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The effects of reaction conditions on block copolymerization of chitosan and poly(ethylene glycol)

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ABSTRACT

A novel injectable in situ gelling thermosensitive chitosan-block-poly(ethylene glycol) formulation was synthesized for drug delivery applications. Block copolymerization of monomethoxy–poly(ethylene glycol) onto chitosan using potassium persulfate as an initiator was carried under a nitrogen atmosphere in aqueous solution. The effects of potassium persulfate and poly(ethylene glycol) concentrations, reaction time and reaction temperature on block polymerization were studied by determining the yield of reaction (%*Y*), polymerization efficiency (%*E*) and add-on percentage (%Add-on). Keeping the other conditions constant, the optimum reaction conditions were found to be initiator = 0.01 M, reaction temperature = 60 °C and reaction time = 6 h.

1. Introduction

Hydrogels are a unique class of macromolecular networks that can contain a large fraction of aqueous solvent within their structure. The ability of hydrogels to simulate biological tissues makes them suitable for biomedical and pharmaceutical applications (Hoare & Kohane, 2008; Peppas, Hilt, Khademhosseini, & Langer, 2006). Interestingly, the aqueous solutions within some hydrogels undergo a sol–gel transition in response to certain stimuli. Therapeutic agents such as drugs, cells or proteins could be mixed into a sol state and injected using a syringe into the subcutaneous layers of a diseased site to form a depot system (Ganji & Vasheghani-Farahani, 2009). These minimally invasive, in situ gelling injection systems are an advantageous alternative to surgical procedures. Therefore, in situ gelling hydrogels have gained considerable interest for pharmaceutical and biomedical applications and have been reviewed from different points of view (He, Kim, & Lee, 2008; Jeong & Gutowska, 2002; Ulijn, 2006; Van-Tomme, Storm, & Hennink, 2008). Thermally reversible hydrogels, which make gels in response to finite temperature changes, have gained the most interest (Ruel-Gariépy & Leroux, 2004; Schmaljohann, 2006; Vermonden, Besseling, Steenbergen, & van Hennink, 2006). Tri-block copolymers of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide), which are available as Poloxamers or Pluronics, are the most widely used thermally reversible hydrogels (Jeong, Kim, & Bae, 2002; Xiong, Tam, & Gan, 2006). The aqueous solution within Poloxamers demonstrates a phase transition from sol to gel between 5 and 30 °C and from gel to sol between 35 and 50 °C, with the temperature increasing monotonically over a polymer concentration range of 20–30 wt%.

Chitosan is the biopolymer that is most widely used as a thermosensitive hydrogel (Muzzarelli et al., 2007). Chitosan is obtained by alkaline deacetylation of chitin, a cellulose-like polysaccharide that is extracted from the shells of crustaceans such as crabs, shrimps and lobsters (Muzzarelli & Muzzarelli, 2009). Although chitin is completely insoluble in aqueous media, chitosan can be dissolved under acidic conditions that provide sufficient protonation of its amino groups. The resulting aqueous solutions are usually stable as long as the pH is below 6.2 (Chenite, Gori, Shive, Desrosiers, & Buschmann, 2006). When the pH exceeds 6.2, chitosan solutions can be neutralized by glycerol phosphate disodium salt solutions, which leads to the formation of a hydrated gel-like precipitate (Chenite, Buschmann, Wang, Chaput, & Kandani, 2001). Chitosan–glycerophosphate solutions exhibit reverse thermogelling properties: they are sols at room temperature and gels at body temperature (Chenite et al., 2000; Ganji, Abdekhodaie, & Ramazany-Sadtabadi, 2007).

A thermosensitive sol–gel solution was also obtained by grafting polymerization of N-isopropylacrylamide (NIPAAm) onto chitosan (Lee, Jung, Park, Park, & Ryu, 2004). The maximum grafted chitosan copolymer was obtained with 0.4 M NIPAAm and 6×10^{-3} M cerium ammonium nitrate as an initiator.

