction pH, and crosslinking conditions, have been studied. Whereas, grafting efficiency of each factor was evaluated. Grafting of alginate has improved both thermal and morphological properties of Arg-g-Alg hydrogel beads. The swelling behavior of Arg-g-Alg beads was determined as a function of pH and compared with virgin calcium alginate beads. The cumulative *in vitro* release profiles of BSA loaded beads were studied at different pHs for simulating the physiological environments of the gastrointestinal tract. The amount of BSA released from neat alginate beads at pH 2 was almost 15% after 5 h, while the Arg-g-Alg beads at the same conditions were clearly higher than 45%, then it increased to 90% at pH 7.2. Accordingly, grafting of alginate has improved its release profile behavior particularly in acidic media. The preliminary results clearly suggested that the Arg-g-Alg hydrogel may be a potential candidate for polymeric carrier for oral delivery of protein or drugs.

1. Introduction

Hydrogels are polymeric materials that have a three-dimensional network structure and can imbibe huge amounts of physiological fluids and considerably in aqueous medium without dissolution (Kamath and Park, 1993). Hydrogels have great interests in controlled release applications because of

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their soft tissue biocompatibility (Kamath and Park, 1993; Pedley et al., 1980; Peppas, 1987; Peppas et al., 2000; Peppas and Mikos, 1987; Pierre and Chiellinin, 1986). The ease is that the drug is dispersed in the matrix, and the high degree of control achieved by selecting the physical and chemical properties of the polymer network (Risbud et al., 2000). A variety of synthetic or natural polymeric hydrogels have been employed as the controlled release system for drug delivery, among them alginate is one of frequently used for pharmaceutical and biomedical needs (Pedley et al., 1980; Peppas et al., 2000; Kimura and Tsuruta, 1993; Mi et al., 2002). Alginate is derived from brown algae, is an anionic linear polysaccharide composed of 1,4-linked β-D-mannuronic acid residues and 1,4-linked α-L-guluronic acid residues of varying properties and arranged randomly along the chain (Ress and Welsh, 1997). Sodium alginate is a water soluble salt of alginic acid, which exhibits a unique property of gel formation in the presence of multivalent cations such as calcium ions in aqueous medium or in lower environmental pH value (Ress and Welsh, 1997). Alginate polymer hydrogels have intrinsic properties such as high hydrophilic, biocompatible, muco-adhesive, easy forming, and relatively economical use, which makes it widely used in different fields of biomedical applications e.g. wound dressing (Kim et al., 2008), scaffolds (Zmora et al., 2002), and dental or surgical impression materials (Nandini et al., 2008).

Several complexes and modifications of alginate attempts have been previously conducted with other polymers and modifier agents, which have been utilized as specific delivery and controlled release for drugs; these polymers and modifiers are for example, chitosan (Anal et al., 2003; Coppi and Iannuccelli, 2009; Goycoolea and Lollo, 2009), *N*-O-carboxymethyl chitosan (Chen et al., 2004), *N*-succinyl chitosan (Dai et al., 2008), hydrophobic modified alginate using long alkyl chain modifier (Leonard et al., 2004), dextran (Khan et al., 2011), heparin (Jeon et al., 2011), amphiphilic alginate-amide using dodecylamine tails (Vallée et al., 2009), and recently polyethylene imine 200 (PEI) (He et al., 2012). PEI was recently used for grafting onto polysaccharide chains of alginate via Shiff-base reaction, where amine groups of PEI quickly formed imine linkage with dialdehyde groups of oxidized alginate, and somewhat cytotoxicity was found by final product of alginate-g-PEI (He et al., 2012).

In the light of such contributions for alginate grafting, L-arginine was chosen to graft onto neat alginate chains due to its non toxicity and fully biodegradability, aside from other intrinsic medical properties as will be described for getting a new amine-grafted alginate for a specific pH-sensitive controlled protein release. Arginine is α-amino acid; the L-form is one of the twenty most common natural amino acids. L-Arginine has many biological activities (Wu, 2004). Furthermore, L-arginine promotes the nitric oxide synthesis in the body and enhances the transportation into the cells, while the biocompatibility and the biodegradability are reported and also important factors in adopting L-arginine in many biomedical matrix developments (Trischitta et al., 2007). Notably, the overdose of L-arginine does not cause any side-effects because the excess is excreted in the urine within few hours (Trischitta et al., 2007). Many researchers reported that the principle advantages of the current hydrogel components such as, sodium alginate (Pedley et al., 1980; Peppas et al., 2000; Kimura and Tsuruta, 1993; Mi et al., 2002) and L-arginine (Wu, 2004; Trischitta et al., 2007) do not show any biologically toxic effect, while p-benzoquinone (PBQ) was used in this study as an activation agent, which has somewhat toxicity toward oral exposure. Accordingly, the activated alginate was thus rinsed many times before crosslinking to remove any excess of PBQ.

The aim of this study is to develop a novel pH-sensitive system from grafted alginate hydrogel-based matrix for protein controlled release applications. Arg-g-Alg hydrogel is composed of L-arginine-grafted-alginate for enhancing sustained protein release particularly in acidic medium, when compared to neat alginate hydrogels. In this study, Arg-g-Alg based hydrogels was synthesized and characterized as well. Swelling behavior of Arg-g-Alg hydrogels as function of pH was assessed. The influence of grafting reaction on the obtained hydrogel bead properties has been highlighted, in terms of thermal, morphological, and physicochemical properties. Likewise, *in vitro* release profiles of model protein (BSA) from Arg-g-Alg hydrogels were studied and compared with neat alginate hydrogel beads, specifically in acidic medium to simulate conditions in the gastrointestinal tract system.

2. Experimental and instrumental measurements

2.1. Materials

Sodium alginate (medium viscosity 200 C.P.), p-benzoquinone, PBQ (purity 99.5%), and albumin from bovine serum (BSA, fraction V, minimum 96% electrophoresis, nitrogen content 16.2%, isoelectric point in water at 25 °C: 4.7) were obtained from Sigma–Aldrich Chemicals Ltd. Germany, L-arginine pure (isoelectric point in water at 25 °C: 10.76), was obtained from Nice chemicals PVT, cochin, India. Calcium chloride (anhydrous Fine GRG 90%) was purchased from Fisher Scientific (Fairlawn, NJ, USA). Fig. 1 represents chemical structures of Arg-g-Alg hydrogel bead components.

