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pH responsive itaconic acid grafted alginate microspheres for the controlled release of nifedipine

Nuran Işıklan*, Murat İnal, Fatma Kurşun, Gülden Ercan

Kırıkkale University, Science and Arts Faculty, Department of Chemistry, Yahşihan, 71450 Kırıkkale, Turkey

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ABSTRACT

A series of pH responsive alginate-g-poly(itaconic acid) (NaAlg-g-PIA) microspheres were prepared as drug delivery matrices of nifedipine cross-linked by glutaraldehyde (GA) in the hydrochloric acid catalyst. Graft copolymers of sodium alginate with itaconic acid were synthesized using ceric ammonium nitrate. The chemical stability of the nifedipine after encapsulation into microspheres was confirmed by FTIR, DSC and X-RD analysis. The preparation conditions of the NaAlg-g-PIA microspheres such as graft yield, GA concentration, exposure time to GA and drug amount were optimized by considering the percentage entrapment efficiency, particle size, swelling capacity and their release data. The results showed that NaAlg-g-PIA microspheres are pH responsive. The release of nifedipine from grafted microspheres was slower for the pH 1.2 solution than that of the pH 7.4 buffer solution. It has been observed that an increase in exposure time, drug amount, GA and NaAlg-g-PIA concentrations causes a decrease in the nifedipine release from the microspheres, whereas an increase in graft yield leads to an increase in the nifedipine release.

1. Introduction

Microspheres have been widely used in biomedical and pharmaceutical applications (Freiberg & Zhu, 2004; Karasulu, Karasulu, Ertan, Kırılmaz, & Güneri, 2003; Kumbar, Soppimath, & Aminabhavi, 2003; Mı, Sung, & Shyu, 2001). For controlled drug release purposes, these systems act as a reservoir of therapeutic agents, with spatial and temporal control of release profiles of the drug leading to desirable therapeutic outcomes. The microparticles used should have some general characteristics, such as the ability to incorporate the drug without loss of activity, tuneable release kinetics, sufficient in vivo stability for function, biocompatibility in terms of lack of toxicity and immunogenicity, degradability and potential to target specific organs and tissues (Wang, Yucel, Lu, Hu, & Kaplan, 2010). Therefore, in recent years significant effort has been put in developing drug delivery microspheres for treating various diseases (Nayak et al., 2009; Oddo et al., 2010; Sultana, Mall, & Maurya, 2010; Wang, Zhang, & Wang, 2009).

Both synthetic and natural polymers have been used in the preparation of drug delivery microspheres. Compared with synthetic polymers, natural polymers, such as pectin, cellulose, alginate, gelatin, and chitosan have good biocompatibility (Agnihotri, Jawalkar, & Aminabhavi, 2006; Babu, Sairam, Hosamani, & Aminabhavi, 2007; Ganji & Abdekhodaie, 2010; Kim, Park, Kim, & Cho, 2003; Rokhade et al., 2006). The main advantages of using natural polymers in these systems are that they can be biocompatible, biodegradable and causing no systemic toxicity during the drug release. The functional groups of these polymers can control the diffusion of the drug and can eliminate the degradation products from the body. However, many natural polymers have some inherent disadvantages such as poor mechanical strength and microbial contamination. To overcome these problems, efforts have been made to develop chemically modified matrices by combining them with synthetic monomers. Graft copolymerization is an easier method to modify the structure of natural polymers as it makes them attractive biomaterials in controlled release applications (Kumbar & Aminabhavi, 2003; Şanlı, Ay, & Işıklan, 2007; Soppirnath & Aminabhavi, 2002).

Polysaccharides are a class of natural carbohydrates polymers and they have been used extensively in the food industry as gelling agents and in the pharmaceutical industry as a matrix for the encapsulation of living cells and for drug delivery systems. Among such polymers, sodium alginate (NaAlg), which is derived from the brown seaweeds, is composed of β-D-mannuronic acid and α-L-guluronic acid (Inal, Yiğitoğlu, & Işıklan, 2008; Oddo et al., 2010). NaAlg has many important properties. It is a biocompatible, biodegradable, non-toxic, chelating able and gelable polysaccharide and it is suitable for chemical modification. Therefore, NaAlg has been used as a carrier material in different controlled release

* Corresponding author. Tel.: +90 318 3574242/1513; fax: +90 318 3572461.
E-mail addresses: nuranisiklan@hotmail.com, nuranisiklan@kku.edu.tr (N. Işıklan).

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Table 1
Preparation conditions and characterization of the nifedipine-loaded microspheres.

Code	Polymer	Graft yield (%)	NaAlg-g-PIA concentration (w/v %)	Concentration of GA (%) and 10 N HCl (%)	Exposure time to GA (min)	Drug amount (w/w %)	Yield value (%)	Entrapment efficiency (%)	Microsphere diameter (mm)	D ($\times 10^8$ cm^2/s)
A_1	NaAlg	–	2.5	1.25 + 2.5	15	10	81.05	69.10 ± 3.65	1.41 ± 0.02	309
A_2	NaAlg-g-PIA$_1$	47	2.5	1.25 + 2.5	15	10	69.95	66.93 ± 3.51	0.77 ± 0.03	85.2
A_3	NaAlg-g-PIA$_2$	52	2.5	1.25 + 2.5	15	10	73.35	81.53 ± 4.93	0.82 ± 0.02	86.8
A_4	NaAlg-g-PIA$_3$	60	2.5	1.25 + 2.5	15	10	70.50	75.05 ± 2.55	0.84 ± 0.02	172
A_5	NaAlg-g-PIA$_4$	106	2.5	1.25 + 2.5	15	10	73.50	88.31 ± 5.25	1.03 ± 0.04	Not calculated
B_1	NaAlg-g-PIA$_1$	47	2.5	1.00 + 2.0	15	10	65.76	91.53 ± 5.25	0.80 ± 0.03	466
B_2	NaAlg-g-PIA$_1$	47	2.5	1.50 + 3.0	15	10	67.22	86.68 ± 3.56	0.75 ± 0.02	76.8
B_3	NaAlg-g-PIA$_1$	47	2.5	1.25 + 2.5	15	20	67.73	92.94 ± 3.15	0.82 ± 0.02	33.3
B_4	NaAlg-g-PIA$_1$	47	2.5	1.25 + 2.5	15	40	73.46	87.33 ± 1.45	0.87 ± 0.02	1.21
B_5	NaAlg-g-PIA$_1$	47	2.5	1.25 + 2.5	30	10	64.22	94.04 ± 2.18	0.75 ± 0.03	80.7
B_6	NaAlg-g-PIA$_1$	47	1.5	1.25 + 2.5	15	10	61.00	60.09 ± 1.78	Pellet	Not calculated
B_7	NaAlg-g-PIA$_1$	47	2.0	1.25 + 2.5	15	10	64.98	66.65 ± 2.25	Pellet	Not calculated

systems and biotechnological applications (Gombotz & Wee, 1998; Shi et al., 2005; Simpson et al., 2006).