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Dal Pozzo et al. (2000) used poly(ethylene glycol) in dialdehyde form to crosslink reacetylated chitosans. They found that the crosslinking of reacetylated chitosan with PEG–dialdehydes results in water-soluble biomaterials that become insoluble upon freeze-drying. Shantha and Harding (2002) studied the preparation and characterization of chemically modified chitosan microspheres obtained by graft copolymerization of PEG–diacrylate macromonomer onto the chitosan backbone. The ceric ion was employed in the synthesis of the graft copolymers. Bhattarai and coworkers synthesized a chitosan-based, injectable thermogel by grafting an appropriate amount of PEG onto the chitosan backbone. They also studied drug release in vitro using bovine serum albumin as a model protein (Bhattarai, Matsen, & Zhang, 2005). Chitosan was first modified with a PEG–aldehyde to yield an imine, which consequently transformed into PEG-g-chitosan during reduction with sodium cyanoborohydride. The Bhattarai group showed that the PEG-g-chitosan copolymers would undergo a thermo-reversible transition from a sol at low temperature to a transparent gel at approximately 25 °C or above. The required time for gelation of their PEG-g-chitosan copolymers varied from 10 min to 1 h, depending on the polymer concentration. They also found that the required amount of grafted PEG to have an injectable thermosensitive copolymer is approximately 36–55 wt%. Below 36 wt% grafted PEG, the obtained copolymers were found to be poorly soluble in water (Bhattarai et al., 2005).

Recently, we synthesized a novel block copolymer of chitosan and PEG at physiological pH values by block copolymerization of monomethoxy–PEG onto the chitosan backbone using potassium persulfate as a free-radical initiator (Ganji & Abdekhodaie, 2008). We demonstrated that the solution of the resultant block copolymer undergoes a thermo-reversible transition from an injectable sol at low temperatures to a gel at body temperature. The gelation time varied from 6 to 11 min. We also found that solutions with high polymer concentrations or low PEG content have a shorter gelation time than those with low polymer concentrations or high PEG contents (Ganji & Abdekhodaie, 2008). Consequently, the amount of PEG grafted onto the chitosan backbone must be an important parameter for controlling the thermosensitivity and injectability of chitosan-b-PEG copolymers. In this work, the effects of reaction conditions like reaction time, reaction temperature, concentration of initiator and concentration of PEG on block copolymerization were investigated.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan was purchased from Sigma–Aldrich Chemical Co. (USA). The degree of deacetylation (DDA) of chitosan was found to be 82.5% by ^{1}H nuclear magnetic resonance analysis. The viscosity-average molecular weight was determined to be 2.5×10^5 using the Mark–Houwink equation (Wang, Bo, Li, & Qin, 1991). Monomethoxy–poly(ethylene glycol) (MPEG, M_w = 2000) was purchased from Fluka Chemical Co. (USA) and was dehydrated thoroughly before use to eliminate traces of moisture. Acryloyl chloride, triethylamine and dried 1,2-dichloromethane were purchased from Merck (Germany). Ethyl ether was purchased from Panreac (E.U.) and was dried carefully. Potassium persulfate (KPS) was purchased from Sigma and was used as received. Other reagents were chemical grade and were used as received.

2.2. Synthesis of block copolymer

Block copolymers of chitosan and PEG (chitosan-b-PEG) were prepared by the method described elsewhere (Ganji & Abdekhodaie, 2008). Briefly, monomethoxy–PEG was first modified with acryloyl chloride to yield a PEG-macromere that was subsequently attached to the chitosan backbone through a free-radical reaction. Then, chitosan was dissolved in 0.1 M acetic acid and was taken into a flask containing mixer and nitrogen inlet. KPS was added to the chitosan solution and the resultant mixture was stirred for 30 min under nitrogen atmosphere at 60 °C. Pre-prepared PEG-macromere was added gradually and the resultant mixture was stirred for 6 h. The mixture was filtered and the filtrate was then precipitated with 5% sodium hydroxide. The precipitate was obtained by centrifugation and was washed with acetone to remove free PEG. Finally, the resultant mixture was dialyzed with a dialysis membrane (M_w = 12,000–14,000 cut) and was dried under atmosphere for 24 h and then in vacuum at 40 °C for 2 days. Block copolymers with different ratio of chitosan to the PEG-macromere were prepared. To study the effects of the principal reaction variables on the copolymerization process, some reaction parameters such as yield of reaction (%Y), efficiency percentage (%E) and add-on percentage (%Add-on) were defined as follows:

$$\%Y = \frac{W_3}{W_1} \times 100, \tag{1}$$

$$\%E = \frac{W_3 - W_1}{W_2} \times 100, \tag{2}$$

$$\%\text{Add-on} = \frac{W_3 - W_1}{W_3} \times 100, \tag{3}$$

where W_1, W_2 and W_3 denote the weight of the initial chitosan, initial PEG-macromere and obtained chitosan-b-PEG copolymer, respectively.

