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Papain Adsorption on Chitosan-Coated Nylon-Based Immobilized Metal Ion (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}) Affinity Membranes

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A novel immobilized metal affinity membrane was prepared for papain adsorption in this article. Higher papain adsorption capacity between 43-67 mg/g was observed and the adsorption isotherm fitted the Freundlich equation. Experimental data were analyzed using two adsorption kinetic models. The pseudo-second-order kinetic model provided better correlation to the experimental results. A significant amount of the adsorbed papain was eluted by 1.0 M NaSCN at pH 5.0 for all affinity membranes. It was concluded that the novel chitosan-coated nylon-based immobilized metal ion affinity membrane could be applied for the large-scale isolation of papain without resulting in enzyme denaturation.

Keywords adsorption; affinity membrane; metal ion; papain

INTRODUCTION

The developments in biotechnology and genetic engineering have accelerated the efforts to develop systems that can be used on a large-scale basis. Over the past decade, applications using affinity membranes for biomolecule separation have gained much attention. This is due to the lower mass-transfer limitations shown for the membrane process than conventional column chromatography (1–4). Various affinity modes have been developed using membrane matrices and their adsorption properties and separation efficiencies have been extensively investigated. Amongst them, the most popular system are immobilized metal affinity membranes due to their high recovery and capacity rates and complete regeneration of the immobilized system. These systems have many advantages over typical methods in affinity chromatography (5–9) namely, different metal ions can be immobilized to the same chelating medium and these can be easily removed for regeneration using stronger chelating agents such as EDTA without any detectable loss of binding properties. The system takes full advantage of metal ion affinities relating to functional groups such as of amino acids located at the surface of proteins/peptides that include the imidazole group of histidine, the thiol group of cysteine, and the indoyl group of tryptophan.

In chromatography, the most frequently used base materials for immobilized metal affinity membranes reported are various microporous or macroporous polymers including nylon, agarose, dextran, cellulose, cellulose derivative, polysulfone, polyethylene, poly(vinylidene difluoride), glass, and synthetic copolymer (8,10–13). Ideally the support for protein separation should have the following requirements: high hydrophilicity, low non-specific adsorption, chemical, thermal and mechanical resistance, large pore size, narrow pore size distribution, bio-compatibility, scalability, non-toxicity enough reactive functional groups. However, for some biotechnological applications hydrophobic and insufficient mechanical strength and porosityfactors influence performance. A possible solution to preventing non-specific binging and to simultaneously present functional groups for covalent modification with ligands is to coat such porous membrane with hydrophilic and functional polymer. For example, poly(vinyl alcohol), hydroxyethyl cellulose, and chitosan (CS) have been coated on different types of microporous/macroporous membrane substrates to introduce a functional surface (12,14,15).

Papain (EC 3.4.22.2) is a thiol enzyme obtained from the latex and unripe fruit of *Carica papaya* (tropical melon or papaw). The enzyme is a carbohydrate free, basic (isoelectric point, 9.6), consists of a single polypeptide chain (212 amino acids) and has a molecular weight 23,350 Da. Papain has a tertiary structure with four disulfide bridges and catalytically activity is associated with cysteine (25) and histidine (158) residues. Compared to other proteases,

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papain is cheap to prepare, has good thermal stability, and has a wide and versatile range of specificity. The enzyme is used for cell isolation, brewing, food (meat tenderizing), pharmaceutical (treating wounds and edemas), leather, cosmetic, and textile industries (16–18). Purification of papain from papaya latex has traditionally been achieved by precipitation (19) However, the purified enzyme is often contaminated with other proteases. An alternative strategy has involved the use of various chromatographic techniques including ion exchange, covalent, or affinity chromatography (20,21).

The work reported here aims to develop systems that evaluate the potential use of immobilized metal ion affinity membrane chromatography for the large scale isolation of papain. In this study a paper, flat sheet of nylon membrane is activated by formaldehyde followed by coupling chitosan onto an activated membrane. Affinity membranes were then prepared by immobilizing various metals ions as ligands on the chitosan-coated nylon system and this was used for papain adsorption. Factors, such as contact time, pH value, enzyme concentration, and ionic strength were also considered.

EXPERIMENTAL

Materials

Nylon membranes filters (diameter 47 mm and aperture 0.8 μm) were purchased from Yadong Resin Co. Ltd., Shanghai. Chitosan (average molecular weight 80,000 and the degree of deacetylation was over 90%) was supplied by the National Pharmaceutical Group Corp., China. $CuSO_4 \cdot 5H_2O$, $NiCl_2 \cdot 6H_2O$, $ZnSO_4$, $CoCl_2 \cdot 6H_2O$, formaldehyde, epichlorohydrin, ethylene diaminetetraacetic acid (Na_2EDTA), citric acid monohydrate, and hydrochloric acid (HCl) were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai. Iminodiacetic acid (IDA) was bought from J&K China Chemical Ltd., Shanghai and papain (EC3.4.22.2) was purchased from Sigma-Aldrich. All other chemicals were of analytical grade and used without further purification.

