International Journal of Pharmaceutics 371 (2009) 16–24

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Preparation of oxidized sodium alginate-graft-poly((2-dimethylamino) ethyl methacrylate) gel beads and *in vitro* controlled release behavior of BSA

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ARTICLE INFO

Article history:
Received 18 June 2008
Received in revised form 7 November 2008
Accepted 11 December 2008
Available online 24 December 2008

Keywords:
Sodium alginate
Poly((2-dimethylamino) ethyl methacrylate)
Oxidation
Protein delivery

ABSTRACT

Graft copolymerization of amino group-terminated poly((2-dimethylamino) ethyl methacrylate) (PDMAEMA-NH_2) onto oxidized sodium alginate (OSA) was reacted without using a catalyst. The structure of the graft was investigated by Fourier transform infrared (FT-IR) spectroscopy. The OSA-g-PDMAEMA gel beads were prepared by dropping the aqueous solution of the graft copolymer into $CaCl_2$ aqueous solution. The effects of pH and ionic strength on the swelling behaviors of the gel beads were studied. The results indicate that the gel beads have pH and ionic strength sensitivity. Bovine serum albumin (BSA) was entrapped in the beads and the *in vitro* drug release profiles were established in buffer solution with pH 1.8 (HCl), pH 7.4 (KH_2PO_4–NaOH), and 0.9% (w/v) NaCl at 37 °C. The results showed that the oral delivery of proteins can be controlled by adjusting the graft percentage (*G*, %), pH and ionic strength. According to this study, the OSA-g-PDMAEMA gel beads could be suitable for the oral delivery of proteins.

1. Introduction

Alginate is an anionic linear polysaccharide composed of (1,4)-linked β-D-mannuronate (M) and its C-5 epomer α-L-guluronate (G) residues arranged in a nonregular blockwise pattern along the linear chain (Liu et al., 2008; Tomme et al., 2008). The most attractive property of alginate is the gel formation simply induced by adding various divalent cations, except Mg^{2+} (Liu et al., 2003). Alginate gel beads are commonly formed by dropping sodium or potassium alginate solution into an aqueous solution of Ca^{2+} typically made from calcium chloride ($CaCl_2$) (Chretien and Chaumeil, 2005; Pasparakis and Bouropoulos, 2006; Sriamornsak and Kennedy, 2006). As the encapsulation method is mild, and done at room temperature in aqueous medium, several sensitive drugs (Grassi et al., 2001; Kim et al., 2005), proteins (Xu et al., 2007), living cells (Stabler et al., 2001), etc. have been successfully released through alginate beads.

However, the only enzyme known to degrade alginate is alginase, a bacterial enzyme which is not synthesized by humans or animals (Bouhadir et al., 1999). So alginate gels are not chemically broken down in mammals. Alginate gel degradation occurs *in vivo*, mainly due to the sensitivity of the gel towards calcium chelating compounds (e.g., phosphate, citrate, and lactate) (Boontheekul et al., 2005). The alginate gels have uncontrollable degradation kinetics and gels dissolve in an uncontrollable manner following the loss of divalent cations releasing high and low molecular weight alginate units (Balakrishnan and Jayakrishnan, 2005). One previously reported approach to control alginate gel degradation involved partial periodate oxidation. Oxidized alginates present more reactive groups and a faster degradation when they are used in supports for drug controlled delivery (Boontheekul et al., 2005; Kong et al., 2004). This approach offers control over the degradation rate by varying the degree of oxidation, as increasing the degree of oxidation can accelerate the rate of degradation.

Poly((2-dimethylamino) ethyl methacrylate) (PDMAEMA) is a water-soluble polymer containing tertiary amino groups which can be protonized in acidic solution (Zha et al., 2002). PDMAEMA has been also shown to be temperature-sensitive (Zhang et al., 2006; Xu et al., 2006). The lower critical solution temperature (LCST) of PDMAEMA is about 45 °C at pH 8.5 and higher than 50 °C at pH 7.0 (Amalvy et al., 2004; Liu and Armes, 2001; Li et al., 2003). It is impractical to utilize such LCST in biomedical applications. Thus, PDMAEMA is often used to prepare pH-sensitive materials, which can be used as controlled drug delivery system (Traitel et al., 2000; Basan et al., 2002), gene transfer agent (Van de Wetering et al., 2000; Kurisawa et al., 2000), etc.

In this study, amino group-terminated PDMAEMA-NH_2 was grafted onto oxidized sodium alginate, and the beads of OSA-g-PDMAEMA were prepared in an aqueous solution of Ca^{2+}. The main idea is that the side chain, PDMAEMA, is a cationic polyelectrolyte for slow rate of drug release. The releases from beads were observed at 37 °C. BSA was used as a model drug.

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doi:10.1016/j.ijpharm.2008.12.013

2. Materials and methods

2.1. Materials

Sodium alginate (the viscosity of 2% solution is 3200 mPa s at 25 °C) was obtained from Qingdao Haiyang Chemical Co. (China). (2-Dimethylamino) ethyl methacrylate (DMAEMA, Acros, 99%) was distilled under vacuum. 2-Aminoethanethiol hydrochloride (AESH-HCl) was purchased from Aldrich. N,N-Azobisisobutyronitrile (AIBN) was recrystallized with 95% ethanol. The other reagents were A.P. grade and used without further purification.

2.2. Synthesis of amino group-terminated PDMAEMA (PDMAEMA-NH_2)

Amino group-terminated PDMAEMA was synthesized as described (Ju et al., 2001). DMAEMA (5 mL), AESH-HCl (5.9×10^{-4} mol), AIBN (2.95×10^{-4} mol) were dissolved in N,N-dimethylformamide (DMF) (2.5 mL). Dried nitrogen was bubbled into the solution for 30 min to remove oxygen. Polymerization was carried out at 70 °C for 6 h. And then, the reactant was precipitated into an excess of petroleum ether. The product was dissolved in distilled water, precipitated in hot water, and dried to constant weight under vacuum at room temperature.

2.3. Preparation of oxidized sodium alginate (OSA)

Partially oxidized sodium alginate was prepared according to previously reported method (Balakrishnan and Jayakrishnan, 2005). Into 10 g sodium alginate dispersed in 80 mL ethanol, sodium periodate (3.2408 g) in 80 mL distilled water was added and stirred magnetically in the dark at room temperature for 6 h to obtain OSA. An equimolar amount of ethylene glycol to periodate was added after 6 h to stop the oxidation reaction. The resultant solution was filtered, washed with ethanol/water (1:1, v/v) and dried under vacuum at room temperature.