2.3. Statistical analysis

All measurements were collected in triplicate, and the average of the independent runs is reported here. The figures show the mean value and the standard deviation among triplicates.

3. Results and discussion

The goal of this study was to characterize the effects of some reaction conditions such as time, temperature, feed mole ratio and initiator concentration on block polymerization of chitosan and PEG. To achieve this goal, chitosan-b-PEG copolymers were synthesized at different conditions and the results were analyzed as described below. Moreover, scanning electron micrographs of chitosan and its block copolymer were obtained using SEM XL-series from Philips (The Netherlands) at 15 kV (Figs. 1 and 2). Apparently, copolymerization modified the surface morphology of chitosan, wherein great morphological differences were visible in the surface topography of the chitosan and its copolymers. The flaky nature of chitosan was clearly changed to a clustered, irregular structure of chitosan-b-PEG.

3.1. Effect of initiator concentration

It is well known that, when KPS is heated in aqueous solution, the persulfate initiator ($S_2O_8^{-2}$) decomposes into sulfate anion radicals ($SO_4^{-\bullet}$). These radicals attack the C_4 carbon of the chitosan chain, break the C–O–C bond and, finally, depolymerize chitosan into two shorter chains. The reactive free-radical end of one chitosan chain could act as a powerful nucleophile that could readily attack the unsaturated carbon–carbon double bond in the PEG-macromere. Therefore, the PEG-macromere could grow on the chitosan chain and produce the block copolymers with a range of PEG content (Scheme 1).

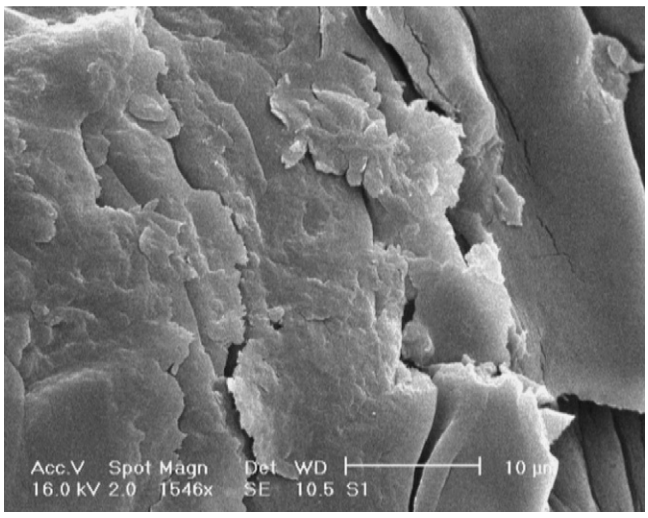


Fig. 1. A SEM microphotograph of chitosan.

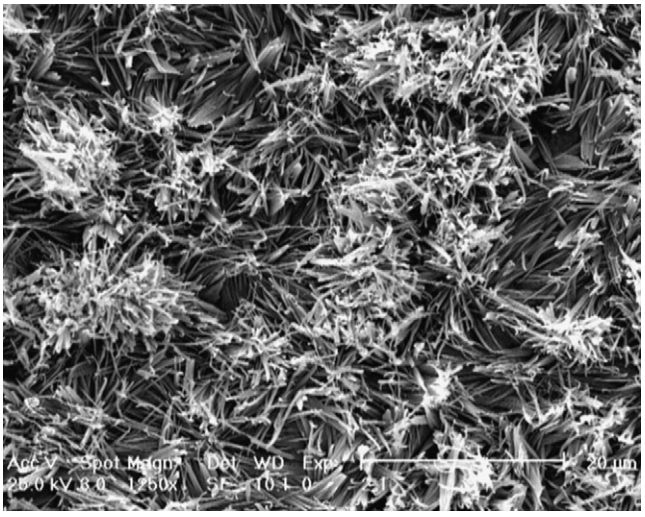


Fig. 2. An SEM microphotograph of chitosan-b-PEG copolymer.