A peristaltic pump (DD-50B, Dandong, China) was used for applying papain solutions and the concentration of the enzyme was determined using a UV-2102PC spectrophotometer (UNICO Instrument Co. Ltd., Shanghai, China). The membrane cartridge (donated from the Dalian Chemical and Physical Institute, China) was used to load the membrane stack.

Preparation of Activated Membrane

The procedure for preparing activated membranes has been described in details in our previous study (22). In brief, covalent coupling of chitosan to activated nylon membrane was performed after reacting the microporous nylon membrane with formaldehyde, and then chemically modified by cross-linking with epichlorohydrin and imanodiacetic acid grafting.

Immobilization of Metal Ion

The membranes were immersed into 100 ppm Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} solution for 2 h at 25°C. In this article, Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} solution were used by $CuSO_4$, $NiCl_2$, $ZnSO_4$, and $CoCl_2$, respectively. After washing with deionized water, chitosan-coated nylon-based immobilized metal ion affinity membranes were prepared.

Characterization of Membranes

SEM Studies

Microscopic observations were performed by using a scanning electron microscope (Jeol, JSM-5600 LV, Japan).

Metal Ion Capacity

The metal ion capacities on membranes were measured with inductively coupled plasma atomic emission spectrometric (Prodigy, Leeman, USA).

Dynamic Adsorption Procedure

Adsorption of papain on the immobilized metal ion affinity support was performed in dynamic adsorption experiment. A membrane cartridge was used to load the membrane stack. The solution containing 1.0 mg/ml papain was pumped through the membrane cartridge with a peristaltic pump at a flow rate of 2.0 ml/min. During the adsorption test, 30 ml permeate samples were withdrawn and recycling adsorbed for 2 h.

According to reference (23), the adsorption of papain on the immobilized metal ion affinity system was studied at various pH values in either Tris-HCl (0.05 M, pH 5.0–10.0) buffer or carbonate buffer (0.05 M, pH 11.0). The initial papain concentration is 1.0 mg/ml in each corresponding solution and adsorptions were conducted through the membrane cartridge at 25°C for 2 h.

The effects of contact time were determined at various times and the initial papain concentration was set at 1.0 mg/ml in buffer. The adsorption experiments were conducted as before using the membrane cartridge.

The influence of ionic strength on papain adsorption were determined in Tris-HCl buffer (30 ml, 0.05 M, containing 1.0 mg/ml of papain) at four different NaCl concentrations (0.25, 0.5, 1.0, 2.0 M). To determine non-specific adsorption properties the initial concentration of enzyme changed between 0 and 2.0 mg/ml.

The amount of adsorbed papain on the affinity membrane was determined by measuring the initial and final concentrations of enzymes in adsorption medium using UV spectroscopy at 280 nm. The UV-2102PC spectrophotometer was set at 280 nm for the measurement of the protein's aromatic residues according to the reference (12). The amount of adsorbed papain was calculated using the following equation:

$$q = (c_i - c_t)Vs/m \qquad (1)$$

where q is the amount of papain adsorbed onto unit mass of the membrane (mg/g); c_i and c_t are the concentrations of the papain in the initial and in the final solution after adsorption, respectively (mg/ml); V_S the volume of the papain solution (ml); m is the mass of the membrane (g). All assays were carried out in triplicate and only mean values were presented.

Breakthrough Curve Measurement

The membrane cartridge containing 10 sheets of immobilized metal ion membranes was first equilibrated in 0.05 M Tris-HCl buffers (at optimal pH for each ion membrane). Papain, 100 ml (1.0 mg/ml, 0.05 M Tris-HCl, the same optimal pH) was loaded at 2 ml/min into the cartridge and the unbound protein was removed by thoroughly washing the system with buffer. The bound enzyme was eluted with 1.0 M NaSCN in Tris-HCl, pH 5.0) and fractions (4 ml) were collected and enzymic activity and protein concentration were analyzed.

Analytical Procedures

Protein Concentration

Protein concentration was measured with the Bradford Protein Assay using bovine serum albumin as standard (24).

Enzyme Activity

The papain solution (1.0 mg/ml) was obtained by first dissolving enzyme in 0.05 M Tris-HCl, pH 8 (0.7 ml), then the papain activator (0.2 ml) consisting of 0.5 M L-cysteine and 0.02 M EDTA (pH 8.0) and the substrate 1% casein pH 8.0, (0.1 ml) were added. Enzyme assays were carried out at 37°C for 10 min stirring throughout before adding 3 ml of 5% trichloroacetic acid (3 ml), the reaction mixture was statically equilibrated at 25°C for 1 h, and then filtered. The absorbance of the filtrate was measured at 280 nm (25). One unit (U) of enzymatic activity was defined as the amount of enzyme increasing 0.001 absorbance/min at 280 nm in this condition (26).

