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Journal of Chromatography B, 794 (2003) 67–76

JOURNAL OF CHROMATOGRAPHY B

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Purification of penicillin G acylase using immobilized metal affinity membranes

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Received 11 March 2003; received in revised form 12 May 2003; accepted 12 May 2003

Abstract

The immobilized metal affinity membrane (IMAM) with modified regeneration cellulose was employed for purification of penicillin G acylase (PGA). For studying PGA adsorption capacity on the IMAM, factors such as chelator surface density, chelating metal, loading temperature, pH, NaCl concentration and elution solutions were investigated. The optimal loading conditions were found at 4 °C, 0.5 *M* NaCl, 32.04 μmol Cu^{2+} per disk with 10 m*M* sodium phosphate buffer, pH 8.5, whereas elution conditions were: 1 *M* NH_4Cl with 10 m*M* sodium phosphate buffer, pH 6.8. By applying these chromatographic conditions to the flow experiments in a cartridge, a 9.11-fold purification in specific activity with 90.25% recovery for PGA purification was obtained. Meanwhile, more than eight-times reusability of the membrane was achieved with the EDTA regeneration solutions.
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Keywords: Immobilized metal affinity membranes; Penicillin G acylase; Enzymes

1. Introduction

Penicillin G acylase (PGA) is an important biocatalyst which could hydrolyze penicillin G to 6-aminopenicillanic acid (6-APA) for further production of semi-synthetic penicillins. To develop an efficient and economic production process, purifying large quantities of PGA at a low cost is required [1–3]. In the literature, different PGA purification methods were suggested and the efficiencies were studied and evaluated [4–9]. Among them, immobilized metal affinity chromatography (IMAC) had been demonstrated to be a promising technique for PGA purification as compared to other methods such as pseudo-affinity chromatography, aqueous two-phase systems, and hydrophobic interaction chromatography [10–14].

IMAC is a chromatographic method applying the chelators (usually multidentates) coupled on the solid supports to immobilize metal ions (such as Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, or Al^{3+}) [15,16], which could specifically interact though nonbonding lone pair electron coordination with exposed electron-donating amino acid residues (such as histidine, cysteine, tryptophan, aspartic acid, or glutamic acid) on the protein surface. Since there are quite a lot specific amino acid residues located on PGA including 13 histidines, 28 tryptophans, 43 aspartic acids, and 36 glutamic acids [17], some of them are very possibly exposed on the protein surface. Therefore,

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doi:10.1016/S1570-0232(03)00417-3

IMAC should be an appropriate approach for PGA purification. Recently, the immobilized metal affinity membranes (IMAMs) process was developed and had gained much attention owing to the lower mass-transfer limitations for the membrane process [18–27]. In general, the membrane process could offer some advantages such as no intraparticle diffusion, short axial-diffusion path, low pressure drop, no bed compaction, easier scale up, which are usually limited in the conventional packed-column chromatographic systems.

In this paper, the modified regenerated cellulose-based IMAM was applied to PGA purification and the optimal operation conditions were evaluated. Moreover, the flow adsorption process for PGA purification using a membrane cartridge was also investigated.

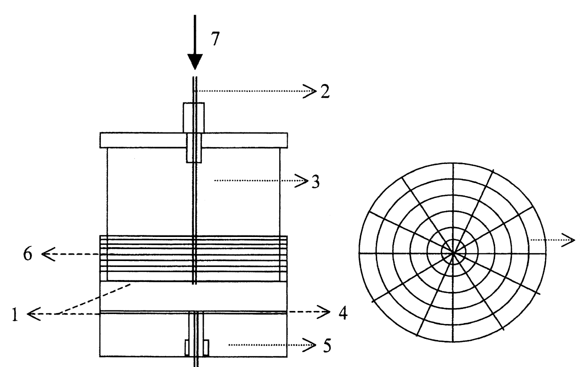

Fig. 1. Schematic representation of the chromatographic cartridge used in this study.

2. Experimental

2.1. Materials and equipments

The regenerated cellulose membranes, with a diameter of 47 mm, an average pore size of 0.45 μm, and a thickness of 160 μm, were purchased from Sartorius (Germany). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) and epichlorohydrin were purchased from Tedia (Fairfield, OH, USA). Iminodiacetic acid (IDA) and imidazole were purchased from Acros Organics (Geel, Belgium). Other reagents were of analytical degree.

