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Electrospun polyacrylonitrile-glycopolymer nanofibrous membranes for enzyme immobilization

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ABSTRACT

A biocatalyst was fabricated by the covalent immobilization of the enzyme catalase on a polyacrylonitrile (PAN) based nanofibrous membrane incorporating a glycopolymer. The glycopolymer poly-(6-O-vinylsebacoyl D-glucose) [poly-OVSEG] was synthesized by a water phase precipitation homopolymerization process, and its structure was characterized by Fourier transform infrared spectroscopy (FT-IR) and NMR. Composite membranes of PAN/poly-OVSEG nanofibers containing varying amounts (50–70%) of poly-OVSEG were subsequently prepared using electrospinning. The nanofibers were studied by scanning electron microscopy (SEM) and FT-IR. Their hydrophilicity was investigated by measuring water contact angles. With increasing content of poly-OVSEG, the contact angle decreases from $65.5 \pm 2.5^\circ$ to $15.2 \pm 1.1^\circ$. Catalase was immobilized on the composite nanofibrous membranes by covalent binding. The maximum catalase adsorption capacity of the polyacrylonitrile-based nanofibrous membranes was observed to be *ca.* 46.5 mg/g. Over 50% of catalyst activity was retained and increased thermal stability observed post-immobilization (with maximum activity at pH 7.5 and 50 °C). This study demonstrates the potential of using electrospun membranes to improve the thermal and storage stabilities of biological catalysts.

1. Introduction

Enzymes have been investigated extensively because of their chemo-, regio-, and stereoselectivity, efficacy under mild reaction conditions, and high level of catalytic efficiency [1–3]. These properties endow them with significant potential for applications in fine chemical or pharmaceutical synthesis, the textile industry, food processing, biosensor fabrication, bioremediation, and protein digestion in proteomic analysis [4–7]. However, the practical applications of free enzymes are limited, because they are unstable, causing toxic reactions and having a short lifetime in the systemic circulation [8]. In practical applications, enzymes are always attached or incorporated onto or into an inert, insoluble material: this overcomes the limitations of instability and non-reusability of the free enzymes [9–11]. In general, the results of immobilization, including the performance of immobilized enzymes, strongly depend on the microstructures and properties of the supports [12].

In recent years, there has been increasing interest in the use of nanoscale materials as supports for the immobilization of enzymes. Such materials have extremely high surface areas and porous structures, which can permit relatively high enzyme loading and catalytic efficiency for large-scale operations and applications [13–15]. It appears to us that electrospun nanofibrous membranes have significant potential as supports [16]. These membranes have low hindrance for mass transfer owing to their high porosity and the interconnectivity of the fibers comprising the support. It is important to consider the precise properties of any nanofibrous support, because its interaction with the enzyme may have significant influence on the latter's stability and activity. Many nanofibrous membranes lack a biocompatible surface, which leads to rapid denaturation and sharply decreased activity of surface-bound enzymes [17–19]. The surface modification of supports is often used to increase their biocompatibility, and provide appropriate environments for enzyme immobilization [20–22].

Saccharides play a central role in biological recognition signals and as functional biomolecules in living systems. Their expanding applications include as delivery systems [23], for tissue engineering [24] and, most pertinently, in enzyme immobilization: saccharides have excellent biocompatibility and biological activity. Inspired by the chemical and physical structures of natural polysaccharides, artificial saccharides known as glycopolymers have been synthesized and widely studied over the past 35 years [25,26]. They are emerging as important tools for the investigation of

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carbohydrate-based biological processes and for simulating various carbohydrate functions [27]. However, unlike natural polysaccharides, which have complex structures and compositions, synthetic glycopolymers are prepared from highly purified monomers. Therefore their molecular structures can be easily evaluated and designed [28]. Containing saccharide residues as side groups, glycopolymers inherit the high hydrophilicity of natural polysaccharides. These characteristics make glycopolymers appropriate biomaterials for enzyme immobilization. They can provide a fitting microenvironment for the immobilized enzyme, permitting it to maintain high biological activity and stability.

Catalase is a tetrameric haeme-containing metalloenzyme which catalyzes the decomposition of H_2O_2 into molecular oxygen and water. It is one of the most common enzymes in plant and animal tissues, [29] being useful for eliminating H_2O_2 produced in various enzymatic oxidation reactions (*e.g.* the air oxidation of glucose catalyzed by glucose oxidase) [30,31]. The activity of catalase is, however, difficult to maintain in realistic reaction conditions. This is because the enzyme can decompose by dissociation into its subunits, a process which is facilitated at high temperatures and at low enzyme concentrations [32].

For enzyme immobilization, a biofriendly interface on the support surface can reduce non-specific interactions between enzyme and support, and further retard protein denaturation, thereby creating a specific microenvironment which benefits enzyme activity [33]. In this research, a facile and efficient strategy for the preparation of electrospun polyacrylonitrile-based nanofibrous membranes incorporating glycopolymer was first developed. Poly-(6-O-vinylsebacoyl D-glucose) [poly-OVSEG] was initially prepared, and incorporated into a nanofibrous membrane using electrospinning. Second, the catalase enzyme was immobilized onto the nanofibrous membranes, using epichlorohydrin as coupling agent. Various characteristics of the immobilized catalase bionanocomposite including enzyme loading, activity, optimum catalysis pH and temperature, thermal and storage stabilities were determined. The results demonstrate that the nanofibrous membranes provide an excellent platform for enzyme immobilization.

2. Experimental

2.1. Materials

Alkaline protease, EC 3.4.21.62, was purchased from the Wuxi Xue Mei Technological Co. Ltd. (Wuxi, China). Catalase (hydrogen peroxide oxidoreductase, EC1.11.1.6 from bovine liver) was obtained from Sigma–Aldrich (Shanghai, China). Polyacrylonitrile (PAN) with a molecular weight of 50,000 was sourced from the Jinshan Petrochemistry Co. Ltd. N,N-dimethylformamide (DMF) was provided by the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals used were analytical grade, and water was distilled before use.

