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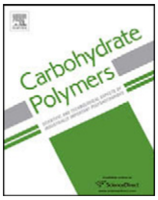

Studies on graft copolymerization of 3-acrylamidopropyl trimethylammonium chloride on pullulan

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ABSTRACT

Graft-polymerization of (3-acrylamidopropyl)-trimethylammonium chloride onto pullulan using potassium persulfate as an initiator was carried out in an aqueous solution. Evidence of grafting was obtained by comparing ^{1}H NMR, FT-IR spectra, SEM and TGA analysis of pullulan and the grafted copolymer. The effects of reaction conditions on the grafting parameters (such as initiator, monomer, and pullulan concentrations, reaction time and temperature) were investigated, and the optimal conditions for the grafting reaction were established. A plausible mechanism for the free-radical grafting was suggested. The obtained graft copolymer was designed to be used in waste water treatment processes and drug delivery.

1. Introduction

Chemical modification of natural polymers is a promising method for production of new biomaterials with specific properties (solubility, biodegradability, chemical or thermal stability, and mechanical behavior). Cationic polysaccharides such as cationic cellulose (Burke & Palepu, 2001), guar (Rodriguez, Alvalez-Lorenzo, & Concheiro, 2003), starch (Granö, Yli-Kauhaluoma, Suortttti, Käki, & Numi, 2000), chitosan (Paulino, Simionato, Garcia, & Nozaki, 2006), chitosan derivatives (Babak, Desbrières, & Tikhonov, 2005; Lee, Jo, Kwon, Kim, & Jeong, 1998; Wu, Seo, Sasaki, Irie, & Sakurai, 2006) and dextran (Wang, Xu, Zhou, & Tan, 2007) are large-scale commercial products with hydrophilicity, biodegradability and antibacterial properties.

Pullulan, a linear, non-ionic polysaccharide with a repeated unit of maltotriose condensed meanly through a $(1 \rightarrow 6)$-α-D linkage, prepared by culturing of *Aureobasidium pullulans* with starch and sugar (Wallenfells, Bender, Keilich, & Bechtler, 1961) has been used extensively in the food and pharmaceutical industries. Notably, pullulan accumulates in the liver in a significantly higher amount than other water-soluble polymers (Yamaoka, Tabata, & Ikada, 1993; Yamaoka, Tabata, & Ikada, 1995). Chemically modified pullulans with improved properties are gaining interests mainly because the polysaccharide portion of the product is flexible in structure, water soluble and biodegradable. Their applications may relate to agriculture, industry, medical treatment and sanitation, and so on (Leathers, 2003; Singh, Saini, & Kennedy, 2008).

Grafting of synthetic polymers on natural polysaccharides has been widely used as one of the most convenient ways to combine the advantages of natural and synthetic macromolecules. A number of papers have been published on the grafting polymerization of N-isopropylacrylamide (Masci, Bontempo, & Crescenzi, 2002), etylenic compound (Seizo, Kozo, Nobuhiro, & Fumio, 1975), lactide (Ohya, Maruhashi, & Ouchi, 1998), ethylene glycol (Jiao, Fu, & Jiang, 2004), butyl acrylate (Tian, Gao, Yu, & Duan, 1992), p-hydroxystyrene (Chen, Oshima, & Kumanotani, 1985), methyl methacrylate (Wu, Jin, Kim, Tong, & Chen, 2009) onto pullulan.

In order to find an inexpensive and efficient initiator, in this study we used an initiator system based on potassium persulfate. This system was found to be an efficient redox initiator in this specific system in aqueous medium. This approach is one of the most interesting methods of grafting vinyl monomers on polymer possessing oxidable functional groups, in which free radicals are formed on backbone of polymer.

This paper deals the synthesis of pullulan grafted with (3-acrylamidopropyl)-trimethylammonium chloride using peroxydisulfate as initiator with the aim to develop a hybrid product which could be used in waste water treatment or as a matrix for drug delivery. The influence of initiator, monomer and polymer

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concentration, reaction time and temperature on grafting parameters was studied.

2. Experimental and methods

2.1. Materials

Pullulan (Pul), Mw = 200,000 g/mol, was purchased from Hayashibara Lab. Ltd. (Okoyama, Japan). (3-acrylamidopropyl)-trimethylammonium chloride (APTAC) (75 wt. % solution in water) (Aldrich, Chemical Corp., Milwaukee, WI, USA) and potassium peroxydisulfate (KPS) (Fluka, Buchs, Germany) have been used as such.

2.2. Methods

2.2.1. Graft copolymerization

The grafting reaction was carried out under nitrogen atmosphere in a three-necked flask equipped with a nitrogen inlet and a reflux condenser immersed in a thermostated oil bath.

In a typical reaction, pullulan (1 g) dissolved in 30 ml distilled water was purged for 30 min with nitrogen. A required amount of initiator (KPS) was added. After 15 min supposed to be enough to induce free radicals onto pullulan chains, a predetermined amount of monomer (APTAC) was charged to the flask under nitrogen atmosphere and the reaction was continued for a predetermined temperature and time. The grafting homogenous mixture was precipitated with vigorous stirring in a large excess of acetone and the grafted copolymer separated through filtration. Thereafter, the homopolymer was extracted from the crude material with methanol for 72 h using a soxhlet apparatus. Finally, the grafted pullulan was solubilized in distilled water, dialysed for 5 days at 20 °C for removal of water soluble species, and recovered by freeze-drying.

The synthesis of homopolymer (pAPTAC) was carried out using the same conditions as those of the graft reaction without the addition of the pullulan. The concentration of monomer was fixed at 0.16 M.