IA is one of the monomers which is obtained from renewable resources by microorganism fermentation with Aspergillus terrus and Pseudozyma antarctica using carbohydrate materials as molasses and hydrolyzed starch at low cost (Levinson, Kurtzman, & Kuo, 2006; Mahdavian, Abdollahi, Mokhtabad, Bijanzadeh, & Ziaee, 2006). It is very hydrophilic and is expected to show high biocompatibility because it is derived from natural sources. The double ionization of IA at different pH values provides the stepwise release behaviour of specially adsorbed drugs or other adsorbents by controlling the pH of the medium (Işıklan, Kurşun, & İnal, 2009). Doubly ionized carboxylic groups bring additional capability of chelate formation under certain cases. A few workers have carried out grafting reactions of IA onto chitin, sisal fibers, and cellulose fibers (Mostafa, Naguib, Saba, & Mokhtar, 2005; Naguib, 2002; Sabaa & Mokhtar, 2002). It was therefore decided to graft IA onto NaAlg in order to develop pH responsive alginate based microspheres. For this purpose, in the previous studies, IA was grafted onto sodium alginate using ceric ammonium nitrate (CAN) and the preparation conditions were optimized considering the effects of the reaction variables such as the reaction time, temperature, percentage of sodium alginate, monomer and initiator concentrations (Işıklan et al., 2009).

Nifedipine, which is a calcium channel blocker, has been widely used in the treatment of hypertension, angina and myocardial infarction. It is poorly water-soluble and has a plasma lifetime of 2 h (Bittar, 1989; Shelke & Aminabhavi, 2007). Self-poisoning with a calcium channel blocker is a common cause of in-hospital death from self-poisoning (Buckley, Dawson, & Whyte, 2007; Olson et al., 2005). Doses of only two to three times the therapeutic dose may cause profound toxicity and side effects in susceptible individuals. Therefore, it is desirable to develop nifedipine controlled-release dosage forms to reduce the side effects, to prevent toxicity, to extend a half-time and to improve patient compliance (Buckley et al., 2007; Huang, Wigent, & Schwartz, 2006). Recently, nifedipine has been used in such controlled-release studies as alginate-methyl cellulose blend microspheres, chitosan-graft-acrylamide microspheres, alginate-chitosan hydrogel beads (Babu et al., 2007; Dai, Li, Zhang, Wang, & Wei, 2008; Kumbar & Aminabhavi, 2003).

The objective of the present study is to design, characterize, and evaluate the pH responsive NaAlg-g-PIA microspheres of nifedipine with high entrapment efficiency. NaAlg-g-PIA solution containing nifedipine was cross-linked by glutaraldeyde which is most frequently used to prepare polymeric microspheres. However, GA is known carcinogenic agent. For this reason, concentrations of GA and exposure times to GA were kept too low in the study. The particle size, microsphere yield and entrapment efficiency of the microspheres were investigated. Equilibrium-swelling degree of the microspheres and nifedipine release was carried out at pH 1.2 and pH 7.4. The effect of such factors as the grafting of IA, extent of cross-linking and the amount of the drug on the swelling behaviour of microspheres, drug loading and release of nifedipine from the microspheres were studied in detail and discussed.

2. Materials and methods

2.1. Materials

Sodium alginate with a viscosity of 3.500 cps (2% solution, 25 °C) and nifedipine were purchased from Sigma Chemical Co. (Louis, USA). IA and CAN were supplied from Fluka Chemie AG (Buchs, Switzerland). Other reagents were Merck (Darmstadt, Germany) and were used as received.

2.2. Graft copolymerization

The grafting reactions were carried out under a nitrogen atmosphere in a 250 mL three-necked flask equipped with a reflux condenser, a stirrer, and a gas inlet system, immersed in a constant temperature bath as described previously (Işıklan et al., 2009). Briefly, NaAlg was dissolved in distilled water (50 mL) at room temperature with constant stirring. The solution was immediately placed into the water bath adjusted to the polymerization temperature. The required amount of IA was dissolved in 10 mL of distilled water and neutralized with saturated NaOH solution. After that, this solution was mixed with NaAlg solution and stirred accompanied by a slow stream of nitrogen for 30 min. Then, CAN at the required concentration in distilled water was added slowly to the reaction mixture and the total volume of the reaction mixture was made up to 100 mL with distilled water. A continuous supply of nitrogen was maintained throughout the reaction period. The grafting reactions were carried out for different periods of time (2–5 h) with different IA concentrations (0.0575–0.46 M). At the end of the predetermined polymerization time, the reaction was terminated by adding a saturated solution of hydroquinone. The products were precipitated in an excess of acetone, separated by filtration, and then extracted with methyl alcohol to remove the homopolymer poly(itaconic acid) (PIA) for 24 h. After the complete removal of PIA, the pure graft copolymer was dried at 40 °C under a vacuum to a constant weight. The graft yield (GY) was calculated as follows:

$$\mathrm{GY}(\%) = \left[\frac{w_g - w_o}{w_o}\right] \times 100 \quad (1)$$

where w_o and w_g denote the weights of the original (ungrafted) NaAlg and grafted NaAlg, respectively. Codes of the graft copolymers used in the experiments are shown in Table 1.

2.3. Preparation of the NaAlg-g-PIA microspheres

NaAlg-g-PIA solution containing nifedipine in various drug amounts was prepared and stirred for 12 h to form a homogenous solution. 20 mL of polymer solution containing nifedipine was added drop wise into the 50 mL water containing glutaraldehyde and HCl under gentle agitation (50 rpm), using a peristaltic pump at a flow rate of 3 mL/min (Masterflex, L/S Digital Economy Drive, USA), which has pump tubing with 0.8 mm of inside diameter. Distance between needle and GA aqueous solution was kept to be 3 cm. The formed microspheres were then removed from the cross-linking solution at selected time intervals of 15 and 30 min and were washed with distilled water repeatedly to remove the adhered glutaraldehyde and acid. After that, the microspheres were dried completely at 40 °C under a vacuum to a constant weight. Unloaded microspheres were prepared in a similar way without nifedipine to determine the equilibrium swelling degree. Preparation conditions of the nifedipine-loaded microspheres are shown in Table 1. In order to estimate the size of the microspheres, 10 samples of the completely dried microspheres from different formulations were selected and their sizes were measured using an electronic digital caliper (Mitutoyo IP.65, Japan).

2.4. Fourier transform infrared measurements (FTIR)

The FTIR spectra of NaAlg-g-PIA copolymer, empty NaAlg-g-PIA microsphere, nifedipine and nifedipine-loaded NaAlg-g-PIA microspheres were taken with a Jasco FTIR-480 Plus spectrometer (Japan). The spectra were taken in the wavelength region 400–4000 cm^{-1} at ambient temperature.

2.5. Differential scanning calorimetry (DSC)

The thermal analysis was performed with a differential scanning calorimeter (PerkinElmer, Sapphire DSC, USA). The sample weights ranged from 5 to 8 mg. The samples were heated from 30 °C to 300 °C at a heating rate of 10 °C/min.

2.6. Scanning electron microscope (SEM)

SEM photographs were taken with JSM 5600 Scanning Microscope (Japan) to examine the morphology and surface structure of the microspheres at the required magnification at room temperature. The microspheres were deposited on a brass holder and sputtered with a thin coat of gold under vacuum. The acceleration voltage used was 20 kV with the secondary electron image as a detector.

2.7. X-ray diffractometry (X-RD)

The X-RD patterns of nifedipine, nifedipine-loaded microsphere and empty microspheres were recorded using a Rigaku D/MAX2200/PC model diffractometer equipped with Cu Kα radiation at a generator voltage of 40 kV and a generator current of 40 mA. Dried microspheres were triturated to get fine powder before taking the scan. The samples were mounted on a sample holder and X-RD patterns were recorded in the range of 0–50° at the speed of 2° min^{-1}.