2.2. Homopolymerization of OVSEG

Polymerizable glucose derivative (OVSEG) was synthesized according to a previously published protocol in anhydrous pyridine at 50 °C, with stirring at 250 rpm for 4 days [34–36]. Alkaline protease was employed as a catalyst. Poly-OVSEG was subsequently synthesized *via* a water-phase precipitation homopolymerization process with ammonium persulfate as an initiator. Reactions were performed by dissolving 1.0 g of OVSEG in 5.0 mL H_2O under an atmosphere of N_2. The resulting product was repeatedly precipitated in acetone and then dried under vacuum. The reaction was performed for 5 h, giving a yield of 81%. The structure of the glycopolymer was confirmed by FT-IR and NMR.

2.3. Electrospinning procedures

Polyacrylonitrile (PAN) and poly-(6-O-vinylsebacoyl D-glucose) [poly-OVSEG] were dissolved in DMF at ambient temperature under gentle stirring for 12 h, forming a homogeneous solution. Initial experiments were performed to optimise the PAN concentration. Subsequently, the concentration of PAN was fixed at 6 wt%, and fibers were synthesized with different quantities of poly-OVSEG in the blend membranes: solutions were prepared with 50, 55, 60, 65 and 70 wt% poly-OVSEG. These preparations were then used for electrospinning.

The electrospinning process was carried out at ambient conditions (T = 25 °C, and relative humidity of 65 ± 3%). The homogeneous spinning solutions were loaded in a 5 mL syringe fitted with a stainless steel capillary needle with an internal diameter of 0.5 mm. A piece of aluminum foil was used to collect the fibers. An electrical potential of 15 kV was applied across a fixed distance (15 cm) between the syringe tip and the collector. The feed rate of solutions was maintained at 1.5 mL/h using a single syringe pump. The collected fibers were dried for 24 h at 37 °C under vacuum (320 Pa) in a DZF-6050 Electric Vacuum Drying Oven to remove the residual organic solvent and moisture.

2.4. Measurement and characterization

The morphologies and diameters of the nanofibers were determined by scanning electron microscopy (SEM; JSM-5600 LV microscope, JEOL, Tokyo, Japan). The diameters of the fibers were analyzed using the Adobe Photoshop image visualization software: the diameters of >100 fibers were determined and mean values calculated. ^{1}H NMR spectra were recorded using a Bruker DRX 400 MHz spectrometer (Bruker, Rheinstetten, Germany). TMS was employed as an internal standard. FT-IR spectra of the electrospun nanofibers were recorded on a Nicolet 17 DSX FT-IR Spectrometer (Thermo-Nicolet, Madison, WI, USA).

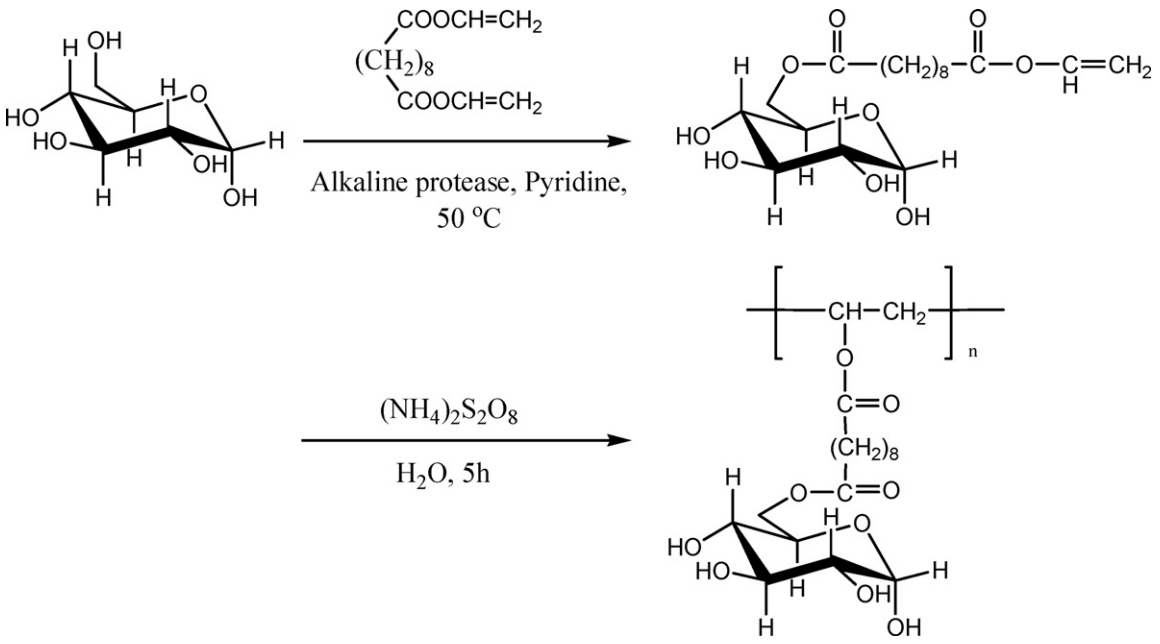
2.5. Hydrophilicity measurements

The hydrophilicity of the membrane surface was characterized on the basis of water contact angles, which were measured using the sessile drop method at room temperature on a contact angle goniometer equipped with video capture (DSA10-MK instrument, KRUESS, Hamburg, Germany). A total of 5 mL of deionized water was dropped onto a dry membrane in an atmosphere of saturated water vapor, and the contact angle was measured when the drop age was around 5 s.

2.6. Immobilization of catalase

Approximately 5 mg of the PAN – polyOVSEG fibrous membrane was immersed in 5 mL of epichlorohydrin and shaken at 37 °C for 3 h. The activated membrane was washed several times with acetone to remove excess epichlorohydrin, and then washed with a phosphate buffer solution (pH 7.0) before enzyme immobilization. A catalase solution was prepared by dissolving an appropriate amount of catalase powder in the phosphate buffer. The activated nanofibrous membrane was submerged in the enzyme solution, the suspension placed in a rotary shaker for 3 h at 4 °C. Finally, the nanofibrous membrane was recovered and rinsed with PBS until no soluble protein was detectable in the washings. Protein concentration was determined with the Coomassie Brilliant Blue reagent following Bradford's method; BSA was used as a standard to construct the calibration curve.