A membrane chromatographic system was comprised of an acrylic cartridge (laboratory made, of 45 mm×47 mm I.D.×60 mm O.D., as shown in Fig. 1), a peristaltic pump (MP-3N, Eyela, Japan), a fraction collector (SF-2100W, Advantec, USA) and a UV–Vis spectrophotometer (V-530, Jasco, Japan).

2.2. Preparation of regenerated cellulose-based IMAMs

The commercial regenerated cellulose membranes were modified by a series of chemical reactions to bind IDA as chelator. All the reactions were carried out in a 200-ml glass bottle. A piece of regenerated cellulose membrane was immersed in 20 ml of 1 *M* NaOH and 5 ml epichlorohydrin, and shaken in a reciprocal shaker at 60 °C for 2 h. The membrane was rinsed with DI (deionized) water, and then immersed in 25 ml of 0.2 *M* IDA solution (pH adjusted to 11.0 with 1 *M* carbonate buffer), and shaken at 80 °C for 12 h. Reaction conditions such as shaking rate and reaction time were changed to study their effects on the coupled chelator density. Each modified membrane was immersed in 10 ml of 0.1 *M* cupric sulfate solution for 6 h, then washed with DI water to remove the unbound copper ions [23,28]. To quantify the immobilized copper density, the membrane was washed with 10 ml of 0.1 *M* EDTA. The eluent was collected and the concentration of Cu^{2+} was determined by a spectrophotometer (Turner Model 340) at 800 nm [29].

2.3. Preparation of crude PGA extract

Fermentation broth from *Escherichia coli* cultivation [30] was obtained. The cells were harvested by centrifuging 100 ml of crude broth at 14 000 *g* for 20 min (centrifuge: Z323K, Hermle, Germany). The pellet obtained was washed twice with DI water, then resuspended in lysis buffer (0.1 *M* KH_2PO_4, 1 m*M* EDTA, pH 7.5), and sonicated for 12 min.

The solution was centrifuged at 18 000 *g* for 20 min and the supernatant was collected [13,14].

2.4. Batch adsorption experiments

For batch adsorption experiments, one piece of IMAM was put into a glass bottle and 10 ml of crude enzyme extract in 10 m*M* phosphate buffer was loaded. The incubation was carried out at a certain temperature for 6 h. Then, elution was conducted by using the elution buffer. Different adsorption and elution conditions were tested in this work.

2.5. Flow adsorption experiments

For flow experiments, 10 pieces of IMAM (under the optimal preparation conditions, metal ion capacity: 31.45±0.97 μmol Cu^{2+}/disk) were stacked in the cartridge. A 100-ml volume of crude PGA extract was loaded at 1.2 ml/min to the cartridge already equilibrated with loading buffer (0.5 *M* NaCl, 10 m*M* phosphate buffer, pH 8.5). Unbound protein was washed out with 72 ml of washing buffer (0.02 *M* NH_4Cl, 0.5 *M* NaCl, 10 m*M* phosphate buffer, pH 6.8). Then, bound enzyme was eluted with 63 ml of elution buffer (1 *M* NH_4Cl, 0.5 *M* NaCl, 10 m*M* phosphate buffer, pH 6.8). Every 3 ml of fraction was collected by the fraction collector, and the enzyme activity and protein concentration were analyzed.

2.6. Analytical procedures

2.6.1. Protein concentration

Protein concentration was measured with the Bio-Rad protein assay using bovine serum albumin as standard [31].

2.6.2. Enzyme activity

Penicillin G acylase activity was determined using the colorimetric method proposed by Balasingham et al. [32], and 1 unit (IU) of enzyme activity was defined as the amount of enzyme required to produce 1 μmol 6-APA/min at 37 °C, pH 8.0.