2.2.2. Grafting parameters

The grafting polymerization process was followed gravimetrically. The increase in weight of the grafted pullulan over that of the pullulan, indicated the grafting of APTAC onto pullulan. The graft yields were characterized by the following parameters (Gao, Yu, Wang, Chang, & Tian, 1998):

$$\text{Grafting ratio } \%\, G = \frac{\text{Weight of grafted chains}}{\text{Weight of pullulan}} \times 100$$

$$\text{Grafting efficiency } \%\, E = \frac{\text{Weight of grafted chains}}{\text{Weight of polymer formed}} \times 100$$

$$\text{Conversion } \%\, C = \frac{\text{Weight of polymer formed}}{\text{Weight of monomer charged}} \times 100$$

$$\text{Homopolymer } \%\, H = 100 - \%\, E$$

All data were averaged from three measurements.

2.2.3. Grafted copolymer composition

The copolymer composition was determined by nitrogen analysis and ^{1}H NMR spectroscopy. ^{1}H NMR spectra were recorded on a Bruker Avance DRX 400 NMR, using deuterated water as the solvent.

2.2.4. Infrared (IR) spectral analysis

The grafted and un-grafted pullulan samples were dried under vacuum at 50 °C for 24 h, then were prepared accordingly to the KBr technique and analysed with a Fourier transform infrared spectrophotometer (VERTEX 70, Bruker, Austria) in the frequency ranges of 400–4000 cm^{-1}.

2.2.5. Scanning electron microscopy (SEM)

The morphology of un-grafted and grafted pullulan, in powdered form, was examined using an environmental scanning electron microscope (ESEM) type Quanta 200, operating with secondary electrons in low vacuum, at 25 kV.

2.2.6. TGA analysis

Thermogravimetric analysis (TGA) was performed under nitrogen flow (20 cm^3 min^{-1}) at heating rate 10 °C min^{-1} until 700 °C, with a Mettler Toledo model TGA/SDTA 851 (Mettler Toledo Corporation, Switzerland).

3. Results and discussion

3.1. Determination of optimum reactions conditions

The effect of variation of potassium persulfate, (3-acrylamidopropyl)-trimethylammonium chloride and pullulan concentration, along with time and temperature on grafting parameters has been studied.

3.1.1. Effect of temperature

The effect of reaction temperature on graft copolymerization of APTAC onto pullulan was investigated by changing the temperature from 30 to 90 °C, while keeping other reaction variables constant. The results given in Fig. 1a show that by increasing the temperature up to 80 °C, the graft yield, grafting efficiency and monomer conversion increase continuously, then levels off. The positive effect of temperature on grafting is due to the fact that both the production rate of free radicals and access of APTAC molecules to pullulan reaction sites increases. However, above this temperature, it was noticed a decrease of all grafting parameters, probably due to a greater probability of chain termination and chain transfer reactions (Abdel Razik, 1997; Li, Li, Li, Ding, & Zong, 2004).

The rate of graft copolymerization (k) was evaluated by measuring the rate of monomer disappearance, using the following equation (Pourjavadi & Zohuriaan-Mehr, 2002):

$$k(\text{mols/Ls}) = \frac{1000 \times W_{\text{polymer formed}}}{M \times t \times V}$$

where M is the molar mass of the monomer (g/mol), t and V are the total reaction time (s) and total volume (mL) of the reaction mixture, respectively.

The apparent first-order APTAC disappearance rate constants were then used to determine the apparent activation energy as shown in Fig. 1b. Based on the slopes of the Arrhenius plot, the activation energy for the graft copolymerization of APTAC onto pullulan backbone was found to be 42 kJ/mol. Similar values for the overall activation energy for the grafting of 4-vinylpirydine onto carboxymethylated cotton (Leza, Casinos, & Guzman, 1989), 2-(dimethylamino)ethyl methacrylate onto starch (Zhang & Chen, 2001) were obtained.

3.1.2. Effect of monomer concentration

The increase in monomer concentration would be expected to increase both the percentage of grafting and the molecular weight of grafting copolymer (Abdel Razik, 1997; Aggour, 2001). Indeed, the results presented in Fig. 2 indicate that as the APTAC concentration increases from 0.075 to 0.25 mol/L both % G and % H diminishes. However, the grafting efficiency % E, and monomer conversion % C, decreases. When the monomer concentration increases over 0.25 mol/L, the grafting ratio, the grafting efficiency and the

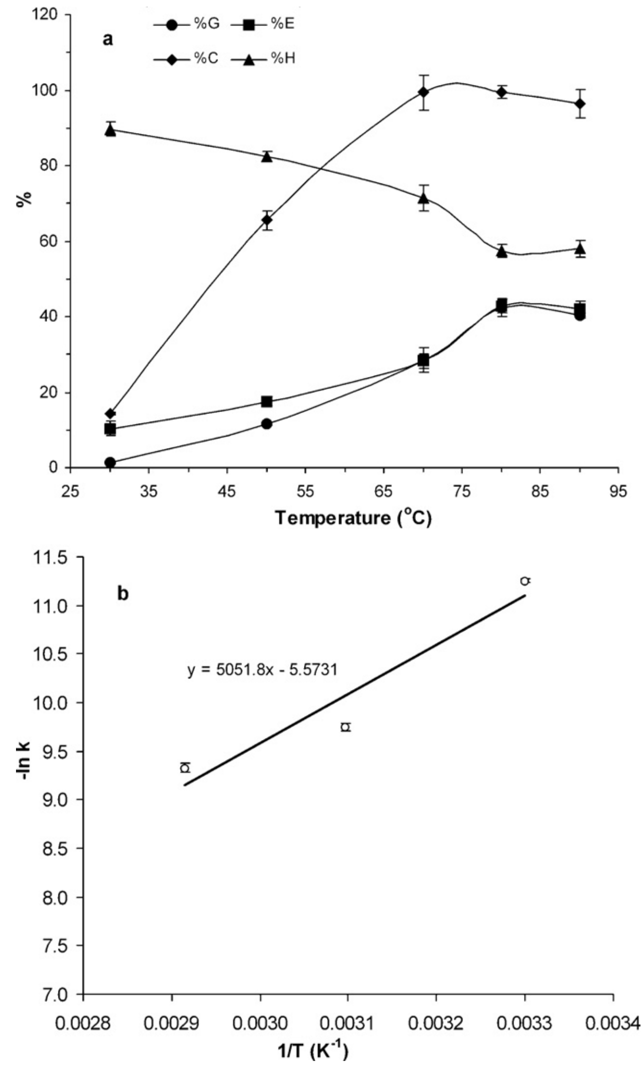

Fig. 1. Effect of temperature on the grafting parameters (a). The Arrhenius plot of grafting rate versus reciprocal of grafting temperature (b) (for the initial portion of the curve related to the temperature series in (a). The values are means of three independent measurements that deviated 1–5%. Reaction conditions: [APTAC] = 0.16 mol/L; [KPS] = 12.33 mmol/L; t = 5 h.