The amount of immobilized protein was calculated from the protein mass balance between the initial and final catalase solutions and washings. The catalase immobilization capacity was



Scheme 1. An outline of the chemoenzymatic synthesis used to produce poly-OVSEG.

defined as the amount of protein (mg) per gram of nanofibrous membrane. Each reported value is the mean of at least three experiments, and the standard deviation was within *ca.* ±5%. The amount of bound protein (*Ae*; mg protein per g membrane) was calculated using the following equation:

$$Ae(\%) = \frac{(C_0 - C) \times V - C_w \times V_w}{W} \times 100$$

where C_0 and C are the initial and equilibrium protein concentrations in solution (mg/mL) C_w is the mean protein concentration in the washings, V and V_w are the solution and washings volumes (mL), and W is the mass of nanofibrous membrane (g).

2.7. Activity assay

The catalytic activity of the free and immobilized catalase was investigated in aqueous media according to a previous report [37]. 3.6 mL of hydrogen peroxide solution (24.4 mM) was equilibrated at 37 °C, and the reaction initiated by adding 30 μl of catalase solution (0.1 mg solid/mL). Activity was determined spectrophotometrically by direct measurement of the decrease in hydrogen peroxide absorbance at 240 nm as a function of time. The rate of change in absorbance was calculated from the initial linear section of the decay curve. One "unit" of enzyme activity was defined as the decomposition of 1.0 μmol hydrogen peroxide per min at 37 °C and pH 7.0. Analogous experiments were subsequently performed with catalase immobilized on PAN/poly-OVSEG nanofibers (5 mg). This latter reaction was terminated after 2 min by removal of the fibers from the reaction mixture. The absorbance of the reaction mixture was determined, and the immobilized catalase activity calculated.

Activity assays were carried out over the pH range of 4.0–9.0 and in the temperature range of 15–55 °C. The results of pH and temperature variation experiments are presented in a normalized

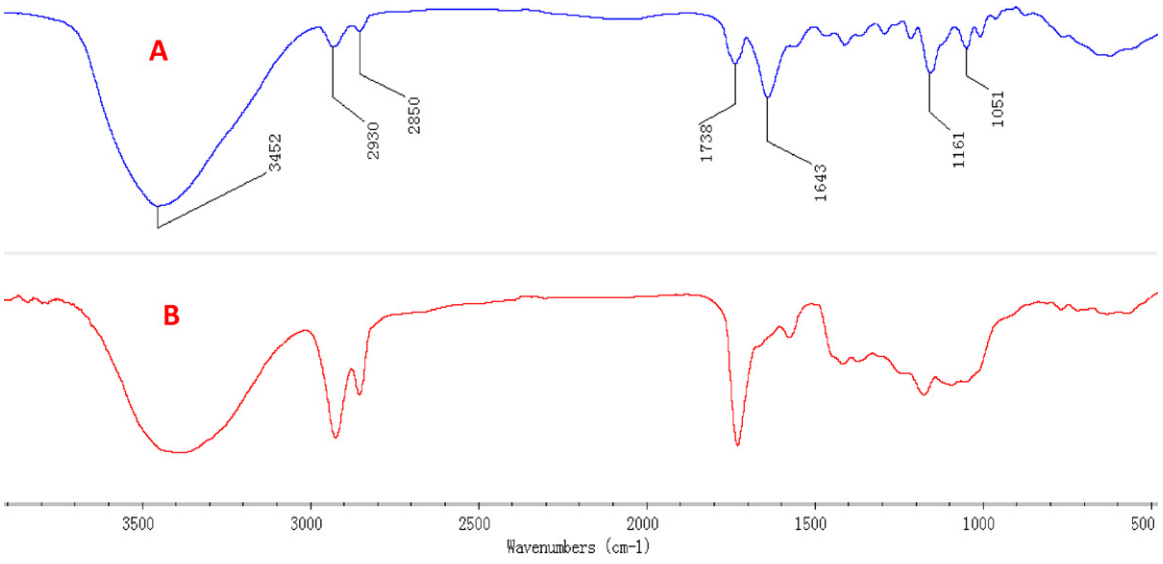


Fig. 1. IR spectra of (A) OVSEG; (B) poly-OVSEG.