The degree of oxidation (DO, %) was defined as the number of oxidized guluronate residues per 100 guluronate units, and followed by determining the concentration of periodate left unconsumed by iodometry after 6 h (Balakrishnan and Jayakrishnan, 2005). Before adding the quencher into oxidation reaction, a 5 mL aliquot of the reaction mixture was neutralized with 10 ml of saturated sodium bicarbonate solution. Iodine was liberated by the addition of 20% (w/v) potassium iodide solution (2 mL). This was kept under dark for 15 min and the liberated iodine was titrated with standardized sodium thiosulphate solution using starch as the indicator. The DO value of OSA was determined by the following equation (Wu et al., 2007):

$$\mathrm{DO}\,(\%) = \frac{198n}{m} \times 100 \tag{1}$$

where m is the weight of sodium alginate (g), 198 is the molecular weight of sodium alginate unit (g/mol), n is the consumed amount of substance of periodate (mol).

The molecular weights of sodium alginate and OSA were calculated from viscosity measurements. The viscosity measurement was performed at 30 °C with Ubbelohde viscometer. Aqueous solutions of 0.2% (w/v) sodium alginate and OSA with 0.1 M sodium chloride were used. Viscosity-average molecular weight (M_η) was calculated by the following equation (Smidahod and Haug, 1968):

$$[\eta]\,(100\,\mathrm{mL/g}) = 2.0 \times 10^{-5}\,\mathrm{M}_\eta \tag{2}$$

Table 1
Preparation of graft copolymers and their graft percentage.

Sample	OSA (g)	PDMAEMA-NH_2 (g)	N (wt%)	G (%)
OSA	2.0	0	0	0
OSA-g-PDMAEMA (9:1)	1.8	0.2	0.34	4.55
OSA-g-PDMAEMA (7:3)	1.4	0.6	0.96	17.73
OSA-g-PDMAEMA (5:5)	1.0	1.0	1.71	37.87

2.4. Preparation of OSA-g-PDMAEMA copolymer

To form Schiff's base between aldehyde group in OSA and amino group in PDMAEMA-NH_2, OSA and PDMAEMA-NH_2 with various proportions (the mass ratio of OSA to PDMAEMA-NH_2 were 9:1, 7:3, 5:5, respectively) were respectively dissolved in water (the total volume was 50 mL). The solution mixture was continuously stirred at room temperature for 24 h. The graft copolymer was purified by precipitation with the addition of ethanol. The production was again dissolved in water and re-precipitated with ethanol. Finally, the precipitate was washed with ethanol and freeze-dried. The composition and designation of each sample are listed in Table 1.

2.5. Characterization

FT-IR spectra of sodium alginate, OSA, PDMAEMA-NH_2 and OSA-g-PDMAEMA were taken in KBr pellet using Nicolet NEXUS 670 FT-IR Spectrometer. The ^{1}H NMR spectrum of PDMAEMA-NH_2 was investigated on Mecury Plus-300 ^{1}H NMR instrument. Elemental analysis of nitrogen by combustion method was used to determine the graft percentage (G, %) of OSA-g-PDMAEMA. The G (%) is calculated from the following equation:

$$G\,(\%) = \frac{W_\mathrm{p}}{W_\mathrm{o}} \times 100 \tag{3}$$

where W_p and W_o are the weights of PDMAEMA-NH_2 and OSA, respectively.

2.6. Preparation of OSA and OSA-g-PDMAEMA gel beads

OSA (0.5 g) was dissolved in 10 mL distilled water. The solution was dropped through 0.9 mm in diameter syringe needle into the 30 mL solution of 2% (w/v) calcium chloride at a flow rate of 1 mL/min. The smooth and spherical beads were formed under magnetic stirring for 30 min. The beads were washed three times with distilled water and dried under vacuum at room temperature. The OSA-g-PDMAEMA gel beads were prepared as the same processes.

2.7. Preparation of the BSA loaded gel beads

BSA was loaded by dissolving it in OSA or OSA-g-PDMAEMA aqueous solution, up to a concentration of 1 mg/ml and subsequently crosslinking during beads synthesis. The other processes were the same as Section 2.6 preparation of OSA and OSA-g-PDMAEMA gel beads.

2.8. Equilibrium swelling ratio studies

The dried beads were immersed in different buffer solutions with constant ionic strength (I = 0.1 M) at 25 °C. The ionic strength of each buffer solution was kept constant at 0.1 M using NaCl. Different formulations of buffer solutions at a wide range of pH values were prepared as described (Chen et al., 2008). The weight of equilibrium swollen samples was measured after removing the water on their surface with filter paper. The equilibrium swelling ratio

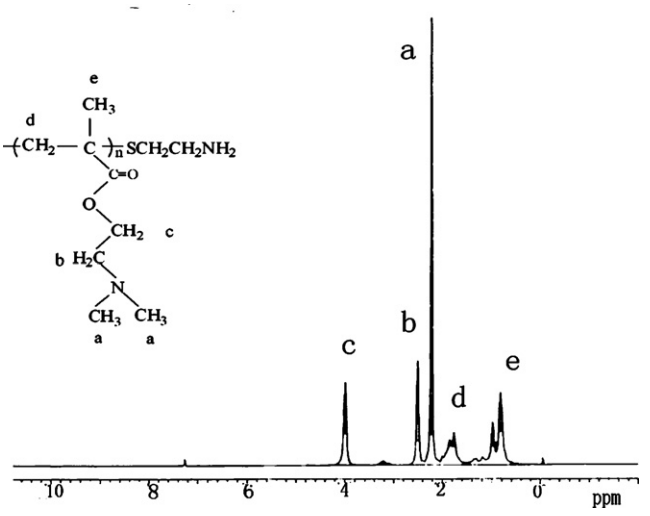


Fig. 1. The ^{1}H NMR ($CDCl_3$) spectrum of PDMAEMA-NH_2.

(ESR) was defined as:

$$\mathrm{ESR} = \frac{W_\infty - W_\mathrm{d}}{W_\mathrm{d}} \quad (4)$$

where W_∞ and W_d are the weight of equilibrium swollen and dried beads, respectively.