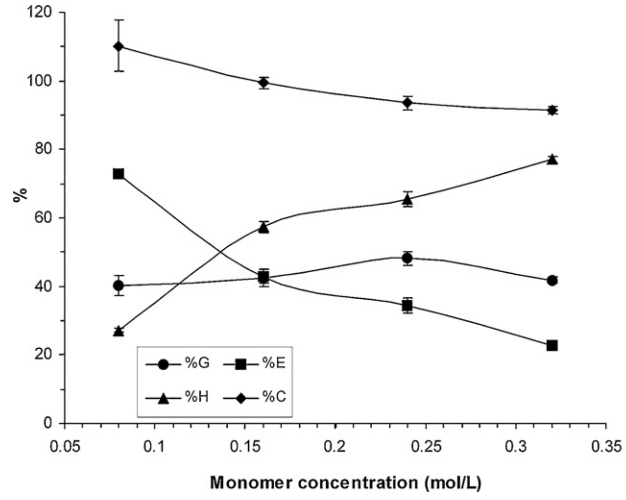

Fig. 2. Effect of monomer concentration on the grafting. The values are means of three independent measurements that deviated 1–7%. [Pul] = 0.2 mol/L; [KPS]= 12.33 mmol/L; T = 80 °C; t = 5 h.

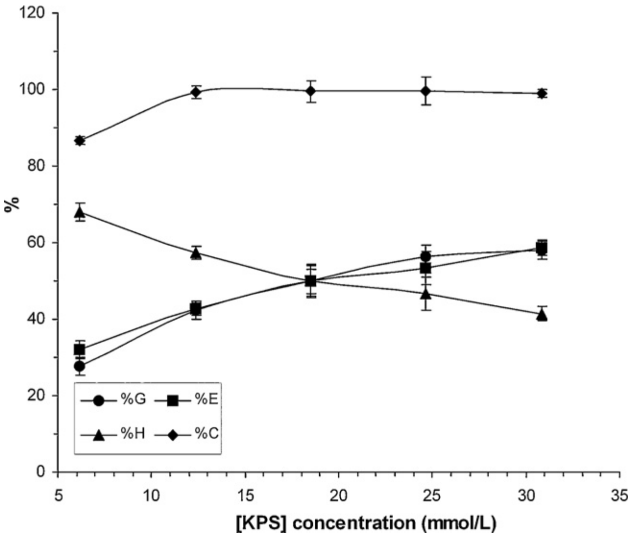

Fig. 3. Effect of initiator concentration on the grafting. The values are means of three independent measurements that deviated 1–5%. [Pul] = 0.2 mol/L; [APTAC] = 0.16 mol/L; T = 80 °C; t = 5 h.

monomer conversion progressively decrease. This may be due to an increase in homopolymer formation, since the homopolymerization is in competition with graft copolymerization. Moreover, the presence of the homopolymer in the reaction mixture, increases its viscosity, and therefore slows down monomer diffusion to the pullulan macroradicals. Another explanation for the observed behavior could be the substantial amount of polymer grafted onto pullulan backbone, which creates steric hindrance for further grafting. A similar trend has been found in various copolymerization systems (Guthrie & Tune, 1991; Nishioka, Matsumoto, & Kosai, 1983).

3.1.3. Effect of initiator concentration

As it can be easily seen in Fig. 3, the increase of the potassium persulfate concentration up to 2.46×10^{-2} mol/L is accompanied by a significant enhancement in grafting ratio and grafting efficiency. This can be the result of the enriched reaction medium in radical ions ($SO_4^{-\cdot}$) and $OH^{\cdot}$ radicals which attack the pullulan backbone immediately, creating a high number of active sites to which the monomer addition takes place (Hsu, Don, & Chiu, 2002). However, a relatively high concentration of initiator may cause a reduction of grafting due to an increase in the number of backbone radicals terminated prior to APTAC addition (McDowall, Gupta, & Stannett, 1984).

3.1.4. Effect of reaction time

The effect of reaction time on graft copolymerization has been studied by varying the time interval from 1 to 24 h (Table 1). It can be easily observed that the grafting ratio, monomer conversion and grafting efficiency increase with reaction time, until 8 h. In fact, the addition of monomer molecules to the growing grafted chain gives

Table 1
Effect of reaction time on grafting of APTAC onto pullulan.

Time (h)	% *G* (w/w)	% *E* (w/w)	% *C* (w/w)	% *H* (w/w)
1	24.76 ± 1.98	27.58 ± 0.714	89.76 ± 0.714	72.42 ± 5.05
2	34.06 ± 0.79	35.45 ± 1.28	96.06 ± 1.287	64.55 ± 1.329
5	42.44 ± 2.39	42.68 ± 1.73	99.44 ± 1.72	57.32 ± 1.68
8	47.39 ± 0.92	51.85 ± 2.39	91.39 ± 2.39	48.15 ± 2.616
20	48.31 ± 2.814	52.9 ± 3.825	91.31 ± 3.82	47.1 ± 1.428

[Pul] = 0.2 mol/L; [APTAC] = 0.16 mol/L; [KPS] = 12.33 mmol/L; T = 80 °C.
Data are the results of three independent experiments ± SD.