2.8. Equilibrium swelling study of the microspheres

The equilibrium swelling degree (ESD) of the cross-linked empty microspheres was determined by measuring gravimetrically the extent of their swelling in 0.1 N HCl solution and buffer solution at pH 7.4 at 300, 310 and 320 K. To ensure complete equilibration, the samples were allowed to swell for 24 h. The excess surface-adhered liquid drops were removed by blotting paper and the swollen microspheres were weighed using an electronic balance (Precisa XB 220A, USA). The microspheres were then dried in an oven at 40 °C until constant in weight. The percentage equilibrium swelling degree was calculated as follows:

$$\text{equilibrium swelling degree}\,(\%) = \frac{M_s - M_d}{M_d} \times 100 \quad (2)$$

where M_s and M_d are the mass of swollen microspheres and mass of dry microspheres, respectively.

2.9. Determination of nifedipine content of the microspheres

The known mass of the microspheres were crushed in an agate mortar with a pestle and then polymeric powder was refluxed with a mixture containing 30% methanol, 70% pH 7.4 phosphate buffer solution and 0.01% (w/w) Tween-80 for 3 h to ensure the complete extraction of nifedipine from the microspheres. The solution containing nifedipine was filtered to remove the pieces of polymer. After that, the absorbance of the solution containing the extracted amount of nifedipine was taken at a wavelength of 238 nm in a UV spectrophotometer (Unicam UV2-100, UK), using pure 30% methanol–70% pH 7.4 phosphate buffer solution as a blank. The percentage of entrapment efficiency was then calculated as follows:

$$\text{entrapment efficicney}\,(\%) = \frac{\text{practical nifedipine loading}}{\text{theoretical infedipine loading}} \times 100 \quad (3)$$

2.10. In vitro drug release

In vitro drug release from the microspheres firstly was studied in 250 mL conical flasks containing 0.1 N HCl solution (pH 1.2) and incubated in a shaking water bath (Medline BS-21, Korea) at 37 °C, with a speed of 100 rpm. At the end of 2 h, the nifedipine release medium was changed to pH 7.4 phosphate buffer to contain 0.01% (w/w) Tween-80 from 0.1 N HCl. 3 mL solution was withdrawn at specific time intervals and nifedipine content was determined by UV spectrophotometer at 238 nm. Equal volume of fresh HCl or phosphate buffer solution was replaced into the release medium to maintain a constant volume. Experiments were performed in triplicate in order to minimize the variational error. Standard deviations from the average values were calculated.

3. Results and discussion

3.1. Characterization of the NaAlg-g-PIA microspheres

The FTIR spectral data was used to confirm the cross-linking of the NaAlg-g-PIA matrix and the chemical stability of the nifedipine in the grafted NaAlg microspheres. The same data was also utilized to characterize the NaAlg-g-PIA copolymer. Fig. 1 compares the FTIR spectra of the NaAlg-g-PIA copolymer (A), nifedipine (B), nifedipin-loaded NaAlg-g-PIA microspheres (C), and empty microspheres (D). The spectrum of the NaAlg-g-PIA shows the peaks at around 2925, 1609, 1417, and 1032 cm^{-1}, indicating the stretching of aliphatic C–H, COO–(asymmetric), COO–(symmetric), and C–O, respectively, as reported in the previous study (Işıklan et al., 2009). Moreover, strong bands at around 1563 and 1385 cm^{-1} were assigned to carboxylate ions as sodium salt of NaAlg-g-PIA, which confirmed the grafting of the monomer. NaAlg-g-PIA also showed a broad band at around 3409 cm^{-1}, which was attributed to O–H stretching vibrations. This band in the empty microspheres narrowed and shifted to around 3420 cm^{-1}(Fig. 1A and D). The cross-linking process of

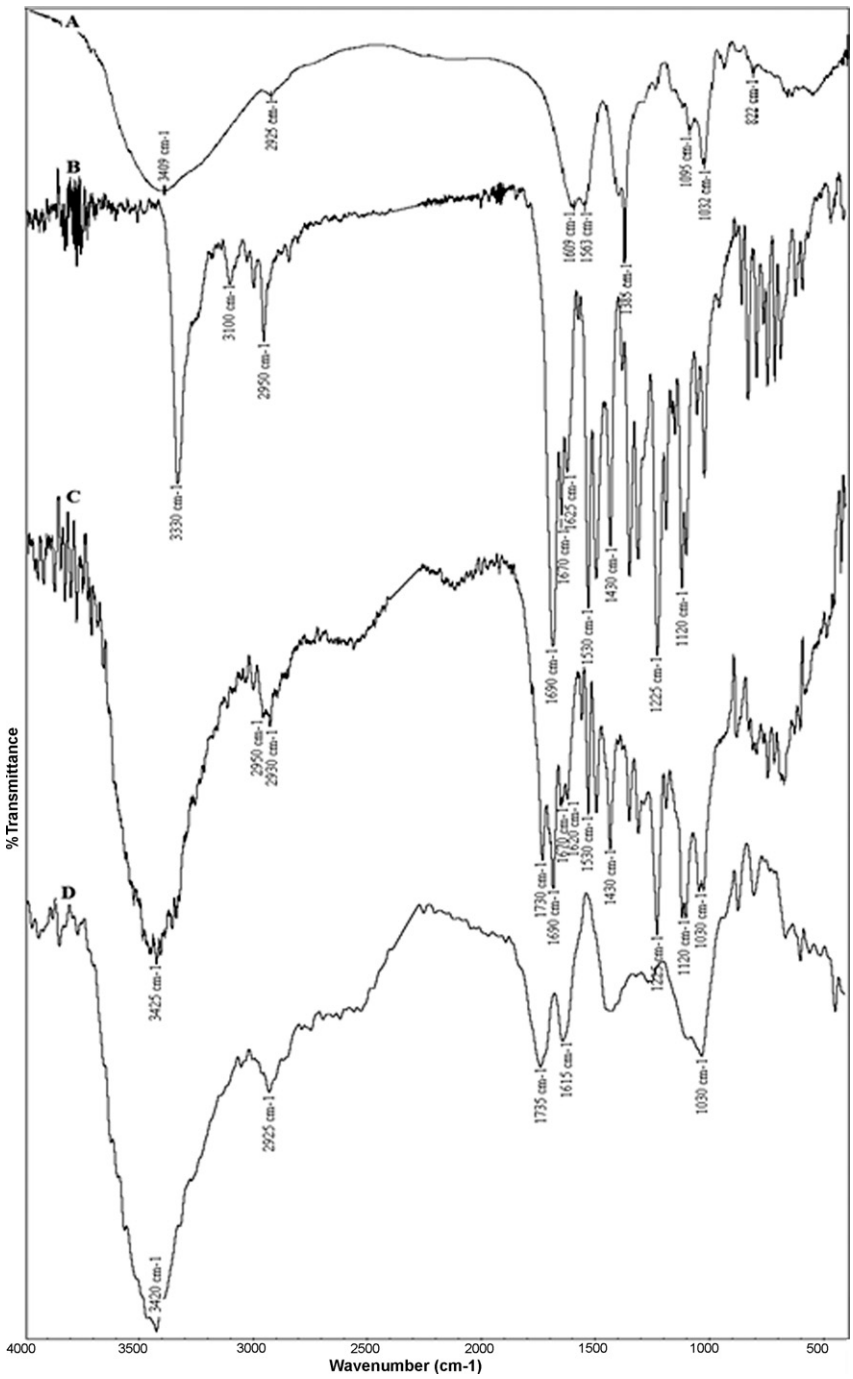

Fig. 1. FTIR spectra of the NaAlg-g-PIA$_1$ copolymer (A), nifedipine (B), nifedipine-loaded NaAlg-g-PIA$_1$ microspheres, (C) and empty NaAlg-g-PIA$_1$ microspheres (D).