Desorption and Regeneration Studies

Papain desorption experiments were performed using a membrane cartridge with buffer solution containing 1.0 M NaSCN at pH 5.0 and protein concentration was determined by spectrophotometry. The desorption ratios of papain were calculated as follows:

$$\textit{Desorption ratio} = [\text{amount of papain desorbed}/\text{amount of papain adsorbed}] \times 100 \qquad (2)$$

To determine the reusability of the affinity membranes the adsorption and desorption cycle was repeated three times using the same membranes. It was noted that after papain desorption some retained protein and this was removed by washing the membranes in 0.1 M EDTA according to reference (23). Next the immobilized metal ion affinity membranes were placed in solutions (5 ml) containing various concentrations of metal ions (0.1 M), these included $CuSO_4$, $NiCl_2$, $ZnSO_4$, and $CoCl_2$ for 30 min. This step was to compensate for the leakage of immobilized metal ions that may have occurred previously. Membranes were finally rinsed in deionized water to remove the rest metal ion on the surface of membranes.

RESULTS AND DISCUSSION

Physical and Morphological Properties of Activated Membrane

SEM micrographs (Fig. 1) show the surface structure of nylon membrane containing immobilized copper, nickel, zinc, cobalt ion affinity membranes. It was noted that after chelation the pore size was reduced and was smaller than that of the nylon membrane because of coupling chitosan and immobilizing various metals ions as ligands. However, the affinity membrane still retained large pores and rough surfaces, pore sizes were 0.1–3.0 μm. The presence of large pores will reduce diffusional resistance and so facilitate mass transfer due to the high internal surface area.

The Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} ion capacities on the immobilized metal ion membranes were measured with inductively coupled plasma atomic emission spectrometric, respectively 4402.1, 658.1, 339.6, and 1258.3 μg/g.

Papain Adsorption

Effect of Contact Time

The effect of contact time on the adsorption of papain by immobilized metal ions affinity membrane at pH 8.5 is shown in Fig. 2. As can be seen from the graph, the effect of contact time on the adsorption process initially showed a rapid increase but this became slower after 60 min. The nature of metal ion and its available adsorption sites affected the time required to reach the equilibrium. For Cu^{2+} and Zn^{2+} affinity membranes, maximum adsorption occurred after 60 min however in the case of Ni^{2+} the maximum adsorption was reached after 50 min. For the other adsorbent, namely Co^{2+}, the maximum adsorption occurred at 30 min. Thus, 60 min was the fixed equilibrium time for all other studies.

Effect of pH

The optimal pH values for adsorption of papain on the metal ions immobilized nylon-based membranes were investigated in the pH range 5–11. The concentration of enzyme in the medium was 1.0 mg/ml for all samples.