3. Results and discussion

3.1. Regenerated cellulose-based IMAM

An ideal membrane carrier for protein and enzyme separations must have most of the following characteristics: high hydrophilicity and low nonspecific binding, fairly large pore size and a narrow pore size distribution, chemical and mechanical resistances, as well as enough reactive functional groups. Regenerated cellulose membranes are considered to meet most of the required characteristics and should be a good choice for IMAM preparation. In a previous study [23], the basic adsorption properties for regenerated cellulose-based IMAMs have been extensively investigated and the possible binding mechanisms discussed. However, practical applications for the regenerated cellulose-based IMAMs have not been explored yet. Therefore, an IMAM-preparation method similar to that adopted in the previous work [23] was employed in this work and the resulting regenerated cellulose-based IMAMs were applied to the practical PGA purification process to evaluate their separation efficiency. Moreover, it may be worthy to note that the pore size of 0.45 μm used in this study is large enough to allow the convective transport of proteins and enzymes and should be able to facilitate the mass transfer efficiency.

In the design of IMAC, a key point is to select an appropriate chelator for the metal ion immobilization. As reported by Wu et al. [23] for the regenerated cellulose-based IMAMs, different chelators such as IDA, *N*,*N*,*N*-tri(carboxymethyl)ethylene diamine (TED), Cibacron blue 3GA and Cibacron red 3BA were used and compared. The highest copper ion capacity and protein adsorption capacity occurred when IDA was adopted as the chelator. Consequently, IDA was selected in the following studies. According to the literature [33,34], the coupled IDA density was greatly influenced by various reaction conditions such as pH, temperature, and coupling time. To find the optimal conditions for coupling IDA on the regenerated cellulose membranes, different reaction conditions were tested. The immobilized copper ion capacity was measured to stand for the coupled IDA density, as usually done in the literature [33,34].

First, the effect of shaking rate was investigated

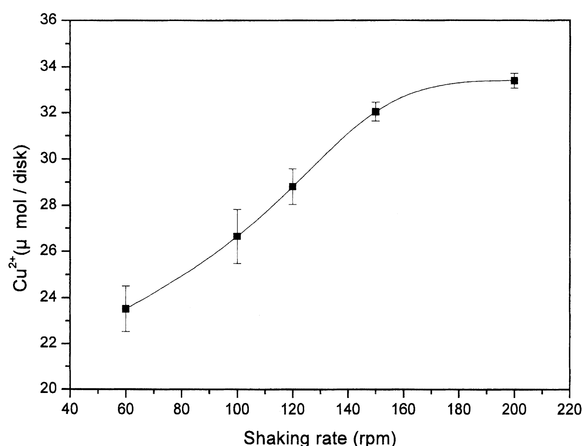

Fig. 2. Effect of shaking rate used in the procedure of IDA coupling on the preparation of regenerated cellulose-based IMAMs. Each point represents the average of three trials.

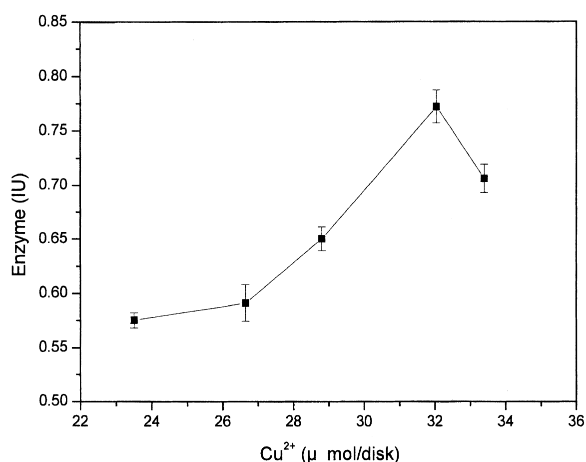

Fig. 3. Effect of copper ion capacity on PGA adsorption capacity. Loading protein: 10 ml, 0.2133 mg/ml; activity: 0.1211 IU/ml; loading buffer: 0.5 *M* NaCl, 10 m*M* phosphate, pH 7.5. Each point represents the average of two trials.

and the results are shown in Fig. 2. The results showed that the immobilized copper ion capacity increased while raising the shaking rate from 60 to 200 rpm. This implied that, with the increasing shaking rate, the diffusional resistance for IDA or metal ions to pass the porous membrane and touch the internal surface may be reduced and hence the coupled IDA density, as well as the immobilized copper ion capacity, was increased. The optimal shaking rate obtained was 150 rpm since the result of 200 rpm was only slightly higher.

