A New Specific Metal Ion Chelated-Poly(N-vinylimidazole) Gel Sorbents for Albumin Adsorption-Desorption

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SUMMARY: Poly (N-vinylimidazole) (PVIm) hydrogels were prepared by γ-irradiating binary mixtures of N-vinylimidazole-water in a ^{60}Co-γ source having 4.5 kGy/h dose rate. These affinity gels having different swelling ratio of Cu(II)-chelated, Co(II)-chelated and plain PVIm in acetate buffer were used in the albumin adsorption studies. Bovine serum albumin (BSA) adsorption on these gels from aqueous solutions containing different amounts of BSA at different pH adjusted with acetate and phosphate buffer was investigated in batch reactors. The adsorption capacities of BSA on/in the gels were decreased dramatically by increasing the ionic strength (I) adjusting with NaCl. BSA adsorption capacities of the metal ion-chelated gels were higher than the plain PVIm gel even if the swelling ratio of the metal ion-chelated gels was very low comparing to the PVIm gel. The rigidity of the metal ion-chelated gel is very high and it can be used for the column applications. More than 95% of BSA were desorbed in 3 h in the desorption medium containing KSCN for PVIm gel and EDTA for metal ion-chelated gels. These results indicate that PVIm and metal ion-chelated PVIm gels are very efficient to remove BSA and the different metal ion-chelated PVIm gels show different affinity for BSA or biomolecules.

Introduction

Separation of proteins requires several processes involving methods, which select on the basis of size, charge, hydrophobicity or specific affinity[1]. Metal chelate affinity chromatography of proteins, with metal chelate linked to Sepharose, was first introduced by Porath et al[2]. They reported a model system using Zn(II) and Cu(II) chelating polymeric material packed columns in tandem for the fractionation of human serum proteins. Metal chelate affinity chromatography offers a new possibility for selectively extracting materials on the basis of their affinities for chelated metal ions. This method, also known as immobilized metal ion adsorption, exploits the affinity shown between certain proteins and metal ions[3,4]. The separation is based on differential binding abilities of the proteins or enzymes to interact with chelated metal attached to a solid carrier[5-7]. Various different types of polymers were used in protein purification either in their plain form, affinity ligand-attached form, and metal-chelated form. In all cases, solid polymeric matrices had low amount of affinity functional groups. Therefore, maximum protein adsorption capacities of

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these type polymeric sorbents were very low. If the affinity functional group containing monomer were used and polymerized, the adsorption capacity of the solid carrier could be increased. There is another important parameter arising from the diffusion of the proteins into the solid matrices which causes an increase in adsorption time and the adsorption capacity is being controlled by diffusion. To overcome these problems, polymeric matrices can be synthesized by the affinity functional group monomer such as N-vinyl 2-pyrrolidone, N-vinylimidazole and non-toxic gel type polymers can be used. In the presence of water, hydrogels absorb a significant amount of water to form elastic gels. So that, in the swollen form of the hydrogels, high molecular weight biopolymers can get inside the hydrogels and interact with suitable affinity group on the matrix and the rate of diffusion being fast because of the open structure of the gel.

The purpose of the present study is to prepare an affinity gel containing imidazole functional group and Cu(II) and Co(II) ions (in chelate form) for separation of proteins. BSA was selected as a model protein. In this article, we present BSA adsorption and desorption properties of PVIm and metal ion chelated form of the gels (i.e. properties of gel and metal ion incorporation), the adsorption conditions (i.e. initial concentration of BSA, pH medium and ionic strength) and adsorption-desorption behaviour of BSA.

Experimental

Materials

Bovine Serum Albumin (BSA, lyophilized, Fraction V) was purchased from Sigma Chemical Co. (St Louis, MO, USA) and used as received. N-vinylimidazole (VIm) was obtained from Merck AG (Darmstadt, Germany) and distilled under reduced pressure in order to remove hydroquinone inhibitor and stored at 4°C until use. All other chemicals were of reagent grade and were purchased from BDH (England).

Preparation of PVIm hydrogel

N-vinylimidazole-water mixture (10 mL) containing 8mL VIm-2mL water was placed in PVC straws of 3mm diameter and irradiated in a ^{60}Co-γ source at a fixed dose rate of 4.5 kGy/h. The gel was taken out from straws and washed several times with distilled water, dried in air and vacuum, ground and stored until use. In order to exibit swellabilities of the gels, swelling ratios were obtained as follows: 1 g of dry gel was placed in a cylindirical

glass tube (10 ml). 0.02 M of acetate buffer solution (pH=5.3) was added in to the tube, and the gels were allowed to swell at room temperature for 48 h (ie, the predetermined preequilibrium swelling time) with accasional shaking, and then the weight of the swollen gels was measured. Swelling ratio was calculated by the following equation.

