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Elaboration, characterization and study of a novel affinity membrane made from electrospun hybrid chitosan/nylon-6 nanofibers for papain purification

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Abstract Electrospun hybrid chitosan/nylon-6 nanofibrous mats with fiber diameters in the range of 80–310 nm were successfully fabricated using an electrospinning method. Nanofibrous membranes were prepared by nucleophilic reaction of the chitosan's hydroxyl and amidocyanogen with the triazinyl chloride of Cibacron Blue F3GA (CB) ligand. This system was used to study the purification of papain. Physical and chemical properties of the affinity membrane were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (ATR-FTIR), differential scanning calorimetry (DSC), contact angle (CA) and element analysis (EA). The equilibrium adsorption capacity (from Langmuir isotherm data) for papain was 93.46 mg/g affinity membrane. Fifteen layers of the composite affinity membrane were packed into a spin column to separate papain from raw material. Significant amount of the adsorbed papain (about 90.4%) was eluted by 1.0 M NaSCN at pH 9.0, and 4.8-fold purification was achieved in a single step. Experiments on regeneration and dynamic adsorption were also performed. It is shown that this system has the potential to be developed for the industrial purification of the papain.

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Introduction

Nowadays biotechnological industry devotes huge efforts in production of highly purified protein due to their wide applications in scientific research [1–3]. Affinity chromatography is a traditional technique which is often employed in the later stages of protein purification. Traditional affinity chromatography purification uses gel beads column chromatography [4, 5]. However, this system is subject to high-pressure drop and low throughput unit operation that exhibits flow rate-dependent dynamic capacities for biomacromolecules. For these reasons, membrane chromatography has been promoted as a promising alternative to packed-bed chromatography [6]. Affinity membrane is thus developed to permit the purification of molecules based on differences in physical/chemical properties or biological functions rather than in molecular weight or size. In affinity membrane, ligand molecules were introduced into the inner surfaces to specifically capture target molecules, while allowing other molecules to pass through. Combining both the high productivity associated with membranes and the outstanding selectivity of the chromatography resins, affinity membrane chromatography is now an attractive and competitive method for purifying proteins or other biomolecules from biological fluids [7–12].

The primary objective of this study is to create novel affinity membranes with ultrahigh protein binding capacities. Dye-ligand chromatography has played an important role in the separation, purification and recovery of proteins, because many inexpensive, stable and group specific dyes are available and they can be used for the separation of a large number of proteins and enzymes [13]. Cibacron Blue F3GA (CB), a monochlorotriazinyl with the enzyme binding anthraquinone and benzene sulfonate rings, is a general purpose ligand for purification of many enzymes.

CB has since been covalently bonded to various hydroxyl or amino containing polymers, such as poly(styrene-co-divinylbenzene) microparticles [14], sepharose or chitosan microspheres or beads [15–17], polyamide [18], cellulose membranes [13, 19], for enzyme separation and immobilization. For these purposes, fibrous materials are among the most suitable matrices due to their intrinsically high specific surfaces, porous structures, reasonable mechanical strengths and superior flexibility for easy handling.

The electrospining technique is a well-known process for making continuous sub-micron to nano-size fibers in the nonwoven mat form. Electrospun nanofibrous membranes possess several attractive attributes that make them very attractive in separation technology, such as high porosity, pore sizes ranging from nanometer to several micrometers, interconnected open pore structure, high permeability for gases and a large surface area per unit volume. These properties make fibers ideal candidates in applications such as filtration [20–23], tissue engineering [24–26], manufacture of protective clothing, pharmacy and functional materials [27–29]. In particular, they also have been highly successful in developing affinity membrane.

Recently, the nylon-6/chitosan complex nanofibers have been successfully prepared by electrospinning in our laboratory. Similarly nylon polymers have many uses as an engineered material due to their strong mechanical properties such as high tensile and impact strengths and abrasion resistance properties [30–32]. Electrospinning of nylon-6 and chitosan blends nanofibers, which may combine the advantages of nylon together with those of chitosan that has high hydrophilicity, biocompatibility, biodegradability, antibacterial and antifungal activity. The blending of these materials could be developed for affinity membrane applications.

In the present study, we outline the CB-attached electrospun nylon-6/chitosan composite nanofiber as a novel affinity membrane for the first time. Physical and chemical properties of the affinity membrane were characterized by SEM, ATR-FTIR, and DSC. The composite membranes were directly used for papain purification from raw papain as apposed from solubilized papain extracts. The performance of the membrane stack was also evaluated.