When the shaking rate was fixed at 150 rpm and other reaction conditions such as epichlorohydrin concentration, IDA concentration, and coupling time were increased, the resulting copper ion capacities on the membranes remained the same (data not shown). It could therefore be concluded that the shaking rate dominated the whole IDA coupling reaction and a 150 rpm shaking rate could achieve an optimal metal ion capacity.

3.2. Effect of copper ion capacity on PGA adsorption capacity

Fig. 3 presents the results of PGA adsorption capacity for the IMAM with different copper ion capacities. The enzyme adsorption capacity reached a maximum of 0.772 IU at 32.0 μmol Cu^{2+}/disk, then decreased to 0.706 IU at 33.4 μmol Cu^{2+}/disk. This decreasing capacity was probably caused by the steric hindrance on a membrane full of crowded immobilized ligands. The optimal copper ion capacity, 32.0 μmol Cu^{2+}/disk, for achieving the maximum enzyme adsorption capacity was the result obtained using the 150 rpm shaking rate. It is proved again that 150 rpm was the optimal shaking rate for coupling chelator procedure and it was adopted in the following experiments.

3.3. Effect of different metal ions

Selection of a suitable immobilized metal ion for regenerated cellulose-based IMAMs was performed in the membranes coupled with IDA under the shaking rate of 150 rpm. Seven metal ions, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, and Al^{3+}, were tested. The metal ion concentration was 0.1 *M* and the incubation time was 6 h. After the successful preparation of IMAMs with different metal ions, the batch PGA adsorption experiments were conducted. The loading buffer was 0.5 *M* NaCl, 10 m*M* sodium phosphate, pH 8.0, and the elution buffer was 10 m*M* imidazole, 0.5 *M* NaCl, 10 m*M* sodium phosphate, pH 6.8. The results are shown in Table 1.

In Table 1, Fe^{3+} gave the best purification factor

Table 1
PGA purification results using IMAMs with different metal ions

Metal	Enzyme (IU)	Protein (μg)	Specific activity (IU/mg)	Purification factor (-fold)
Co^{2+}	0.32±0.02	179.5±15	1.783	3.93
Fe^{3+}	0.41±0.05	174.3±21	2.335	5.14
Ca^{2+}	0.28±0.11	252.8±18	1.106	2.44
Zn^{2+}	0.24±0.02	225.5±10	1.046	2.30
Al^{3+}	0.30±0.10	167.3±12	1.793	3.95
Ni^{2+}	0.54±0.02	549.0±5	0.964	2.12
Cu^{2+}	1.30±0.05	672.3±17	1.934	4.26

Loading protein: 10 ml, 0.301 mg/ml, activity: 0.1364 IU/ml, specific activity: 0.454 IU/mg. Loading buffer: 0.5 *M* NaCl, 10 m*M* phosphate, pH 8.0, stirred for 6 h at 4 °C. Elution buffer: 10 m*M* imidazole, 0.5 *M* NaCl, 10 m*M* phosphate, pH 6.8, stirred for 6 h at 4 °C. Each value was obtained with three trials.

of 5.14, whereas Cu^{2+} yielded the best adsorption capacity of 1.30 IU. The adsorption capacity of Cu^{2+} was triple that of Fe^{3+}. From these results, it is likely that more Cu^{2+} was immobilized on the IMAM than other metals and resulted in higher protein and enzyme adsorption. On the other hand, Fe^{3+} had the best specific affinity to the enzyme. According to the literature [35–38], the immobilized metal affinities to the retained proteins are in the following order: $Cu^{2+} > Ni^{2+} > Zn^{2+} \geqq Co^{2+}$, when applying IDA as the chelator. The protein adsorption results in Table 1 exactly match this order. In contrast to these commonly used metal ions, which have preference for extra-nitrogen-containing amino acids, hard Lewis metal ions such as Ca^{2+}, Fe^{3+}, Al^{3+}, and Yb^{3+} prefer oxygen-rich groups such as aspartic acid, glutamic acid or phosphate groups [16,37]. According to the gene map, PGA contains 13 histidines, 28 tryptophans, 43 aspartic acids, and 36 glutamic acids. This may provide an explanation for why it had a better affinity to Fe^{3+} than Cu^{2+}. Nevertheless, from economic viewpoint, Cu^{2+} was selected as the metal ion for the following IMAM experiments.