NaAlg-g-PIA with GA provided a shift of lower intensity of COO^- stretching peak at 1563 and 1609 cm^{-1} to higher wavenumbers (1615 and 1735 cm^{-1}) (Fig. 1A and D). These results were attributed to a cross-linking reaction between O–H groups of NaAlg-g-PIA and –CHO groups of GA molecules. Similar results were found in the previous studies (Işıklan, 2006; Işıklan, İnal, & Yiğitoğlu, 2008; Şanlı et al., 2007).

The FTIR spectrum of the nifedipine has shown characteristic bands. The bands at 3330, 3100 and 2950 cm^{-1} are due to N–H, aromatic C–H and aliphatic C–H stretching, respectively. Nifedipine has also shown characteristic bands, which is attributed to C=O stretching at around 1670 and 1690 cm^{-1}, C=C stretching at around 1625 cm^{-1}, N–O stretching in NO_2 group at around 1430 and 1530 cm^{-1}, C–CH_3 stretching at around 1225 cm^{-1} and C–O stretching at around 1120 cm^{-1} in ester groups (Soppimath, Aminabhavi, Agnihotri, Mallikarjuna, & Kulkarni, 2006). The characteristic bands of the nifedipine maintain their positions even after being encapsulated in the NaAlg-g-PIA matrix, which indicates the chemical stability of the drug in the grafted NaAlg matrix.

DSC analyses were performed to understand the thermal behaviour of the drug loaded microspheres and the results are illustrated in Fig. 2a. DSC analyses also showed the presence of cross-linking. After cross-linking with GA, the endothermic peak of the NaAlg-g-PIA microsphere shifted to a higher temperature value indicating the cross-linking reaction (Fig. 2C and D). Hence, the polymer matrix is more rigid and therefore shifts the endothermic

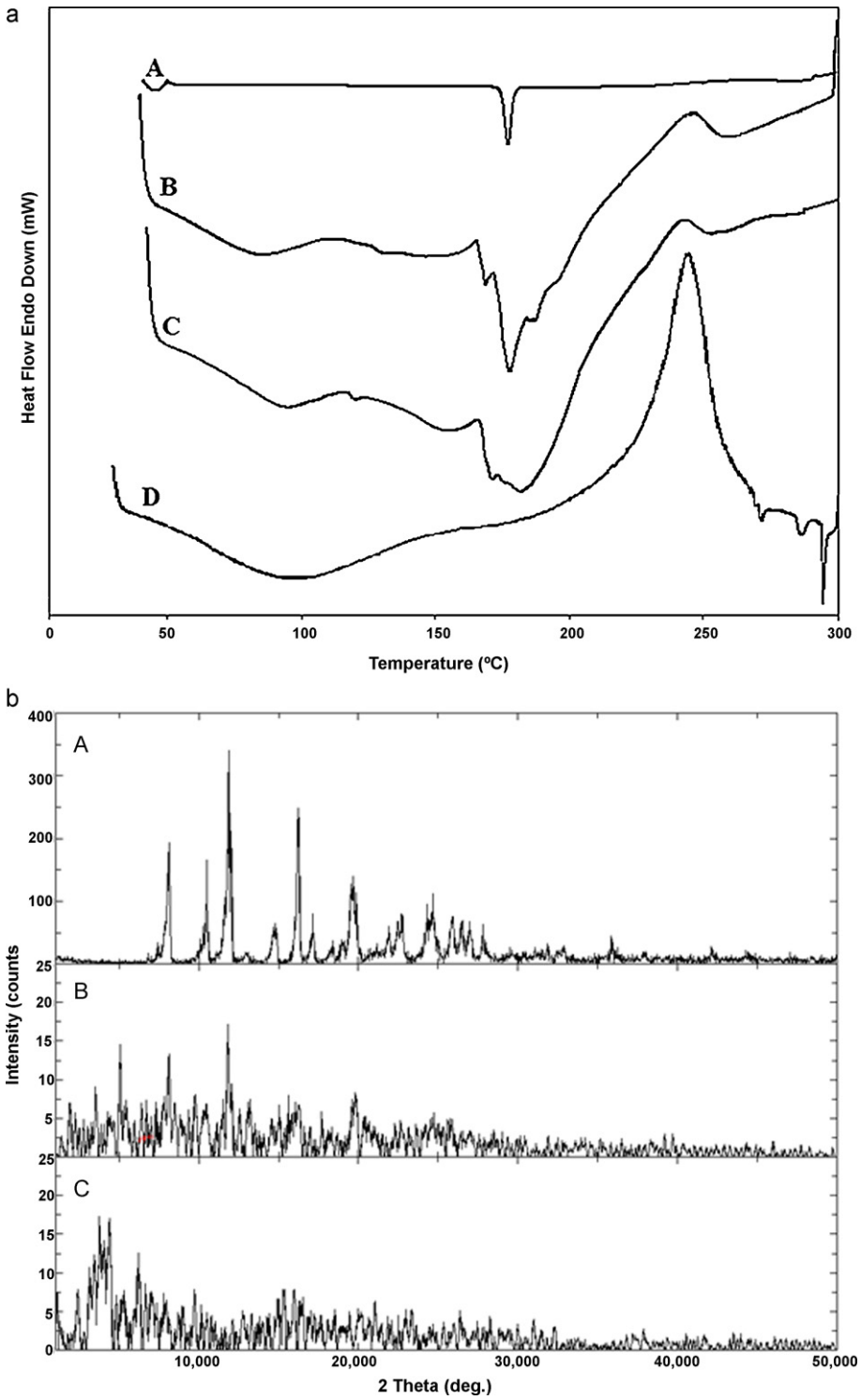

Fig. 2. (a) DSC termogram of the nifedipine (A), nifedipine-loaded NaAlg-g-PIA$_1$ microspheres (B), empty NaAlg-g-PIA$_1$ microspheres (C) and NaAlg-g-PIA$_1$ copolymer (D). (b) X-ray diffraction patterns of nifedipine (A), nifedipine-loaded microspheres (B) and empty microspheres (C).

peak to a higher temperature. The melting point of the nifedipine has been found to be 172 °C from the thermogram of the nifedipine and nifedipine-loaded NaAlg-g-PIA microspheres, as reflected Fig. 2a (A and B). This indicates that the drug is dispersed in the form of crystalline structure in the polymeric matrix.

X-ray diffractograms of nifedipine (A), nifedipine-loaded microspheres (B) and empty microspheres (C) are displayed in Fig. 2b. These studies are useful to investigate the crystallinity of the drug in cross-linked microspheres. Nifedipine has shown the characteristic intense peaks at 2θ of 6°, 12°, 16°, 20°, 25°, and 27° due to its crystalline nature as reported in the literature (Babu et al., 2007). Many of these characteristic peaks also appeared in the X-ray diffraction spectrum of the nifedipine-loaded microspheres, whereas a few of them overlapped with the noise of the coated polymer itself. This indicates that drug particles are dispersed at crystalline structure in the drug-loaded polymer matrices.