Experimental

Materials

Nylon-6 (weight-average molecular weight ~20,000 g/mol) was purchased from Shanghai Chemical Fibers Institute. Chitosan (molecular weight average 80,000 with over 85% deacetylation) was supplied by National Pharmaceutical Group Corp., China. Two solvents, 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) from Fluorochem Ltd. (UK) and formic acid (FA) from National Pharmaceutical Group Corp., China, were used to dissolve the blends of chitosan and nylon-6.

Electrospinning procedures

The procedure for the preparation of nylon-6/chitosan nanofiber has been detailed in our previous study [33]. Nylon-6 and chitosan blends were dissolved in HFIP/FA (v/v, 90/10) at a concentration of 6 wt.% (g/mL). Nylon-6 was mixed with chitosan in weight ratios of 85/15 and was then used for electrospinning at room temperature. The polymer solution was placed in a 5 mL syringe with a metal needle of 0.6 mm in diameter. A power supply (ES40P-20W/DAM) was used to provide a high voltage, 16 kV, to the syringe needle tip and a metal collector. The fibers were collected on an aluminum foil. With a tip-to-collector distance of 15 cm and a solution flow rate of 1 mL/h before drying in a vacuum oven for 24 h at 60 °C.

Immobilization of the Cibacron Blue F3GA

CB was immobilized onto the membranes through the nucleophilic reaction between the chloride of its triazine ring and the hydroxyl or amino group of the chitosan molecule under mild alkaline conditions. The coupling procedure was followed by the method described previously [34]. Briefly, 20 membranes were immersed together into a 200 mL dye solution (10 mg/mL) for 30 min at 60 °C. This was followed by the addition of 5 mL NaCl aqueous solution (20 wt.%) in order to stimulate the deposition of the dye on the internal surface of the membranes. After 30 min, 2 mL Na_2CO_3 aqueous solution (25 wt.%) was added to accelerate the reaction between the dye and the membrane which took place for 4 h at 80 °C. Finally, the dyed membrane was washed with hot water, methanol, 2 M NaCl, 6 M urea and distilled water, successively, until no dye could be detected in the eluate. The membranes were stored in 0.05 M Tris–HCl (pH 8.0) containing 0.02 wt.% sodium azide at 4 °C.

Measurement and characterization

The morphology and diameter of the electrospun nanofibers were determined by scanning electron microscopy (SEM) (JeoL JSM-5600 LV, Japan). Prior to scanning, samples were sputter coated for 90 s with gold using a JEOL JFC-1200 fine coater. The diameters of fibers were analyzed using image visualization software Adobe Photoshop.

FT-IR spectra of the electrospinning nanofibers and the films were recorded using a Nicolet 17DSX FT-IR Spectrometer.

The thermal properties of the electrospun fibers were measured by a TA Instruments DSC-822 Differential Scanning Calorimeter (Mettler Toledo Company, Switzerland) over a temperature range of 20–300 °C at a heating rate of 10 °C/min.

The amount of CB attached on the electrospun hybrid chitosan/nylon-6 nanofibrous membrane was evaluated by using elemental analysis instrument (Elementar, VarioELIII, Germany) by considering the sulphur stoichiometry.

Using a sessile drop method, static water contact angle was measured at room temperature on a contact angle goniometer (KRUSS DSA10-MK) equipped with video capture.

Adsorption studies

Adsorption of papain from solution on the CB-attached electrospun nylon-6/chitosan nanofiber affinity membrane was studies at five different papain concentrations (0, 0.5, 1.0, 1.5, and 2.0 mg/mL) in Tris–HCl buffer (10 mL, 0.05 M, pH 8.0). The adsorption experiments were conducted at 25 °C for 3 h, with continuous stirring. After this period, the affinity membranes were removed from the solution. The adsorbed papain on the CB-attached electrospun nylon-6/chitosan nanofiber affinity membranes was determined by measuring the initial and final concentrations of the enzyme within the adsorption medium at 280 nm by UV-spectrophotometry. The amount of papain adsorbed was obtained by using the following equation:

$$q = \frac{(C_0 - C)V}{M} \quad (1)$$

where q is the amount of papain adsorbed onto membranes (mg/g), C_0 and C are the concentrations of the papain in the initial solution and in the aqueous phase after adsorption, respectively (mg/mL), V is the volume of the aqueous solution (mL), and M is the mass of the membrane in the adsorption medium (g).

