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Studies on graft copolymerization of 2-hydroxyethyl acrylate onto chitosan

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Abstract

Graft-polymerization of 2-hydroxyethyl acrylate (HEA) onto chitosan (CS) using ammonium persulfate (APS) as an initiator was carried out in an aqueous solution. Evidence of grafting was obtained by comparing ^{1}H NMR and FT-IR spectra and scanning electron microscopy images of chitosan and the grafted copolymer as well as solubility characteristics of the products. The effects of APS, HEA concentration, reaction temperature and duration of graft-polymerization were studied by determining the grafting parameters, such as grafting percentage and grafting efficiency. The HEA-grafted chitosan product is soluble in a wide pH range, while the original unmodified chitosan is water-soluble only in a narrow pH range. A mechanism for the free-radical grafting was proposed.

Keywords: Chitosan; Dynamic viscosity; FT-IR; Graft-polymerization; 2-Hydroxyethyl acrylate; NMR

1. Introduction

Chitosan is the product obtained at *N*-deacetylation of chitin with strong alkali. Chitin, the naturally occurring polysaccharide [1], is the main substance in carapaces of Crustacea such as shells, crabs, lobsters, and shrimps. It is the second most abundant polysaccharide on the earth next to cellulose. Although chitosan and its derivatives are widely used in pharmaceutics [2], biomaterials technology [3], and agriculture [4], their poor solubility in water is still quite an obstacle for application in, for instance, a rapidly developing green food and health additives industry.

Chemical modification of natural polymers is a promising method for production of new biomaterials with specific properties. A number of papers have been published on the grafting polymerization of

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acrylonitrile, dimethylaminoethyl methacrylate, acrylamide, and vinylpyrrolidone onto chitosan using ceric ammonium nitrate (CAN) as an initiator [5–9]. γ-Ray and photo-induced graft-polymerization of acrylamide, methyl methacrylate, 2-hydroxyethyl methacrylate, styrene and acrylonitrile onto chitosan also have been reported [9–13]. Potassium persulfate has been used as redox initiator for the grafting onto chitosan of methyl methacrylate, methyl acrylate, butyl acrylate, 2-acrylamido-2-methylpropane sulfonic acid, and acrylic acid [7,13–15].

In the present work, chemical modification of chitosan was performed by means of graft-polymerization of 2-hydroxyethyl acrylate. Improved water-solubility of the modified polymer was observed compared to the original chitosan. The effect of the principal reaction variables on the grafting process was investigated.

2. Experimental

2.1. Materials

Water-soluble chitosan (hydrochloride form) was obtained from Jakwang Co., Korea. The weight–average molecular weight of the sample was 200,000 g/mol. The deacetylation degrees of the chitosan as determined by FT-IR spectroscopy [16] (Satellite 3000 spectrometer, Mattson, USA) and by ^{1}H NMR spectroscopy (Brucker 300 NMR spectrometer, USA) were 85% and 88%, respectively. Ammonium persulfate ($(NH_4)_2S_2O_8$) ($\geqslant$98%) and 2-hydroxyethyl acrylate (96%) were obtained from Aldrich Chemical Co. All the chemicals and reagents were used as received.

2.2. Synthesis

In a typical grafting reaction, dry chitosan was first dissolved in distilled water (pH of the resulting solution was 3.6). Then, HEA and APS were added to the chitosan solution and well stirred ($T = 25$ °C). The reaction mixture was transferred in glass ampoules (20 ml) and bubbled with argon for 10 min. Then the ampoules were sealed and placed into a water bath with a magnet stirrer and a digital temperature controller. After the graft-polymerization was accomplished, the reaction mixture was precipitated in acetone, filtered, and rinsed with acetone to remove unreacted monomer. The powder was allowed to dry under vacuum to constant weight. Exhaustive extraction of the product with ethanol allowed separation of poly(hydroxyethyl acrylate) (PHEA) homopolymer formed during the grafting reaction from chitosan-graft-HEA. The degree of purification was controlled by detection of the PHEA in the alcohol solution using FT-IR spectroscopy. Solubility of polymers in water was controlled by measuring optical density (D) of the solutions at various pH-values on UV–vis spectrometer (UV-2401 PC Shimadzu, Japan) at 400 nm.