Table 2
Effect of the polymer concentration on grafting of APTAC onto pullulan.

[Pul] (mol/L)	% *G* (w/w)	% *E* (w/w)	% *C* (w/w)	% *H* (w/w)
0.123	42.53 ± 1.88	45.96 ± 0.815	92.53 ± 2.58	54.04 ± 0.816
0.205	42.44 ± 2.39	42.68 ± 1.73	99.44 ± 1.72	57.32 ± 1.68
0.308	29.36 ± 2.44	29.36 ± 2.74	99.87 ± 1.07	70.6 ± 2.74

[APTAC] = 0.16 mol/L; [KPS] = 12.33 mmol/L; $T = 80\,^{\circ}C$; $t = 5$ h.
Data are the results of three independent experiments ± SD.

low percentage of homopolymer. However, when the reaction time overcomes 8 h, a reduction of the grafting parameters occurs (Sun, Xu, Liu, Xue, & Xie, 2003)

3.1.5. *Effect of pullulan concentration*

The effect of pullulan concentration on grafting parameters has been studied by varying the concentration of pullulan from 0.12 to 0.31 mol/L. The results, presented in Table 2, reveal that the grafting ratio and efficiency decrease on increasing the concentration of pullulan. This behavior could be explained on the fact that the viscosity of reaction medium increases, reducing both the mobility of the free macroradicals and monomer. Similar results were observed in other work (Behari, Kumar, Tripathi, & Pandey, 2001).

3.2. *Mechanism of grafting process*

On the basis of experimental results, a tentative mechanism has been proposed for graft copolymerization of APTAC onto pullulan, using potassium persulfate as initiator (Fig. 4). Free radicals ($R^{\cdot}$) are generated with increasing in temperature, by the dissociation of persulfate ions to sulfate radical ions (Abdel Razik, 1997; Hunkeler, 1991; Kolthoff & Miller, 1952). Then, the sulfate radical ions react with water to produce hydroxyl radicals, the polymerization been initiated either by $SO_4^{-\cdot}$ radical ions or by $OH^{\cdot}$ radicals. The free radicals abstract hydrogen atom from pullulan and produce $PulO^{\cdot}$ macroradicals at the OH sites of the pullulan molecule. The APTAC molecules which are in close vicinity of the reaction sites become acceptor of pullulan radical resulting in chain initiation of graft copolymer and thereafter themselves become free radical donor to neighbouring molecules. In this way the grafted chains grow. These chains are terminated by combination to give graft copolymer.

3.3. *Evidence of grafting*

3.3.1. *FTIR spectroscopy of pullulan and Pul-g-pAPTAC*

The existence of the grafting was also confirmed by observing the difference between the IR spectra of pullulan and the graft copolymer. As it can be seen in Fig. 5, the spectrum of grafted copolymer with a *G* of 41% shows not only the characteristic pullulan bands at 3430 cm^{-1} (–OH stretching frequency of anhydroglucose units), 2925 cm^{-1} (sp_3 C–H bond of alkane compounds), 850, 758 and 920 cm^{-1}(characteristics of D-glucosidic bonds) (Thirumavalavan, Manikkadan, & Dhanasekar, 2009), but also the additional adsorption bands of the C=O stretching vibration at 1729 cm^{-1}, the quaternary ammonium group at 1480 cm^{-1}, secondary amide N–H stretching, amide II band and amide I band at 1560 cm^{-1} and 1655 cm^{-1}, respectively, $–CH_3$ symmetric stretching at 2850 cm^{-1} and the asymmetric $–CH_2–$ stretching at 2925 cm^{-1}. C–O–C stretching vibration at 1110 cm^{-1} confirms the attachment of monomer molecules to substrate through ether linkage at OH sites of substrate.

3.3.2. *NMR spectroscopy of pullulan and Pul-g-pAPTAC*

The chemical structure of the graft copolymer (Fig. 6) was also examined by ^1H NMR spectroscopy (Fig. 7). In the pullulan spectrum (Fig. 7a) the peaks at 4.96 ppm, 5.36 and 5.40 ppm were assigned to the (1 → 6) and (1 → 4) anomeric protons. Hydrogen atoms in the pullulan rings give signals around 3.44–4.04 ppm (Bruneel, Schacht, & De Bruyn, 1993). ^1H NMR spectrum of the pAPTAC is shown in Fig. 7e. The –CH and $–CH_2$ backbone protons of pAPTAC resonate at 2.22 ppm and 1.6–1.7 ppm region, respectively. The other signals are assigned as follows: 2.06 ppm to the $–CH_2$ protons of the propyl group, 3.18 ppm to the protons of the methyl groups on the quaternary ammonium salt, 3.24 ppm to the $NH–CH_2–$ protons, 3.39 ppm to the $–CH_2–N^+$ and 8.29 ppm to the –NH– protons, respectively. These data agree with those obtained by Utsel, Malmström, Carlmark, and Wågberg (2010). In the ^1H NMR spectra of the graft copolymer Pul-g-pAPTAC (Fig. 7b–d), all typical peaks were found at corresponding range. By keeping con-