Dye affinity chromatography of papain

Fifteen sheets of CB-attached electrospun nylon-6/chitosan nanofiber affinity membranes were constructed into the patented membrane cartridge which consisted of two round plates holding the membranes, a cylinder with an internal diameter of 47 mm holding the two plates and membranes with glue. Another cylinder connected with the former cylinder by screw thread and a ring for adjusting the distance between the two cylinders. The peristaltic pump that consisted of a membrane cartridge and a digital control pump, containing was equilibrated in 0.05 M Tris–HCl buffer (pH 8.0), the flow rate is 2.0 mL/min. The bed volume of the membrane chromatogram is 25 mL. It was then loaded with 15 mL of 1.0 mg/mL papain solution, the flow rate is 0.8 mL/min. After adsorption, the membrane stack was washed with 0.05 M Tris–HCl (pH 8.0) to remove unbounded protein for 30 min, the flow rate is 0.8 mL/min. The elution experiments were then performed with 0.05 M Tris–HCl (pH 6.0) containing 1.0 M NaSCN which are the conditions proposed by Clontech Laboratories user manual [35].

Analytical procedures

Protein concentration

Protein concentration was measured with the Bradford Protein Assay using bovine serum albumin as standard [36].

Enzyme activity

Papain solution (1.0 mg/mL) was obtained by papain dissolved in 0.05 M Tris–HCl (pH 8.0). Then, 0.7 mL of Tris–HCl (pH 8.0), 0.2 mL of papain activator consisting of 0.5 M L-cysteine and 0.02 M EDTA (pH 8.0), 0.1 mL of 1 wt.% casein solution (pH 8.0) were added to a 10 mL with a magnetic stirrer. The enzyme reaction was carried out at 37 °C for 10 min with stirring, 3 mL of 5% trichloroacetic acid solution was added into the beaker flask, the reaction mixture was statically equilibrated at 25 °C for 1 h, and then it was filtered. The absorbance of the filtrate was measured at 280 nm. One unit (U) of enzymatic activity was defined as the amount of enzyme increasing 1 absorbance/min at 280 nm in this condition.

Electrophoresis of papain

The samples were first dialyzed against 10 mM Tris–HCl pH 8.0 buffer containing 1 mM EDTA, and then mixed with an equal volume of an aqueous solution containing 5.0 wt.% SDS, 10 vol.% β-mercaptoethanol and 0.02 wt.% bromophenol blue solution. The mixtures were heated at 100 °C for 5 min and then filtered to remove any insoluble material before they were used for electrophoresis.

Membrane regeneration

Papain-saturated membranes were regenerated by washing and recycling with sodium hydroxide and then washing with 0.5 M NaSCN, the adsorbed papain was released in 2 h when 0.5 M NaSCN was used as a desorption agent, before they were successively regenerated with 6 M urea,

1% Tween 80 and water. Regenerated membranes were then reused for papain equilibrium adsorption.

Results and discussion

Physical properties

The prepared dye affinity membrane is a hydrophilic matrix. It was found that the contact angle was 19.2 ± 0.4° after the 4th second the droplet had contact with the electrospun affinity membrane. Chitosan has four hydroxyl groups, an amine group, and a minor proportion of amide groups, which are, in general, partially hydrolyzed. All these functional groups enhance the hydrophilic properties of the natural biopolymer chitosan and so improve the mechanical strength when complexed with hydrophobic material such as nylon-6. The hydroxyl or amino groups in chitosan of the composite membranes can react with the chloride of the triazine ring of the CB under alkaline conditions, thus forming covalent bonds.

Dead-end filtration was done with stirred a cell with effective area of 0.0038 m^2. It was thermostated at 25 °C. Flux values of pure water at different transmembrane pressures (ranging 0–0.4 MPa) were measured at steady-state condition using Eq. 2:

$$J_v = \frac{\Delta V}{S\Delta t} \tag{2}$$

where J_v is the volume flux (L/(m^2 h)), ΔV is the variation of permeate volume (L), S is the membrane effective area (m^2), and Δt the time interval (h). The hydraulic permeability was calculated from the Poiseuille's law as represented with Eq. 3:

$$P_m = \frac{J_v}{\Delta P} \tag{3}$$

where P_m *i*s the hydraulic permeability (L/(m^2 h MPa)) and ΔP is the transmembrane pressure (MPa). For the retention of heavy metal ions, all filtration experiments were done with 150 mL of solution at stirrer speed of 10 rps (600 rpm). The first 7 mL permeate was discarded and the subsequent volumes were collected for analysis. Before each dead-end filtration experiment under magnetic stirring, the membrane was exposed to a higher pressure (5 bar) to stabilize them. The main physical and morphological properties of affinity membranes are presented in Table 1.