3.4. Selection of batch adsorption conditions

The loading buffer for the batch adsorption experiments was 10 m*M* phosphate buffer. Different adsorption conditions were conducted and the results will be presented in the following sections. The elution buffer used was 1 *M* NH_4Cl, 0.5 *M* NaCl, 10 m*M* sodium phosphate, pH 6.8.

3.4.1. Effect of temperature

The pH of the phosphate buffer was kept at 8.0 with 0.5 *M* NaCl in the adsorption process. The temperatures tested were 4, 8, 18, 28, 38, 48 °C, respectively. As shown in Fig. 4, the equilibrium adsorption of PGA onto the IMAM significantly decreased with increasing temperature. There are two possible explanations. First, it was reported previously [39,40] that conformational change of protein was observed when the temperature increased from 4 °C to greater than 37 °C. This effect results in variation in the accessibility of the histidinyl residues of the

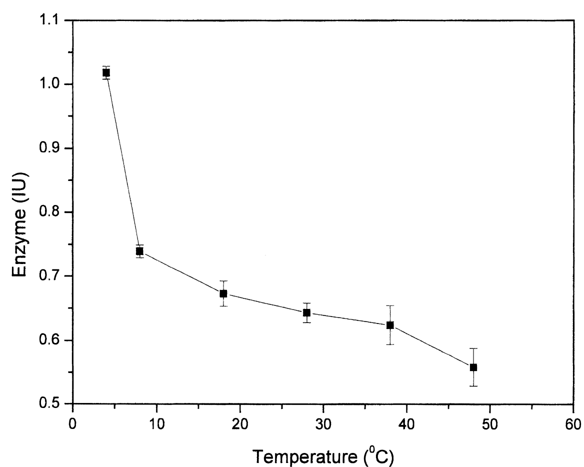

Fig. 4. Effect of temperature on PGA adsorption capacity. Loading protein: 10 ml, 0.20154 mg/ml; activity: 0.01602 IU/ml; loading buffer: 0.5 *M* NaCl, 10 m*M* phosphate, pH 8.0. Each point represents the average of two trials.

PGA to the ligand. The second possibility is that the decrease in affinity at higher temperature could also be due to reduction in the degree of ionization of amino acid residues associated with binding site, thus causing the lone pair electron donor properties of the solvent-exposed histidinyl residues to change [40]. Since low temperature is preferred for the PGA adsorption onto the regenerated cellulose-based IMAM, 4 °C was employed for the following experiments.

3.4.2. Effect of pH

Fig. 5 displays the results of different adsorption pH conditions (6.0, 7.0, 8.0, 8.5, and 9.0, respectively). At pH 8, the adsorbed PGA activity level reached its maximum value of 0.616 IU. The important amino acid residues of protein such as histidine, cysteine, and tryptophan can coordinate to Cu^{2+} at higher pH. When pH is decreased, the protonation effect on these amino acid residues will be increased and hence their coordination ability to immobilized metal ions will be reduced [15,16]. This could explain the phenomenon from pH 6.0 to 8.0. As to the tendency of worse adsorption at pH value greater than 8.0, it is possible that the impurity protein can compete with PGA to bind with copper ion, making PGA adsorption decrease. Summing up these results, pH 8.0 was the optimal pH for PGA adsorption without the addition of salts.

3.4.3. Effect of salt concentration

The effect of different NaCl concentrations (0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1.0 *M*) on PGA adsorption at pH 8.0 and 4 °C was investigated. In Fig. 6, the maximum PGA adsorption capacity (1.15 IU) was achieved for the addition of 0.5 *M* NaCl. At concentrations greater or less than 0.5 *M* NaCl, the PGA adsorption capacity decreased. During adsorption, an addition of salt at suitable concentration (such as 0.5 *M*) may promote PGA adsorption by the shielding effect to suppress ion–ion interactions and leave more opportunity for PGA to adsorb onto the IMAM [28,41,42]. This meant that proper amount of NaCl could enhance the strength of hydrophobic interaction between the enzyme and the IMAM, or reduce the competition from the impurity protein. The enzyme adsorption capacity may accordingly be improved. However, when the salt concentration was further increased, it may start to impede the binding