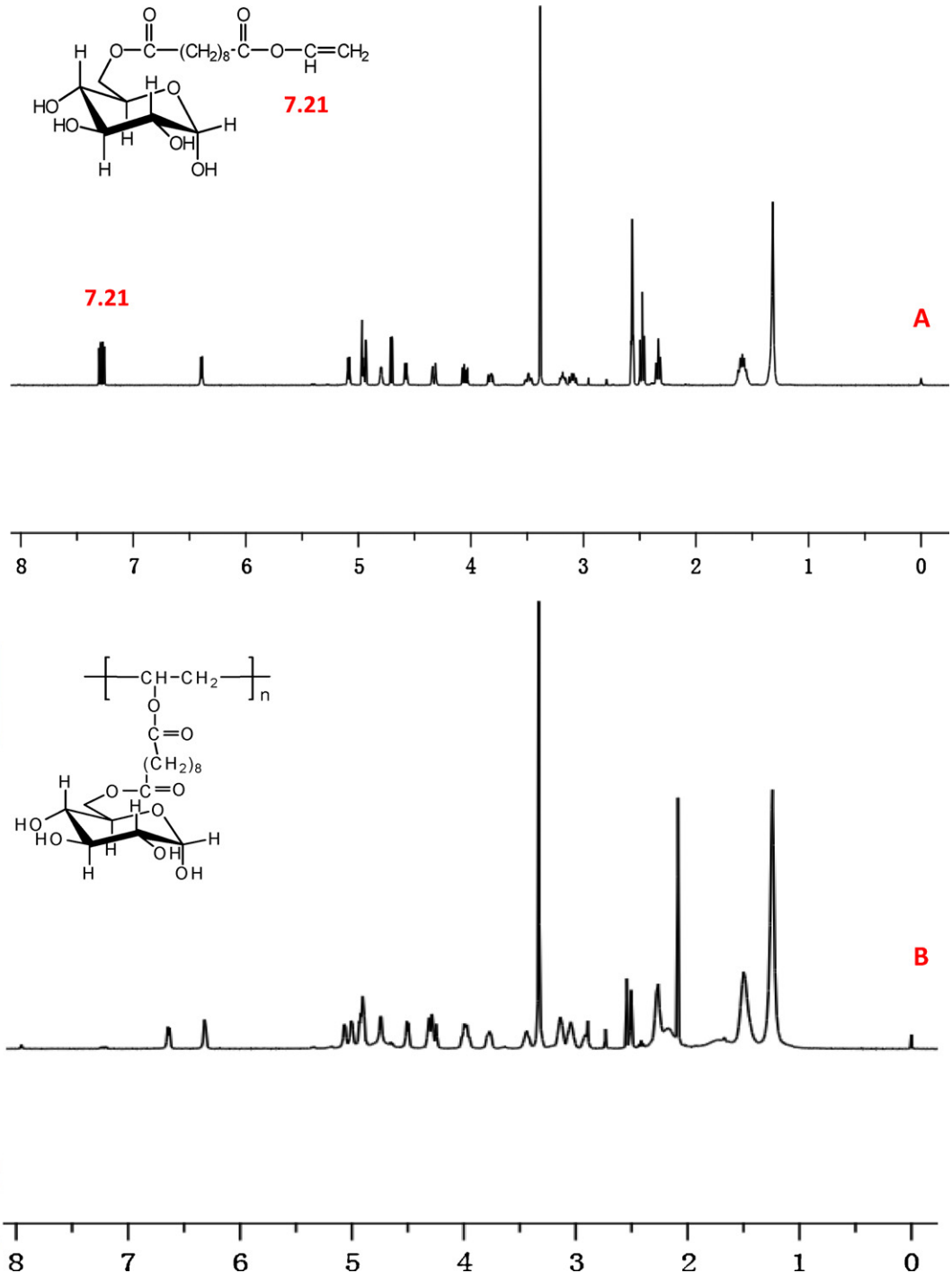


Fig. 2. ^{1}H NMR spectra of (A) OVSEG; (B) poly-OVSEG.

form, with the highest value of each set being assigned as 100% activity.

2.8. Stability measurements

The thermal stabilities of free and immobilized catalase were determined by measuring the activity of the enzyme after a heat treatment. The free and immobilized catalases were stored in PBS at 50 °C and 60 °C for 2 h and then periodically withdrawn for activity measurement. The residual activities were determined as described in Section 2.7.

Storage stabilities of catalase immobilized nanomats and free catalase were determined by means of storing them in PBS buffer (pH 7.0) at 4 °C for 30 d. The residual activities of the samples were measured for a month. Activity retention was given as percentage of activity taken as 100% for the initial activity before storage.

3. Results and discussion

3.1. Preparation and characterization of poly-OVSEG

The chemoenzymatic synthesis employed to produce poly-OVSEG is shown in Scheme 1. Its products were analyzed by FT-IR

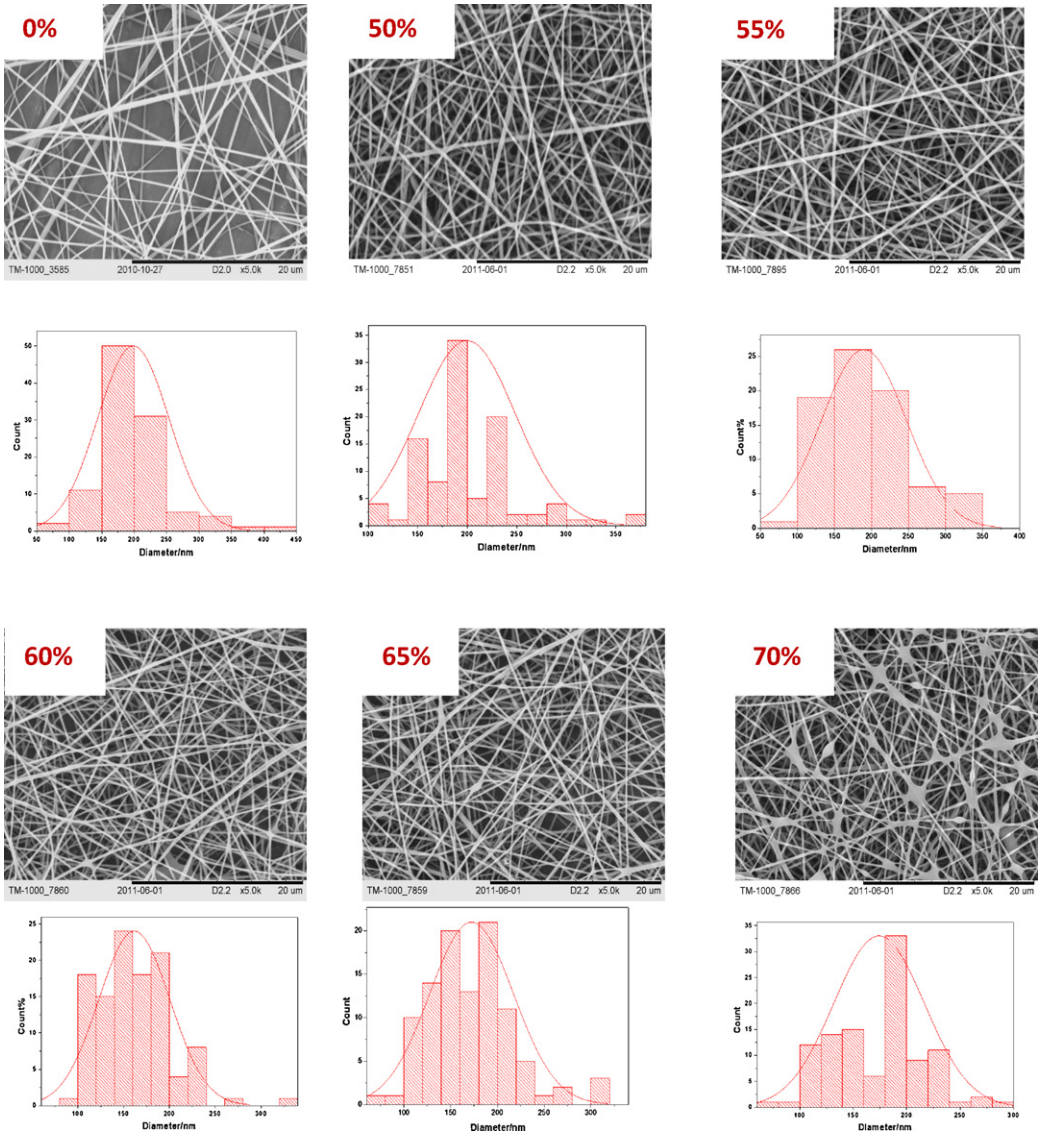


Fig. 3. SEM images of nanofiber mats prepared with different weight ratios of poly-OVSEG to PAN, and diameter distribution (PAN concentration, 6 wt%; voltage, 15 kV; distance to collector, 15 cm).