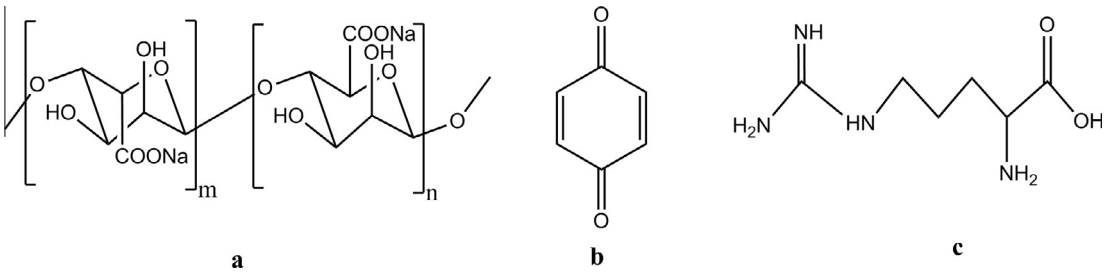
2.2. Preparation of L-arginine-grafted- Alginate (Arg-g-Alg) hydrogel beads

2.2.1. Activation of Na-alginate using PBQ

Sodium alginate was first activated using -benzoquinone (PBQ) as a coupling agent, which has been described elsewhere with slight modification (Eldin, 2011). The procedure is based on mixing Na-alginate solution (0.4 g i.e. 4%, w/v) with equal volume of -benzoquinone (PBQ) solution (0.02 M, in deionized alkaline water, pH 10) and keeping them under continuous stirring for 4 h at 25 °C to get a final concentration 2% (w/v) of alginate and 0.01 M of (PBQ).

2.2.2. Grafting Alg-PBQ with L-arginine (grafting technique)

Factors affecting on coupling efficiency between Alg-PBQ and L-arginine e.g. concentration of L-arginine and coupling reaction temperature/time, were studied to adjust the grafting step. Varied concentrations of L-arginine (0.1–5%, w/v) were added to the mixture of Alg-PBQ and stirring at 27–60 °C for different coupling times 15–75 min. Homogeneous graft copolymer solution was formed. The mixture was added dropwise using peristaltic pump (flow rate 5 ml/min), through 10 cm plastic syringe. Uncrosslinked Alg-PBQ-Arg was dropped in calcium chloride solution (3%, w/v) and left for crosslinking time of



a b c

Figure 1 Chemical structure of Arg-g-Alg hydrogel bead components, sodium alginate (a), PBQ (b), and L-arginine (c).

10 min at room temperature. The wet beads (2 mm diameter) were washed 10 times using a mixture of hot distilled water/ethanol (1:4) to remove the excess of calcium chloride, free PBQ and un-reacted L-arginine and to ensure the safety of obtained hydrogel beads. Other method was used to remove free PBQ completely from polymer, as follows. Typically, crosslinked activated alginate (Alg-PBQ) beads were dissociated using phosphate buffer overnight. The obtained mixture of Alg-PBQ polymer was precipitated using ethanol. Alg-PBQ was rinsed ten times using absolute ethanol to remove any excess or free PBQ. Pure Alg-PBQ was then grafted with L-arginine and followed by crosslinking again with $CaCl_2$ solution as shown in the conditions above. The obtained Arg-g-Alg beads were dried under vacuum for 24 h at 30 °C. Schematic diagram presented in Fig. 2, describes the proposed pathway for synthesis of alginate-g-arginine (Arg-g-Alg) hydrogel beads.

2.3. Grafting efficiency determination

The grafting efficiency percentage, GE% of obtained Arg-g-Alg polymers was evaluated in terms of the factors affecting on the grafting reaction, such as L-arginine concentration, pH value of grafting reaction, time, and temperature of grafting reaction, where one factor is varied while other variables are kept constant. GE% of Arg-g-Alg polymer was calculated by the following Eq. (1): (Liu et al., 2005)

$$\mathrm{GE}(\%) = [(W_{Arg-g-Alg} - W_{Alg}/W_{Arg})] \times 100 \quad (1)$$

where $W_{\text{Arg-g-Alg}}$ is the weight of obtained vacuumed dried Arg-g-Alg beads, W_{Alg} is the initial weight of neat alginate, and W_{Arg} is the initial weight of L-arginine that has been introduced in the grafting reaction.

2.4. Water uptake behavior

In order to measure the water uptake degree of Arg-g-Alg hydrogel beads, hydrogel beads either neat alginate or grafted alginate, pre-weighed piece 0.1 g of hydrogels was first soaked for 4 h in distilled water to remove uncrosslinked materials, then dried for swelling test. The hydrogel samples were immersed in aqueous reservoir samples and incubated in freshly prepared buffer solution at 37 °C (with varied pHs 2–11, HCl–KCl buffer, acetate buffer, and Tris–HCl buffer; the ionic strength of buffer was adjusted by NaCl diluted solution). Hydrogel samples were allowed to swell at specific interval times (W_s), till equilibrium swelling state, and then dried at 30 °C under vacuum for 24 h. The weight of dried sample was precisely determined (W_e) Kamoun and Menzel, 2010. The water uptake of hydrogel samples was determined using the following Eq. (2) Yang and Robinson, 1998.

$$\textit{Water uptake ratio}\ (\mathrm{WU})\% = [(W_s - W_e)/W_e] \times 100 \quad (2)$$

The sample, which had the best swelling characteristics for protein drug delivery among all studied groups, was subsequently selected for the BSA release profile study.

2.5. Preparation of BSA loaded hydrogel beads

The grafted alginate beads were disintegrated using phosphate buffer pH 7.2 to obtain a homogenous solution, the ability of alginate and grafted alginate hydrogels to entrap and release the drug or protein e.g. BSA with final concentration 20 mg/mL to Arg-g-Alg solution, was studied. The Arg-g-Alg solution containing BSA (~20 mg/ml) was crosslinked in $CaCl_2$ as described above. The BSA loaded beads were rinsed once with distilled water to remove the excess drug on bead surface. Finally, the hydrogel BSA-beads were dried under vacuum at 30 °C, for 24 h. Dried beads containing BSA are ready for determination of the release profile of BSA from beads as described below.

2.6. Protein release in vitro

Precisely weighed amounts of BSA loaded beads (250 mg, in 10 ml of polymer solution) with final BSA concentration of 20 mg/ml after subtracting the lost amount of BSA in $CaCl_2$ solution, were placed in conical flasks containing the release medium, (100 ml of phosphate buffer solution at 37C, pH 7.6). The samples were incubated at 37 °C under shaking at 50 rpm. At predetermined time intervals, samples of 1 ml were collected from the release medium and replaced by an equal volume of fresh buffer solution. The amount of BSA released from the hydrogel beads was assayed by UV spectroscopy at 280 nm.