2.3. Characterisation

FT-IR spectra of the chitosan, PHEA and PHEA-grafted chitosan (CS-g-PHEA) were recorded as KBr pellets on a FT-IR-Satellite 3000 spectrometer (Mattson, USA). The spectra were taken as average over 120 scans from 4000 to 400 $cm^{-1}$with resolution 4 cm^{-1}. H^1 NMR spectra of the polymers in D_2O solution were obtained on a Bruker 300 NMR spectrometer.

The dynamic viscosities of the polymer solutions were measured with a Rheotest 2.1 (Germany) at the shear rate 0.123 s^{-1}, rotation rate 0.25 rpm, and at temperature 60 ± 0.05 °C.

The morphology of the chitosan and CS-g-PHEA samples were observed by SEM. For that, the polymers were precipitated in acetone from 2 wt.% water solutions, and dried in vacuum at room temperature to constant weight. After coating with gold, the samples were analyzed with a JEOL® SUPERPROBE 733 electron probe micro analyser. All micrographs were the product of secondary electron imaging used for surface morphology identification with energy dispersion spectrometer INCA ENERGY (Oxford Instruments).

Grafting percentage ($\%G$), grafting efficiency ($\%E$), homopolymer content ($\%H$) were determined as follows [17]: $\%G = (W_1 - W_0)/W_0 \times 100\%$, $\%E = (W_1 - W_0)/W_2 \times 100\%$ and $\%H = (W_3 - W_1)/W_2 \times 100\%$, where W_0, W_1, W_2, and W_3 stand for the weight of chitosan in the initial load, weight of HEA-grafted chitosan after purification, monomer load, and weight of grafted chitosan before extraction PHEA, respectively.

3. Results and discussion

3.1. Synthesis

The grafting reaction of 2-hydroxyethyl acrylate onto chitosan was studied by varying the initial concentrations of the monomer, initiator, and chitosan,

as well as reaction time and temperature. The effect of the reaction variables on the grafting percentage (*%G*), grafting efficiency (*%E*), homopolymer content (*%H*), and dynamic viscosity are shown in Figs. 1–3.

As seen from Fig. 1, both *%G* and *%E* increased gradually with increase in the reaction time up to 60 min, when reached a plateau, while the dynamic viscosity increased continuously during the process of grafting (curve 4 in Fig. 1). With the increase in reaction time, the concentration of monomer and free-radicals in the system is reduced, and thus chain growth probability is reduced as well. As typical for graft-polymerization, a homopolymer HEA was formed along with the graft-copolymer and with increasing of reaction time yield of homopolymer HEA enhanced. Its yield however did not exceed 40% at these reaction conditions.

As it might be expected, *%G*, *%H,* and *%E* increased continuously with HEA content in feed, because of the increased availability of monomer for grafting. As it reaches 0.7 mol/L, the formation of hydrogels was observed at very early stages of the reaction (data not shown).

As seen from Fig. 2, the grafting percentage, grafting efficiency and the dynamic viscosity of the reaction mixture showed extreme dependences on the initiator ammonium persulfate (APS) concentration in feed. Namely, the parameters increased steeply reaching maximum value, and then slowly decreased. A similar phenomenon was described by Hsu et al. [18]. They found that taken in a small amount, APS radicals first attack a carbon atom in

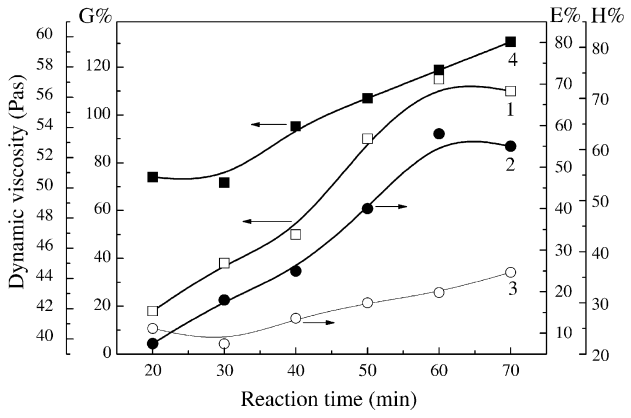

Fig. 1. Effect of the reaction time on the grafting percentage (1), grafting efficiency (2), homopolymer content (3) and dynamic viscosity (4). Reaction conditions: [Chitosan], 2 wt.%; [APS], 1×10^{-2} mol/L; [HEA], 0.35 mol/L; and temperature, 60 °C. Dynamic viscosity of solutions was measured during the reaction.