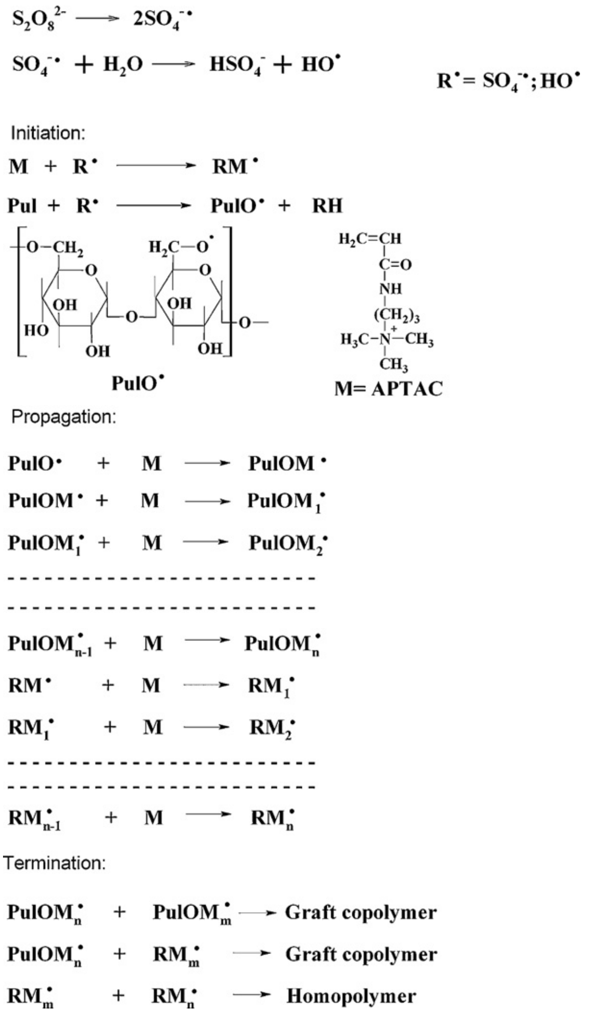

Fig. 4. Mechanism of the graft copolymerization of APTAC onto pullulan.

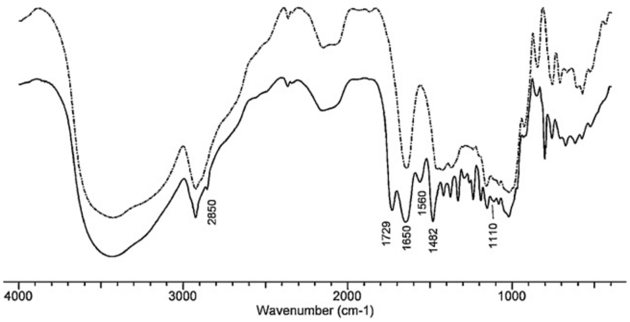

Fig. 5. FTIR spectra of pulluan (——) and Pul-g-pAPTAC (------) copolymer.

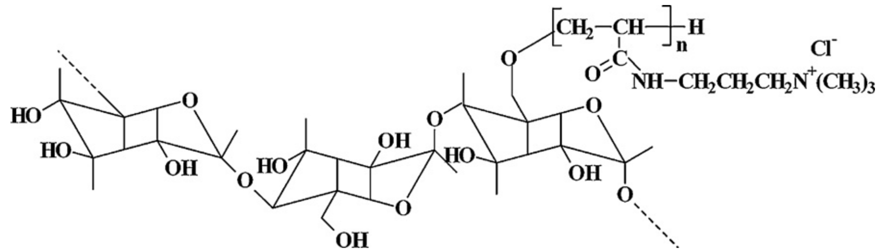

Fig. 6. Chemical structure of Pul-g-pAPTAC copolymers.

stant the intensity of the pullulan signals, the intensity of the peaks ascribed to APTAC increased with increasing the grafting percentage. The molar ratio of APTAC units grafted on structural unit of pullulan was calculated from the rapport of the integral value of methylene protons at 1.9–2 ppm which correspond to the APTAC divided by a factor of 2, and that of H-1 protons of pullulan moieties observed at 5.6 and 5.2 ppm.

This content was confirmed from the nitrogen analysis by the Keldhal method. The content of APTAC in grafted copolymers was calculated from N content using the following equation:

$$\%\,\text{grafted APTAC} = \frac{\%N}{14.01} \times 206.72$$

where 206.72 represent molar mass of the monomer

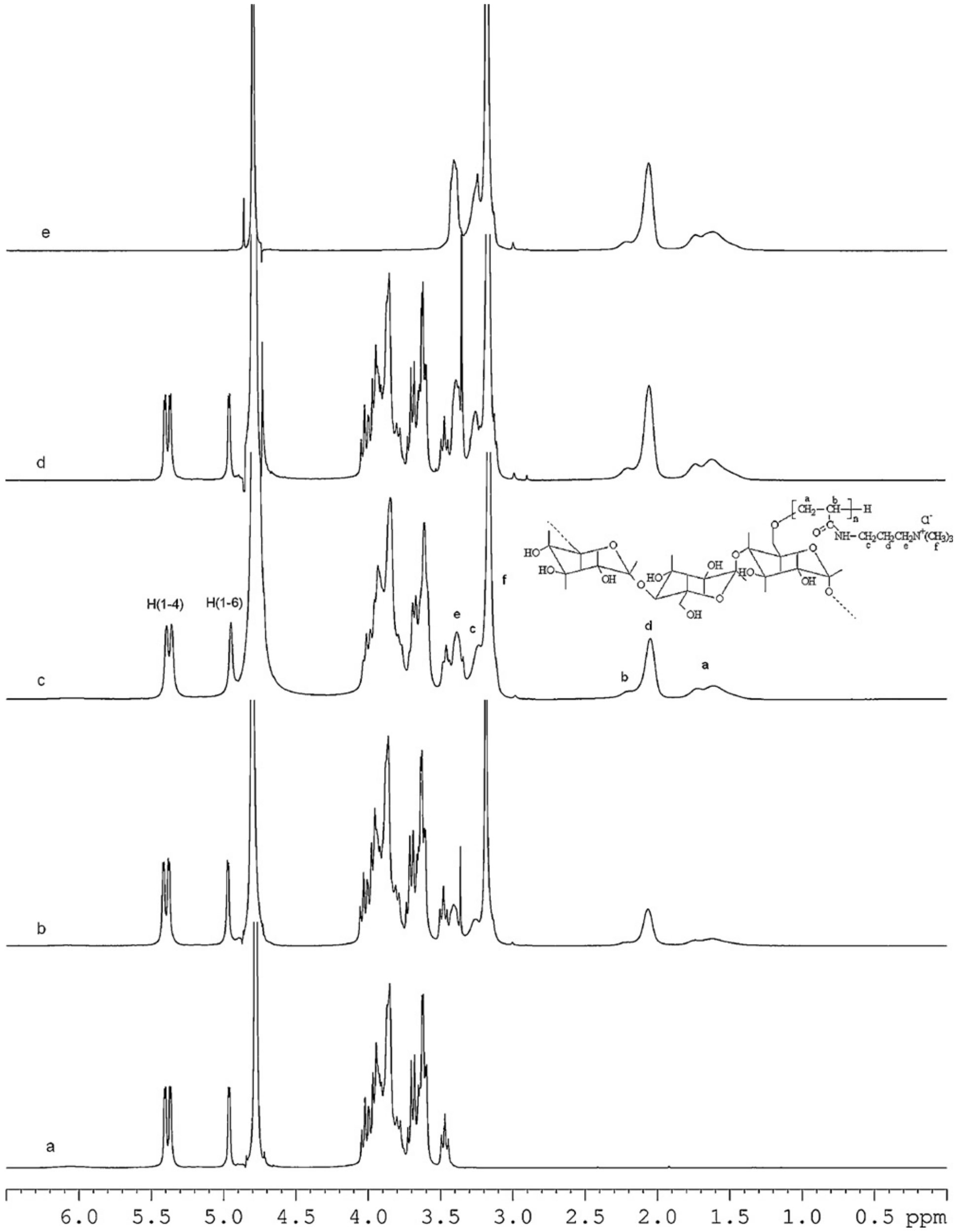

Fig. 7. ¹H NMR spectra of pure pullulan (a), Pul-g-pAPTAC copolymer with different grafting ratio: 29% (b), 41% (c), 60% (d), and of pure pAPTAC (e).

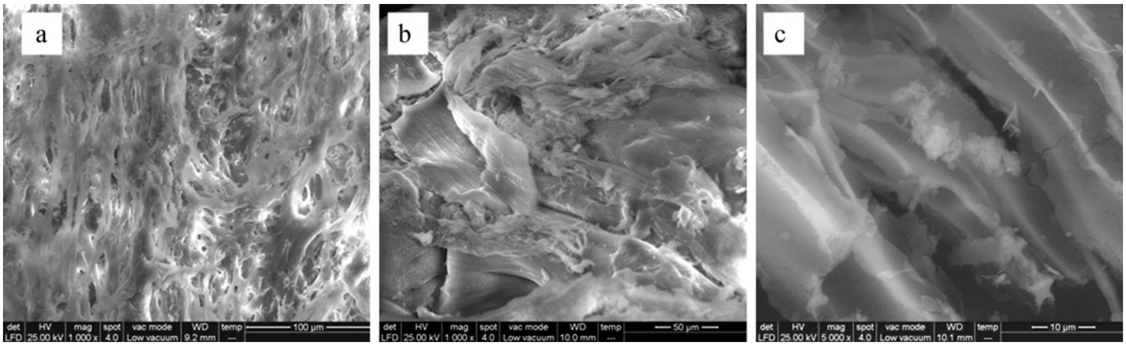

Fig. 8. Scanning electron micrographs of pullulan (a), and Pul-g-pAPTAC copolymer with different grafting ratio: 29% (b) and 41% (c).

3.3.3. Scanning electron microscopy of pullulan and Pul-g-pAPTAC

The scanning electron photomicrographs of pullulan and the graft copolymers Pul-g-pAPTAC with different grafting percentages (G 29%, G 41%) are shown in Fig. 8. The introduction of pAPTAC onto pullulan backbone destroyed its original porous structure. The porous morphology of pullulan was changed to a lamellar structure; the higher is the percentage of grafting, the more evident appear the structured lamellas.

3.3.4. Thermal analysis of pullulan and Pul-g-pAPTAC

The characteristic TG and DTG curves for pullulan, pAPTAC and grafted copolymers are presented in Fig. 9. The primary thermograms of polymers were obtained at a scan rate of 10 °C/min in an inert atmosphere. All samples involve only two steps of degradation, after the first stage loss of moisture at low temperature. Table 3 illustrates the thermal decomposition data of pullulan, pAPTAC and Pul-g-pAPTAC copolymers, respectively.

In the second stage, pullulan (Fig. 9a) begins to decompose at 261 °C, rapidly loses 44.66% of its weight up to 311 °C, which could be attributed to degradation of the saccharide rings and disintegration of macromolecule chains of pullulan. Above 311 °C, the weight loss is slow and gradual up to 487 °C. The maximum rate of weight loss occurred at 288 °C. pAPTAC showed higher thermal stability than pullulan (Fig. 9d), the homopolymer presenting a major weight loss of 55% in the range 280–332 °C at $T_{\max}$ = 304 °C. Furthermore, pAPTAC showed very little ash (~9%) after thermal degradation, whereas pullulan showed 24% of ash.

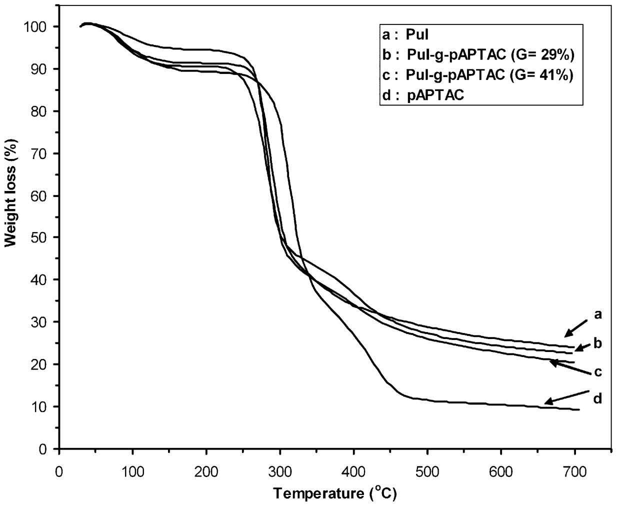

Fig. 9. TGA thermograms of pullulan (a), Pul-g-pAPTAC copolymers with different grafting ratio: 29% (b) and 41% (c), and of pAPTAC homopolymer (d).