and NMR. In the IR spectrum of OVSEG (Fig. 1), there is a distinct absorption band at 1643 cm^{-1}, which corresponds to the double bond in the monomer. This band is no longer present in the spectrum of poly-OVSEG. Other absorptions at *ca.* 3420–3475 cm^{-1}, 1161 cm^{-1}, and 1061 cm^{-1} are assigned to the OH group of glucose, and are present in both spectra. Similarly, the band at *ca.* 1738 cm^{-1}, attributed to the sebacyl O—C=O group can be observed in both spectra.

Successful polymerization is also evidenced from ^{1}H NMR spectra (Fig. 2). The OVSEG monomer shows resonances at 7.21 and 4.88 ppm, corresponding to the vinyl group: these peaks are absent in the spectrum of poly-OVSEG. The full NMR data of poly-OVSEG may be assigned as follows: δ (ppm): 6.63, 6.32 (d, 1-OH of α-D-glucose); 5.06 (d, H-1 of α-D-glucose); 4.88–4.5 (m, 4-OH, 3-OH, 2-OH of α-D-glucose); 4.26–3.98 (d, H-6, H-6′, of α-D-glucose, sebacyl —CH—); 3.75–2.9 (m, H-5, H-3, H-2, H-4 of α-D-glucose); 1.52, 1.25 (m, decanedioyl —CH_2—).

3.2. SEM

The morphology and diameter of electrospun fibers are dependant on various parameters including applied voltage, electrospinning distance, and solution concentration. The latter is particularly important [38].

Fig. 3 gives SEM micrographs of the nanofiber mats formed using a PAN/poly-OVSEG blend containing varying weight ratios of poly-OVSEG. The average diameter of the fibers changed little with increasing poly-OVSEG concentration, until the poly-OVSEG content was increased above 70 wt%, when electrospinning spinning became impossible under these conditions.

3.3. FT-IR

The FTIR spectra for poly-OVSEG, a pure PAN fiber and PAN/poly-OVSEG fibers are depicted in Fig. 4. The FT-IR spectrum of the D-glucose monomer and poly-OVSEG (Fig. 4(a)) contain characteristic peaks at 2922 cm^{-1} (C—H stretching), 2854 cm^{-1} (—CH_2— stretching), and 1729 cm^{-1} (—C=O of carboxylic acid). The spectrum of the pure PAN fiber (Fig. 4(c)) shows important bands at *ca.* 2848 cm^{-1} (—CH_2— stretching) and 2244 cm^{-1} (—CN). A very broad band is visible at around 3440 cm^{-1}, which is attributed to the presence of water.

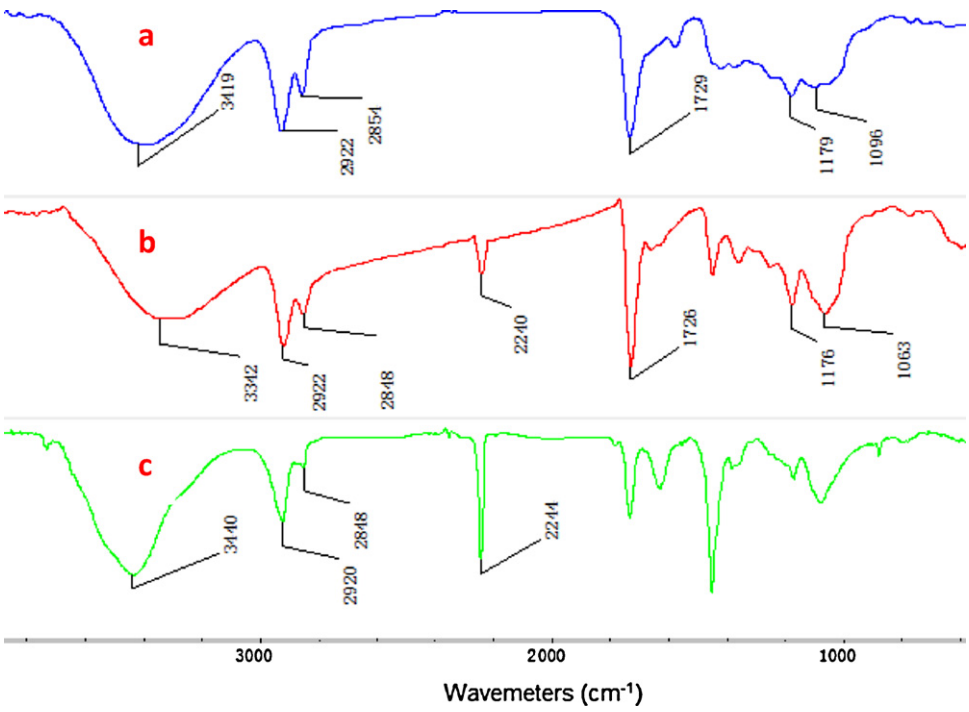


Fig. 4. Infrared spectra of (a) poly-OVSEG; (b) PAN/poly-OVSEG fibers (The quantity of poly-OVSEG was fixed at 55 wt%); and (c) PAN.

The spectrum of the blended fibers (Fig. 4(b)) demonstrates significant differences in the vibrational modes of both poly-OVSEG and PAN. The absorption bands of poly-OVSEG at approximately 2931 and 2851 cm^{-1} and of PAN at 2243 cm^{-1} have lower intensity and are shifted to lower frequency compared to the individual compounds. This is due to hydrogen bonding between poly-OVSEG and PAN in the fibers.