The dried beads were also immersed in NaCl aqueous solution with different ionic strengths at 25 °C. The ionic strength of the medium was altered by changing the amount of NaCl. The ESR was determined by the above procedure.

2.9. Morphology observation

The surface and cross-section morphology of OSA and OSA-g-PDMAEMA beads were examined using scanning electron microscopy (JSM-5600LV SEM, Japan) at an accelerating voltage of 20 kV. The used beads were freeze-dried for 12 h on LABCONCO freeze-dried system (England) after swelling 1 h in buffer solution with pH 6.0 (KH_2PO_4–NaOH).

2.10. In vitro drug release

Release studies were carried out in three different media: buffer solutions with pH 1.8 (HCl), pH 7.4 (KH_2PO_4–NaOH) and 0.9% (w/v) NaCl. *In vitro* BSA release profiles of the beads were determined as follows: the BSA loaded beads were placed into conical flask with 50 mL release medium and incubated at 37 °C. At appropriate intervals, 5 mL of the solution was replaced by fresh medium. The amount of BSA released from the beads was evaluated at 280 nm using a PerkinElmer Lamda 35 UV–visible spectrophotometer (USA). The percent released of BSA is defined as the percentage of the released amount on the basis of the total amount entrapped in gel beads.

3. Results and discussion

3.1. Characterization of PDMAEMA-NH_2

Fig. 1 shows the ^{1}H NMR spectrum of PDMAEMA-NH_2, the signals at 2.2 and 2.5 ppm are attributed to the methyl and the methlyene groups on the tertiary amine, respectively. The signal at 4.0 ppm is assigned to the methylene group adjacent to the carboxylate group of PDMAEMA-NH_2. The signals in the region of 3.0–3.4 ppm correspond to the terminal amide protons. From these results, we could confirm the synthesis of PDMAEMA-NH_2. The

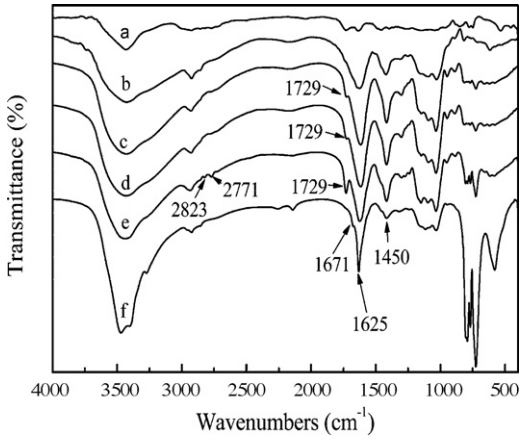


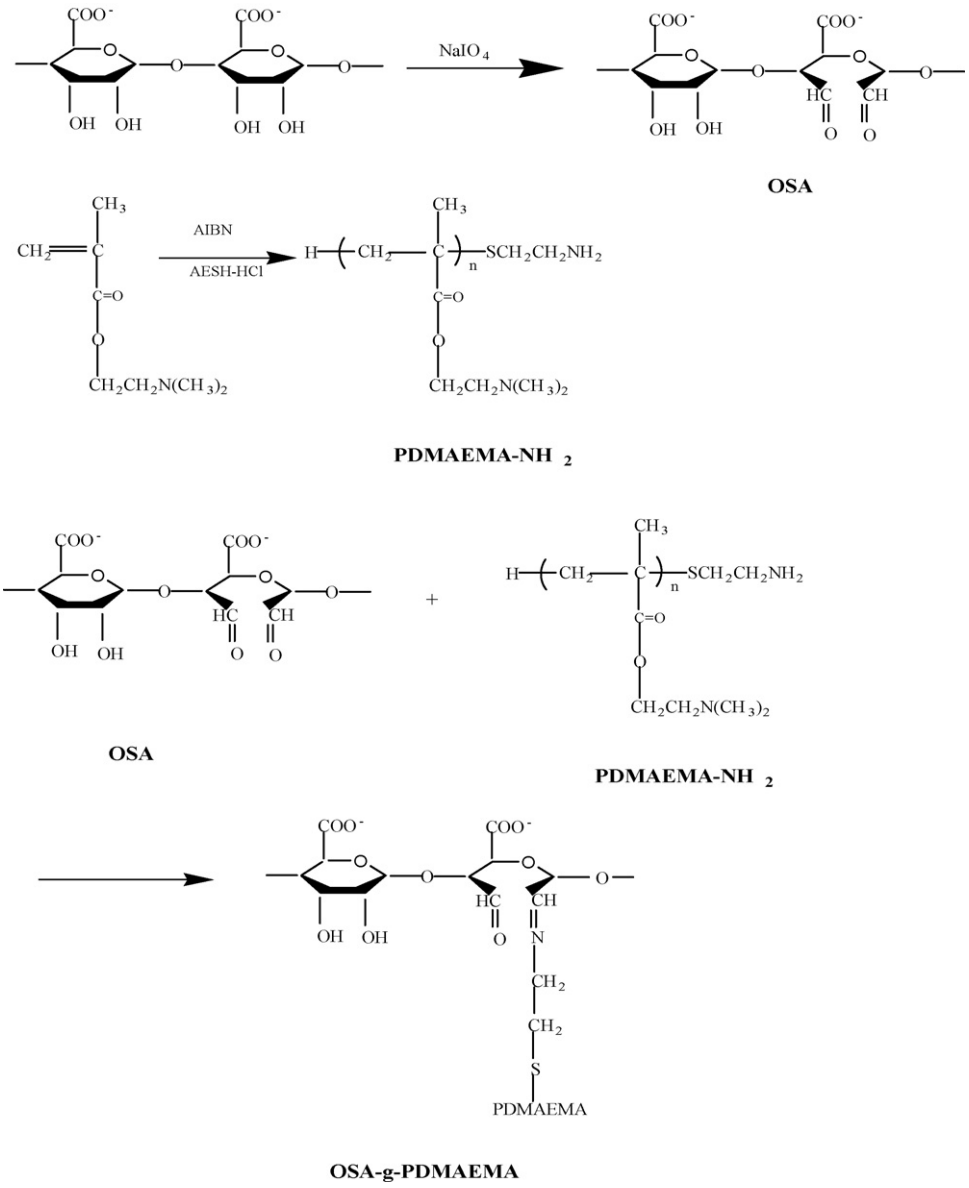
Fig. 2. FT-IR spectra of (a) PDMAEMA-NH_2, (b) sodium alginate, (c) OSA-g-PDMAEMA (9:1), (d) OSA-g-PDMAEMA (7:3), (e) OSA-g-PDMAEMA (5:5) and (f) OSA.

semitelechelic PDMAEMA has an average of one amino-terminated group per polymer chain, so the number-average molecular weight (M_n) of PDMAEMA-NH_2 is calculated to be 2278 determined by elemental analysis.