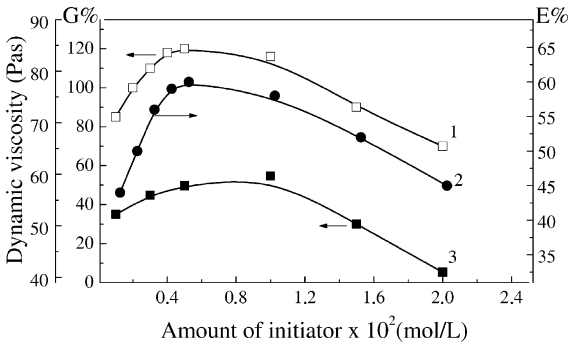

Fig. 2. Effect of the initiator concentration on grafting percentage (1), grafting efficiency (2) and dynamic viscosity (3). Reaction conditions: [Chitosan], 2%; [HEA], 0.35 mol/L; time, 60 min; and temperature, 60 °C. Dynamic viscosity of solutions was measured at the end of the reaction.

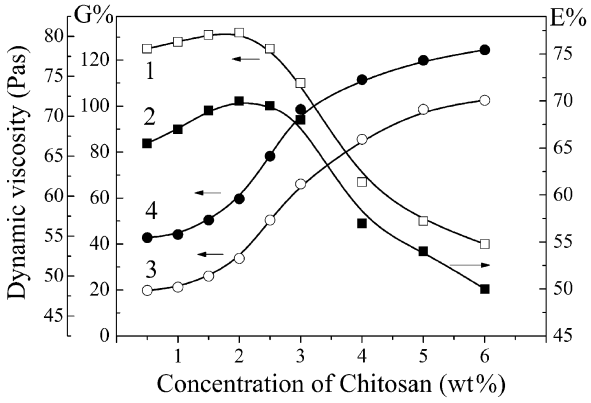

Fig. 3. Effect of the chitosan content on grafting percentage (1), grafting efficiency (2), dynamic viscosity of solutions at the beginning (3) and at the end of the reaction (4). Reaction conditions: [APS], 1×10^{-2} mol/L; [HEA], 0.35 mol/L; temperature, 60 °C; and time, 60 min.

hydroxymethylene group of chitosan. Carrying also a negative charge, the $SO_4^{-\cdot}$ radicals may easily be attracted to the cationic amine groups of the chitosan. With further increase of the initiator in feed over 10 mmol, the anionic radicals attack also ether bonds C–O–C of the chitosan backbone. Consequently, the chitosan chain degraded into shorter chains. Indeed, as shown in Fig. 2, at high concentration of APS the solution viscosity decreased lower than that of the initial solution (51 Pas before reaction starts, and 41 Pas at the end). In this study, dynamic viscosity decreased in a region of APS concentration above 10 mmol, accompanied with decreases in *%G* and *%E*. Decrease of *%G* and *%E* is likely due to bimolecular termination of the growing radicals and interaction with anion-radicals of initiator.

The effect of the chitosan concentration in the reaction mixture onto grafting parameters was studied (Fig. 3). The maximum of *%G* and *%E* were obtained at the chitosan concentration about 2 *wt*.%. It is evident that the dynamic viscosity rises with increasing chitosan concentration [19–21], the trend is similar for both viscosities at the beginning and at the end of the reaction (curves 3 and 4 in Fig. 3). Obviously, at lower concentration, increasing availability of grafting sites plays a major role in the grafting parameters growth. As chitosan concentration reaches the critical value, the grafting efficiency dropped significantly, from 72 to 50% (meanwhile grafting percentage decreases from 135% to 40%). This is probably due to high solution viscosity, i.e. hindered macromolecules' mobility, and deactivation of the macroradicals (e.g., transfer reaction, recombination, and interaction with the primary radicals) dominates soon after their formation.