2.7. Characterizations

2.7.1. FTIR

Vacuumed and dried samples of neat alginate or Arg-g-Alg hydrogel were analyzed by FT-IR on(Shimadzu FTIR, model No. 8400S, Japan). Translucent KBr-disks were prepared by grinding the dried sample materials together with infrared grade KBr and then pressing. The FTIR spectra were obtained by recording 64 scans between 4000 and 400 cm with a resolution of 2 cm. All samples were freeze-dried using liquid nitrogen, crushed to a fine powder (KBr:sample

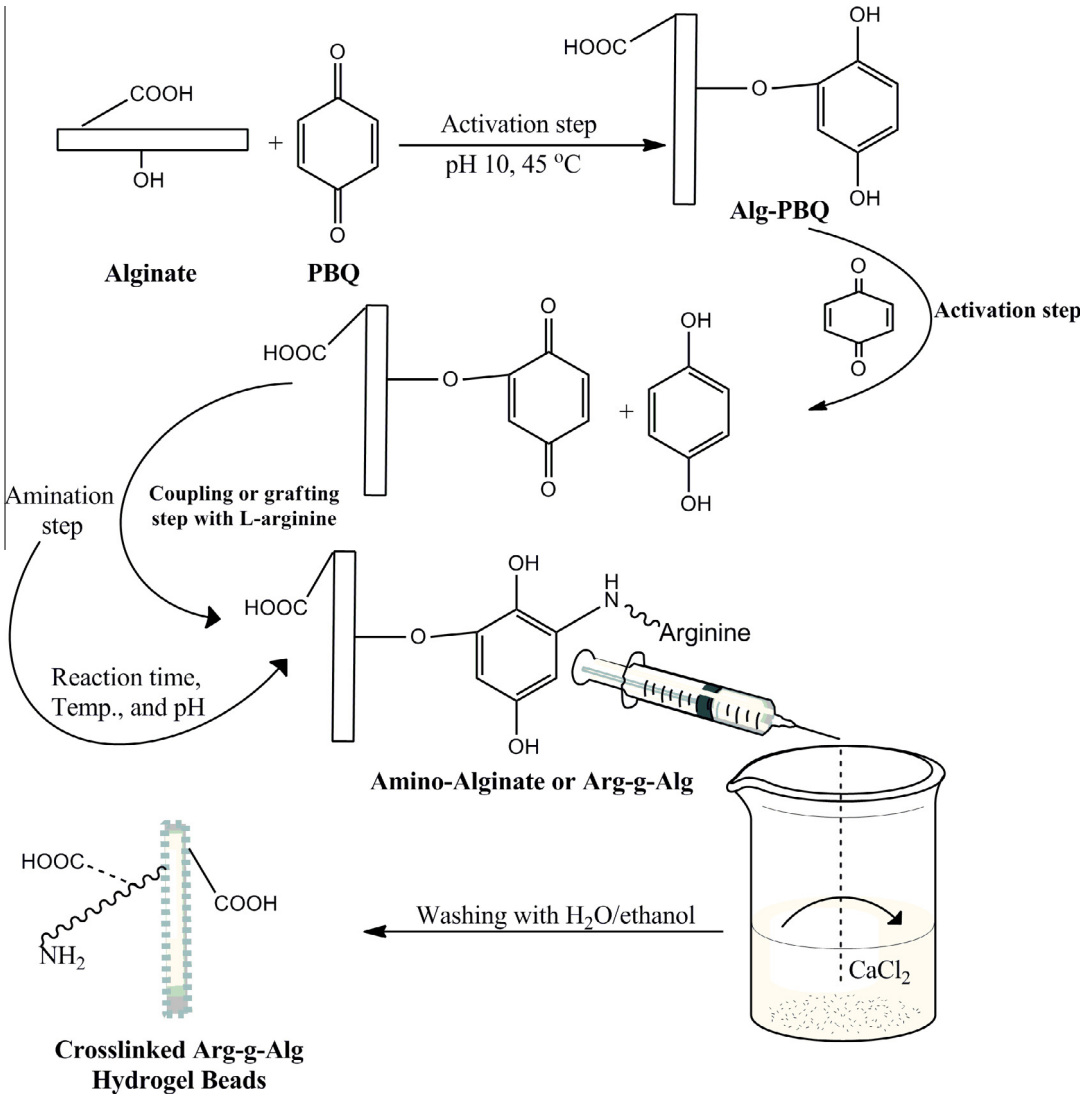


Figure 2 General Strategy for the synthesis route of crosslinked (Arg-g-Alg) hydrogel beads, including activation of alginate using PBQ, coupling of Alg-PBQ with L-Arg, and crosslinking step using $CaCl_2$ solution.

~ 140 mg:2 mg) respectively, and pressed by applying a force 105 N into transparent disk (maximum disk weight ~150 mg) with a diameter 13 mm. All samples were measured in absorbance mode.

2.7.2. SEM

The surface morphology structure of the Arg-g-Alg hydrogel samples was investigated by Analytical-SEM (type: JEOL, JSM-6360LA, Japan) with 15 kV voltage for secondary electron imaging. The hydrogel samples were first soaked in deionized water (after dissolution in medium pH of 7.5) for 24 h to bulge the internal channels and remove any impurities as well. The samples were then dehydrated by suddenly-freezing using liquid nitrogen followed by lyophilization conditions at −90 °C under 0.5 mbar, and coated with Au using an ion sputter coater in (model: 11430, USA, combined with vacuum base unit or SPi module control, model: 11425, USA).

2.7.3. TGA and DSC

The thermal properties of vacuumed-dried crosslinked neat alginate hydrogel beads (Alg.), and crosslinked alginate-grafted-arginine (Arg-g-Alg) hydrogel beads, have been accomplished using TGA and DSC thermogram analysis. The thermogravimetric analysis (TGA) was performed on a TGA instrument (Shimadzu TGA–50, Japan) from 50 to 600 °C at a heating rate 10 °C/min. The degradation temperature (T_d), and onset temperature (T_{onset}) were determined by TGA thermograms. T_{onset} is defined as the temperature at the intersection of the baseline mass and tangent drawn to the mass curve at the inflection point or point of greatest rate of mass loss% (Kamoun and Menzel, 2010).

Glass transition temperatures (T_g) of dried hydrogel were determined using a differential scanning calorimeter, DSC (Shimadzu, DSC 60-A, Japan). All measurements were made at a heating rate of 5 °C min from 25 to 220 °C under nitrogen. The T_g, and T_{onset} temperatures were measured by DSC

thermograms. T_g values were determined as mid-point in the thermograms, as measured from the extensions of the pre- and post-transition baselines.