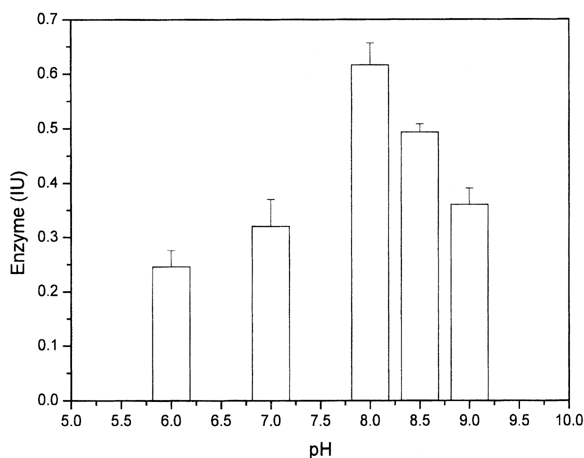

Fig. 5. Effect of pH on PGA adsorption capacity. Loading protein: 10 ml, 0.3362 mg/ml; activity: 0.1873 IU/ml; loading buffer: 10 m*M* phosphate. Each point represents the average of two trials.

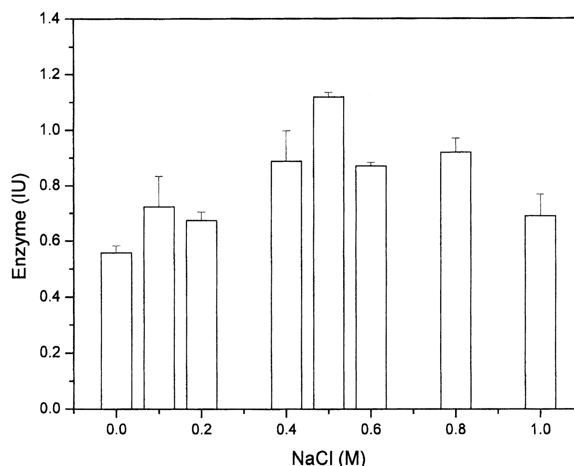

Fig. 6. Effect of salt concentration on PGA adsorption capacity at 4 °C. Loading protein: 10 ml, 0.2133 mg/ml; activity: 0.1211 IU/ml; loading buffer: 10 m*M* phosphate, pH 8. Each point represents the average of two trials.

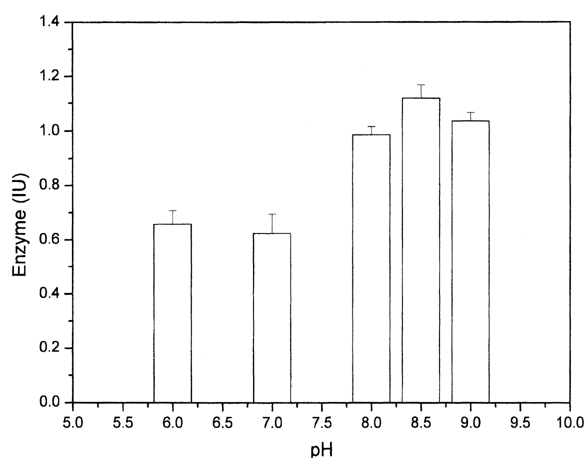

Fig. 7. Effect of pH on PGA adsorption capacity with the addition of 0.5 *M* NaCl at 4 °C. Loading protein: 10 ml, 0.3362 mg/ml; activity: 0.1873 IU/ml; loading buffer: 10 m*M* phosphate. Each point represents the average of two trials.

of PGA with the immobilized cationic copper ions or other nonspecific charged groups on the IMAM.

3.4.4. Effect of pH with the addition of 0.5 M NaCl

To investigate whether the addition of 0.5 *M* NaCl will affect the selection of the optimal adsorption pH or not, the experiment of pH effect was conducted again. The results are presented in Fig. 7. The PGA adsorption capacities in Fig. 7 were greater than those without NaCl addition in Fig. 5. It is verified again that the addition of 0.5 *M* NaCl could help raising the PGA adsorption. Moreover, the enzyme adsorption capacities were very close for pH ranging from 8 to 9, different from the rapidly decreasing phenomenon as shown in Fig. 5. The maximum PGA activity of 1.12 IU occurred at pH 8.5. Therefore, pH 8.5 was selected as the optimal pH for PGA adsorption with the addition of 0.5 *M* NaCl.