Dependence of the grafting parameters on the reaction temperature was studied in the range 30–80 °C. It was found, that both the parameters *%G* and *%E* reached their maximum values at reaction temperature 55–60 °C (data not shown). As one can assume from above, this behavior possibly originates from such factors as diffusion rate, decomposition of initiator, generation of chitosan macroradicals, termination, and chain transfer reactions.

The graft-copolymerization kinetics in chitosan-HEA system was studied from data presented in Fig. 4. The reaction rate in a logarithm form was calculated at low conversion degree, and plotted against the logarithm of the reagents' concentration. Linear dependences were observed for all the reagents. The reaction order was calculated from the slopes of each line. Thus, the rate of graft copolymerization of HEA onto chitosan is expressed with the following equation: $V = k[HEA]^{2.25}[APS]^{0.18}[CS]^{-0.03}$, where k is a constant. The deviation of the reaction order from 0.5 for initiator is in accordance with literature data on grafting copolymerization of water-soluble monomers onto chitosan [22,23]. In the cited paper, the authors refer the phenomenon of possible monomolecular recombination.

3.2. Characterization

The FT-IR spectra of the chitosan, PHEA, and the graft-copolymer CS-g-PHEA were recorded (Fig. 5a–c). The chitosan spectrum shows the characteristic absorption bands at 1636 (Amide I), 1528 ($-NH_2$ bending) and 1381 cm^{-1} ($-CH_2$ bending). The absorption bands at 1156 cm^{-1} (anti-symmetric stretching of the C–O–C bridge), 1084 and 1028 cm^{-1} (skeletal vibrations involving the C–O stretching) are characteristics of the polysaccharide structure. The band at 1726 cm^{-1} of PHEA spectrum is assigned to the carbonyl stretching vibration of carboxylic ester moieties, while the primary

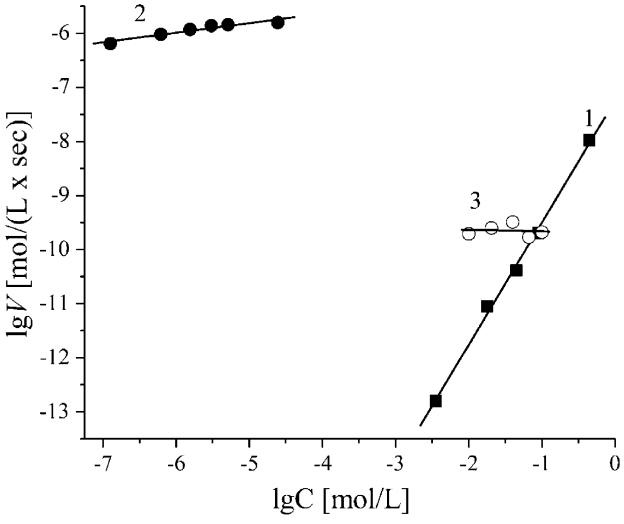

Fig. 4. The logarithm of rate of graft copolymerization versus logarithm of concentration of reagents HEA (1), APS (2), and chitosan (3).

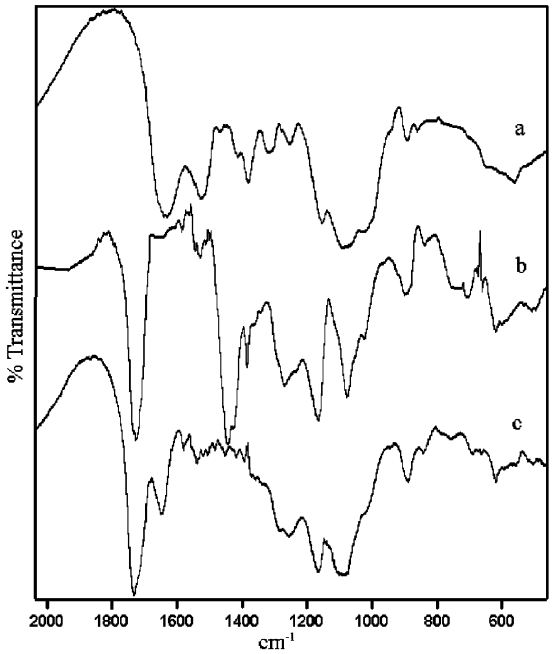

Fig. 5. FT-IR spectra in the region 500–2000 cm^{-1} for pure chitosan (a), PHEA (b), and CS-g-PHEA (c).