3. Results and discussion

3.1. FTIR-analysis

Fig. 3 shows the FT-IR spectra of materials including sodium alginate (Alg), p-benzoquinone (PBQ), L-arginine (Arg), and uncrosslinked alginate-graft-arginine (Arg-g-Alg). The characteristic peaks of alginate appeared at 3450, 1630, and 1417 cm, corresponding to hydroxyl (–OH), carbonyl (C═O), and carboxyl (COOH) groups, respectively, (Fig. 3, Alg) while, two bending peaks of carbonyl (C═O) and benzene ring have been detected at 1624, 1467, and 1213 cm, respectively for p-benzoquinone, (Fig. 3, PBQ). In the same context, peaks at 3316, 3074, 1634, and 1145 cm, corresponding to two amine groups, carbonyl, and imine (C═N) linkage, respectively for L-arginine, are shown in (Fig. 3, Arg). In order to confirm the interaction or grafting between alginate and L-arginine, IR spectra of uncrosslinked alginate-graft-arginine were displayed in Fig. 3, Arg-g-Alg. It was easy to notice that bending peak at 3420 cm corresponds to free hydroxyl (–OH) and amino ($-NH_2$) groups for alginate and L-arginine, respectively. Evidently, imine (C═N) and (C–O–) stretching peak was found simultaneously at 1064 cm for L-arginine and linkage of Alg-PBQ. Also, carbonyl (C═O) and carboxyl (COOH) stretching peaks were observed at 1621 and 1422 cm

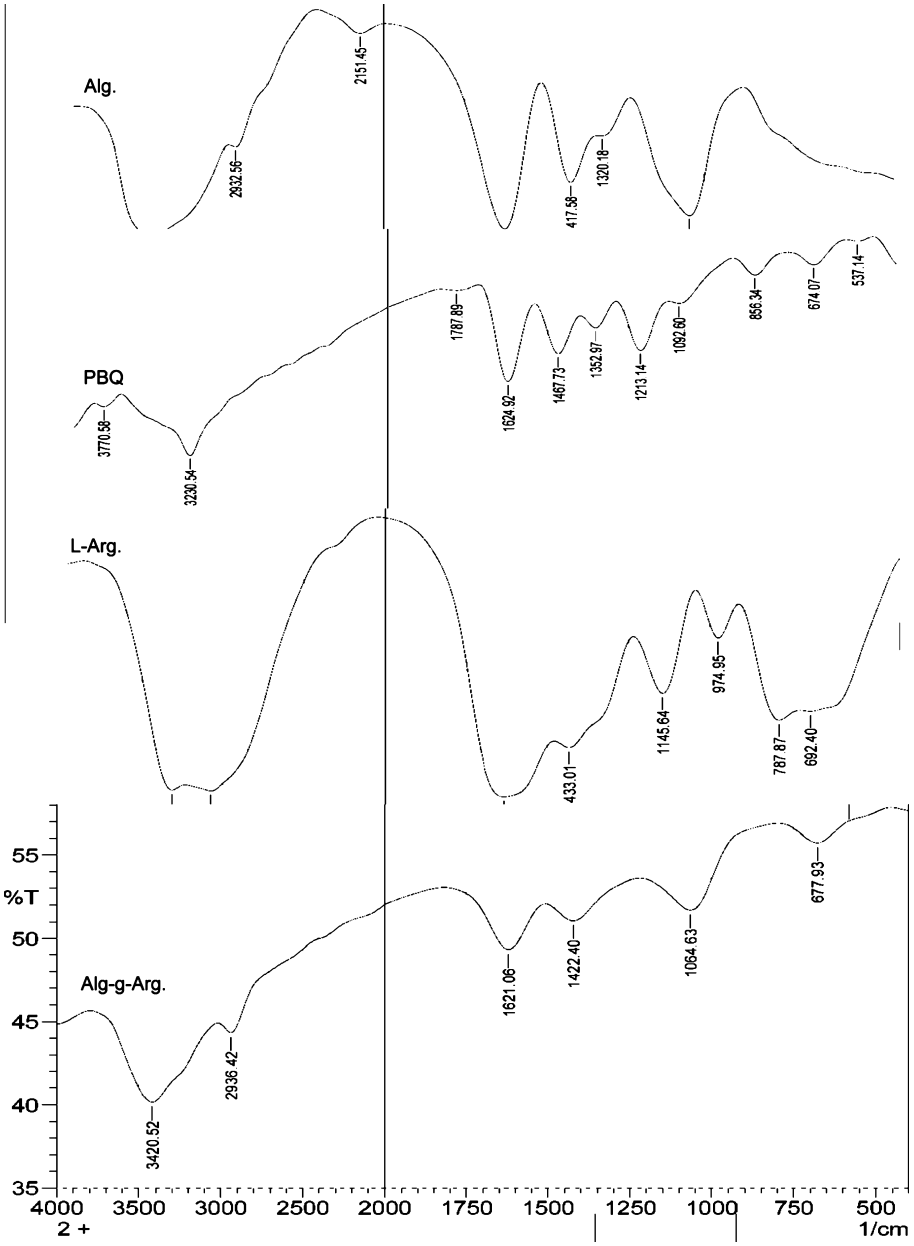


Figure 3 FTIR spectra of neat sodium alginate (Alg.), p-benzoquinone (PBQ), L-arginine (L-Arg.), and uncrosslinked L-arginine-graft-alginate (Arg-g-Alg).

respectively, corresponding to alginate and alginate/L-arginine. According to FT-IR analysis, it is likely that prorogated amines on L-arginine interacting with carboxylate on alginate and linkages of Alg-PBQ and Alg-PBQ-Arg were verified too.