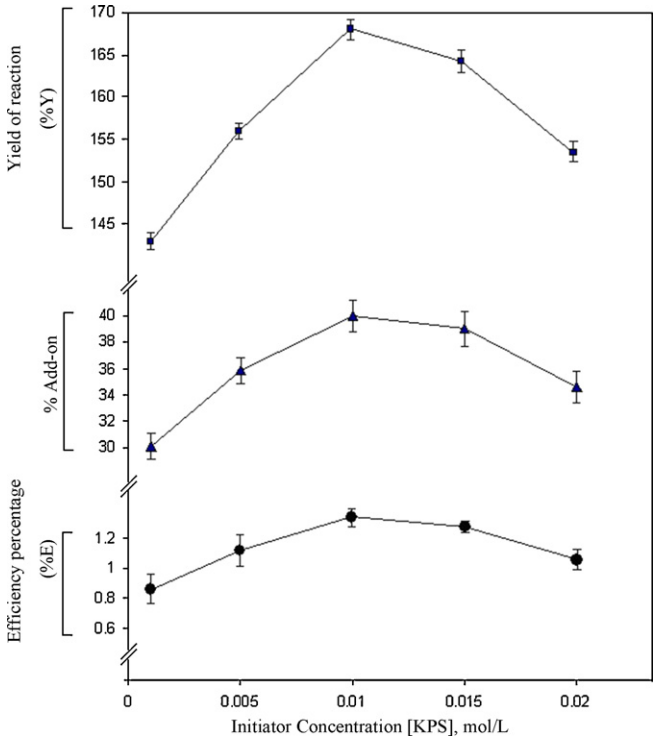
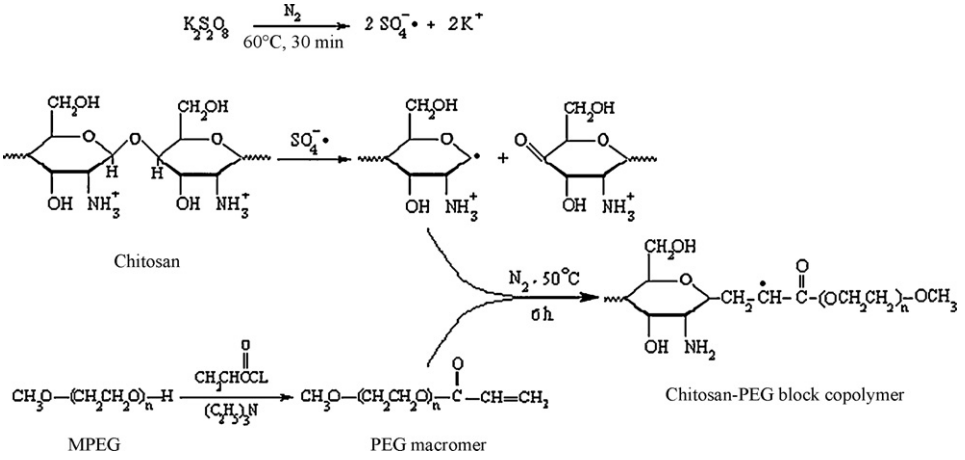


Fig. 3. The effects of initiator concentration on yield of reaction (%*Y*, ■), add on percentage (%Add-on, ▲) and efficiency percentage (%*E*, ●). Reaction conditions: mole ratio of PEG/CH in feed = 0.4; time = 6 h; temp. = 60 °C (n = 3, error bars represent standard deviations).

The importance of the KPS concentration was estimated by studying its effect on the copolymerization parameters. By keeping all of the other reaction variables constant, the KPS concentration was varied from 10^{-3} to 2×10^{-2} M (Fig. 3). The yield of reaction (%*Y*), efficiency percentage (%*E*) and add-on percentage (%Add-on) all increased with increasing KPS concentration until they reached a maximum value at 0.01 mol/L KPS. The reason for this maximum is that the active radicals of the chitosan in the copolymerization increased as the amount of $SO_4^{-\bullet}$ ions increased. Further increases in the KPS concentration led to the decrease of all the reaction parameters. It was also observed that the mole ratio of PEG to chitosan (PEG/CH) in the block copolymers was considerably less at low KPS concentrations (<0.01 mol/L), whereas there was a significant amount of PEG blocked to chitosan beyond this value (Fig. 4). This behavior could be explained by the increase in the formation of free-radical-terminated chitosan chains at high KPS concentrations. In this case, the chitosan macro-radicals have the opportunity to combine with the existing excess of primary free radicals present in the reaction medium. Furthermore, the formation of chitosan



Scheme 1. A schematic representation of block copolymerization of chitosan and PEG-macromere, Reproduced from Ganji and Abdekhodaie (2008), with permission.