SEM photographs of a single NaAlg and NaAlg-g-PIA microspheres taken at 50 and 500 magnifications are shown in Fig. 3.

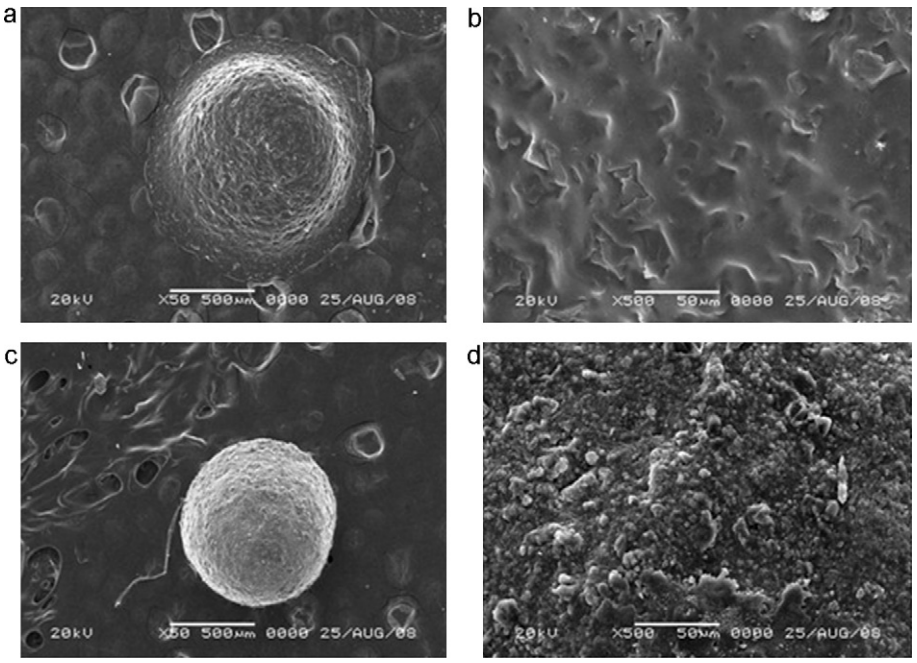

Fig. 3. SEM micrographs at 50 and 500 magnifications of the nifedipine-loaded NaAlg microspheres (a and b) and nifedipine-loaded NaAlg-g-PIA$_1$ microspheres (c and d).

As it is seen from the figure, both of the microspheres are almost spherical in shape and show a roughness on the surface. The roughness on the surface of the microspheres increases with the grafting of IA onto NaAlg.

3.2. Particle size, entrapment efficiency and yield value evaluation of the microspheres

The results of the microsphere diameter, entrapment efficiency (%) and microsphere yield (%) are shown in Table 1. As can be seen from the table, the NaAlg-g-PIA microspheres formed have particle sizes ranging from 0.75 ± 0.03 to 1.03 ± 0.04 mm in diameter. The best NaAlg-g-PIA concentration for formation of spherical microspheres was obtained to be 2.5%. The microspheres prepared with 1.5 and 2.0% NaAlg-g-PIA concentrations collapsed to pellet form due to the decrease in the viscosity of the polymer solutions. The size of the microspheres changed with the grafting of IA, the drug amount, cross-linking concentration and exposure time to GA. The diameter of the NaAlg-g-PIA microspheres is smaller than that of NaAlg microsphere (1.41 ± 0.02 mm). Moreover, an increase in cross-linking concentration and time causes a decrease in the diameters of the microspheres. With an increase in these parameters, the microspheres with a smaller size were produced, probably due to the formation of a more rigid network because of increased cross-link density. Similar results have been found in NaAlg–methylcellulose and polyacrylamide grafted guar gum hydrogel microspheres (Babu et al., 2007; Soppirnath & Aminabhavi, 2002). As it is also seen from Table 1, as the drug amount and graft yield increases, the diameter of the microspheres also increases. As the amount of nifedipine increases from 10% to 40%, the diameter of the microspheres increases from 0.75 ± 0.03 mm to 0.87 ± 0.02 mm, respectively, due to the increase of nifedipine content in the NaAlg-g-PIA matrix. In addition, as the grafting of the IA groups onto the NaAlg increases, the diameter of the microspheres increases, this could be attributed to the formation of the heterogeneous structure. A similar observation was found in the previous study (Işıklan, 2006) and other studies in the literature (Katime, Valderruten, & Quintana, 2001; Kumbar & Aminabhavi, 2003). In the earlier study, it was reported that NaAlg/gelatin beads had a heterogeneous structure with a dense surface layer and loose core, because of the heterogeneous gelation mechanism, which resulted in the collapse of beads during the drying process.

Many factors, such as the nature of the drug, polymer concentration, drug/polymer ratio, and the type of matrix material of the microspheres, affect the percentage of entrapment efficiency and the microsphere yield. The results of the percentage of entrapment efficiency and microsphere yield are also presented in Table 1. As it is seen from the table, values of the microsphere yield of the NaAlg and NaAlg-g-PIA microspheres are quite high. Generally, the percentages of entrapment efficiency of NaAlg-g-PIA microspheres were found to be higher than that of pure NaAlg microsphere. A high percentage of entrapment efficiency of the NaAlg-g-PIA microspheres shows that the NaAlg-g-PIA polymer prepared has a good property for the entrapment of drug. When the IA is grafted, copolymer traps more nifedipine molecules and entrapment efficiency increases. Another reason for the high entrapment efficiency may be the interaction between the drug and PIA groups (Taşdelen, Kayaman-Apohan, Mısırlı, Güven, & Baysal, 2005). Similar results have also been observed in pH and temperature sensitive core–shell microspheres studied by Ma, Liu, Liu, Chen, and Cui (2010). They reported that as the amount of carboxyl groups on microgels increases, which enhances the interaction of the drug and methacrylic acid, the entrapment efficiency of the drug increases, as well. The percentage of entrapment efficiency and the microsphere yield has changed slightly with the preparation conditions of NaAlg-g-PIA microspheres, as can be seen in Table 1.

3.3. Effect of IA grafting on the nifedipine release

To understand the release of nifedipine from the cross-linked NaAlg and NaAlg-g-PIA microspheres, an in vitro release study was carried out in gastric and intestinal pH conditions at 37 °C. Fig. 4 displays the effect of IA grafting on the cumulative nifedipine release of microspheres. From the figure it is observed that the release of nifedipine is higher for the NaAlg-g-PIA microspheres than that of the NaAlg microsphere at pH 7.4. The highest cumulative nifedipine release obtained at the end of 3 h was 100% for the

Table 2a
Equilibrium swelling degree and M_c values of the empty microspheres.