3.2. Characterization of OSA and OSA-g-PDMAEMA

Sodium alginates were oxidized using sodium periodate at room temperature during 6 h in the dark. Hydroxyl groups on carbons 2 and 3 of the repetitive unit were oxidized by sodium periodate which leads, by rupture of carbon–carbon bond, to the formation of two aldehyde groups in each oxidized uronic acid units (Gomez et al., 2007). The oxidation of sodium alginate is confirmed by appearance of a symmetric vibrational band of the aldehyde group at 1671 cm^{-1} (Fig. 2f), which is lower than that obtained by Lee et al. (2002) and Bouhadir et al. (2001). Yamaguchi et al. (2005) investigated the structure of crystalline chitin by two-dimensional FT-IR spectroscopy. In their study, two sharp peaks were observed in the lower frequency region at 1619 and 1581 cm^{-1}, which were assigned to the carbonyl of [C (6) OH···O=C] and [NH···O=C]. The oxygen atom of carboxyl and the hydrogen atom of α-hydroxyl groups or α-amino groups may form hydrogen bond, and the molecule is apt to exist in the form of overlap conformation (Xing et al., 1993). Thus, the peak at 1671 cm^{-1} might be caused by the influence of hydrogen bond. In addition, the degree of oxidation is determined to be 29.01% and the M_η is 9.75×10^4 g/mol.

As we known, the graft reaction is predominantly due to Schiff's base formation between the amino group of PDMAEMA-NH_2 and the available aldehyde of OSA (Scheme 1). Characteristic absorption peaks of OSA appear at 3400 cm^{-1} for hydroxyl group and at 1625 and 1450 cm^{-1} for the asymmetric COO^- stretching vibration and symmetric COO^- stretching vibration, respectively (Fig. 2f). In the case of PDMAEMA-NH_2 (Fig. 2a), a sharp peak observed at 1729 cm^{-1} corresponds to the ester carbonyl stretching vibration. In the FT-IR spectra of OSA-g-PDMAEMA (Fig. 2c–e), these peaks are seen together, which can indicate the presence of DMAEMA in the graft copolymer structure. The characteristic peaks at 2771 and 2823 cm^{-1} of PDMAEMA for the $-CH_2-$ groups which are adjacent to the nitrogen atom are clear (Jin et al., 2004). With an increase in the amount of PDMAEMA in graft, the characteristic peaks at 1729, 2771 and 2823 cm^{-1} of PDMAEMA become much stronger. Probably, the absorption due to C=N stretching, which is weak in aliphatic Schiff's base, is masked by C=O (Vieira et al., 2008). The N (wt %) and G (%) are displayed in Table 1. It can be found that N (wt %) and G (%) increase as PDMAEMA-NH_2 content in the graft copolymer



Scheme 1. Schematic illustration of the synthesis route of OSA-g-PDMAEMA.

increases. Therefore, it is concluded that PDMAEMA-NH_2 is grafted onto OSA.

3.3. *Effect of pH on ESR*

In order to investigate the effect of pH on the ESR of gels, dried samples were allowed to swell equilibrium in buffer solution with various pH, fixed ionic strength (I = 0.1 M) and temperature (25 °C). Fig. 3 shows the changes in the ESR of gels with the changing pH value, the ESR of gel beads with different G (%) increases continuously with increasing pH values. It is known that the pKa of alginate is 3.2 and 4.0 for guluronic and mannuronic acids, respectively (Lee et al., 2006). At a low pH region, most carboxylic acid groups of alginate are in the form of COOH. As the pH of the medium increases, the carboxylic acid groups become ionized, and the resulting electrostatic repulsion caused the gels to swell. With a pKa of 7.0–7.3, PDMAEMA can be protonated and ionized at low pH, while deprotonation above pH 8.0 can render PDMAEMA hydrophobic (Alarcón et al., 2005; Liu and Armes, 2001; Amalvy et al., 2004). At the pH ranged from 3.0 to 6.0, ionization of tertiary amino groups of

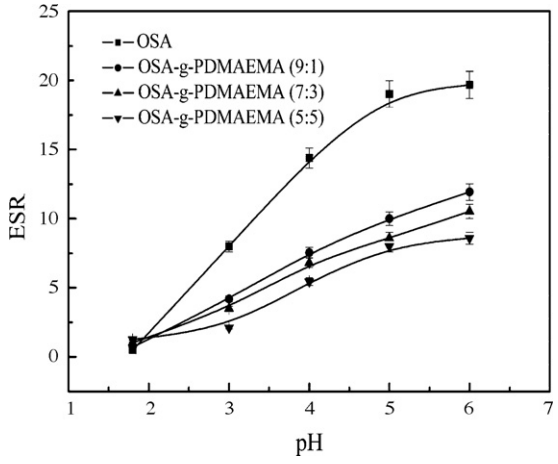
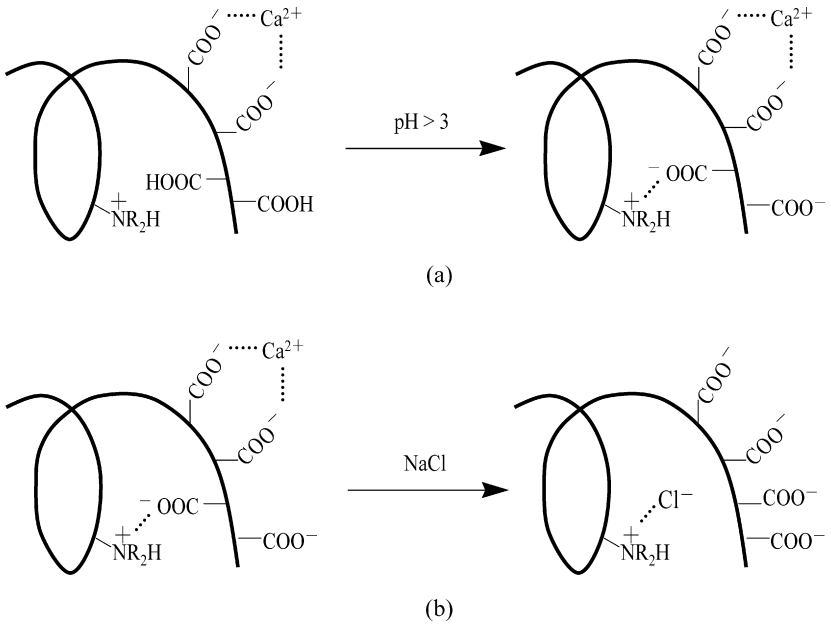


Fig. 3. Effect of pH on the ESR of OSA and OSA-g-PDMAEMA beads at 25 °C.