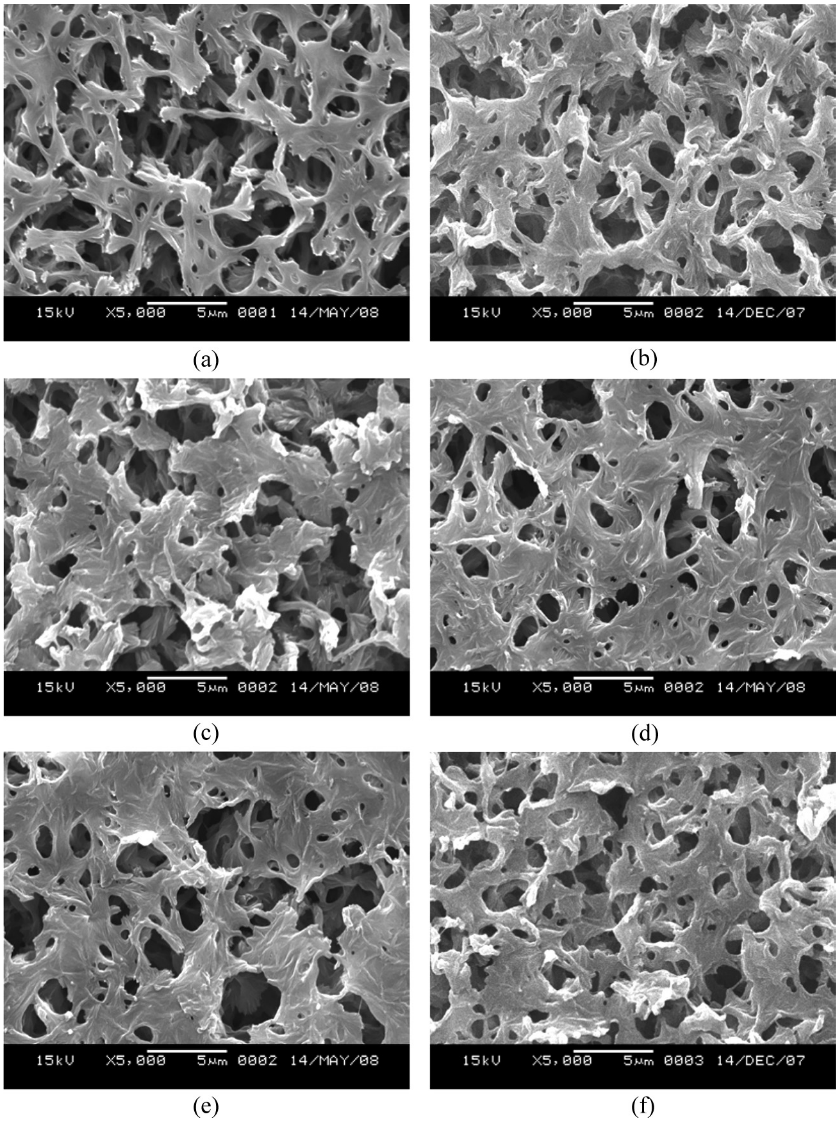

FIG. 1. The SEM micrograph of all types of membrane (5000 ×): (a) plain nylon membrane, (b) chitosan-coated membrane, (c) Cu^{2+}-IDA-nylon membranes, (d) Ni^{2+}-IDA-nylon membranes, (e) Zn^{2+}-IDA-nylon membranes, (f) Co^{2+}-IDA-nylon membranes.

Figure 3 shows the effect of pH on the amount of papain adsorbed on the different metal affinity membranes. Maximum adsorptions were obtained at pH 8.0 with Cu^{2+}-IDA-nylon membranes, at pH 10.0 with Ni^{2+}-IDA-nylon membranes, and at pH 9.0 with Zn^{2+}-IDA-nylon and Co^{2+}-IDA-nylon membranes. These results indicate that proton concentration in the media plays an important role in the adsorption equilibrium of papain. Normally proteins demonstrate maximum adsorption efficiency on or around their isoelectric points (papain $pI = 9.6$) where there is a net zero charge across the structure and therefore the electrostatic repulsion will be diminished. Since proteins are amphoteric, the number of charges on the surface of a protein would vary with the pH of the medium. Amino acid residues of protein such as histidine, cysteine, and tryptophan could coordinate to borderline metal ions such as Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}. As an outcome the divalent ions interact with weak Lewis bases such as nitrogen and sulphur as in the amino acids cysteine and histidine. Considering the limited number of cysteine residues on the surface of proteins, this leaves histidine residues as potentially the major target for borderline metal ions (27–29). At lower pH, partial deprotonation of the exposed histidine residue in the protein would be promoted by increasing pH in the range higher than its p*K*a (about 6–7 (30)), which would increase the possibility of specific binding with immobilized cationic metal ions (31). Under alkaline pH, coordination with amino and hydroxyl groups took place and resulted

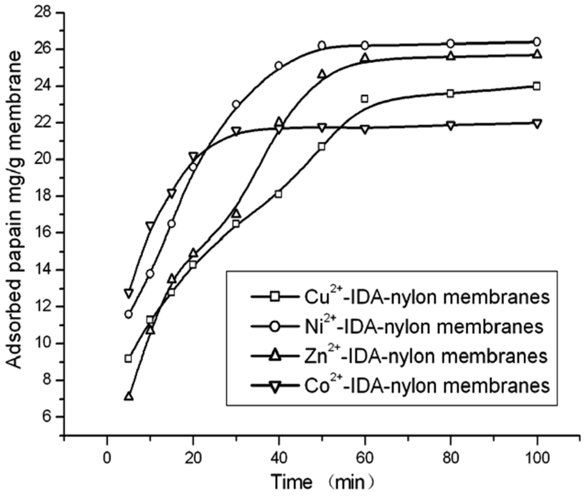

FIG. 2. Effect of constant time on papain adsorption on the metal ion affinity membranes. Initial concentration of papain 1.0 mg/ml, at pH 8.5 and at 25°C. The results were means of duplicate determination on triple independent measurements.

in less effective adsorption of papain. Meanwhile, it would cause metal ion precipitation. The electrostatic interaction may hardly be changed by a higher pH, due to papain becoming less cationic and the residual negatively charged groups on the membranes being more anionic. On the other hand, with a p*I* around 9.6, papain will possess a higher degree of neutrality with higher pH and its hydrophobic interaction with the membrane surface may be enhanced (32). Different adsorption capacity of papain on the four metal ion affinity membranes at different optical pH as shown here may be due to metal ion precipitation. The factors in metal ion precipitation related to the Ksp and adsorption capacity of metal ion on membrane. The Ksp value of Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} was 2.2×10^{-20}, 2×10^{-15}, 1.2×10^{-17}, 2×10^{-15} and was the order of $Cu^{2+} < Zn^{2+} < Co^{2+} \approx Ni^{2+}$. On the other hand, the order of their adsorption capacity on the membrane is $Zn^{2+} < Ni^{2+} < Co^{2+} < Cu^{2+}$. Based on these two factors, the leakage of different metal ions occurred at different pH. Therefore, the adsorption curve versus pH value behaves as the phenomena described above.