alcohol group shows a strong absorption band at 1275 and 1075 cm^{-1}. The main absorption bands appearing in the chitosan and PHEA's FT-IR spectra are present in IR spectra of the graft-copolymer CS-g-PHEA (1726, 1636, 1528 cm^{-1}). The characteristic peak for amide I group of chitosan was slightly shifted to 1653 cm^{-1}. Some shifts of the peaks in the region 1229–915 cm^{-1} are due to the influence of ester groups of PHEA to the polysaccharide. The observed differences between the FT-IR spectra of the chitosan and CS-g-PHEA verify existence of HEA-grafted polymer chains.

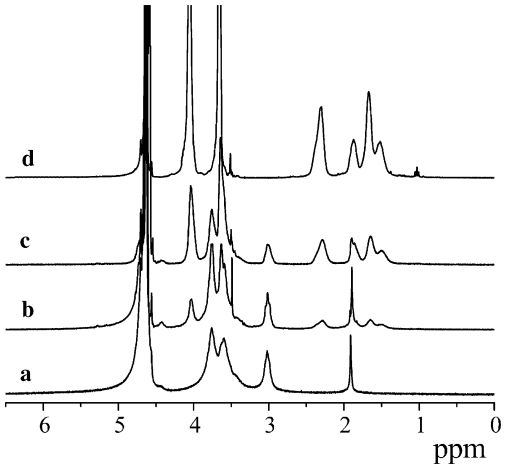

Fig. 6. ^{1}H NMR spectra of pure chitosan (a), CS-g-PHEA with grafting percentage of 25% (b), CS-g-PHEA with grafting percentage of 90% (c), and PHEA (d).

^{1}H NMR spectra of the acetylated chitosan, PHEA and CS-g-PHEA with two different grafting percentages were recorded in D_2O (Fig. 6). In the chitosan spectrum (Fig. 6a), the peak at 1.9 ppm was assigned to methyl protons. The signal 3.1 ppm was attributed to the amine group protons. Hydrogen atoms in the chitosan rings as well as methylene protons give signals around 3.5–3.8 ppm. ^{1}H NMR spectrum of the PHEA is shown in Fig. 6d. The –CH and $–CH_2$ backbone protons of PHEA resonate at 2.25 ppm and 1.51–1.87 ppm region, respectively. Signals at $\delta = 4.14$ and 3.65 ppm are assigned to $–CH_2$ protons of the hydroxyethyl group. These data agree with those obtained by Coca et al. and Mun et al. [24,25]. In the ^{1}H NMR spectra of the graft-copolymer CS-g-PHEA (Fig. 6b and c), all typical peaks were found at corresponding range. The copolymers with higher grafting percentage showed greater area of the peaks responsible for –CH proton in main chain