Table 1 The physical and morphological properties of the affinity nanofibrous membranes

Items	Property
Specific surface area	283 m^2/g
Hydraulic permeability	24 ± 2.6 L/(m^2 h bar)
Porosity	87 ± 2.6%
Thickness	90 ± 8.0 μm
Diameter	47.0 mm
Contact angle (4th second)	19.2 ± 0.4 (θ)
Dye CB content	102 ± 7.2 μmol/g

Scanning electron microscopy examination

SEM photographs of nylon-6/chitosan blend nanofibers as a function of concentration (nylon-6/chitosan = 85/15) are shown in Fig. 1. The fiber diameters of all nanofibrous mats were analyzed by related software. Relatively smooth, defect-free hybrid nylon-6/chitosan nanofibers with fiber diameter of 80–310 nm were fabricated using electrospinning technique shown in Fig. 1a. Also, the fibers obtained had cylindrical morphology and no fiber bundles, indicating that the tip-to-collector distance with 15 cm was adequate for proper evaporation of the solvent.

Figure 1b shows an obvious change after dye CB as a ligand was then covalently immobilized on the nylon-6/chitosan blend nanofibrous membranes. CS is hydrophilic but water resistant, nonporous, but water permeable. The CS mixed into the membranes would allow water to penetrate without losing too much flow rate, while the smooth surface would minimize the blockage problem [37]. The diameters of the nanofibers become coarser than those of unbonded nylon-6/chitosan blend nanofibers due to CB immobilizing. In addition, the pore sizes appear smaller than those of the uncoated nanofibers since the CB coating covers the surface and intersections of the nanofibers, as revealed in Fig. 1b.

FT-IR

The FT-IR spectra of native electrospun hybrid chitosan/nylon-6 nanofibrous membrane, Cibacron Blue F3GA and the dye attached affinity membrane are presented in Fig. 2. The FT-IR spectra of dye affinity membranes (Fig. 2c) have some absorption bands different from the native electrospun hybrid chitosan/nylon-6 nanofibrous membrane (Fig. 2a). The absorption band of the dye affinity membrane at 1230 and 1475 cm^{-1} represents the stretching vibrations of C–N on CB, which are also observed in CB (Fig. 2b). The absorption band in the 1600–1450 cm^{-1} region is characteristic of the benzene ring. On the other hand, the adsorption intensity of dye affinity membrane at 1024 cm^{-1} representing symmetric stretching of S=O is greatly higher than that of the plain membrane. This data indicated that the coupling of CB is successful. The bands at 1600–1230 cm^{-1} region have many characteristic bands