Effect of Ionic Strength

The effect of ionic strength on papain adsorption at optical pH is shown in Fig. 4. Protein adsorption was reduced with increasing salt concentration from 0 to 2.0 M. With increasing salt concentration, the affinity binding between the exposed histidine residues of the protein and the immobilized metal ions would hardly be affected. This is the same phenomena as for the specific binding between the charged protein molecules and the positively charged metal ions. The electrostatic interaction between protein molecules and metal-chelated affinity system could be reduced in the presence of salt due the surface charge of protein being screened. An increase in ionic strength also causes changes in the interaction mechanism due to contributions from hydrophobic and protein–protein interactions (33). As shown in Fig. 4, papain adsorption capacities were significantly decreased after the addition of 0.25 M NaCl, indicating that non-specific electrostatic interaction was effective.

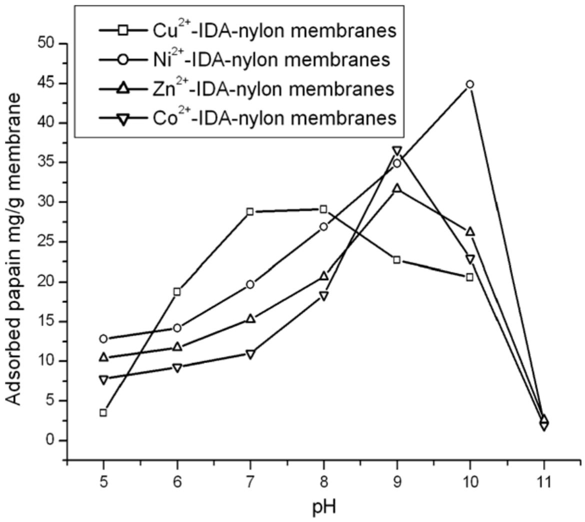

FIG. 3. Effect of pH on papain adsorption on the metal ion affinity membranes. Initial concentration of papain 1.0 mg/ml and at 25°C. The results were means of duplicate determination on triple independent measurements.

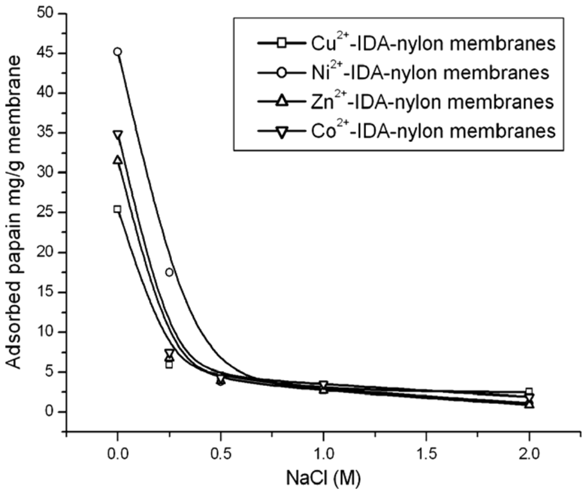

FIG. 4. Effect of ionic strength on papain adsorption on the metal ion affinity membranes. Initial concentration of papain 1.0 mg/ml, at optical pH (pH 8.0 for Cu^{2+}-IDA-nylon membranes; pH 10.0 for Ni^{2+}-IDA-nylon membranes; pH 9.0 for Zn^{2+}-IDA-nylon membranes and Co^{2+}-IDA-nylon membranes) and at 25°C. The results were means of duplicate determination on triple independent measurements.

Non-Specific Adsorption of Papain on Affinity Membranes

The papain adsorption on immobilized metal ion affinity membrane is given in Fig. 5. As noted as the concentration of the enzyme increases resulted in greates adsorption of papain on the Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}-IDA-nylon membranes (50.8, 67.2, 54.1, 42.6 mg/g respectively). It should be noted that a negligible amount of papain was adsorbed non-specifically on the modified nylon membrane by coating chitosan (21.6 mg/g), while the papain adsorption capacity on plain nylon membrane was 28.7 mg/g. Compared with the plain nylon membrane, the nonspecific adsorption capacity of papain on the CS-coated membrane significantly decreased, while the adsorption capacity on immobilized metal ion affinity membrane greatly increased. It is clear that this increase is due to specific interaction between the immobilized metal ion and papain molecules. Thus low non-specific binding to the affinity membrane will be useful factor when considering the purification of papain using the above system.