Scheme 2. Schematic illustration of the swelling of OSA-g-PDMAEMA gels in buffer solution (a) and in NaCl aqueous solution (b).

PDMAEMA takes place, which could lead to the increase in the swelling ratio of OSA-g-PDMAEMA gels. However, the ESR of OSA-g-PDMAEMA gels is lower than that of OSA gel in this pH range. This is due to the low amount of OSA in OSA-g-PDMAEMA gels and the formation of interpolymer polyelectrolyte complex (COO^- in alginate and $N^+(CH_3)H$ in PDMAEMA). The lower the amount of OSA in OSA-g-PDMAEMA gels, the weaker the electrostatic repulsion among COO^- groups. At the same time, complex formation consequently decreased the ESR that the functional groups responsible for swelling are involved in the complex formation (Torre et al., 2003). As a result, the ESR of OSA-g-PDMAEMA gels decreases by increasing the G (%) from pH 3.0 to 6.0. Based on the above analysis, the schematic structure of OSA-g-PDMAEMA gel in buffer solution is brought forward, as shown in Scheme 2a.

Since alginate gel beads crosslinked with Ca^{2+} are not stable at higher pH of 7.0 (Xu et al., 2007), the disruption of Ca^{2+}-alginate hydrogel matrix occurs fast in phosphate buffer solution with pH 7.0 due to the chelating action of phosphate ions. So the ESR of hydrogels at pH 7.0 was not investigated due to their burst disintegration. The swelling profiles in buffer solution with pH 6.8 (KH_2PO_4–NaOH) reveal no time lag (even for beads prepared with pure oxidized sodium alginate) and a higher swelling ratio (Fig. 4a). This phenomenon has also been observed by other authors (Bajpai and Sharma, 2004; Gaudio et al., 2005). In addition, the time of maximum swelling attainment is increased from 4 to 6 h in pH 6.8.

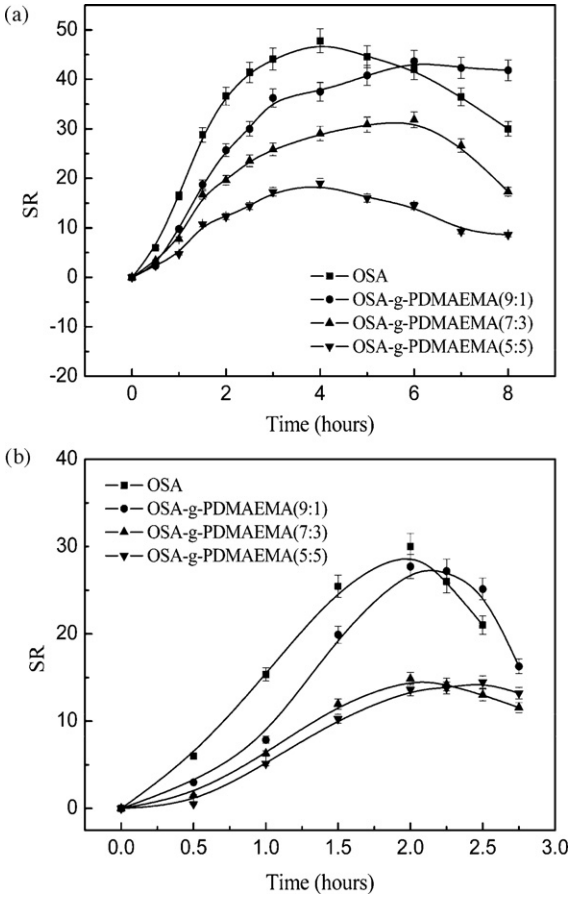


Fig. 4. Swelling kinetics of OSA and OSA-g-PDMAEMA beads in pH 6.8 (a) and pH 7.4 (b).

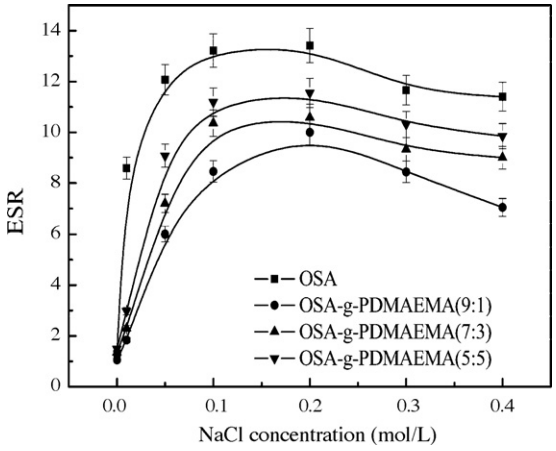


Fig. 5. Effect of ionic strength on the ESR of OSA and OSA-g-PDMAEMA beads at 25 °C.

Subsequently, the bead structure is quickly disaggregated, leading to the dissolution of the swollen bead. It is generally believed that the residence time of drug carriers in the small intestine (pH 6.8) is 3 h (Xu et al., 2007; Liu and Pan, 2008). Through the above analysis, we believe that the beads in the small intestine are stable, which is important to the oral delivery of proteins. The swelling kinetics of different beads in pH 7.4 (KH_2PO_4–NaOH) are also studied (Fig. 4b), which is a guide to the BSA release *in vitro*.

3.4. Effect of ionic strength on ESR

Fig. 5 depicts the ESR of gels with an increase in the ionic strength of the solution. The ionic strength of the medium is adjusted by changing the amount of NaCl in solution.