3.2. Synthesis of Arg-g-Alg and grafting efficiency estimation

The grafting efficiency (GE%) has been studied through monitoring effective factors e.g. L-arginine concentration, pH of grafting reaction, time, and temperature of grafting reaction. Thus, it is of importance to display the GE% variation of Arg-g-Alg polymers, the obtained data have been displayed in Fig. 4a–d. It was observed that GE% of L-arginine onto polysaccharide chains of alginate after coupling with PBQ, dramatically increased with increasing L-arginine concentration, which has been used as a modifier agent, while pH, time, and temperature of grafting reaction are kept constant, (Fig. 4a). The progressive increase in trend of GE% may be due to the fact that an increase in the L-arginine concentration decreases the chance of formation of multi-attachment covalent bonds with L-arginine molecules as a modifier agent (Eldin, 2011), which increases the number of attached L-arginine molecules onto alginate polymer side chain. The maximum GE% (i.e. 65%) was detected at the highest arginine concentration of 5%, (w/v). This leads to both GE% and grafting behavior that tend to grow sharply. Similarly, relative lower rate of GE% progressively enhanced with increasing the time and temperature of grafting reaction, (Figs. 4c and d, respectively) where the maximum GE% reached to 40%, 40%, and 48% for grafting pH 10, grafting reaction time for 75 min, and grafting reaction temperature at 60 °C, respectively. This behavior is due to the faster diffusion and the mobility of the polysaccharide chains. Additionally other reactants from the aqueous phase to form side chains, can be easily detected with an increase in either time or temperature of grafting reaction up to certain limit. Expectedly, the result of GE% has somewhat the same trend with pH variation but with higher rate, where GE% recorded exponential increment and shows

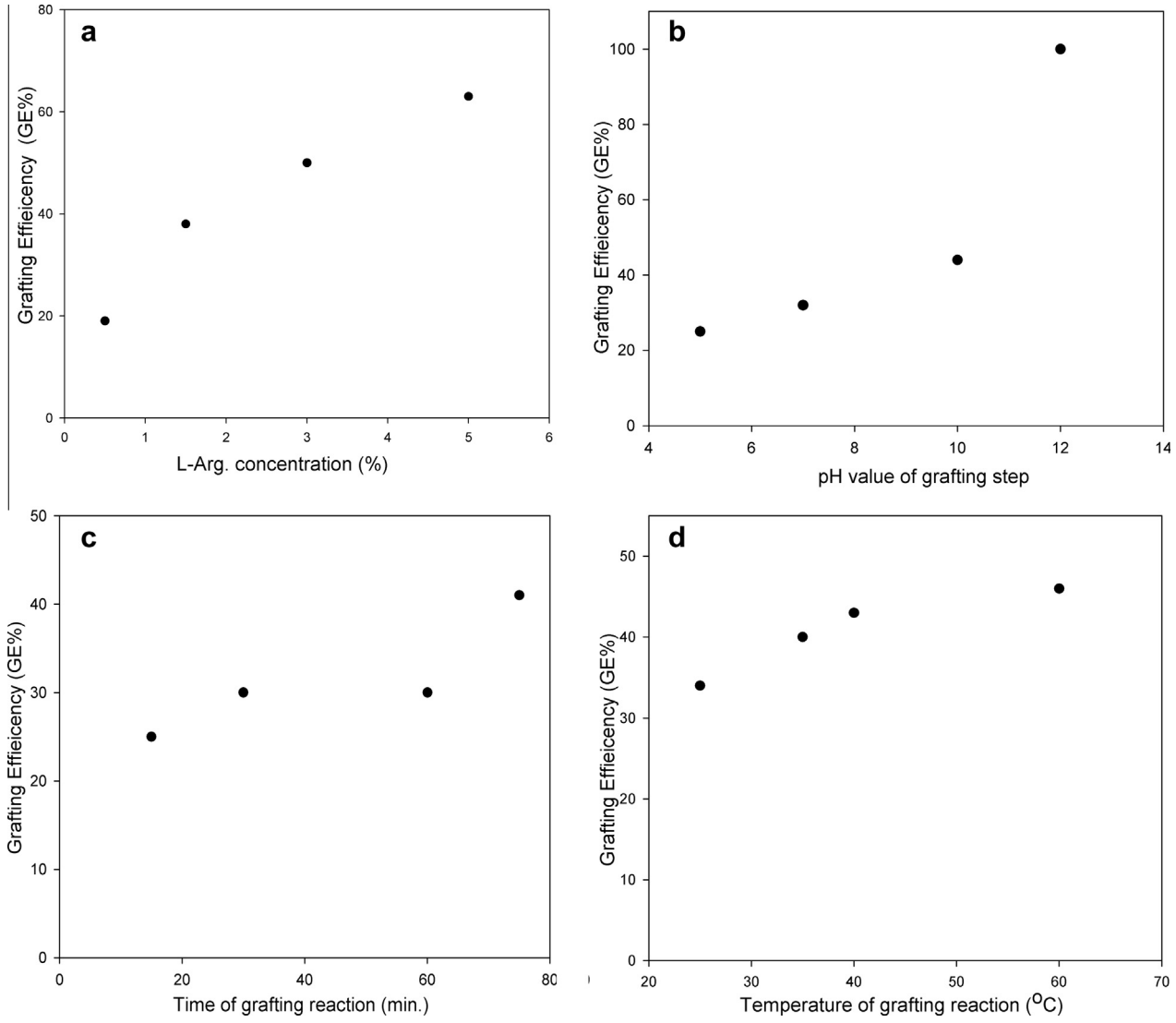


Figure 4 Grafting efficiency percentage (GE%) of obtained Arg-g-Alg uncrosslinked polymer as a function of L-arginine concentration (a), pH value (b), time (c), and temperature of grafting reaction (d). Grafting conditions look Section 2.2.2.

the highest value at pH 12 which is higher than the value of isoelectric point of L-arginine, (Fig. 4b). This behavior can be attributed to the effect of grafting reaction pH which has a strong influence on protonation (NH_3) or de-protonation of free amine groups of arginine, and consequently on its covalent binding with PBQ centers over activated alginate. The obtained results are in agreement with other published results (Mohy Eldin, 2011).

3.3. Thermal properties

The thermal properties of both crosslinked neat alginate and crosslinked arginine-graft-alginate micro-bead xerogels (Arg-g-Alg) have been conducted by TGA and DSC instruments. The thermo-gram data have been depicted in Table 1. According to TGA results, it is evident that the pure alginate shows a weight loss in three thermal-degradation stages. The first degradation stage ranges from 44 to 120 °C and shows about 15% weight loss, which corresponds to the loss of residual or absorbed moisture. However, the first degradation stage of grafted alginate ranges with higher temperature at 89–200 °C and weight loss reached to lower weight 8%. This thermal behavior reveals that graft copolymer alginate is resistant to moisture absorption owing to introduction of bulky structure onto alginate backbone chains.