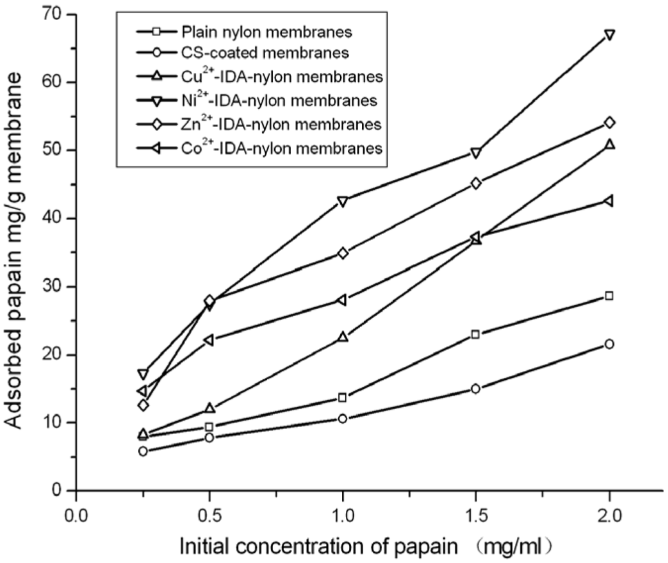

FIG. 5. Effect of initial concentration of papain on papain adsorption on the metal ion affinity membranes at optical pH (pH 8.0 for plain nylon membranes, CS-coated membranes and Cu^{2+}-IDA-nylon membranes; pH 10.0 for Ni^{2+}-IDA-nylon membranes; pH 9.0 for Zn^{2+}-IDA-nylon membranes and Co^{2+}-IDA-nylon membranes) and at 25°C. Initial concentration of papain 1.0 mg/ml. The results were means of duplicate determination on triple independent measurements.

Adsorption Isotherms

In this study, two classical adsorption models were employed to describe the papain adsorption equilibrium, namely the Langmuir and Freundlich models. The Langmuir isotherm is valid for monolayer adsorption onto a surface with a finite number of identical sites (34) and is based on the assumption of adsorption homogeneity, such as equally available adsorption sites, monolayer surface coverage, and no interaction between the adsorbed species. If the papain adsorption follows the Langmuir model, the adsorption process can be expressed as:

$$q_{eq} = \frac{q_m C_e}{K_d + C_e} \tag{3}$$

Eq. (3) can be transformed to a linear form as follows:

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

where C_e (ppm) is the equilibrium concentration of enzyme q_{eq} (mg/g) the adsorption capacity at equilibrium, q_m(mg/g) the maximum adsorption capacity. K_d is the effective dissociation constant.

The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of papain binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. The Freundlich isotherm model is usually adopted for heterogeneous adsorption (29). One limitation of this model is that the amount of the adsorbed solute increases indefinitely with the concentration of solute in solution. This isotherm equation is:

$$q_{eq} = K_F (C_e)^{1/n} \tag{5}$$

Eq. (5) can be transformed to a linear form as follows:

$$\log q_{eq} = \log K_F + (1/n) \log C_e \tag{6}$$

where q_{eq} (mg/g) is the adsorption capacity at equilibrium, C_e (ppm) the equilibrium concentration of papain in solution, and K_F and n are the physical constants of the Freundlich adsorption isotherm. The K_F and n are the indicators of the adsorption capacity and adsorption intensity, respectively. The slope and the intercept of the linear Freundlich equation are equal to $1/n$ and $\log K_F$, respectively.

The Langmuir and Freundlich parameters for the adsorption of papain onto the activated membrane were listed in Table 1. A best-fit straight line with high correlation coefficient was obtained in the case of Freundlich, while the linearity of the plot of the Langmuir model was poor. As shown (Table 1), the magnitude of K_F and n show easy adsorption of papain from aqueous medium. This suggests that the adsorption data was well represented by the Freundlich isotherm model and supports the assumption that adsorption took place on hetergeneous surfaces.

Adsorption Kinetics

In order to determine the potential rate-controlling steps involved in the process of adsorption, kinetic models

TABLE 1
The Langmuir and Freundlich constants and correlation coefficients of papain adsorption on different metal ion affinity membranes at 25°

	Langmuir constants			Freundlich constants		
Type of membranes	q_m (mg/g)	K_d (mg/ml)	R^2	K_F	n	R^2
Cu^{2+}-IDA-nylon	133.3	3.213	0.3869	30.68	1.28	0.9647
Ni^{2+}-IDA-nylon	80.0	0.416	0.9383	55.13	2.18	0.9936
Zn^{2+}-IDA-nylon	93.5	1.27	0.8713	40.10	1.52	0.988
Co^{2+}-IDA-nylon	48.3	0.377	0.9476	33.55	2.76	0.9818

should be established. According to reference (35), two classic kinetic models reference were used to fit the experimental data at different initial concentrations, temperatures at 25°C, optical pH.