$$\text{Swelling Ratio (\%)} = [(W_{swollen}-W_{dry})/W_{dry}] \times 100$$

Fine particles of hydrogels with 800 μm average size were used for the BSA adsorption. For the incorporation of Cu(II) and Co(II) ions to PVIm hydrogel, the following procedure was established: PVIm hydrogels were mixed with aqueous solutions containing 1000 ppm metal ion, at constant pH of 6.0, which was the optimum pH for Cu(II) and Co(II) chelate formation at room temperature[8,9]. The flasks were stirred magnetically for 10 days (sufficient to attain equilibrium). The metal ion adsorption capacities was determined by measuring the initial and final concentrations of metal ions within the adsorption medium, spectrophotometrically. Cu(II) and Co(II) leakage from the metal ion-chelated gel was investigated in BSA adsorption media. The solution containing the metal ion-chelated gel was stirred for 3 days at room temperature. After this period Cu(II) and Co(II) leakage were determined in these solution using Atomic Absorption Spectrophotometer (AAS, GBC 932 AM, Victoria, Australia).

The effect of pH and initial BSA concentration on BSA adsorption

BSA adsorption of the PVIm and metal ion chelated PVIm (PVIm-Cu(II) and PVIm-Co(II)) gels were studied at various pH. The pH of the adsorption medium was changed between 3.0 and 8.0 by using buffer systems (0.02 M CH_3COONa-CH_3COOH for pH 3.0-6.0, 0.02 M K_2HPO_4-KH_2PO_4 for pH 7.0, 8.0). The initial BSA concentration was changed between 5-200 mg/20 mL. The adsorption experiments were carried out for 27 h at 25°C. At the end of the equilibrium period (i.e., 20 h), the gels were separated from the solution. The BSA adsorption capacity was determined spectrophotometrically at 278 nm[10].

Desorption of BSA

Desorption of BSA was echieved by using 0.1 and 0.5 M of KSCN at pH=7.0 for PVIm gels and 50 mM of EDTA at pH=4.7 for Cu(II) and Co(II)-chelated PVIm gels. BSA loaded PVIm gels were placed in these desorption medium and stirred magnetically for 32 h at room temperature. The final BSA concentration in the aqueous phase was determined by UV-visible spectrophotometer. The desorption ratio was calculated from the amount of BSA adsorbed on the gels and the final BSA concentration in the desorption medium.

Results and Discussion

Influence of pH on BSA Adsorption

Figure 1 shows the effect of pH on BSA adsorption. In all cases investigated, the maximum adsorption of BSA was observed at pH=5.3. Significantly lower adsorption capacities were obtained with all gels in more acidic and in more alkaline pH regions. It has been shown that proteins have no net charge at their isoelectric points, and therefore, the maximum adsorption from aqueous solutions is usually observed at their isoelectric point[11,12]. The reasonable interaction occurs between negatively charged site of the BSA and the positively charged site of the imidazole functional group on the gel. At lower pH, C-terminus group and the other deprotonated site groups of the BSA has partially negative charge but imidazole ring has mainly positive charge. In this pH range, amount of the interaction is being controlled by the C-terminus group character. Around isoelectric point, C-terminus group has completely negative charge and imidazole ring has relatively high positive charge. Therefore, BSA adsorption capacity at this pH is very high. If the pH is higher than the isoelectric point of the BSA, C-terminus group of BSA has completely negative charge but imidazole functional group (pK_a=6.2 in the polymeric structure) is losing some part of its positively charge. For this reason, BSA adsorption is decreasing after isoelectric point of BSA. Beyond pH=7.0 imidazole group is mainly in neutral form and the interaction is very low between BSA and the imidazole group in the high pH region. All these explanations show that the nature of the interaction between BSA and PVIm is electrostatically type. In order to find the pH effect on the BSA adsorption for the metal ion-chelated PVIm gel, pH was changed between 3.0-8.0 and found that maximum adsorption attained at pH = 5.3. At this pH, BSA adsorption performs via positively charged sites of imidazole functional group and metal ion that is complexed with imidazole ligand. Because of this interaction, there is no any pH shift for the maximum BSA adsorption onto metal ion-chelated PVIm gels compare to PVIm gels. BSA adsorption mechanism onto metal ion-chelated PVIm gel was shown in the same route. First, metal ions have blocked some active sites of imidazole ring and have been occurred metal chelate form of PVIm. In this case, gel has potentially high positive charge. Therefore, BSA adsorption capacities of metal ion-chelated PVIm gels are very high comparing to plain gel sorbent.

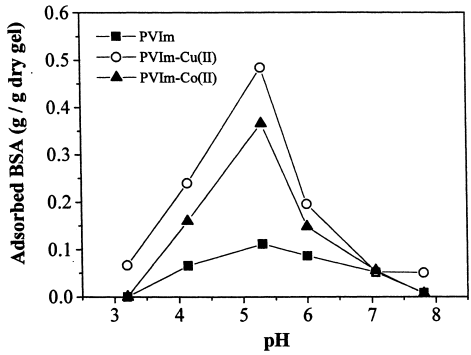


Figure 1. Effects of pH on BSA adsorption for different type of gels; ionic strength: 0.02; initial BSA concentration: 20 mg/20 mL; adsorption time: 20 h.