The second degradation stage referred to as a pyrolysis stage starts at 120 °C and continues up to 276 °C with weight loss 32%, for pure alginate, which corresponds to degrade the macromolecular chains. Whereas, in case of grafted alginate, second pyrolysis stage starts with higher temperature at 200 °C up to 305 °C with mass loss of 34% which corresponds to degrade some un-grafted alginate. The third degradation stage of both alginate and grafted alginate starts at 276–310 °C and 305–500 °C, respectively. In the latter degradation stage, the remained polymer mass is completely thermally-degraded as a result of carbonization or volatilization. On the other hand, T_{onset} values are significantly enhanced with grafting alginate. It is also evident from TGA data that, the degradation temperature at 50% weight loss of grafted alginate is 355 °C, which is higher than that of pure alginate at 282 °C. The presented thermogravimetric data of TGA illustrate that the thermal stability of alginate-g-arginine has been improved by the grafting process. Finally, T_g and T_{onset} extracted from DSC-results confirmed the same thermal stability behavior, where they showed a significant enhancement in the thermal transition behavior, particularly after alginate grafting with L-arginine, (Table 1). In addition introduction of grafted side chains and Ca ions as crosslinkers has tremendous impact to improve T_g values of neat alginate xerogels from 91 to 190 °C of grafted alginate xerogels, (Table 1).

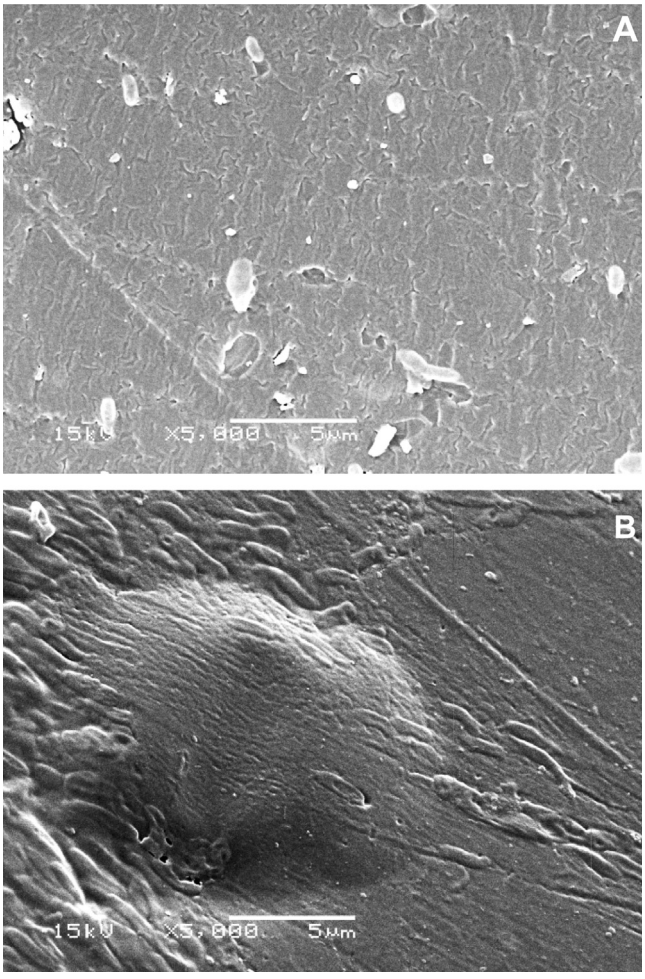


Figure 5 SEM micrographs of crosslinked alginate hydrogel beads (A), and crosslinked (Arg-g-Alg) hydrogel beads (B).

3.4. Morphological investigation of Arg-g-Alg hydrogel beads

The scanning electron micrographs of pure alginate hydrogel beads and arginine-g-alginate (Arg-g-Alg) hydrogel beads are presented in Fig. 5. It is obvious that the morphological surface of pure alginate beads is very rough, rugged, and uniform in shape–structure. Conversely, the surface of grafted alginate beads tends to be smoother, somewhat fibrillar-shape structure, and has almost turned into an entirety, which may be ascribed to the introduction of arginine onto the alginate side chains.

Table 1 Thermal properties of crosslinked neat alginate xerogel beads (Alg.), and crosslinked alginate-grafted-arginine (Arg-g-Alg) xerogel beads according to the DSC and TGA thermogram results.

Sample	DSC results		TGA results		T_d of (Mass loss 50%),C	T_{onset}, °C
	T_g, °C	T_{onset}, °C	T_d, in the second degradation stage, °C (mass lose%)	T_d, in the first degradation stage, °C, (mass lose%)		
Alg	91	40	44–120, (15%)	120–276, (32%)	282	114
Arg-g-Alg	190	179	89–200, (8%)	200–305, (34%)	355	190

3.5. Swelling study of Arg-g-Alg hydrogel beads

The Arg-g-Alg hydrogel crosslinked beads were allowed to swell in 50 mL of tris–HCl buffer solution pH 7.2 at 37 °C. The water uptake percentage, (WU%) of Arg-g-Alg hydrogel beads was compared as a function of different grafting reaction conditions, such as L-arginine concentrations (a), pH (b), time (c), and temperature (d) of grafting reaction. WU% values are shown in Fig. 6. In Fig. 6a-d, one factor was varied for studying with WU%, while other variables were kept constant.

In Fig. 6a, the grafting using L-arginine has a significant impact on WU%, since the whole Arg-g-Alg samples were swelled promptly with a higher water uptake than neat alginate beads (0% of L-Arg). Whereas, WU% values increased monotonically with increasing grafted L-arginine concentration up to 3%. The increase of WU% as L-arginine concentration increased which can be ascribed to the fact that incorporation of further hydrophilic groups like $–NH_2$ and –COOH of L-arginine structure might increase WU% values till certain concentration. Notably, Arg-g-Alg hydrogel samples which have been grafted in different pH mediums, have progressive WU% increment with pH of grafted medium and got the maximum value at pH 2 and 12, (Fig. 6b). This is due to grafting of higher amounts of L-arginine as previously confirmed by GE% results in Fig. 4b. This is due to protonation of primary amino groups ($–NH_3$) on Alg-g-Arg polymer hydrogel network. While, no big differences were detected in the SW% of Arg-g-Alg hydrogels, these differences were limited for polymers grafted in neutral pH medium (pH 5, 7 and 10), which might be due to formation of hydrogen bonds between (–COOH) of L-arginine and (–OH) groups of alginate, which somewhat restrict the limit of swelling of hydrogel network. Furthermore, these results were previously confirmed by GE% results in Fig. 4b, where up to 5% (w/v) of L-arginine concentration the GE% is increased.