Pseudo-First-Order Lagergren Model

The pseudo-first-order rate expression of Lagergren model is generally expressed as follows Eq. (7):

$$\frac{dq}{dt} = k_1(q_{eq} - q) \tag{7}$$

where q_{eq} and q are the amounts of adsorbed Cu^{2+} onto the activated nylon-based membranes at equilibrium and at time t, respectively. The k_1 is the rate constant of first-order adsorption. The integrated form of Eq. (8) is:

$$\log(q_{eq} - q) = \log q_{eq} - k_1 \frac{t}{2.303} \tag{8}$$

The plots of $\log(q_{eq} - q)$ against t for the pseudo-first-order equation gave a linear relationship and k_1 and q_{eq} values can be determined from the slope and intercept of this equation, respectively. However, to fit Eq. (8) to experimental data, the value must be pre-estimated by extrapolating the experimental data to $t = \infty$. Kinetic parameters of the kinetic models are shown in Table 2 along with the corresponding correlation coefficients.

The theoretical value q_{eq} estimated from the *Pseudo-first-order Lagergren* model gave significantly different value compared to the experimental value, and the correlation coefficient was also found to be lower. These results suggest that the Pseudo-first-order Lagergren model is improper for this affinity membrane system.

Pseudo-Second-Order Kinetic Model

If the adsorption rate is pseudo-second-order, the pseudo-second-order kinetic rate equation is expressed as:

$$\frac{dq}{dt} = k_2(q_{eq} - q)^2 \tag{9}$$

where k_2 is the rate constant of second-order adsorption. After integrating, the following equation is obtained:

$$\frac{t}{q} = \frac{1}{k_2 q_{eq}^2} + \frac{t}{q_{eq}} \tag{10}$$

It should be noticed that for the utilization of this model, the experimental value of q_{eq} is not necessary to be pre-estimated. The second-order rate constants k_2 and q_{eq} values presented in Table 2 were determined from the slopes and intercepts. The results in Table 2 show that the correlation coefficients for the *Pseudo-second-order kinetic* model is close to 1.0 for all cases, and the calculated q_{eq} values are closer to the experimental data than the

TABLE 2
Constants and correlation coefficients of the first-order and second-order kinetics for papain adsorption on different metal ion affinity membranes at 25°

		First-order kinetics			Second-order kinetics		
Type of membranes	Experimental q_{eqex} (mg/g)	$k_1 \times 10^2$ (min^{-1})	q_{eq} (mg/g)	R^2	$k_2 \times 10^3$ ($g \cdot mg^{-1} min^{-1}$)	q_{eq} (mg/g)	R^2
Cu^{2+}-IDA-nylon	27.82	1.428	23.49	0.9846	1.44	30.40	0.9988
Ni^{2+}-IDA-nylon	32.53	2.234	24.88	0.9793	2.04	33.90	0.9985
Zn^{2+}-IDA-nylon	33.21	1.612	28.42	0.9831	1.06	36.63	0.9979
Co^{2+}-IDA-nylon	24.21	3.570	14.54	0.9867	7.44	25.45	0.9991