Code	Swelling temperature (K)	Equilibrium swelling degree in pH 1.2 (%)	Equilibrium swelling degree in pH 7.4 (%)	ϕ	N	χ	M_c
A_1	300	103.47 ± 1.26	459.05 ± 14.34	0.3449	−1.0671	0.6412	14,763
A_1	310	135.16 ± 1.26	636.38 ± 11.39	0.3057	−1.0974	0.6191	19,641
A_1	320	152.73 ± 1.12	806.69 ± 10.37	0.2858	−1.1155	0.6098	25,274
A_2	300	122.21 ± 1.94	841.00 ± 27.18	0.3554	−1.0600	0.6506	17,293
A_2	310	145.48 ± 0.83	991.98 ± 16.91	0.3158	−1.0890	0.6272	22,255
A_2	320	162.63 ± 2.09	1317.95 ± 18.69	0.2949	−1.1070	0.6168	28,795
A_3	300	139.10 ± 3.01	1361.13 ± 18.04	0.3257	−1.0811	0.6316	18,868
A_3	310	163.68 ± 0.66	2340.31 ± 16.65	0.2909	−1.1107	0.6129	25,249
A_3	320	182.99 ± 2.27	3255.50 ± 16.65	0.2646	−1.1372	0.6002	33,903
A_4	300	161.78 ± 3.24	2274.76 ± 27.40	0.2940	−1.1077	0.6125	20,967
A_4	310	186.88 ± 1.94	2919.46 ± 32.87	0.2653	−1.1364	0.5987	28,525
A_4	320	220.55 ± 4.05	3628.26 ± 38.86	0.2328	−1.1755	0.5838	41,019
A_5	300	172.91 ± 2.52	3836.60 ± 36.02	0.2804	−1.1207	0.6086	30,426
A_5	310	188.38 ± 5.28	Degradation	0.2628	−1.1391	0.6008	40,312
A_5	320	243.29 ± 5.87	Degradation	0.2157	−1.1995	0.5783	63,162
B_1	300	132.54 ± 0.96	968.01 ± 9.80	0.3405	−1.0702	0.6453	27,328
B_1	310	155.21 ± 2.89	1066.26 ± 18.10	0.3020	−1.1006	0.6229	34,708
B_1	320	175.66 ± 3.47	1562.40 ± 14.24	0.2759	−1.1253	0.6095	46,261
B_2	300	108.56 ± 7.56	499.61 ± 1.10	0.3829	−1.0431	0.6699	17,069
B_2	310	124.08 ± 1.50	627.56 ± 12.36	0.3511	−1.0628	0.6498	20,890
B_2	320	140.26 ± 6.15	828.05 ± 12.95	0.3229	−1.0833	0.6336	26,406
B_5	300	110.46 ± 5.28	522.95 ± 7.64	0.3789	−1.0454	0.6635	12,924
B_5	310	120.64 ± 4.80	628.01 ± 16.83	0.3576	−1.0586	0.6512	16,116
B_5	320	138.13 ± 4.57	1012.96 ± 3.04	0.3201	−1.0855	0.6290	20,564

NaAlg-g-PIA_4 microsphere. On the other hand, the least cumulative nifedipine release obtained was 41.4% for the NaAlg microsphere at the end of 8 h. As the grafting of IA groups increases, the cumulative release of nifedipine increases. This result is quite expected since the hydrophilic character of the polymeric matrix increases with the increase in IA groups and the grafting of IA groups onto NaAlg brings many –COOH groups of hydrophilic nature. The results obtained are also consistent with the swelling results. Equilibrium swelling experiments were performed in 0.1 N HCl (pH 1.2) and in pH 7.4 phosphate buffer solutions for various empty microsphere formulations and were presented in Table 2a. As can be seen from the table, the equilibrium-swelling degree generally increases with the grafting of IA onto NaAlg and with the increase in temperature for all the microspheres. When the swelling degree of the microspheres increases, amorphous regions of the matrix produce free volumes that are suitable for penetration of the liquid molecules to the microsphere, which ensures the diffusion of the drug to the external medium. Therefore the cumulative release of nifedipine increases with the grafting of the IA groups. Similar observations were found in the literature. Katime et al. (2001) studied the controlled release of aminophylline from poly(N-isopropylamide-co-itaconic acid) hydrogels and they found that the percentage cumulative release increased with the increasing amount of IA in the hydrogels.

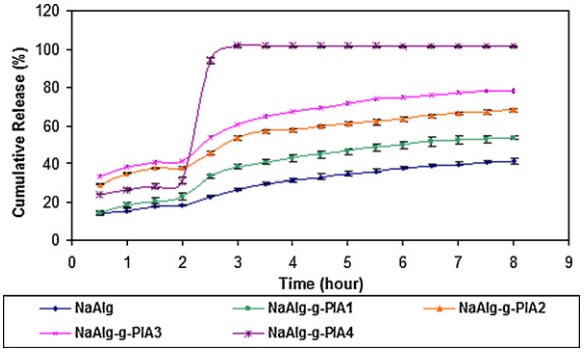

Fig. 4. Effect of IA grafting on the nifedipine release of the microspheres.

3.4. Effect of the drug amount on the nifedipine release

Another parameter that affects the nifedipine release from the microspheres is the drug amount. The effect of the drug amount on nifedipine release is shown in Fig. 5a. The figure illustrates that nifedipine release from the NaAlg-g-PIA microspheres with 10% of the drug is higher than that of microspheres with 20% and 40% of the drug. The highest nifedipine release for the microspheres with 10% of the drug obtained is 53.73% while the lowest nifedipine release for the microspheres with 40% of the drug obtained is 16.30% at the end of 8 h. When the amount of drug decreases from 40% to 10%,

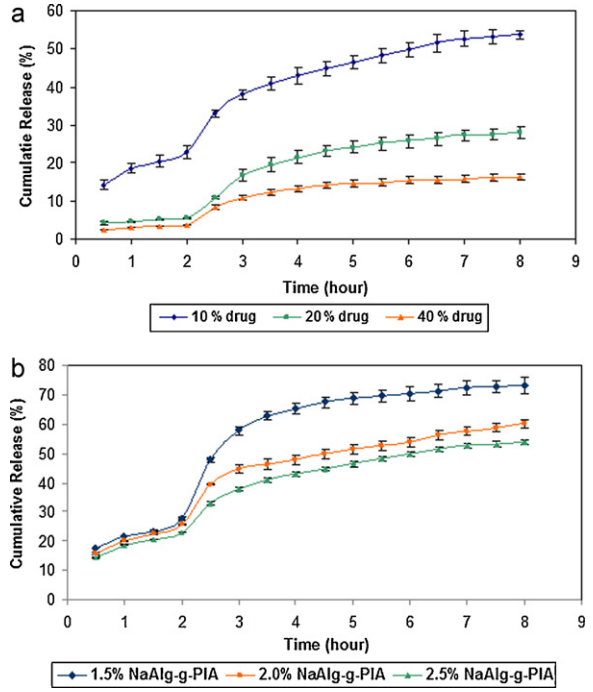

Fig. 5. (a) Effect of the drug amount on the nifedipine release of the microspheres. (b) Effect of NaAlg-g-PIA concentration on the nifedipine release of the microspheres.

nifedipine content of the microspheres decreases as well. Lower nifedipine content might lead to the easier penetration of the liquid through the microspheres and then nifedipine diffusion from the microspheres gains speed. Similar observations were also found in the literature (Işıklan et al., 2008; Mc Gann, Higginbotham, Geever, & Nugent, 2009; Soppimath, Kulkarni, & Aminabhavi, 2001). In addition, as the amount of nifedipine increases drug–copolymer interaction increases. Therefore, the release of nifedipine from the NaAlg-g-PIA microspheres decreases.