Table 3
Thermogravimetric (TGA and DTG) data of the pullulan, pAPTAC and grafted copolymers.

Polymer	Decomposition stage	Temperature range (°C)	DTG maximum (°C)	Weight loss (%)	Char yield at 700 °C (%)
Pul	1	54–133	76	9.35	
	2	261–311	288	44.66	
	3	323–487	365	22.76	24
Pul-g-APTAC (G = 29%)	1	56–116	78	10	
	2	250–305	285	50.12	
	3	387–445	411	20.15	20.4
Pul-g-APTAC (G = 41%)	1	50–126	88	6	
	2	257–300	282	50.2	
	3	374–465	404	21.6	22.6
pAPTAC	1	58–133	85	10.8	
	2	280–332	304	55.18	
	3	388–459	418	26.9	9.4

The TGA thermograms of graft copolymers contains two well-identified stages (Fig. 9b and c). The first stage is the range of 250–300 °C with $T_{\max}$ = 285 °C and the second is at the range of 374–465 °C with $T_{\max}$ = 404 °C. The pullulan characteristics played a major role in the thermograms being the major component of the graft copolymer. The evidence of APTAC chains grafted to pullulan was only exhibited at the temperature range of 375–465 °C, similarly to the homopolymer. The % weight loss of pullulan fraction in the copolymer is 50%, whereas the % weight loss of APTAC fraction is 20%, which indicates that the ratio of pullulan to pAPTAC is 2.5:1. Initial decomposition temperature (T_i) and the temperature at the maximum rate of weight loss ($T_{\max}$) of grafted copolymers are lower that of pullulan indicating that the pAPTAC grafting lowers, but no significantly, the thermal stability of pullulan.

4. Conclusions

Graft copolymers of pullulan and poly((3-acrylamidopropyl)-trimethylammonium chloride) were successfully synthesized using potassium persulfate as the redox initiator under N_2 atmosphere. Reaction conditions such as temperature, time, initiator concentration, monomer and pullulan concentrations have shown a great influence on grafting.

The maximum % grafting was obtained at 80 °C and a reaction time of 5 h, 0.0246 mol L^{-1} of KPS, 0.16 mol L^{-1} of APTAC and 0.123 mol L^{-1} of pullulan.

Graft polymerization of hydrophilic monomers (such as APTAC) onto pullulan is a versatile tool for preparing polysaccharide-based advance multifunctional materials for wide application in medicine and pharmaceutics.

Acknowledgements

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References

Abdel Razik, E. A. (1997). Aspects of thermal graft copolymerization of methyl methacrylate onto ethyl cellulose in homogeneous media. *Polymer-Plastics Technology and Engineering*, *36*, 891–903.

Aggour, Y. A. (2001). Reaction kinetics of graft copolymerization and thermochemical studies of the degradation of poly(vinyl alcohol) graft copolymer. *Polymer International*, *50*, 347–353.

Babak, V. G., Desbrières, J., & Tikhonov, V. E. (2005). Dynamic surface tension and dilational viscoelasticity of adsorption layers of a hydrophobically modified chitosan. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *255*, 119-30.

Behari, K., Kumar, R., Tripathi, M., & Pandey, P. K. (2001). Graft copolymerization of methacrylamide onto guar gum using a potassium chromate/malonic acid redox pair. *Macromolecular Chemistry and Physics*, *202*, 1873–1877.

Bruneel, D., Schacht, E., & De Bruyn, A. (1993). Structural analysis of succinoylated and chloroformate activated pullulan: NMR study in DMSO solution. *Journal of Carbohydrate Chemistry*, *12*, 769–778.

Burke, S. E., & Palepu, R. (2001). Interactions of a hydrophobically modified cationic cellulose ether derivative with amphiphiles of like charge in an aqueous environment. *Carbohydrate Polymers*, *45*, 233–244.

Chen, Y. Y., Oshima, R., & Kumanotani, J. (1985). A morphological analogue of Japanese lacquer. Grafting of *p*-hydroxystyrene onto pullulan by ammonium persulfate initiation in dimethylsulfoxide. *Journal of Polymer Science Part A: Polymer Chemistry Edition*, *23*, 1283–1292.

Gao, J., Yu, J., Wang, W., Chang, L., & Tian, R. (1998). Graft copolymerization of starch-AN initiated by potassium permanganate. *Journal of Applied Polymer Science*, *68*, 1965–1972.

Granö, H., Yli-Kauhaluoma, J., Suortti, T., Käki, J., & Numi, K. (2000). Preparation of starch betainate: A novel cationic starch derivative. *Carbohydrate Polymers*, *41*, 277-83.

Guthrie, J. T., & Tune, P. D. (1991). The preparation, characterization, and application of cellulose-MMA graft copolymers. I. The aqueous-based preparation of cellulose-MMA graft copolymers. *Journal of Polymer Science Part A: Polymer Chemistry*, *29*, 1301–1312.

Hsu, S. C., Don, T. M., & Chiu, W. Y. (2002). Free radical degradation of chitosan with potassium persulfate. *Polymer Degradation and Stability*, *75*, 73–83.

Hunkeler, D. (1991). Mechanism and kinetics of the persulfate-initiated polymerization of acrylamide. *Macromolecules*, *24*, 2160–2171.

Jiao, Y., Fu, Y., & Jiang, Z. (2004). The synthesis and characterization of poly(ethylene glycol) grafted on pullulan. *Journal of Applied Polymer Science*, *91*, 1217–1221.