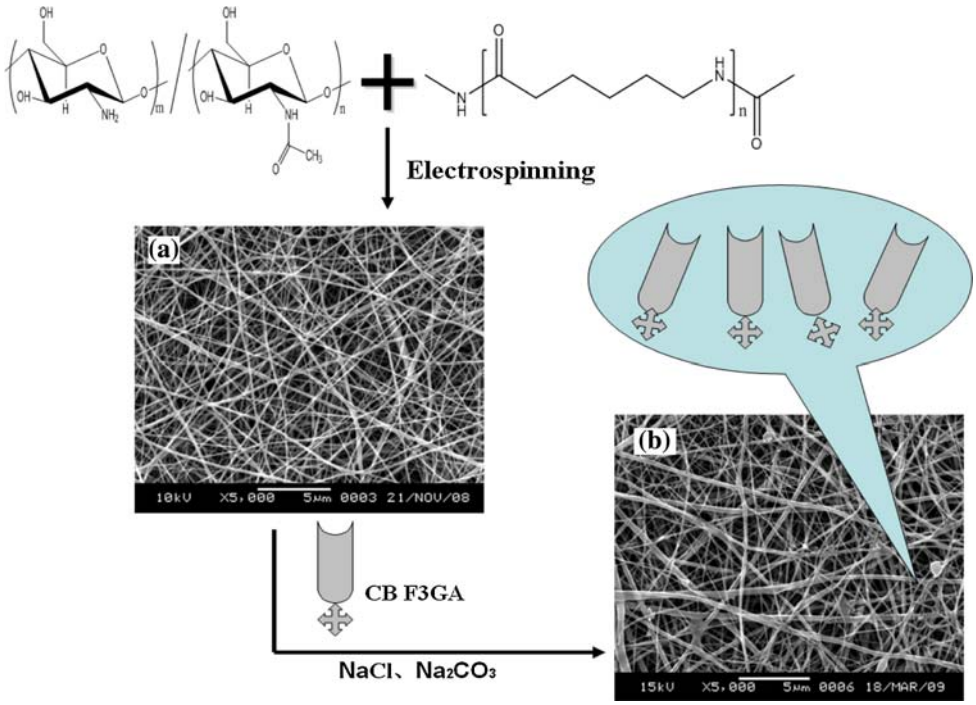

Fig. 1 Schematic representation for Cibacron Blue F3GA attached on the electrospun hybrid chitosan/nylon-6 nanofibrous membrane

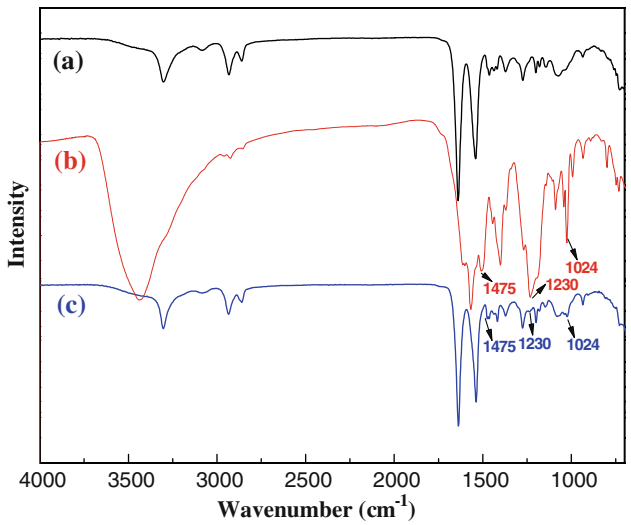

Fig. 2 The FT-IR spectra: *a* native electrospun hybrid chitosan/nylon-6 nanofibrous membrane; *b* CB F3GA; *c* dye affinity membrane

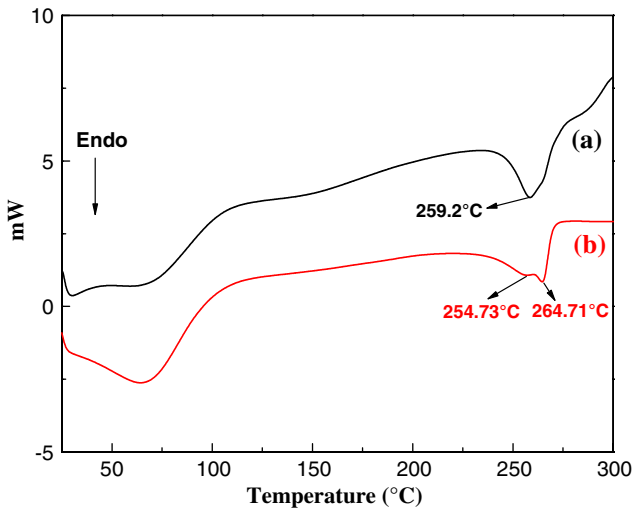

Fig. 3 DSC curves of *a* native electrospun hybrid chitosan/nylon-6 nanofibrous membrane; *b* dye affinity membrane. The heating speed was 10 °C/min

of CB; however, they do not appear on the dye membranes due to plain electrospun hybrid chitosan/nylon-6 nanofibrous membrane also has some absorption bands in the same region. Thus, absorption bands of plain electrospun hybrid chitosan/nylon-6 nanofibrous membrane overlap with those of CB around these wavenumbers. These observations indicate that the dye CB was successfully bonded to the hybrid chitosan/nylon-6 nanofibrous membranes.