Soppimath et al. (2001) studied the controlled release of nifedipine from polyacrylamide grafted guar gum microgels. They reported that nifedipine release decreased from 55% to 17% in 0.1 N HCl solution and decreased from 75% to 37% in pH 7.4 phosphate buffer solution as drug loading was increased from 5% to 20%.

Mc Gann et al. (2009) investigated pH-sensitive poly(vinyl alcohol)-poly(acrylic acid) composite hydrogels for the controlled release of aspirin. They observed that aspirin release decreases with the increase in the amount of aspirin from 0.5% to 1%.

3.5. Effect of NaAlg-g-PIA concentration on the nifedipine release

Three different concentration of NaAlg-g-PIA (1.5%, 2.0% and 2.5%) were utilized for the preparation of the microspheres. The drug release behaviours are shown in Fig. 5b. The results of the release study indicated that the amount of drug release decreases with an increase in the concentration of NaAlg-g-PIA. NaAlg is a polysaccharide that contains different type of hydrophilic functional groups. As increase in the concentration of the NaAlg-g-PIA, hydrophilic functional groups in the microsphere preparation solution increase. Therefore, more functional groups cross-linked with the –CHO groups of the GA, which result in decrease of nifedipine release. A similar type of result was also obtained by Sultana et al. (2010) in the study of alginate microspheres containing diltiazem hydrochloride.

3.6. Effect of exposure time to GA and concentration of GA on the nifedipine release

The drug release from the microspheres is subjected to a number of physical and chemical parameters including those related directly to the release medium, the release conditions (temperature, pH), preparation conditions and those resulting from the change in the characteristics of the microspheres. One of the most effective ways to change the release rate of microspheres is to change the cross-link density of the matrix by employing varying times of exposure to cross-linking agent and concentrations of the cross-linking agent. Fig. 6a and b presents the release profiles of the NaAlg-g-PIA microspheres, which are prepared with different concentrations of GA and exposure times. As it is seen from the figure, the release of nifedipine decreased when the GA concentration and exposure time of the GA were increased. As the GA concentration was increased from 1.00% to 1.50%, nifedipine release decreased from 79.0% to 44.7% in pH 7.4 at the end of 8 h, as shown in Fig. 6a. As the exposure time to GA was increased from 15 to 30 min, nifedipine release decreased from 54.5% to 45.0% in pH 7.4, at the end of 8 h (Fig. 6b). The observed decreases in the cumulative release are due to the fact that increasing exposure time and concentration of GA result in an increase in the cross-link density of the microsphere which gives rise to a compact network of the polymer. Consequently, the free volume reduces and the penetration of water molecules and diffusion of nifedipine molecules become difficult. The nifedipine release results were also supported by the equilibrium swelling degree data presented in Table 2a. Nifedipine release is significantly influenced by the equilibrium swelling degree of the cross-linking polymeric microspheres. Drug release from the microspheres increased as the

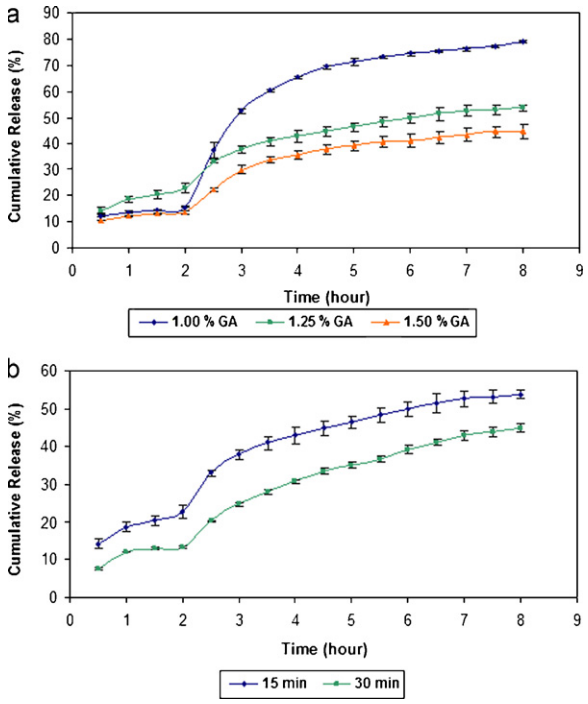

Fig. 6. (a) Effect of GA concentration on the nifedipine release of the microspheres. (b) Effect of exposure time to GA on the nifedipine release of the microspheres.

equilibrium swelling degree increased due to the easy diffusion of the drug molecules to the outside of the microspheres. As seen from Table 2a, the swelling percentage of the microspheres at all the temperature and pH values decreases with the increase in concentration of GA and exposure time to GA. When the concentration of GA in the microsphere preparation solution was increased from 1.00% to 1.50%, the ESD value of the NaAlg-g-PIA microspheres (B1 and B2) significantly decreased from $1066.26 \pm 18.10\%$ to $627.56 \pm 12.36\%$, respectively, in the pH 7.4 phosphate buffer medium at 310 K. In the same way, when the exposure time to the GA was increased from 15 to 30 min, the ESD value of the NaAlg-g-PIA microspheres (A2 and B5) decreased from $991.98 \pm 16.91\%$ to $628.01 \pm 16.83\%$, respectively, in the pH 7.4 phosphate buffer at 310 K because of the increase in the cross-linking degree. Similar results were reported in many studies (Babu et al., 2007; Babu, Hosamani, & Aminabhavi, 2008; Şanlı, Biçer, & Işıklan, 2008; Şanlı, Karaca, & Işıklan, 2009).

Babu et al. (2007) studied the controlled release of the nifedipine from NaAlg-methylcellulose blend microspheres. They reported that the microspheres cross-linking with 2.5 mL of GA showed 393% equilibrium swelling degree, whereas when prepared with 7.5 mL of GA, the microspheres showed 337% equilibrium swelling degree. They also found that when the GA concentration of 10% drug-loaded microspheres was changed from 2.5 mL to 7.5 mL, cumulative drug release decreased from 95% to 82%.

Table 2a also displays the effect of the medium temperature on the swelling degree. The effect of medium temperature on the swelling degree for the various microsphere formulations was investigated at three different temperatures (300 K, 310 K and 320 K) and the obtained results are given in Table 2a. As can be seen from the table the equilibrium swelling degree increases with an increase in the temperature.

According to the free volume theory, the thermal motion of polymer chains in the amorphous regions randomly produces free volume (Mark, Eisenberg, Grtaessly, Maldelkern, & Koening, 1984). As the temperature increases, the frequency and amplitude of the chain jumping increase and resulting free volumes become larger.

The penetration of water molecules through these free volumes and then swelling of the polymeric matrix become easier at a higher temperature. Hence, the swelling degree of the cross-linked NaAlg-g-PIA microspheres is high when the temperature is high. Similar results concerning the effect of temperature on the swelling degree of the beads have been reported in the literature (Bajpai & Giri, 2002; Bajpai & Bhanu, 2003).