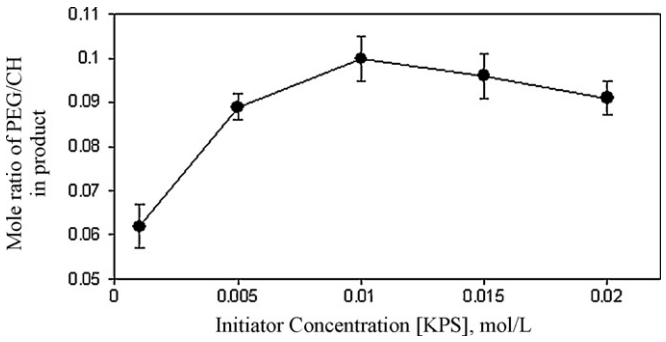


Fig. 4. The effect of initiator concentration on [PEG/CH] mole ratio in product. Reaction conditions: mole ratio of PEG/CH in feed = 0.4; time = 6 h; temp. = 60 °C (n = 3, error bars represent standard deviations). [PEG/CH] mole ratio in product is determined by ^{1}H NMR spectra analysis.

homo-polymers can be expected due to the unavailability of sites on short-length free-radical-terminated chitosan chains, which are produced at high KPS concentrations. As a result, unused KPS can lead to an increase in the rate of homo-polymerization. Similar results were published for emulsion polymerization of poly(methyl methacrylate) (PMMA) in the presence of chitosan and KPS (Hsu, Don, & Chiu, 2002). The polymerization rate was shown to decrease as the pre-degradation time of chitosan solution by KPS increased. These results indicated that the excess amount of degraded chitosan chains could inhibit the overall reaction. Also, the extent of deactivation was related to the extent of pre-degradation of chitosan. The terminal ring structure with the carbonyl group in the degraded chain was assumed to inhibit the free radicals (Hsu et al., 2002). Joshi and Sinha also described the same behavior for grafting reaction of acrylic acid onto carboxymethyl chitosan in the presence of ceric ammonium nitrate (CAN) as a free-radical initiator (Joshi & Sinha, 2007). Based on their studies, formation of homo-polymer was considerably less at low initiator concentration, whereas there was a significant homo-polymer formation beyond a certain value of CAN.

3.2. Effect of reaction temperature

The effect of temperature was studied by changing the reaction temperature from 40 to 90 °C and keeping the other reaction conditions constant. Fig. 5 shows that %*Y*, %*E* and %Add-on increased as the temperature rose from 40 to 60 °C, were constant from 60 to 70 °C and, then, decreased as the temperature rose above 70 °C. At low temperatures, the dissociation reaction of KPS and the chitosan chain degradation was slow; therefore, a small amount of radicals were generated and the efficiency was low. At temperatures above 70 °C, the reaction occurred with poor selectivity and various undesired and secondary reactions accelerated, which led to a decrease in reaction parameters. Furthermore, the decrease in %*Y*, %*E* and %Add-on at elevated temperatures could be explained by the acceleration of the termination reaction, which leads to the formation of more homo-polymers. The same results were published for the graft copolymerization of 2-hydroxyethylmethacrylate (HEMA) onto carboxymethyl chitosan (CMCH) (Joshi & Sinha, 2006). At high temperatures, various hydrogen abstractions and chain-transfer reactions were accelerated, which led to a decrease in grafting efficiency of HEMA onto CMCH. Soroush, Grady, and Kalfas (2008) presented an evaluation of systems challenges in free-radical solution homo-polymerization as the temperature was increased. They claimed that the classical chain polymerization kinetic models (accounting for initiation, propagation, chain transfer to solvent and monomer, and termination reactions) are unable to describe the dynamics of these processes. The dynamics of homo-