Differential scanning calorimetry (DSC) studies

Differential scanning calorimetric was conducted to study the pre-melting behavior. DSC results in Fig. 3 showed that the two melting peaks of the dye attached affinity membrane unlike the native electrospun hybrid chitosan/nylon-6 nanofibrous membrane. The strong broad absorption peak between 50 and 100 °C is the dehydration peak due to loss of water molecules which is strongly adsorbed in the

Table 2 Thermal properties (melting points, transition enthalpies) of native electrospun hybrid chitosan/nylon-6 nanofibrous membrane and dye affinity membrane

Samples	T_{m1} (°C)	T_{m2} (°C)	ΔH_{m1} (J/g)	ΔH_{m2} (J/g)
Native electrospun membrane	259.20	n/a	55.91	n/a
Dye attached affinity membrane	254.73	264.71	1.65	5.03

Thermograms of electrospun composite fibers were measured at a scanning rate of 10 °C /min at a temperature range of 25–300 °C

material by hydrogen bond. The native electrospun hybrid chitosan/nylon-6 nanofibrous membrane was rigid, showing a relatively high melting temperature T_m value at 259.20 °C and ΔH_m was 55.91 J/g, respectively.

Attaching of the dye CB on the affinity membrane slightly influenced the thermal behavior of the membranes. The effect of incorporation of dye CB on the thermal properties of electrospun hybrid chitosan/nylon-6 nanofibrous membrane is shown in Fig. 3b and Table 2. The melting temperatures (T_m) of dye attached affinity membrane were around 254.73 and 264.71 °C, respectively, compared to 259.0 °C of plain electrospun hybrid chitosan/nylon-6 nanofibrous membrane (Fig. 3a). Their melting enthalpies (ΔH_m) were 1.65 and 5.03 J/g, respectively. The appearance of multiple melting peaks of dye attached affinity membrane was possibly ascribed to the different extent of alignment and orientation in the electrospun membranes [38]. Both plain electrospun hybrid chitosan/nylon-6 nanofibrous membrane and dye attached affinity membrane showed clear characteristic dehydration endotherms. The slightly lower melting temperature of the dye attached affinity membrane suggested its strong interaction between CB and the electrospun membrane. These results were consistent with the FTIR studies reported here and it further supports that coupling of CB on the electrospun membrane was successful.

Adsorption studies

The non-specific adsorption of papain was obtained from batch experiments in a Tris–HCl (pH 8.0) at 25 °C. The papain adsorption on the CB attached composite membrane is presented in Fig. 4a. As seen from the figure, an increase in the papain concentration in the adsorption medium led to an increase for papain adsorption on the membrane (70.13 mg/g). The adsorption isotherm fitted the Langmuir model well. The equilibrium adsorption capacity (from Langmuir isotherm data) for papain was 93.46 mg/g affinity membrane. The Langmuir model is based on the assumption of adsorption homogeneity, representing equally available adsorption sites, monolayer surface coverage, and no interaction occurs between adsorbed species. The adsorption process can be expressed as:

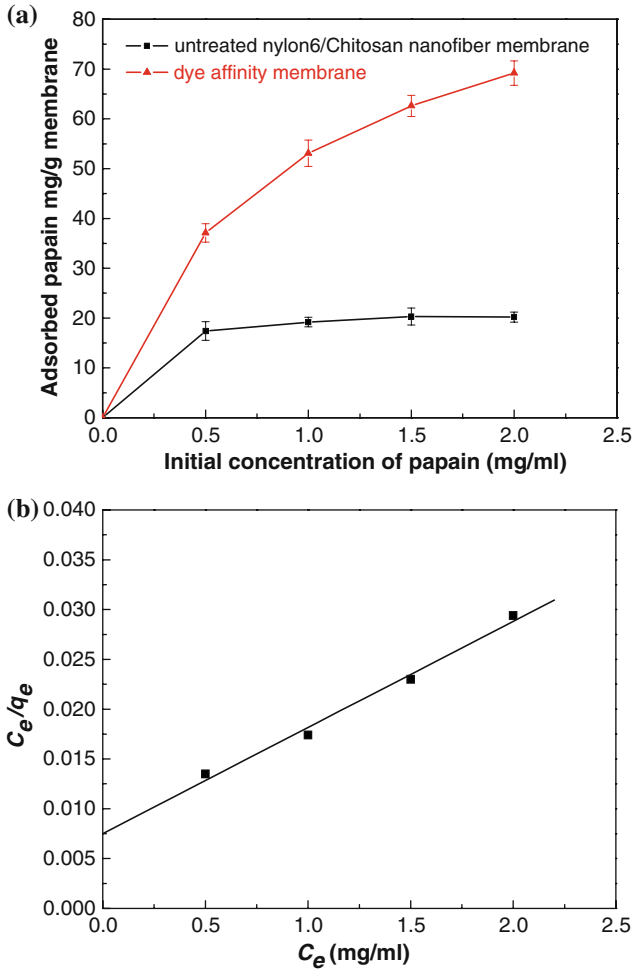

Fig. 4 **a** Adsorption of the effect of initial concentration of papain on papain adsorption on the different membranes (pH 8.0 and $T =$ 25 °C). **b** Langmuir model