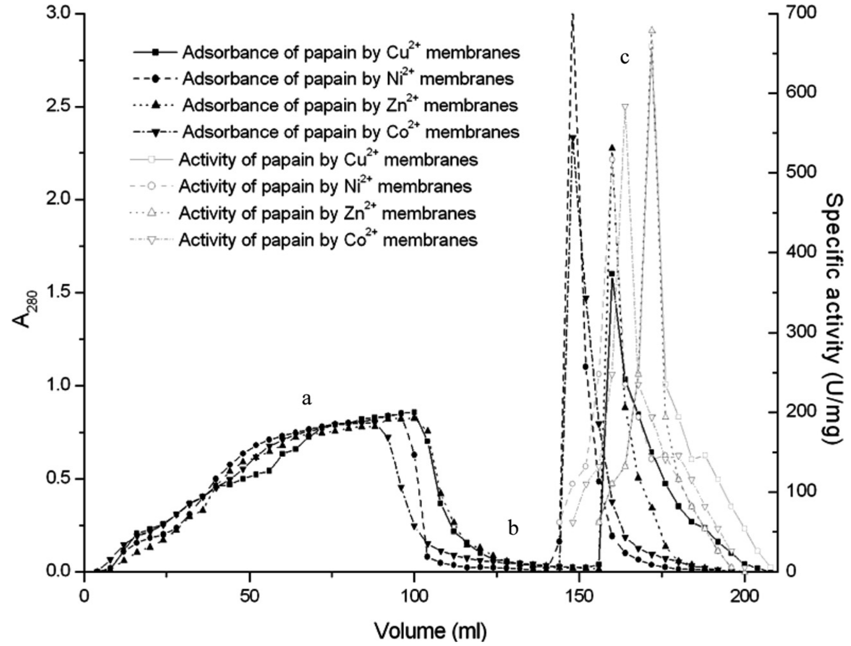

FIG. 6. Separation of papain by metal ion chelating membrane affinity chromatography: (a) adsorption of papain (the concentration of papain: 1.0 mg/ml) at optical pH Tris-HC1 (pH 8.0 for Cu^{2+}-IDA-nylon membranes; pH 10.0 for Ni^{2+}-IDA-nylon membranes; pH 9.0 for Zn^{2+}-IDA-nylon membranes and Co^{2+}-IDA-nylon membranes), volume:100 ml, flow rate:2.0 ml/min; (b) washing : same optical pH Tris-HC1, Volume : 40 ml, flow rate :2.0 ml/min; (c) elution of papain (1.0 mol/L NaSCN pH 5.0 Tris-HC1, Volume: 60 ml, flow rate: 1.0 ml/min.

calculated values of pseudo-first-order *Lagergren* model. On the other hand, the correlation coefficients for the pseudo-first-order *Lagergren* model were lower than those for the pseudo-second-order *kinetic* one. Therefore, the adsorption of papain onto the immobilized metal ion affinity membranes is more favorably suited by the pseudo-second-order kinetic model.

Breakthrough Curve Measurement

The crude papain solution used for papain purification using a membrane disc cartridge system above showed good separation of the protein as noted from enzymic activity and protein concentration (Fig. 6). After applying 1.0 M NaSCN a peak containing papain activity was evident and the specific activity and purification parameters are given in Table 3. The Zn^{2+}-IDA-nylon membrane providing the greatest level of purification amongst all the membranes assayed. However, the difference in purification efficiency by four of the tested membranes is not significant, probably because the selected ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}) are all divalent borderline metal ion and their properties are similar. The desorption for all the tested membranes of papain was high (about 98.6%, 94.3%, 91.5%, 93.5% for Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}-IDA-nylon membranes respectively). Up to 90% of the adsorbed papain was effectively desorbed by using for all the tested membranes. Therefore, these membranes can be used repeatedly.

TABLE 3
The purification (fold)parameter of different metal ion affinity membranes

Type of membrane	Volume (ml)	Fraction number	Protein content (mg)	Specific activity (U)	Specific activity (U/mg)	Purification (-fold)[a]
Papaya powder	100	–	112.5	2824	25.1	–
Cu^{2+}-IDA-nylon	4	43	2.571	1693	658.5	26.23
Ni^{2+}-IDA-nylon	4	40	0.768	397	516.9	20.59
Zn^{2+}-IDA-nylon	4	43	1.372	932	679.3	27.06
Co^{2+}-IDA-nylon	4	41	0.605	353	583.5	23.25

[a] The papain purify was defined as the activity per mg protein, and the purification was calculated by dividing the purify of the final product by the purity of papaya powder.

In this membrane process, the high recovery and purification of papain activity was obtained in a single step as compared to other purification method (19). Besides this, the immobilized metal ion membrane method offers some advantages over other separating systems such as the large surface area, short diffusion path, low pressure drop, and short residence time. These factors are often contributed to enhancing systems that are to be developed further for large scale use. Therefore, based on the above evidence, we believe that papain purification with immobilized metal affinity membranes has the potential for future commercial applications.

Regeneration of Immobilized Metal Ion Affinity Membranes

In order to show reuse and stability of the membranes, the adsorption elution cycle was repeated three times using the same membrane stack. There was no significant reduction in the adsorption capacity of papain, the papain adsorption capacity decreased only by a small amount (6.2, 9.8, 9.5, 7.6% for Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}-IDA-nylon membranes) thus indicating that the membranes developed in this study can be reused and easily recycled. Such phenomenon was also reported in the literature (23).

CONCLUSION

The present study describes the preparation and application of chitosan-coated nylon membrane for metal ion (Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}) chelated affinity chromatography for protein isolation and purification. The affinity membranes were prepared by activation of nylon membranes with formaldehyde. The chelating agent was then covalently attached to the membrane as affinity and metal chelating ligand. The borderline metal ions (Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}) were immobilized on membranes. Experiment revealed between 43–67 mg/g papain could be adsorption and that factors such as the pH, the incubation time, and the ionic strength contributed to the process. The papain adsorption process showed a Freundlich isotherm at the given range of papain concentration. Regeneration of the membrane suggested good mechanical and chemical stability. Moreover, this system was rapid, efficient, inexpensive, and convenient to operate. These features make the affinity membranes good candidates for use in metal-chelated affinity separation of papain and this would be effective in industrially processing large amounts of the enzymes.

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