Kolthoff, I. M., & Miller, I. K. (1952). The chemistry of persulfate. III. The reaction of persulfate with mercaptans solubilized in sodium laurate solutions in the presence of unsaturated compounds. *Journal of the American Chemical Society*, *74*, 4419–4422.

Leathers, T. D. (2003). Biotechnological production and applications of pullulan. *Applied Microbiology and Biotechnology*, *62*, 468–473.

Lee, K. Y., Jo, W. H., Kwon, I. C., Kim, Y. H., & Jeong, S. Y. (1998). Physicochemical characteristics of self-aggregates of hydrophobically modified chitosans. *Langmuir*, *14*, 2329–2332.

Leza, M. L., Casinos, I., & Guzman, G. M. (1989). Graft copolymerization of 4-vinylpyridine onto cellulosics: Effect of temperature. *European Polymer Journal*, *25*, 1193–1196.

Li, G. J., Li, Q., Li, A. P., Ding, R., & Zong, M. H. (2004). Study on the kinetics for enzymatic degradation of a natural polysaccharide, *Konjac Glucomannan*. *Macromolecular Symposia*, *216*, 165–178.

Masci, G., Bontempo, D., & Crescenzi, V. (2002). Synthesis and characterization of thermoresponsive *N*-isopropylacrylamide/methacrylated pullulan hydrogels. *Polymer*, *43*, 5587–5593.

McDowall, D. J., Gupta, B. S., & Stannett, V. T. (1984). Grafting of vinyl monomers to cellulose by ceric ion initiation. *Progress in Polymer Science*, *10*, 1–50.

Nishioka, N., Matsumoto, K., & Kosai, K. (1983). Homogeneous graft copolymerization of vinyl monomers onto cellulose in a dimethyl sulfoxide–paraformaldehyde solvent system II. Characterization of graft copolymers. *Polymer Journal*, *15*, 153–158.

Ohya, Y., Maruhashi, S., & Ouchi, T. (1998). Graft polymerization of L-lactide on pullulan through the trimethylsilyl protection method and degradation of the graft copolymers. *Macromolecules*, *31*, 4662–4665.

Paulino, A. T., Simionato, J. I., Garcia, J. C., & Nozaki, J. (2006). Characterization of chitosan and chitin produced from silkworm chrysalides. *Carbohydrate Polymers*, *64*, 98–103.

Pourjavadi, A., & Zohuriaan-Mehr, M. J. (2002). Modification of carbohydrate polymers via grafting in air 1. Ceric-induced synthesis of starch-*g*-polyacrylonitrile in presence and absence of oxygen. *Starch*, *54*, 140–147.

Rodriguez, R., Alvalez-Lorenzo, C., & Concheiro, A. (2003). Interactions of ibuprofen with cationic polysaccharides in aqueous dispersions and hydrogels: Rheological and diffusional implications. *European Journal of Pharmaceutical Sciences*, *20*, 429–438.

Seizo, N., Kozo, T., Nobuhiro, T., Fumio, F. (1975). *Polymer composition of ethylenic compounds and pullulan*. US Patent Office. Pat. No. 4 029 616.

Singh, R. S., Saini, G. K., & Kennedy, J. F. (2008). Pullulan: Microbial sources, production and applications. *Carbohydrate Polymers*, *73*, 515–531.

Sun, T., Xu, P., Liu, Q., Xue, J., & Xie, W. (2003). Graft copolymerization of methacrylic acid onto carboxymethyl chitosan. *European Polymer Journal*, *39*, 189–192.

Thirumavalavan, K., Manikkadan, T. R., & Dhanasekar, R. (2009). Pullulan production from coconut by-products by *Aureobasidium pullulans*. *African Journal of Biotechnology*, *8*, 254–258.

Tian, R.-C., Gao, J.-P., Yu, J.-G., & Duan, M.-L. (1992). Graft copolymerization of butyl acrylate onto pullulan using manganic pyrophosphate as initiator. *Journal of Applied Polymer Science*, *45*, 591–595.

Utsel, S., Malmström, E. E., Carlmark, A., & Wågberg, L. (2010). Thermoresponsive nanocomposites from multilayers of nanofibrillated cellulose and specially designed *N*-isopropylacrylamide based polymers. *Soft Matter*, *6*, 342–352.

Wallenfells, K., Bender, H., Keilich, G., & Bechtler, G. (1961). Über pullulan, das glucan der schleimhülle von *Pullularia pullulans*. *Angewandte Chemie*, *73*, 245–246.

Wang, M., Xu, J., Zhou, X., & Tan, T. (2007). Modification with DEAE-dextran, an alternative way to prepare anion-exchange monolithic column with lower pressure drop. *Biochemical Engineering Journal*, *34*, 76–81.

Wu, Y., Seo, T., Sasaki, T., Irie, S., & Sakurai, K. (2006). Layered structures of hydrophobically modified chitosan derivatives. *Carbohydrate Polymers*, *63*, 493–499.

Wu, S., Jin, Z., Kim, J. M., Tong, Q., & Chen, H. (2009). Graft copolymerization of methyl acrylate onto pullulan using ceric ammonium nitrate as initiator. *Carbohydrate Polymers*, *76*, 129–132.

Yamaoka, T., Tabata, Y., & Ikada, Y. (1993). Body distribution profile of polysaccharides after intravenous administration. *Drug Delivery*, *1*, 75–82.

Yamaoka, T., Tabata, Y., & Ikada, Y. (1995). Comparison of body distribution of poly(vinyl alcohol) with other water-soluble polymers after intravenous administration. *Journal of Pharmacy and Pharmacology*, *47*, 479–486.

Zhang, L.-M., & Chen, D.-Q. (2001). Grafting of 2-(dimethylamino)ethyl methacrylate onto potato starch using potassium permanganate/sulfuric acid initiation system. *Starch*, *53*, 311–316.