$$q_{eq} = \frac{q_m C_m}{K_d + C_m} \tag{4}$$

Equation 4 can be transformed to a linear form as follows:

$$\frac{C_e}{q_{eq}} = \frac{C_e}{q_m} + \frac{K_d}{q_m} \tag{5}$$

where C_e (mg/mL) is the equilibrium concentration of bromelain in solution, q_{eq} (mg/g) the adsorption capacity, q_m (mg/g) the maximum adsorption capacity and K_d is the effective dissociation constant. The data of the adsorption

Table 3 The Langmuir constants and correlation coefficients for papain adsorption on the dye affinity membranes

Temperature (K)	q_m (mg/g)	K_d	R^2
298	93.46	0.7010	0.9944

isotherm is presented in Fig. 4b. The Langmuir constants are presented in Table 3. From Table 3, it can be seen that the corresponding semireciprocal plots (C_e/q_e versus C_e) of the experimental data gave a linear plot ($R^2 = 0.9944$) for the dye affinity membrane. Thus, the heterogeneous adsorption of papain on the dye-attached affinity membrane can be modeled using Langmuir isotherm.

It should be noted that a negligible amount of papain was adsorbed non-specifically on the native electrospun hybrid chitosan/nylon-6 nanofibrous membrane about 18.61 mg/g. Compared with the native electrospun hybrid chitosan/nylon-6 nanofibrous membrane, the adsorption capacity (about 3.8-fold) of papain on the affinity membrane greatly increased. It was clear that this increase is due to the specific interaction between the immobilized CB and papain molecules. It seems that the dye ligand would make a good candidate specifically isolating this enzyme.

Dye affinity chromatography of papain

In this study, a single step batch purification of papain from raw papain media is possible and wide short cartridges (bundle of membranes) were used for separation instead of thin long columns in conventional chromatography. The membrane cartridge containing 15 sheets of the dye affinity membrane was first equilibrated with 0.05 M Tris–HCl buffer (pH 8.0), then loaded with 25 mL of 1.0 mg/mL raw papain solution for 30 min. After the adsorption process, the membrane stack was washed with 0.05 M Tris–HCl (pH 8.0) to remove the unbounded protein until the absorbance detected at the wavelength of 280 nm was <0.1. The elution process was performed with 0.05 M Tris–HCl (pH 6.0) containing 1.0 M NaCl. The flow rate of the mobile phase was 2.0 mL/min. The schematic representation for dye affinity chromatography of papain is shown in Fig. 5.

Figure 6 shows the elution curve of the membrane stack in a membrane container. The outlet concentration reached the feed concentration rapidly in the non-adsorption breakthrough curve, indicating that the dead volume of the filter holder is small and its mixing effect is weak. While in the adsorption breakthrough curve, the outlet concentration increased slowly and reached the feed concentration until the elution volume is 24 mL. It is known that an ideal affinity membrane should possess such effective performance that when the outlet concentration reach 10% of the feed