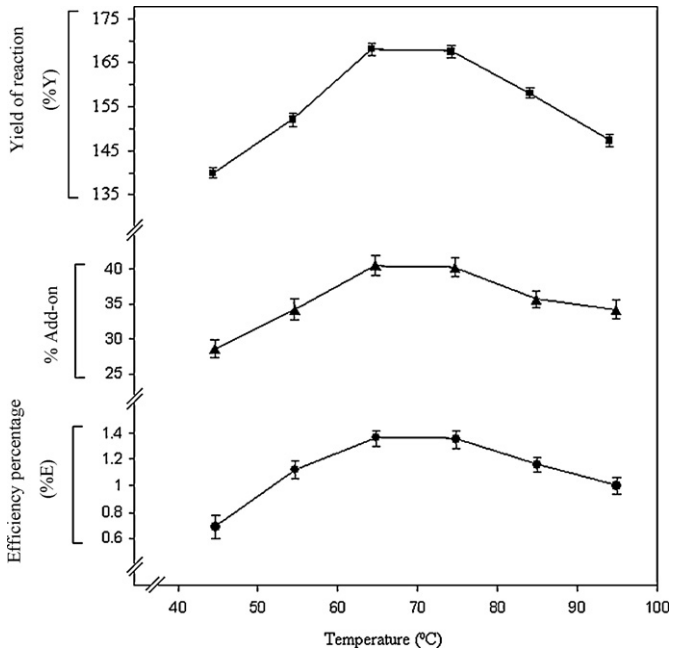


Fig. 5. The effects of temperature on yield of reaction (%*Y*, ■), add-on percentage (%Add-on, ▲) and efficiency percentage (%*E*, ●). Reaction conditions: [KPS], 0.01 M; [PEG/CH] mole ratio in feed = 0.4; time = 6 h (n = 3, error bars represent standard deviations).

polymerization at high temperatures are complicated by significant contributions of secondary reactions, such as de-propagation, self-initiation, β-scission, and inter/intra-molecular chain-transfer reactions. Soroush et al. (2008) concluded that these secondary reactions have less effect on the polymerization at low temperatures.

3.3. Effect of reaction time

Fig. 6 shows the effects of varying the reaction time while keeping the other reaction conditions constant. The maximum for %*Y*, %*E* and %Add-on was attained after 6 h; then, the reaction parameters decreased slightly. The increase in the reaction parameters may be due to a better reaction environment being produced and the time for polymerization being extended. Prolonging the reaction time clearly results in favorable effects on the diffusion and absorption of the reactants and induces better contacts between the monomers (Bhattacharyya, Singhal, & Kulkarni, 1995). The decrease in the reaction parameters after 6 h could be explained by the decrease in initiator concentration and in the number of chitosan free radicals as well as by the reduction in the amount of PEG-macromeres available for the reaction. In a similar study, the observed decrease in the grafting efficiency for longer reaction times of poly(acrylic acid) grafted onto chitosan was attributed to the gradual liberation of retained impurities or small molecules from grafted chitosan macromolecules (Yazdani-Pedram, Retuert, & Quijada, 2000).

3.4. Effect of PEG/CH mole ratio in feed

Fig. 7 shows that, by increasing the relative amount of PEG-macromere to chitosan in feed, the %*Y*, %*E* and %Add-on increases markedly due to the greater availability of macromeres for polymerization. Fig. 8 shows that the mole ratio of PEG/CH in the product increases when this ratio is increased in the feed. This behavior could be explained by the fact that an increase of PEG-macromere concentration leads to the accumulation of PEG molecules in close proximity to the chitosan backbone, which, in

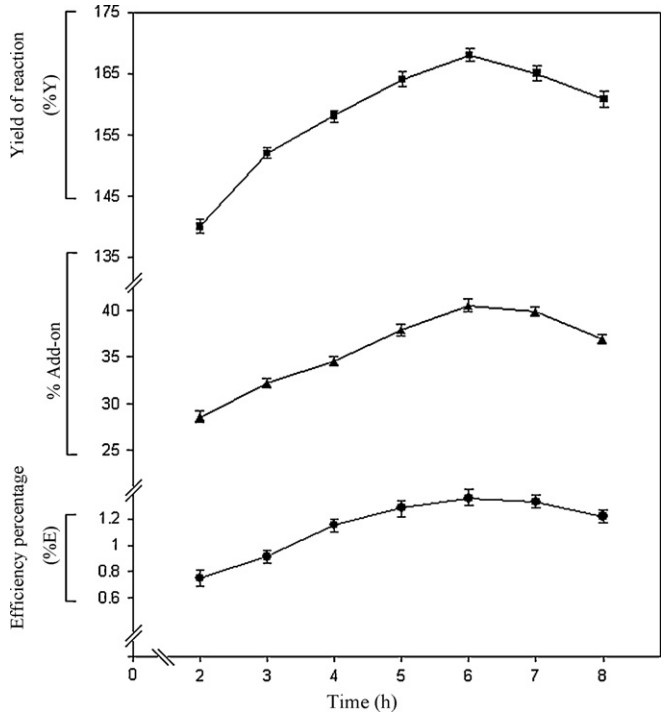


Fig. 6. The effects of time of reaction on yield of reaction (%*Y*, ■), efficiency percentage (%*E*, ●) and add-on percentage (%Add-on, ▲). Reaction conditions: [KPS], 0.01 M; [PEG/CH] mole ratio in feed = 0.4; temp. = 60 °C (*n* = 3, error bars represent standard deviations).