For gels crosslinked with Ca^{2+}, unlike chemically crosslinked gels, the increase of ionic strength in solution increase the number of the charged groups in the gel. The ionic strength of the medium increases as the increase of ion exchange between Ca^{2+} and Na^+ ions. A large number of the charged groups decrease the crosslinking density and increase the hydrophilicity of the network (Ju et al., 2002, 2001). Wang et al. (2007) also reported that the decrease of osmotic pressure inside the fiber with the increase of the salt concentration and the weakened salt-bond between Ca^{2+} and alginate, alginate and fiber matrix by Na^+. In addition, the stability of polyelectrolyte complex depends on the amount of free

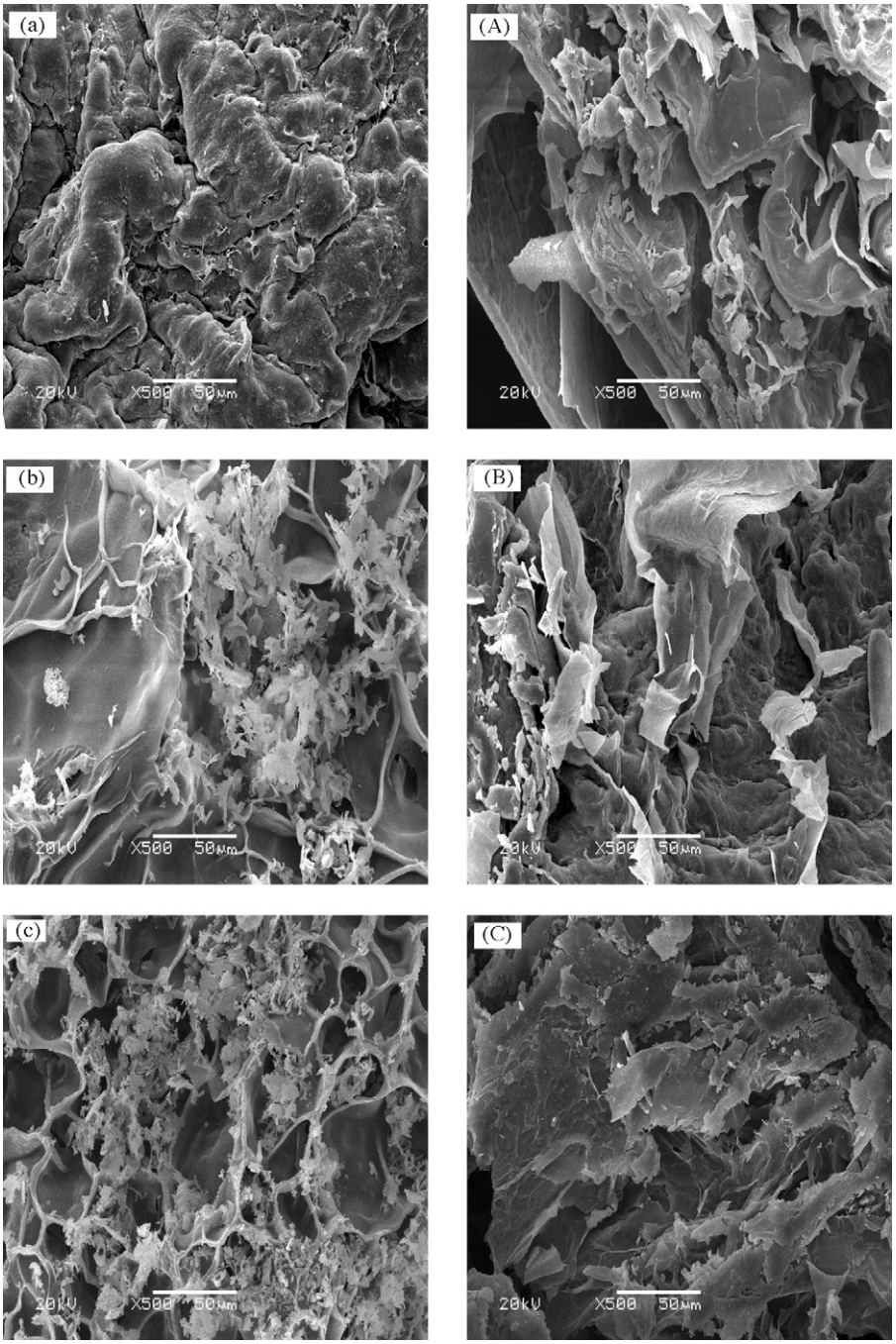


Fig. 6. SEM photos and macroscopic photos of different beads. Surface morphology of (a) OSA bead, (b) OSA-g-PDMAEMA (9:1) bead, (c) OSA-g-PDMAEMA (7:3) bead, (d) OSA-g-PDMAEMA (5:5) bead; cross-section morphology of (A) OSA bead, (B) OSA-g-PDMAEMA (9:1) bead, (C) OSA-g-PDMAEMA (7:3) bead, (D) OSA-g-PDMAEMA (5:5) bead. Macroscopic photos of OSA beads: (e) dried beads and (E) swollen beads.