Fig. 5 Schematic representation for dye affinity chromatography of papain

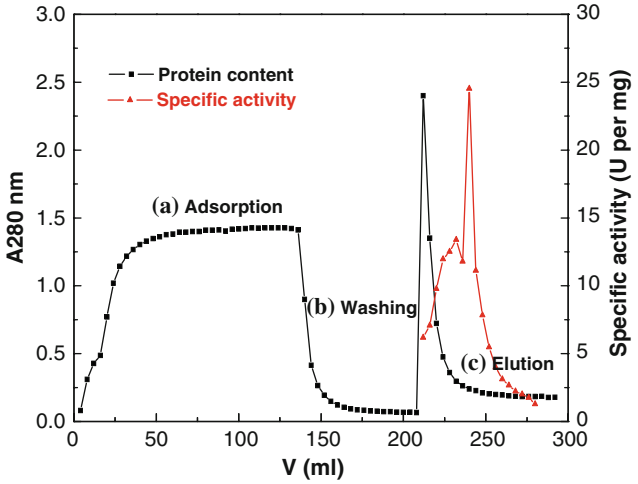

Fig. 6 The adsorption and desorption profile of papain on CB F3GA attached affinity membranes: *a* adsorption from a total flow of 25 L papaya powder (1.0 mg/mL) at flow rate of 1.0 mL/min; *b* washing with 50 mL the Tris–HCl buffer (0.05 M, pH 8.0) at flow rate of 2 mL/min; *c* elution with a total flow of 60 mL Tris–HCl buffer (0.05 M, pH 6.0) containing 1.0 M NaCl at flow rate of 1.3 mL/min (*filled triangle* specific activity of papain; *filled square* protein content). The results were means of duplicate determination on triple independent measurements. (Color figure online)

concentration, the membrane should already capture 90% ligate of its saturated capturing capacity [39]. This novel dye affinity membrane system showed that over 90.4% of the adsorbed papain was eluted, and the extent of separation was determined by both protein content and enzymic activity assays. It should be noted that the papain was purified over 4.8-fold (Table 4) in a single step, as determined by protein content and enzyme activity assays. The quality of the purification achieved was determined by electrophoresis (Fig. 7). The results show that the purified papain exhibits a single band. Results show that this electrospun affinity

Table 4 Purification of papain obtained from raw papain using dye electrospun affinity membrane

Eluted papain from eluted solution: 1# 212–220 mL; 2# 220-228 mL; 3# 228–236 mL; 4# 236–244 mL; 5# 244–252 mL; 6# 252–260 mL

Name	Volume (mL)	Protein content (mg)	Specific activity (U/mg)	Purification (-fold)
Raw papain	150	15.105	6.67	–
1#	8	1.2918	6.17	0.93
2#	8	1.0972	7.08	1.06
3#	8	0.6756	9.76	2.46
4#	8	0.3976	16.41	4.79
5#	8	0.2734	31.95	2.87
6#	8	0.1956	13.27	1.99

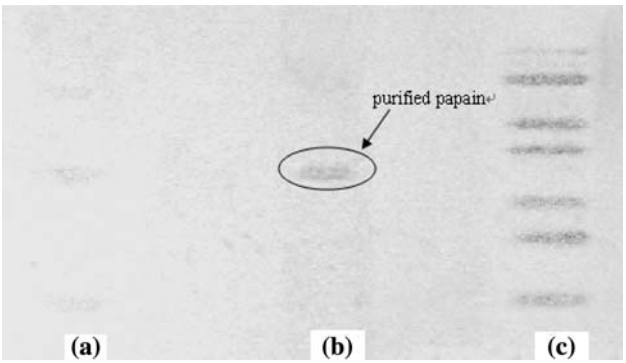

Fig. 7 SDS–PAGE analysis of papain purified from crude papain with dye affinity membranes stack. **a** Crude papain; **b** papain purified from crude papain with dye affinity membranes stack; **c** marker

membrane system is highly suitable for papain purification process. It is known that affinity membrane's separation efficiency can be made to be ideal only if parameters such as membrane microstructure, total thickness of the stacked membranes, ligand capacity, flow rate of the feeding solution, ligand–ligate association and dissociation coefficients are optimized [39]. Therefore, the electrospining fabrication processes and the ligand attached method should be improved in the future to prepare affinity membranes with higher chromatographic separation efficiency.

Desorption and reusability of adsorbents

In order to demonstrate the reusability of the affinity membrane, we have evaluated the dye electrospun affinity membrane to purify papain under the same condition more than 3 cycles, there was no significant reduction in the adsorption capacity of papain, the papain adsorption capacity decreased only 4%, indicating that the affinity membranes developed in this study can be reused and recycled easily.

Conclusions

In this research, electrospun hybrid chitosan/nylon-6 nanofibrous mats with fiber diameters in the range of 80–310 nm were successfully fabricated using electrospinning method. A dye ligand, Cibacron Blue F3GA, was covalently attached to the electrospun hybrid chitosan/nylon-6 nanofibrous membrane. Batch experiments revealed a high papain adsorption capacity and the adsorption process showed a Langmuir isotherm at the given range of the papain concentration. Dynamic experiments indicated a higher papain adsorption and desorption rate. Regeneration of the membrane suggested good mechanical properties and chemical stability. Additionally, this system was inexpensive and easy to operate. It is shown that this system has the potential to be developed for larger scale purification of papain.

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