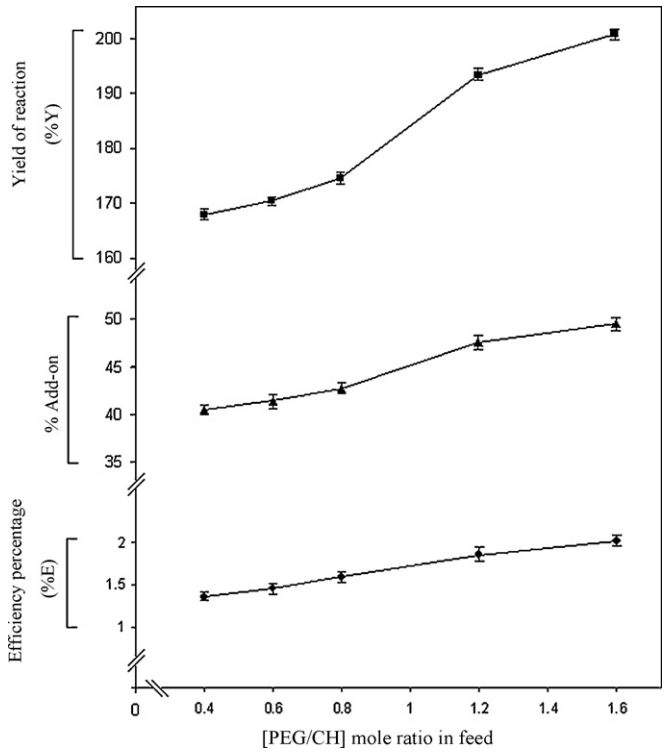


Fig. 7. The effects of mole ratio of [PEG/CH] in feed on yield of reaction (%*Y*, ■), add-on percentage (%Add-on, ▲) and efficiency percentage (%*E*, ●). Reaction conditions: [KPS], 0.01 M; [PEG/CH] mole ratio in feed = 0.4; temp. = 60 °C (*n* = 3, error bars represent standard deviations).

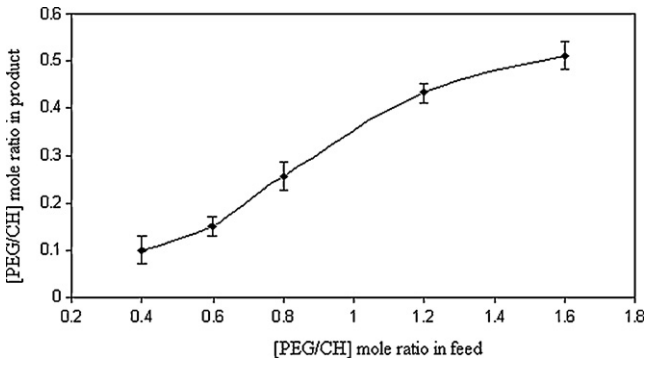


Fig. 8. The effect of mole ratio of [PEG/CH] in feed on [PEG/CH] mole ratio in product. Reaction conditions: [KPS], 0.01 M; temp. = 60 °C; time = 6 h. [PEG/CH] mole ratio in product is determined by ^{1}H NMR spectra analysis (*n* = 3, error bars represent standard deviations).

turn, increases the mole ratio of PEG/CH in the obtained block copolymer.

4. Conclusion

This work demonstrated the feasibility of block copolymerization of PEG onto chitosan using KPS as an initiator. The study of FTIR spectra and SEM analysis confirms the success of block copolymerization. The optimal reaction temperature and reaction time for the copolymerization were 60 °C and 6 h and the optimal amount of KPS was 0.01 mol/L. This thermosensitive, chitosan-based hydrogel has potential applications in controlled drug delivery systems.

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