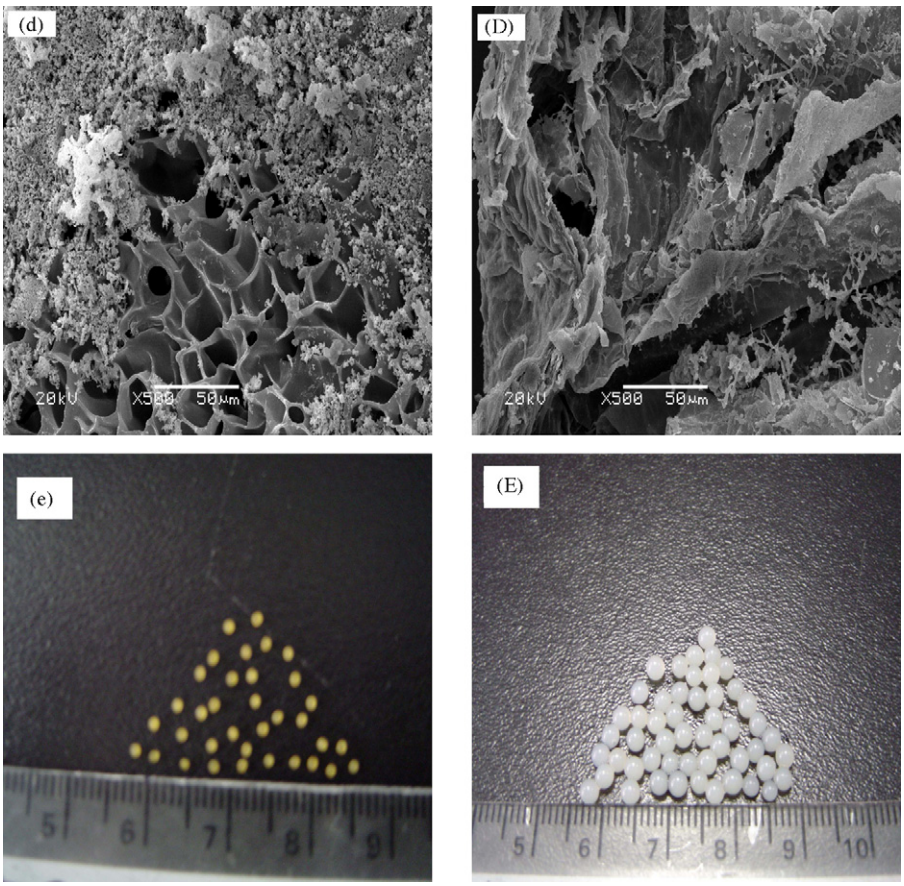


Fig. 6. (*Continued*).

ions because the electrostatic interaction between polyannions and polyactions can be destroyed by salt ions owing to electrostatic screen (Chen et al., 2008). Based on these considerations, we can propose a model swelling mechanism about OSA-g-PDMAEMA gel beads in NaCl aqueous solution (pH 6.0–7.0), which is shown in Scheme 2b.

It can be seen from Fig. 5 that the ESR of gels increases up to about 0.2 mol/L NaCl but decreases at higher NaCl concentrations. Similar results were observed by Ju et al. (2002). The decrease of the swelling ratio at high ionic strengths is caused by the decrease of the crosslinking density or destruction of the alginate network (Ju et al., 2002).

3.5. Morphology of OSA and OSA-g-PDMAEMA beads

Fig. 6 shows the SEM photos of the surface and cross-section morphology of OSA and OSA-g-PDMAEMA beads after swelling 1 h in buffer solution with pH 6.0. It can be seen from the surface morphology observations that the floccule increases by increasing the amount of PDMAEMA-NH_2 in the graft, which results from the chelating action of phosphate ions. Cross-section observations (Fig. 6A–D) reveal that there are some pores in the beads. In addition, some dendritic dangling chains, which result from the entanglement of the freely mobile PDMAEMA chain, are observed in the SEM image of OSA-g-PDMAEMA beads. These dendritic dangling chains would limit the release of drug. Macroscopic observation shows that the beads have a spherical shape and smooth surface (Fig. 6e and E). Generally, the diameter of wet beads is about 3.1–3.5 mm. After drying, the diameter of the beads is reduced to 1.2–1.5 mm.

3.6. In vitro drug release

Percent of BSA release as a function of time was determined in pH 1.8, pH 7.4 and 0.9% (w/v) NaCl at 37 °C to investigate the influence of pH and ionic strength on drug release.

Fig. 7a shows the BSA release profiles from the gel beads at pH 1.8 buffer solutions at 37 °C. The sequence of drug release percent follows the sequence of ESR at pH 1.8. It implies that the *G* (%) is higher in high ESR gel, leading to a result of higher percent of drug release. At pH 1.8, tertiary amino groups of PDMAEMA can be protonized, which cause the beads to swell. Bouhadir et al. (2000) reported that the C=N bond under acidic conditions would hydrolyze. The ionization of tertiary amino groups and the hydrolysis of C=N bonds make the pore size of diffusion path became large. Among the four gel beads, highest percent of BSA release is occurred in OSA-g-PDMAEMA (5:5) (61.24% in 9 h) due to the larger pore size of diffusion path.

The release profiles of BSA from the gel beads at pH 7.4 at 37 °C are given in Fig. 7b. The BSA released from the OSA gel beads is much faster than that from the others and the BSA total release is up to 98.4% in 7 h. The affinity of phosphate for calcium is higher than that of alginate (Xu et al., 2007), phosphate ions interact with calcium ions to form calcium phosphate, and the gel structure becomes to lose gradually (Bajpai and Sharma, 2004). BSA is, at neutral conditions, a negatively charged protein that could be bound by positively charged PDMAEMA grafted chains (Savina et al., 2005; Kusumo et al., 2007). Sparse long grafted polymer chains are preferable for binding BSA molecules. They are more flexible and could protrude in solution performing as "tentacles" capable of multipoint interactions with negatively charged BSA molecules. The higher the *G* (%), the higher the BSA binding capacity. As