3.4. Hydrophilicity of the membranes

The surface hydrophilicity of electrospun nanofibers will play an important role in their overall performance as biomaterials [39]. We studied this by measuring water contact angles. For membranes with comparable structures, lower contact angle values normally mean increased hydrophilicity. It can be seen from Fig. 5 that the water contact angles of the membranes decreases from $65.5 \pm 2.5^{\circ}$ to $15.2 \pm 1.1^{\circ}$ as the poly-OVSEG content was increased from 0 to 70 wt%. Poly-OVSEG has many hydroxyl groups which, in general, enhance the hydrophilic properties of the composite membranes.

3.5. Immobilization of catalase

Catalase was covalently immobilized onto the polyacrylonitrile-based nanofibrous membranes using epichlorohydrin as coupling agents. Fig. 6 outlines the effect of immobilization time on enzyme loading and activity. Beyond a 3 h reaction time, the enzyme content leveled off, indicating that after this time the crosslinking process had reached equilibrium. The enzyme loading peaked at *ca.* 50 mg enzyme per gram of fiber mat. There was a close correlation between enzyme loading and activity up to a 3 h reaction time: after 3 h the enzyme activity was reduced. It is possible that

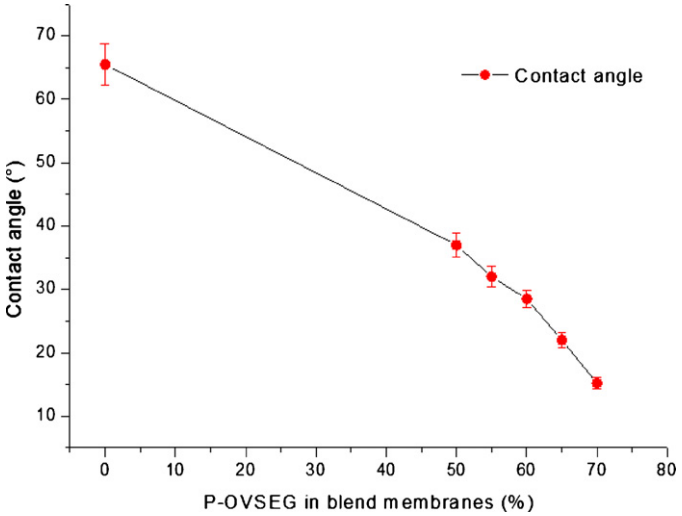


Fig. 5. Water contact angle variation of electrospun PAN/poly-OVSEG nanofibers generated with different poly-OVSEG contents. ●, contact angle.

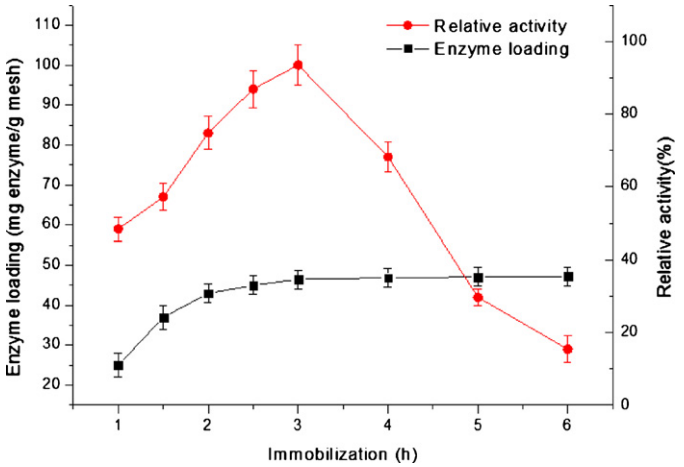


Fig. 6. The effect of immobilization time on enzyme loading and activity. ●, Relative activity; ■, enzyme loading.

Table 1
Enzyme loading and activity of catalase immobilized on PAN/poly-OVSEG nanofibers.

Mass of poly-O VSEG in the nanofibrous mats (wt%)	0	50	55	60	65	70
Bound enzyme (mg/g fiber)	2.1	34.3	39.6	42.3	44.6	46.5
Activity retention (%)	–	49.5	49.6	50.2	48.6	49.2

this behavior is caused by the cross-linking agent epichlorohydrin, which can be harmful to enzyme activity.

The amount of bound protein (*Ae*) and the enzyme activity for fibers containing different amounts of poly-OVSEG are detailed in Table 1. The amount of bound enzyme was greatest with fibers comprising 70 wt% poly-OVSEG, at about 46.5 mg/g. The overall enzyme activity of the immobilized system was invariant with poly-OVSEG content, at approximately 50%. This effect arises because the poly-OVSEG content provides a biomimetic microenvironment for the catalase, allowing it to retain its specific activity.

3.6. Stability of the immobilized catalase

The results presented thus far show that electrospun polyacrylonitrile-based nanofibrous membranes incorporating glycopolymer represent a facile and efficient strategy for catalase immobilization. For industry applications, the stability of the immobilized enzyme crucially important: enhanced stability can render an immobilized enzyme superior to one free in solution. Hence, the effect of pH on the activity of the free and immobilized catalases was assessed over the pH range of 4.0–9.0 at 37 °C. Fig. 7 illustrates the effect of pH on catalase activity. Overall results, the relative activity of catalase increased from pH 4 to pH 7, and then decreased dramatically at higher pHs. Maximum activity is at pH 7.0 for the free enzyme, and pH 7.5 for the immobilized catalase. This change may be caused both by the immobilization itself, and also by the basic character of the support material. It has been previously been noted that the immobilization of catalase can improve its stability, and broaden the range of pH over which it is effective [37].

Catalase in the immobilized state is found to have greater temperature stability than its free form (Fig. 8). The immobilizated enzyme has maximum activity at 50 °C, a 10 °C increase in stability over free catalase (which has maximum activity at 40 °C). It is presumed that immobilization of the enzyme confers some additional stability on the enzyme in its folded state, protecting it to some extent against temperature increases.