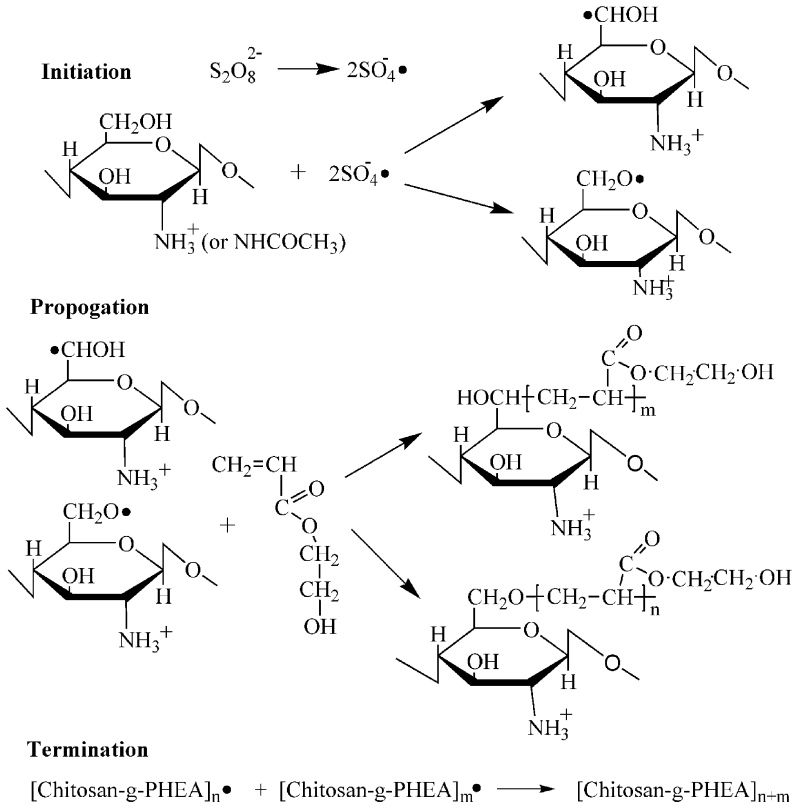

Fig. 7. The mechanism of the graft copolymerization of HEA onto chitosan.

of HEA ($\delta = 2.25$ ppm) and $-CH_2$ protons of hydroxyethyl group ($\delta = 4.14$ ppm). On the basis of the above results, the grafting contents of HEA part to chitosan in the copolymers were determined with the signal intensities for the HEA unit and the chitosan unit. The signal of H-2 proton from chitosan at 3.1 ppm is clearly differentiated from the protons of the HEA unit that appear at 4.14 ppm. So grafting contents of HEA branches could be calculated as follows: HEA-grafting content% = $(116 \times I_{4.14\ \mathrm{ppm}})/\ (161 \times 6I_{3.1\ \mathrm{ppm}}) \times 100\%$, where 116 and 161 are the molecular weights of HEA and chitosan unit, respectively. Data calculated from ^{1}H NMR spectra are in good agreement with those ones obtained by gravimetrical method.

A possible mechanism of grafting HEA onto chitosan by means of APS-initiated radical polymerization is proposed in Fig. 7 in analogy with one mentioned by Ding et al. [26]. It can traditionally be presented by three main steps – initiation, propagation and termination. After thermal dissociation of the initiator, the formed anionic radicals attack H-atoms in α-methylene (CH_2) or hydroxyl groups (OH) of the hydroxymethylene group of the chitosan, as was previously showed for high molecular alcohols [27]. At those sites, polymer chain of HEA starts and propagates as regular radical polymerization of polyarylates.

It is known that original chitosan is not soluble in distilled water but only in dilute organic acids and hydrochloric acid. In this work, solubility of the acetylated chitosan and HEA-grafted chitosan with different percentage of grafting was observed as a function of optical density versus pH (Fig. 8). As seen, the acetylated chitosan is soluble in aqueous solutions up to pH 5.5 (Fig. 8, curve 1). At higher pH range the optical density rapidly rises and the polymer precipitates showing so-called pH-sensitivity. Grafting of hydrophilic polymers onto chitosan is supposed to improve solubility in water and at alkaline pH range. Indeed, the HEA-grafted chitosan with grafting percentage 12, 34, and 50% behave in analogy with the chitosan except for precipitation over pH 6–7. As %G reaches 78% and over, the CS-g-PHEA polymers become soluble even at alkaline pH, however losing their pH-sensitivity.

Fig. 9 shows scanning electron micrographs of the chitosan (a) and the CS-g-PHEA (b). Due to high rigidity, the chitosan particles typically present a porous morphology. A non-porous fibrous structure was observed for the CS-g-PHEA polymer with %G ~ 100%. Our graft-polymers are similar to other kinds of grafted chitosans, such as poly(L-lactic acid)-graft chitosan and polyion fiber of chitosan with gellan [28,29]. They can be recommended for