3.5. Selection of washing and elution conditions

Two eluents, NH_4Cl and imidazole solutions, with various concentrations were employed in PGA elution process and the results are listed in Tables 2 and 3, respectively. With regard to imidazole as eluent, it is found that 5 m*M* imidazole achieved the highest purification (4.08-fold) and 15 m*M* imidazole resulted in the best recovery (99.0%), while using NH_4Cl as eluent, 1.0 *M* NH_4Cl gave the best purification (4.63-fold) and recovery (97.9%). Since both high recovery and purification could be performed under the use of 1.0 *M* NH_4Cl, it was chosen as the eluent. In addition, it is worth noting that, by adding 0.02 *M* NH_4Cl, no PGA was desorbed whereas some of impurity protein was eluted out. This result is useful for designing the washing process, where desorption of the desired product should be minimized. Consequently, 0.02 *M* NH_4Cl was adopted in the washing process and 1.0 *M* NH_4Cl in the elution process for the following flow purification experiments.

Table 2
Effect of imidazole concentration in elution buffer on PGA purification

Imidazole concentration (m*M*)	Enzyme (IU)	Protein (μg)	Specific activity (IU/mg)	Purification (-fold)	Recovery (%)
1	0.1±0.012	143.2±7			9.1
5	0.920±0.03	504.8±21	1.823	4.08	83.3
7.5	0.952±0.009	621.6±15	1.531	3.21	86.2
10	1.092±0.015	639.8±30	1.707	3.58	98.8
15	1.116±0.17	702.0±17	1.590	3.33	99
20	1.084±0.007	782.2±21	1.386	2.91	98.1

Loading protein: 10 ml, 0.302 mg/ml, activity: 0.1351 IU/ml, specific activity: 0.477 IU/mg. Loading buffer: 0.5 *M* NaCl, 10 m*M* phosphate, pH 8.5, stirred for 6 h at 4 °C. Elution buffer: 10 m*M* phosphate buffer, pH 6.5, stirred for 6 h at 4 °C.
Each value was obtained with two trials.

Table 3
Effect of NH_4Cl concentration in elution buffer on PGA purification

NH_4Cl concentration (*M*)	Enzyme (IU)	Protein (μg)	Specific activity (IU/mg)	Purification (-fold)	Recovery (%)
0.01[a]	0	62.0±5	0	0	0
0.02	0	84.0±5	0	0	0
0.04	0.180±0.004	101.2±7	1.779	2.73	16.8
0.06	0.350±0.010	158.8±5	2.204	3.38	32.8
0.08	0.624±0.032	211.6±10	2.949	4.53	58.4
0.1[b]	0.634±0.010	299.7±15	2.115	4.41	47.5
0.3	0.722±0.010	412.2±20	1.752	3.65	66.2
0.5	0.920±0.031	440.8±17	2.087	4.50	84.3
0.7	0.980±0.021	443.6±25	2.201	4.37	89.8
1.0	1.068±0.011	480.7±31	2.222	4.63	97.9
1.5	1.054±0.010	592.4±13	1.779	3.7	97.8

Loading buffer: 0.5 *M* NaCl, 10 m*M* phosphate, pH 8.5, stirred for 6 h at 4 °C. Elution buffer: 10 m*M* phosphate buffer, pH 6.5, stirred for 6 h at 4 °C.
Each value was obtained with two trials.
[a] Loading protein: 10 ml, 0.204 mg/ml; activity: 0.1329 IU/ml; specific activity: 0.651 IU/mg.
[b] Loading protein: 10 ml, 0.278 mg/ml; activity: 0.1335 IU/ml; specific activity: 0.480 IU/mg.

3.6. Flow adsorption experiments

Flow experiments for PGA purification using a membrane disc cartridge were performed and the results are displayed in Fig. 8. When applying 0.02 *M* NH_4Cl in the washing buffer, no PGA but some impurity protein was washed out. Whereas using 1.0 *M* NH_4Cl in the elution process, a peak of PGA activity appeared, which comprising 90.25% of the total applied PGA activity with an overall specific activity of 4.854 IU/mg protein. This meant a 9.11-fold purification is attained in this chromatographic process.