3.7. pH-responsive characteristics of microspheres

pH is a key factor in the oral drug delivery systems which determines the release of a drug in pH-responsive microspheres. It is known that pH is about 1.2 in the stomach and is about 7.4 in the intestine. It is also observed from Figs. 4–6 that NaAlg-g-PIA microspheres have demonstrated a slower release of nifedipine in the acidic pH as compared to medium of pH 7.4. A difference is observed in the release data between pH 1.2 and pH 7.4 conditions, which can be also attributed to less swelling of NaAlg-g-PIA in acidic medium (as it can be seen from Table 2a). For instance, the equilibrium swelling degree of A2 at 37 °C is found to be 145.48 ±0.83% at pH 1.2 whereas it is found to be 1991.98 ± 16.91% at pH 7.4. At acidic pH, nifedipine release is low for 2 h. At low pH values, the less swelling should reduce the matrix permeability and limit the drug diffusion. At this pH, alginate is protonated into insoluble form of alginic acid, which is a polymer that displays the properties of less-swelling matrix, and this explains why the release amount is low. In addition, at acidic pH, amine group of nifedipine gains proton and nifedipine in this form dissolves easily in the aqueous solution. At pH 7.4, a rapid increase of the release occurs since the alginate and poly(itaconic acid), which swell in water, convert to sodium salt. The deprotonation of alginic acid and itaconic acid causes a break of hydrogen bands and the generation of the electrostatic repulsion among polymer chains. Therefore, a higher swelling degree could be obtained at high pH, which speeds the release of the nifedipine (Fernandez-Herva's, Holgado, Fini, & Fell, 1998; Wang et al., 2009; Yotsuyanagi, Yoshioka, Segi, & Ikeda, 1991).

3.8. Determination of diffusion coefficient

Diffusion coefficient, D, can be calculated for water absorption or drug release by microspheres using the equation as follows (Agnihotri & Aminabhavi, 2006):

$$D = \left(\frac{r\theta}{6M_\infty}\right)^2 \pi \tag{4}$$

where θ is slope of the linear portion of the plot of M_t/M_∞ vs. $t^{1/2}$, r is radius of the microspheres and M_∞ is the maximum drug release. The D values that depend on nifedipine release in pH 7.4 phosphate buffer solution are shown in Table 1. The D values change between 1.21×10^{-8} and 4.66×10^{-6} cm^2/s. As it is seen from the table, the D value of the microspheres increased with the increase in IA grafting yield, whereas it decreased with the increase in GA concentration, exposure time to GA and drug amount. These results are in good agreement with the release data. Similar findings were also obtained in the previous studies (Işıklan et al., 2008; Inal et al., 2008).

3.9. Molar mass between the cross-links

The drug release from the polymer matrix is a function of the extent of cross-linking. In order to understand the cross-linking of the polymer network, it is important to calculate the molar mass, M_c, between the cross-links of the network polymer. The magnitude of M_c significantly affects the physical and mechanical properties of the cross-linked polymer and its determination has great practical significance. Equilibrium swelling is widely used to determine M_c. Flory and Rehner's equation in the following form was used to calculate M_c values (Flory, 1953).

$$M_c = -\rho_p V_s \phi^{1/3} [\ln(1-\phi) + \phi + \chi\phi^2]^{-1} \tag{5}$$

The volume fraction, ϕ of the swollen polymer was calculated as follows:

$$\phi = \left[1 + \frac{\rho_p}{\rho_s}\left(\frac{M_a}{M_b}\right) - \frac{\rho_p}{\rho_s}\right]^{-1} \tag{6}$$

In the above equations, ρ_p and ρ_s are the densities of polymer and solvent, respectively; M_b and M_a, are the mass of polymer before and after swelling, respectively. V_s is the molar volume of the solvent. The interaction parameter, χ, was calculated using the following equation, a procedure published by Aithal and Aminabhavi (1990).

$$\chi = \left[\phi(1-\phi)^{-1} + N\ln(1-\phi) + N\phi\right] \times \left[2\phi - \phi^2 N - \phi^2 T^{-1}\left(\frac{d\phi}{dT}\right)^{-1}\right]^{-1} \tag{7}$$

where N is calculated in the following equation:

$$N = \left(\frac{\phi^{2/3}}{3} - \frac{2}{3}\right)\left(\phi^{1/3} - \frac{2\phi}{3}\right)^{-1} \tag{8}$$

and $d\phi/dT$ is the slope of the line obtained by plotting the volume fraction vs. temperature (in K).

The M_c values were calculated from the equilibrium swelling data at three different temperatures and presented in Table 2a. The M_c values varied in the range from 12,924 to 63,162. This data indicates that M_c values increase with the increasing temperature and grafting yield due to the decrease in cross-link density while they decrease with the increasing GA concentration and exposure time, since the network would become denser, which supports the nifedipine release results. Similar types of results have also been reported elsewhere (Agnihotri & Aminabhavi, 2006; Bajpai & Sharma, 2005; Işıklan et al., 2008).

3.10. Drug release kinetics

In order to find out the mechanism of drug release through the polymeric matrices, we have analyzed the release kinetics data using the diffusion equation: $M_t/M_\infty = Kt^{0.5}$ proposed by Higuchi (Roseman & Higuchi, 1970) to evaluate the values of K, the kinetic rate constant, which is independent of geometrical and structural properties of the polymer. Here, M_t is the amount of drug released at time, t and M_∞ is the amount of drug released after (longer time) infinite time. Obtained results are presented in Table 2b. High correlations were observed in the Higuchi plots. The release of drug from the microsphere was proportional to square root of time,

Table 2b
Release kinetics data for drug-loaded NaAlg-g-PIA microspheres.

Polymer codes	Polymer	K	R^2
A_1	NaAlg	0.001	0.996
A_2	NaAlg-g-PIA$_1$	0.002	0.991
A_3	NaAlg-g-PIA$_2$	0.001	0.994
A_4	NaAlg-g-PIA$_3$	0.002	0.981
A_5	NaAlg-g-PIA$_4$	Not calculated	
B_1	NaAlg-g-PIA$_1$	0.003	0.979
B_2	NaAlg-g-PIA$_1$	0.001	0.996
B_3	NaAlg-g-PIA$_1$	0.001	0.993
B_4	NaAlg-g-PIA$_1$	0.001	0.990
B_5	NaAlg-g-PIA$_1$	0.002	0.998
B_6	NaAlg-g-PIA$_1$	0.002	0.970
B_7	NaAlg-g-PIA$_1$	0.002	0.976

suggesting that the drug release from the microspheres followed diffusion-controlled mechanism.

4. Conclusions

In this study, graft copolymers of sodium alginate with itaconic acid were synthesized in aqueous solution using ceric ammonium nitrate and pH-responsive NaAlg-g-PIA microspheres were successfully prepared. Nifedipine, a calcium channel blocker drug, was encapsulated into the NaAlg-g-PIA matrix with a high percentage of entrapment efficiency. The microspheres formed were almost spherical in shape and showed a roughness on the surface. The release of nifedipine was found to be much higher at high pH value compared to low pH value. The equilibrium swelling measurements of the microspheres clearly demonstrated the pH-responsive nature of the materials. It was found from the release study that nifedipine release from the NaAlg-g-PIA microspheres decreased with the increase of exposure time to GA, drug amount, GA and NaAlg-g-PIA concentrations while it increased with the increase of itaconic acid graft yield. The equilibrium swelling degree, diffusion constant, and M_c value of all the formulations were found to be consistent with the release results. To conclude, the nifedipine release from the alginate-based microspheres can be changed depending on the preparation conditions of the microspheres. These results suggest that NaAlg-g-PIA microspheres have the potential to be used as an effective pH-responsive drug delivery system in the biomedical field.

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