In Fig. 6c and d it can be seen that, the swelling ability of Arg-g-Alg hydrogels beads was apparently improved by an increase in either time prolonged up to 75 min or temperature

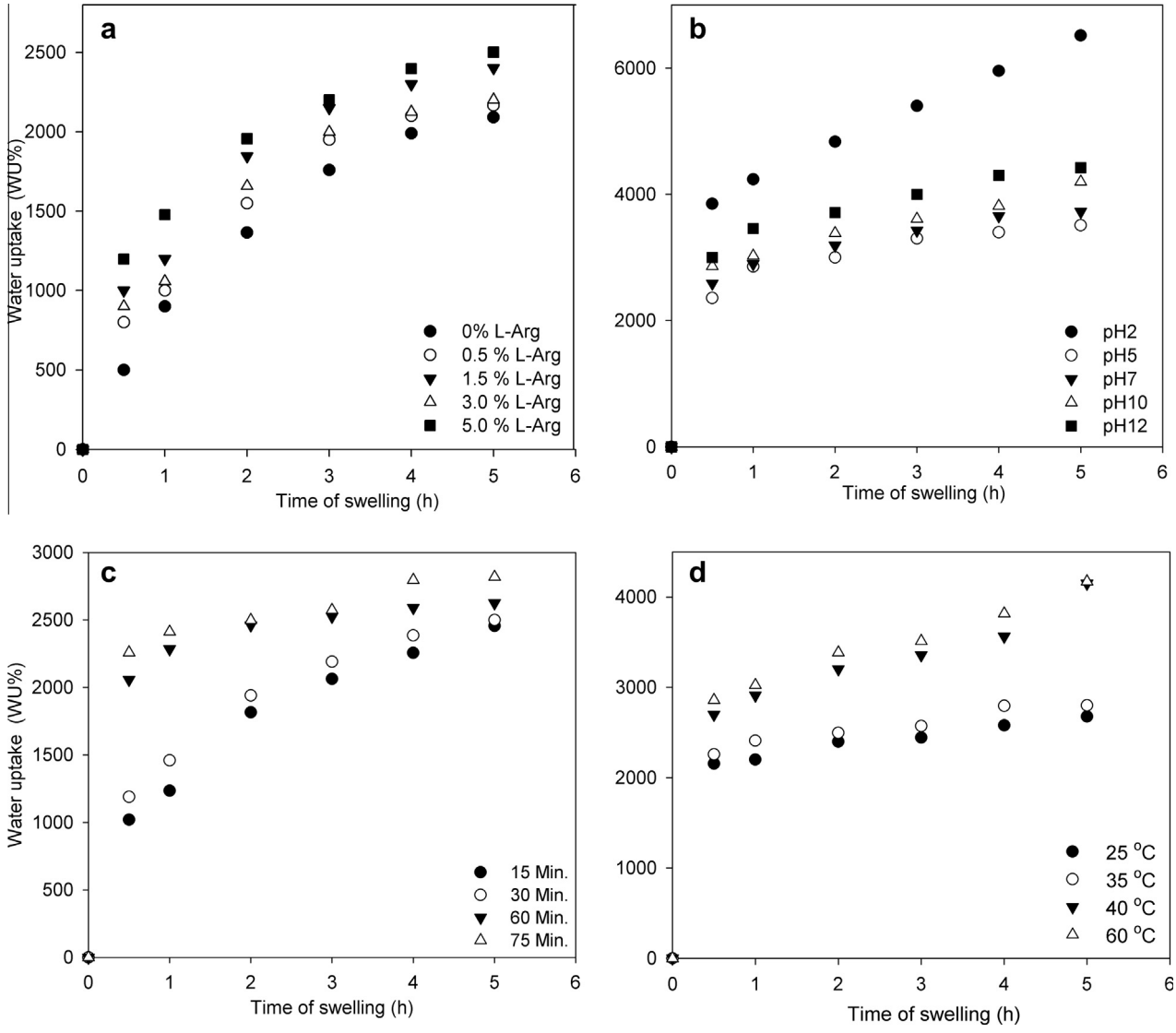


Figure 6 Swelling characteristics of (Arg-g-Alg) hydrogel beads as a function of polymers have different grafting conditions e.g. L-arginine concentration (a), pH (b), time (c), and temperature of grafting reaction (d); (swelling media: tris–HCl buffer solution pH 7.2, at 37 °C), (hydrogel beads samples have been crosslinked by 3% of $CaCl_2$, for 10 min., at RT).

reaching 60 °C of grafting reaction. It is likely that, WU% values increase steadily as time and temperature of grafting reaction increase is due to the increase of the time and temperature grafting reaction might allow further L-arginine amount to graft which in turn increases the swell-ability characteristics of grafted polymers. Additionally, these results are fully consistent with the obtained results of GE% (Fig. 4c and d).

3.6. In vitro release profile of BSA from Arg-g-Alg hydrogel beads

Fig. 7 shows the BSA release profiles *in vitro* from the Arg-g-Alg hydrogel beads obtained with different grafting reaction variables e.g. L-arginine concentrations (Fig. 7a), grafting times (Fig. 7b), and grafting temperatures (Fig. 7c). The release profiles were carried out in buffer solution at 37 °C, as used in swelling test formulation (Section 2.4). Generally, the cumulative BSA released from Arg-g-Alg hydrogel beads are depending outright upon the comparatively swelling ability of hydrogels, regardless the kind of grafting reaction variable. Accordingly, the amount of released BSA increases feasibly with increasing the swelling characteristic of hydrogel network. Similarly, the same result trend which has been obtained in Fig. 6a is consistent completely with the current release results in Fig. 7a, where the amounts of released BSA from Arg-g-Alg hydrogels increase, as L-arginine concentration increased in the grafting reaction of Arg-g-Alg (Fig. 7a). In the same way, the amounts of BSA profiles released from Arg-g-Alg hydrogel beads increase progressively whether with prolonging the grafting reaction time (Fig. 7b), or high temperature degrees of grafting reaction (Fig. 7c). This may correspond to the release profile that has strong relativity to GE% results in Fig. 4a and swelling ability of hydrogels as presented in Fig. 6c and d, where the maximum cumulative BSA released from Arg-g-Alg hydrogel beads, was obtained from polymers grafted with the highest L-arginine concentration (5%, w/v). This may correspond to; the release profile has strong relative with swelling ability of hydrogels as presented in Fig. 6c and d. This increase can be further explained by "diffusion-effect", where swelling degree and GE% are increased; the diffusion or profile of release must improve forward release behavior.

For further simulating the gastrointestinal tract (GIT) conditions, the release profiles were evaluated in pH 2 for neat alginate hydrogel beads and grafted alginate hydrogel beads (Arg-g-Alg) simulating gastric media, while at pH 6.5 and 7.5 for only grafted alginate hydrogel beads simulating intestinal media, the total release profiles in GIT are shown in Fig. 8.