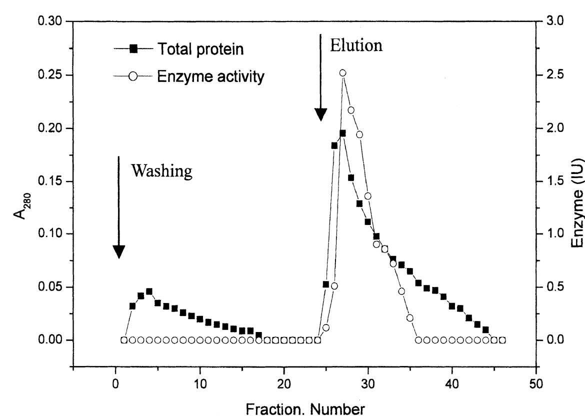

Fig. 8. Flow adsorption results of PAC using a membrane disc cartridge with 10 pieces of IMAM. Loading protein: 100 ml, 0.245 mg/ml; activity: 0.1305 IU/ml; specific activity: 0.533 IU/mg. Loading buffer: 0.5 *M* NaCl, 10 m*M* phosphate, pH 8.5; washing buffer: 0.02 *M* NH_4Cl, 0.5 *M* NaCl, 10 m*M* phosphate, pH 6.8; elution buffer: 1 *M* NH_4Cl, 0.5 *M* NaCl, 10 m*M* phosphate, pH 6.8. Washing begins from fraction 1, elution from fraction 25.

As reported in the literature [10–14], the purification of PGA by IMAC gave the highest purification than other purification methods. Fitton and Santarelli [13] obtained a 12.36-fold purification in specific activity and a 97% enzyme recovery by using Cu^{2+}-chelating Sepharose packed column with three steps of NH_4Cl elution. In this membrane process, the high recovery and purification of PGA activity were obtained in a single step as compared to other purification methods [10–14]. Besides, the IMAM offered some advantages such as large surface area, short diffusion path, low pressure drop and short residence time, which the packed column process must overcome while in scale-up. Therefore, PGA purification with immobilized metal affinity membrane is a potential approach in industry.

3.7. Regeneration of the chelated membrane

The reusability of regenerated cellulose-based IMAMs was investigated. After PGA desorption from the IMAM, some retained protein needed to be

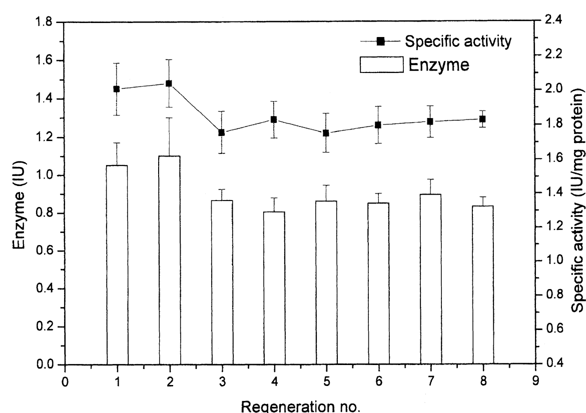

Fig. 9. Reusability of the IMAM (regeneration by 10 ml of 0.1 *M* EDTA). Each point represents the average of three trials.

removed for regenerating the IMAM. A stronger eluent such as EDTA is used for this purpose. In this work, 10 ml of 0.1 *M* EDTA was adopted for membrane regeneration. The regeneration was repeated eight times and the results are shown in Fig. 9. It was found that the enzyme capacity decreased slightly on the second regeneration, yet was stable from the third to the eighth times. It was possible that some active sites in the membrane were irreversibly blocked with the protein causing the reduction of the enzyme purification capacity [43].

4. Conclusions

An immobilized metal affinity membrane with modified regeneration cellulose as matrix for purification of penicillin G acylase was investigated. Factors such as chelator surface density, chelating metal, loading temperature, pH, NaCl concentration and elution buffers were studied consequently. Our investigation provided the optimal conditions for PGA purification using the IMAM process. A 9.11-fold purification in specific activity in a single step with 90.25% recovery for PGA purification was obtained. More than eight-times reusability of the membrane was realizable with the EDTA regeneration process. From the economic viewpoint, the membrane process with the advantage of large surface area, short diffusion path, and low-pressure drop is easy to be scaled up industrially. This implied that IMAM provided another feasibility while the column process was difficult to meet the aim in large-scale purification process.

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