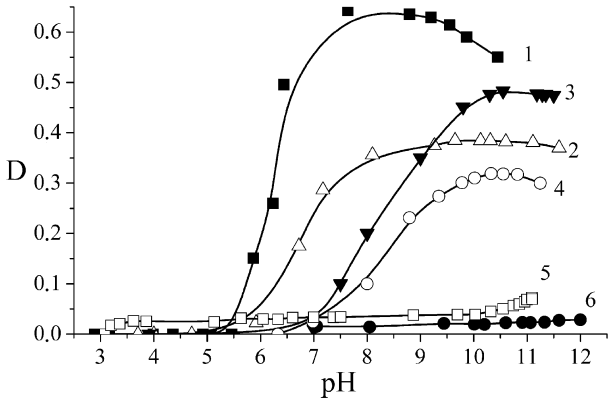

Fig. 8. Dependence of optical density of chitosan (1) and CS-g-PHEA (2–6) on pH. Experimental conditions: *%G*: 2–12, 3–34, 4–50, 5–78, 6–116. [Polymers] = 0.01 μmol/L.

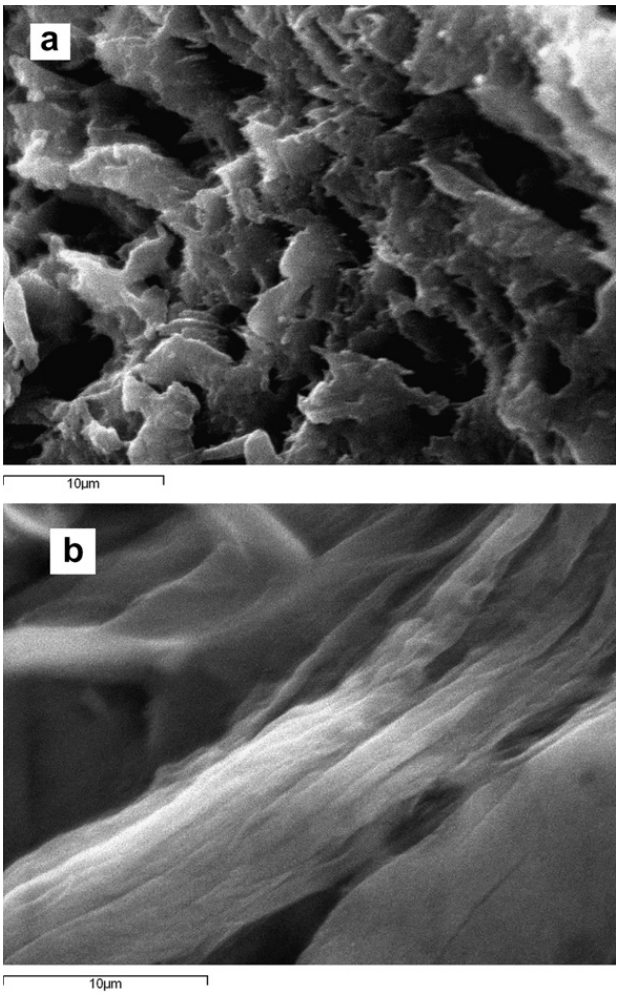

Fig. 9. SEM images of the top view of chitosan (a) and CS-g-PHEA (b) at 3000× magnification.

biomedical application as, for example, nerve-vein implants.

4. Conclusions

2-Hydroxyethyl acrylate was successfully grafted onto chitosan up to 300% in homogeneous phase under inert atmosphere by using ammonium persulfate as the initiator. It was possible to control the grafting parameters by varying the reaction conditions such as concentration of the initiator and the monomer, reaction duration and temperature. The polymerization rate equation $V = k[HEA]^{2.25}[APS]^{0.18}[CS]^{-0.03}$ was calculated empirically. The polymerization rate is much more sensitive to the concentration of the 2-hydroxyethyl acrylate than it is to the concentration of the initiator. The grafted copolymer samples are soluble in water and at alkaline pH, describing an enhanced hydrophilic character as compared with the parent acetylated chitosan. In scanning electron micrographs native chitosan appeared to have porous morphology, whereas CS-g-HEA appeared to have fibrous. Graft polymerization of hydrophilic monomers such as HEA onto chitosan is a versatile tool for preparing polysaccharide-based advance multifunctional materials for wide application in medicine and pharmaceutics.

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