The results showed obviously that, the grafting of L-arginine onto alginate has a prominent impact to change the release profile behavior as compared to that of neat alginate hydrogel beads. The maximum BSA released from neat alginate hydrogel is about 15% in the first 15 min of release time

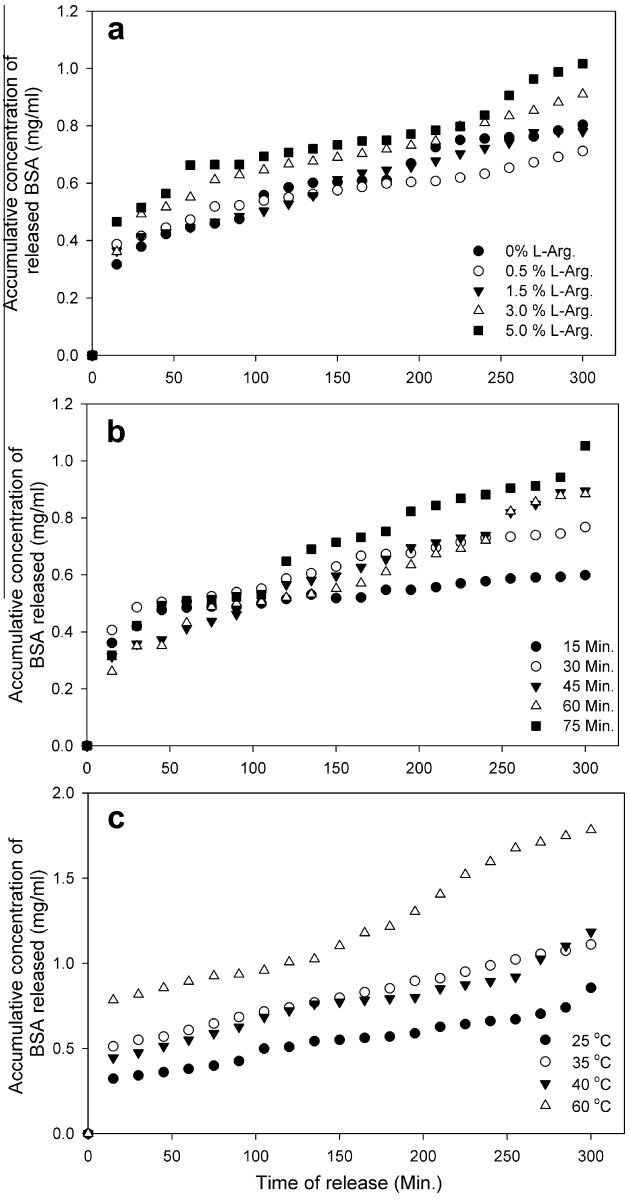


Figure 7 BSA release profiles from (Arg-g-Alg) hydrogel beads as function of polymers have different grafting conditions e.g. L-arginine concentration (a), time (b), and temperature of grafting reaction (c); (hydrogel beads samples have been crosslinked by 3% of $CaCl_2$, 10 Min., at RT).

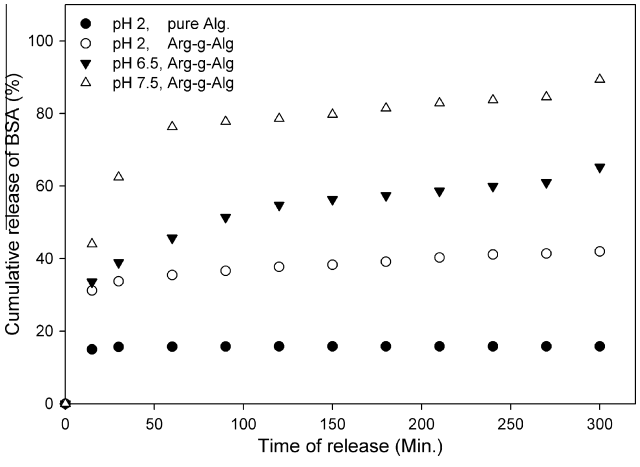


Figure 8 BSA release profile from pure alginate and (Arg-g-Alg) hydrogel beads at different pH values at 37 C for simulating gastric media; (grafting conditions: 3% L-Arg., pH 2, at 60 °C for 75 min.), (crosslinking conditions: 3% of $CaCl_2$, 10 min., at RT).

as called "*burst effect*", [30] and no further BSA release was detected after the burst effect time in pH 2 as release media. On the other hand, higher amount of BSA released from the grafted alginate hydrogels as the burst effect, is about 45%, at the same pH of release media i.e. pH 2. This implies that incorporation of amino groups of arginine during grafting reaction into alginate structure has enhanced its release behavior specifically in acidic media at pH 2, for about triple of release value of (300%). Thus we can report that, the grafted alginate has created a noteworthy improvement in the release profile particularly in acidic media due to incorporation of amine groups in the side chain. Expectedly, the release profiles of BSA from Arg-g-Alg hydrogel beads increase as pH release media tends to alkaline ranges at pH 6.5 and pH 7.5 are almost 66% and 90% respectively, due to the main structure of grafted alginate is carrying –COOH of alginate and little –COOH groups of arginine which have featured characteristics toward the swelling ability, diffusion, and significant release profile in alkaline media conditions.

4. Conclusions

In conclusion, graft polymers of L-arginine onto alginate using different grafting conditions and variables can be carried out successfully. The proof of grafting was verified from IR analysis. The aforementioned results indicated that the grafting efficiency of Arg-g-Alg can be easily adjusted by grafting reaction variables. As shown, increase of L-arginine concentration, pH, time, and temperature of grafting reaction, increase the grafting efficiency and swelling ability of obtained crosslinked Arg-g-Alg hydrogels. Grafting reaction has a clear influence on thermal stability and morphological structure of crosslinked Arg-g-Alg when compared with neat alginate hydrogel beads. Grafted alginate polymers showed an excellent thermal stability; however neat alginate hydrogel beads showed a humble thermal stability. In addition, the grafting via incorporation L-arginine onto alginate backbone changed entirely the release profile of neat alginate hydrogels particularly in acidic media, where Arg-g-Alg showed sufficient release profile which is about 300% in acidic media as compared to pure alginate hydrogel beads. Finally, the results clearly suggested that Arg-g-Alg hydrogel beads could be a suitable polymeric protein carrier for site-specific and dual-gate of acidic and alkaline pH mediums in the gastrointestinal tract system.

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