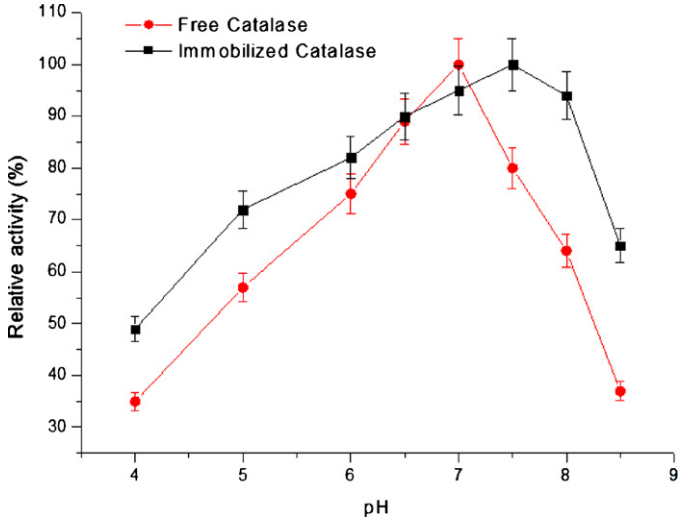


Fig. 7. Effect of pH on the activity of free and immobilized catalase. ●, Free catalase; ■, immobilized catalase.

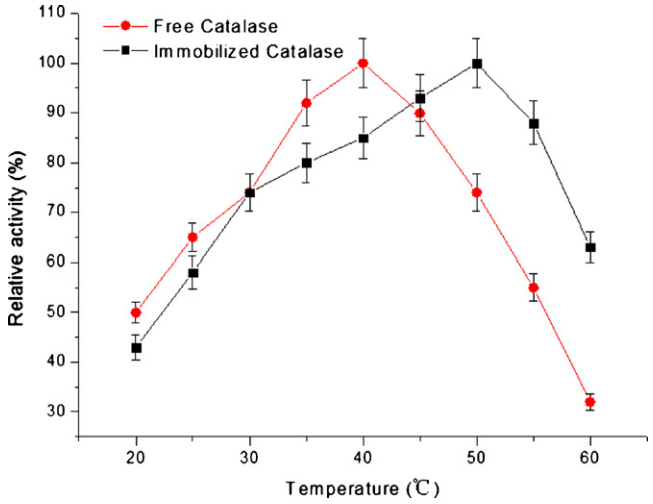


Fig. 8. Effect of temperature on the activity of free and immobilized catalase. ●, Free catalase; ■, immobilized catalase.

Fig. 9 shows the thermal stability of free and immobilized catalase. At 50 °C, the free catalase lost all of its activity within 100 min, while the immobilized enzyme retains 50% of its initial activity after a 120 min heat treatment. With heating at 60 °C, after 20 min the free catalase lost all its initial activity while the immobilized catalase retained about 34% of its initial activity. Clearly, at 60 °C temperature-induced conformational change in the catalase subunits rapidly and detrimentally affects the enzyme's quaternary structure. However, the thermal stability of immobilized catalase is much greater than that of the free enzyme.

The storage stability of the immobilized catalases compared to that of the free one is shown in Fig. 10. Under the same conditions, the free catalase loses of its initial activity within 22.5 days at 4 °C. While the residual activity of catalase immobilized on the polyacrylonitrile-glycopolymer fibrous membranes retained over 70% of its initial activity. These results indicated that

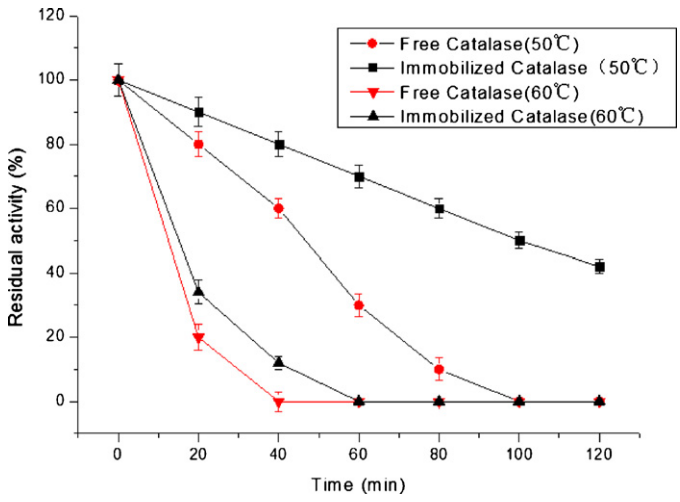


Fig. 9. The residual activity of free and immobilized catalase. ●, Free catalase; ■, immobilized catalase.

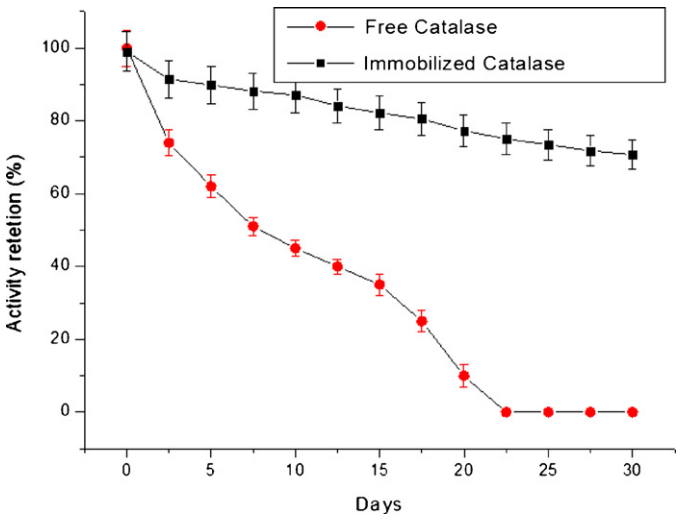


Fig. 10. Storage stability of the free and immobilized catalase at 4 °C. ●, Free catalase; ■, immobilized catalase.

immobilization can be prolonged till the storage period. The good storage stability of catalase immobilized could be attributed to the covalent bonds between catalase and polyacrylonitrile-glycopolymer fibrous membranes support which prevent the structural denaturation of the enzymes.

4. Conclusions

In this work, polyacrylonitrile (PAN)-based nanofibrous membranes incorporating a glycopolymer were fabricated, and employed to covalently immobilize the catalase enzyme. Results from water contact angle measurements revealed that increased poly-OVSEG content enhanced the hydrophilicity of the nanofibers, and the stabilities of the immobilized catalase with high enzyme loading and catalytic efficiency were obviously improved compared with the free enzyme. The nanofibrous membranes would have great potentials as biocatalysts for the applications in a wide range of reactions such as industrial production, diagnostic or clinical use, analytical methods, or biocatalytic remediation due to the specific structure, figure, and physical properties of this new enzyme preparation.

