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

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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

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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.