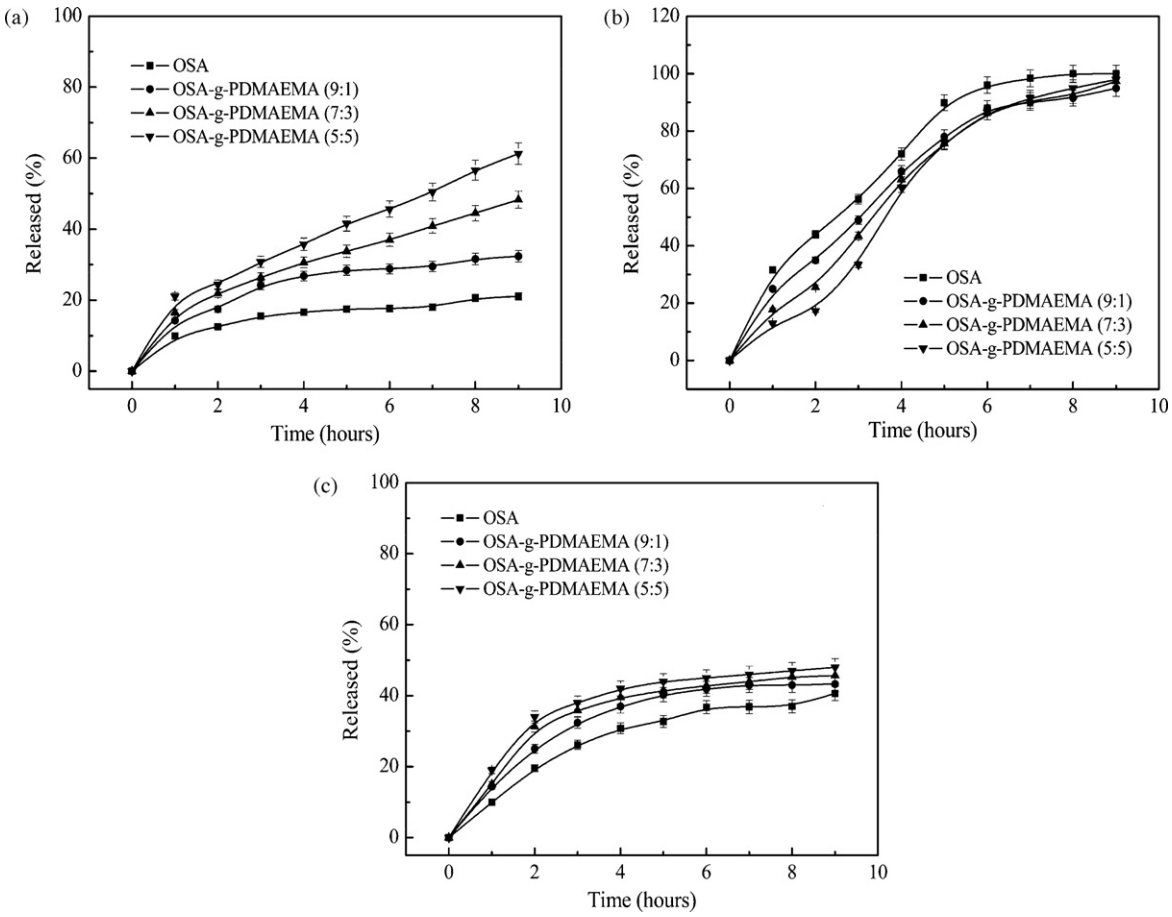


Fig. 7. Release of BSA from the beads of the different formations at pH 1.8 (a), pH 7.4 (b) and 0.9% (w/v) NaCl (c) at 37 °C.

shown in SEM studies, the more the amount of PDMAEMA-NH_2 in the graft, the more the dendritic dangling chains. The positively charged PDMAEMA grafted chains can also couple with COO^- of OSA, which can prevent the beads dissolution. BSA release speed could be influenced concurringly by these factors.

Fig. 7c shows the release profiles of BSA from the gel beads at 0.9% (w/v) NaCl. OSA and OSA-g-PDMAEMA gel beads are formed by ionic bonds between COO^- of alginate and Ca^{2+}. The BSA can be released at 0.9% (w/v) NaCl solution, which is mainly attributed to the ion exchange between Na^+ and Ca^{2+} ions. A larger number of the charged groups decrease the crosslinking density, and increase the pore size of diffusion path. The BSA cumulative release of OSA, OSA-g-PDMAEMA (9:1), OSA-g-PDMAEMA (7:3) and OSA-g-PDMAEMA (5:5) are respectively 40.61, 43.30, 45.62, and 48.00% in 9 h.

3.7. Analysis of release data

Drug release kinetics is analyzed by plotting the cumulative release data versus time and by fitting these data to the exponential equation (Ritger and Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n \tag{5}$$

where M_t/M_∞ represents the fractional drug release at time t, k is a constant characteristic of the drug–polymer system and n is an exponent characterizing the release mechanism.

The n and k values determined from the initial portion of ($0 \le M_t/M_\infty \le 0.6$) the fitted plot of $\ln(M_t/M_\infty)$ versus $\ln(t)$ are presented in Table 2 together with the correlation coefficients (r^2). For $n = 0.5$, the diffusion and release of the drug from the polymer matrix follow a Fickian diffusion. If $n > 0.5$, an anomalous or non-Fickian type drug diffusion occurs. Finally, for $n = 1$, a completely non-Fickian or Case II release kinetics is operative (Ritger and Peppas, 1987).

As could be seen in Table 2, the values of k and n show a dependence on the release medium and the G (%) of the gel beads. Values of n for beads at 0.9% (w/v) NaCl ranging from 0.60 to 0.38, lead to a shift of transport from non-Fickian to Fickian type. The gel beads at pH 1.8 have the n values ranging between 0.33 and 0.49, indicating the drug release obey to Fickian type. At pH 7.4, the values ranging between 0.52 and 0.82 indicate that the release deviates from the Fickian mode. On the other hand, the effect of G (%) on k of the gel beads is more noticeable. The value of k increases at 0.9% (w/v) NaCl and pH 1.8 with increasing G (%), but decreases at pH 7.4.

Table 2
Release kinetics of BSA from gel beads of the different formations.

Formulation		r^2	$k \times 10^2$	n
0.9% (w/v) NaCl	OSA	0.9416	0.12	0.60
	OSA-g-PDMAEMA (9:1)	0.9212	0.17	0.49
	OSA-g-PDMAEMA (7:3)	0.8554	0.19	0.45
	OSA-g-PDMAEMA (5:5)	0.8479	0.23	0.38
pH 1.8	OSA	0.9721	0.10	0.33
	OSA-g-PDMAEMA (9:1)	0.9536	0.15	0.38
	OSA-g-PDMAEMA (7:3)	0.9932	0.16	0.48
	OSA-g-PDMAEMA (5:5)	0.9657	0.19	0.49
pH 7.4	OSA	0.9951	0.31	0.52
	OSA-g-PDMAEMA (9:1)	0.9802	0.25	0.60
	OSA-g-PDMAEMA (7:3)	0.9342	0.17	0.78
	OSA-g-PDMAEMA (5:5)	0.8687	0.12	0.82

4. Conclusions

The OSA-g-PDMAEMA with different *G* (%) was synthesized, and OSA and OSA-g-PDMAEMA gel beads crosslinked with Ca^{2+} were also prepared. The ESR of the gel beads with different *G* (%) increased continuously with increasing pH values. The ESR of all the gel beads was also affected by the ionic strength in solution. The percent of drug release increased at pH 1.8 and 0.9% (w/v) NaCl by increasing *G* (%); and decreased at pH 7.4. The oral delivery of proteins can be controlled by adjusting the *G* (%), pH and ionic strength. From the results obtained, OSA-g-PDMAEMA gel beads seem to be a good candidate for oral protein delivery applications. However, further *in vivo* studies on animal models are necessary to establish the efficiency of the system.

Acknowledgement

The financial support of Special Doctorial Program Funds of the Ministry of Education of China (contract grant number: 20030730013) is gratefully acknowledged.

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