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References

[1] Z.K. Xu, X.J. Huang, A.G. Yu, J. Jiang, C. Pan, J.W. Qian, J. Mol. Catal. B: Enzym. 57 (2009) 250–256.
[2] J.M. Palomo, R.L. Segura, C. Mateo, R. Fernandez-Lafuente, J.M. Guisan, Biomacromolecules 5 (2004) 249–254.
[3] U. Cogan, A. Fishman, J. Mol. Catal. B: Enzym. 22 (2003) 193–202.
[4] L.S. Wan, Z.G. Wang, Z.M. Liu, X.J. Huang, Z.K. Xu, J. Mol. Catal. B: Enzym. 56 (2009) 189–195.
[5] G. Choudhary, S.L. Wu, P. Shieh, W.S. Hancock, J. Proteome Res. 2 (2003) 59–67.
[6] F. Hasan, A.A. Shah, A. Hameed, Enzyme Microb. Technol. 39 (2006) 235–251.
[7] T.D. Sutherland, I. Horne, K.M. Weir, C.W. Coppin, M.R. Williams, M. Selleck, R.J. Russell, J.G. Oakeshott, Clin. Exp. Pharmacol. P 31 (2004) 817–821.
[8] S.H. Wu, J.Y. Ran, S.Y. Jia, Y. Liu, W. Zhang, X.L. Pan, Bioresour. Technol. 101 (2010) 6285–6290.
[9] Z.K. Xu, X.J. Huang, P.C. Chen, F. Huang, Y. Ou, M.R. Chen, J. Mol. Catal. B: Enzym. 70 (2011) 95–100.
[10] Z.X. Liang, S. Pasunooti, W. Surya, S.N. Tan, J. Mol. Catal. B: Enzym. 67 (2010) 98–103.
[11] Y.J. Wang, X. Jian, G.S. Luo, Y.Y. Dai, Bioresour. Technol. 99 (2008) 2299–2303.
[12] R.A. Sheldon, Adv. Synth. Catal. 349 (2007) 1289–1307.
[13] H.F. Jia, G.Y. Zhu, B. Vugrinovich, W. Kataphinan, D.H. Reneker, P. Wang, Biotechnol. Prog. 18 (2002) 1027–1032.
[14] M. Sastry, S. Phadtare, V.P. Vinod, P.P. Wadgaonkar, M. Rao, Langmuir 20 (2004) 3717–3723.
[15] B.C. Kim, S. Nair, J. Kim, J.H. Kwak, J.W. Grate, S.H. Kim, M.B. Gu, Nanotechnology 16 (2005) S382–S388.
[16] Z.K. Xu, X.J. Huang, D. Ge, Eur. Polym. J. 43 (2007) 3710–3718.
[17] Z.K. Xu, P. Ye, J. Jiang, Colloid Surf. B 60 (2007) 62–67.
[18] A. Sadana, Chem. Rev. 92 (1992) 1799–1818.
[19] J.P. Bearinger, D.G. Castner, S.L. Golledge, A. Rezania, S. Hubchak, K.E. Healy, Langmuir 13 (1997) 5175–5183.
[20] Z.K. Xu, P. Ye, A.F. Che, J. Wu, P. Seta, Biomaterials 26 (2005) 6394–6403.
[21] H.T. Deng, Z.K. Xu, X.J. Huang, J. Wu, P. Seta, Langmuir 20 (2004) 10168–10173.
[22] H.T. Deng, Z.K. Xu, Z.M. Liu, J. Wu, P. Ye, Enzyme Microb. Technol. 35 (2004) 437–443.
[23] R. Narain, M. Ahmed, Biomaterials 32 (2011) 5279–5290.
[24] S.H. Kim, T. Hoshiba, T. Akaike, Biomaterials 25 (2004) 1813–1823.
[25] D.M. Haddleton, V. Ladmiral, E. Melia, Eur. Polym. J. 40 (2004) 431–449.
[26] M. Okada, Prog. Polym. Sci. 26 (2001) 67–104.
[27] Q. Yang, Z.K. Xu, M.X. Hu, J.J. Li, J. Wu, Langmuir 21 (2005) 10717–10723.
[28] Z.K. Xu, Z.W. Dai, L.S. Wan, J. Membr. Sci. 348 (2010) 245–251.
[29] S.A. Cetinus, E. Sahin, D. Saraydin, Food Chem. 114 (2009) 962–969.
[30] M. Yoshimoto, H. Sakamoto, H. Shirakami, Colloid Surf. B 69 (2009) 281–287.
[31] M. Yoshimoto, Y. Miyazaki, Y. Kudo, K. Fukunaga, K. Nakao, Biotechnol. Prog. 22 (2006) 704–709.
[32] L. Betancor, A. Hidalgo, G. Fernandez-Lorente, C. Mateo, R. Fernandez-Lafuente, J.M. Guisan, Biotechnol. Prog. 19 (2003) 763–767.
[33] Z.K. Xu, X.J. Huang, A.G. Yu, Bioresour. Technol. 99 (2008) 5459–5465.
[34] (a) Q. Wu, N. Wang, Y.M. Xiao, D.S. Lv, X.F. Lin, Carbohydr. Res. 339 (2004) 2059–2067;
(b) X.F. Lin, J. Quan, Q. Wu, Polymer 48 (2007) 2595–2604.
[35] A. Pollak, H. Blumenfeld, M. Wax, R.L. Baughn, G.M. Whitesides, J. Am. Chem. Soc. 63 (1980) 6324–6336.
[36] R. DiCosimo, C.-H. Wong, L. Daniels, G.M. Whitesides, J. Org. Chem. 49 (1981) 4622–4623.
[37] G. Bayramoglu, B. Karagoz, M. Yilmaz, N. Bicak, M.Y. Arica, Bioresour. Technol. 102 (2011) 3653–3661.
[38] K.H. Lee, H.Y. Kim, Y.M. La, D.R. Lee, N.H. Sung, J. Polym. Sci. Polym. Phys. 40 (2002) 2259–2268.
[39] L.M. Zhu, H.T. Zhang, S.B. Li, C.J.B. White, X. Ning, H.L. Nie, Electrochim. Acta 54 (2009) 5739–5745.