BSA Adsorption Kinetics of PVIm and Metal Ion Chelated-PVIm Gels

Figure 2 shows the adsorption kinetics of BSA on PVIm, PVIm-Cu(II) and PVIm-Co(II) chelated gels. Up to 2.5 h, adsorption increased very slowly then rapidly and reached the equilibrium in 20 h for PVIm gels. For the metal ion-chelated PVIm gels, the equilibrium adsorption time was completed in the first 10 h. This is due to high swellable behaviour of the plain gel and the very low swellable behaviour of the metal ion-chelated gels. To reach the equilibrium, much time is needed for the plain gel and the equilibrium time is being controlled by the swellability time and accompanying the diffusion of the BSA in the gel. There is no similar action for the metal ion-chelated gels.

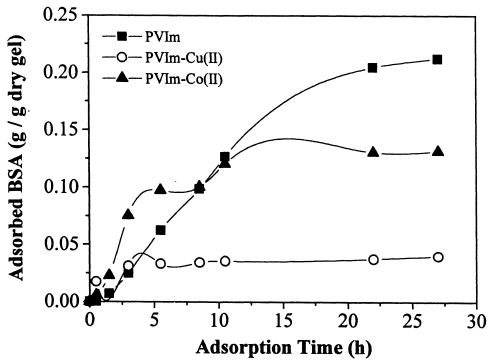


Figure 2. BSA adsorption kinetics of PVIm, PVIm-Cu(II) and PVIm-Co(II) gels; ionic strength: 0.02; pH: 5.3, initial BSA concentration: 100 mg/20 mL

BSA Adsorption Capacities of PVIm and Metal Ion Chelated-PVIm Gels

Figure 3 shows the effects of initial BSA concentration on the adsorption. As shown in this figure, with increasing BSA concentration in solution, the amount of albumin adsorbed by the plain gel increases rapidly at low concentrations, below about 25 mg/20mL, then increases less rapidly and approaches to first plateau. It increases rapidly again and shows the second plateau region between 150-200 mg/20mL. The maximum BSA adsorption of the PVIm gel is 0.55 g/g dry gel at 0.02 M ionic strength. If the ionic strength increased up to 0.1, BSA adsorption capacity of the gel is decreased to 0.28 g BSA / g dry gel. This is the case of the ionic interaction between imidazole ring and negatively charged site of BSA. When the ionic strength increased to 0.4 using NaCl, adsorption capacity of the gels reached almost zero value. Here, all of the electrostatic interactions are broken down and 0.4 M NaCl could be used as desorbing agent (data not shown).

Albumin adsorption capacities of the PVIm gels containing maximum amounts of Cu(II) and Co(II) (0.12 g Cu(II)/g dry gel and 0.04 g Co(II)/g dry gel) were investigated at pH=5.3. The initial concentration of BSA was changed between 5 - 200 mg/20 mL and the adsorption capacity of the metal chelated gel was investigated. Maximum BSA adsorption was 1.00 and 1.06 g BSA/g dry gel for the Cu(II)-chelated gel and for the Co(II)-chelated gel respectively. Adsorption capacity of the metal ion-chelated gel is about 2 folds higher as compared to the plain PVIm gel. Note that the swelling ratio of the metal ion-chelated gel is very low comparing to the plain gel. At pH=5.3, the swelling ratio of the plain gel is about 3000 % and the swelling ratio of the Cu(II)-chelated and Co(II)-chelated gels are 13% and 46%, respectively (Table 1). Metal ion-chelated form of the gel has highly compact structure because of the internal interaction between metal ion and the imidazole ring causing the shrinkage. Due to this shrunken structure, BSA molecules are not getting easily inside the gel and making fewer interactions with the imidazole group located inside the gel. However, there is a higher interaction between BSA and chelate form of the metal ion on the exterior surface of the gel comparing to the plain gel because of the chelated-metal ion affinity. The maximum BSA adsorption capacities for the Cu(II) and Co(II) derivatized gels are 1.00 and 1.06 g BSA/g dry chelated form of the gels. This is very high adsorption capacity for the BSA comparing to the other results given in the literature[13-16]. Maximum BSA adsorption capacities for the metal ion-chelated poly (N-vinylimidazole) gel is shown in Figure 3, and the swellability and the adsorbed BSA on different gels are given in Table 1 at two different ionic strength. The maximum Co(II) complexing capacity of PVIm gel is about three times lower than Cu(II) complexing capacity, but BSA adsorption capacities of both metal ion-

chelated PVIm gels are almost the same. This is probably due to the different coordination numbers of these two metal ions. One coordination site of Cu(II)-PVIm complex in the four coordination state is occupied by one BSA molecule in the presence of BSA in the solution. But Co(II) has a coordination number of six in its complexes. In the presence of C(II)-chelated PVIm form, BSA could be coordinated more than one mole to one mole Co(II) complexed ion onto the PVIm gel. So that 0.04 g Co(II) and 0.12 g Cu(II) /g dry PVIm gel could adsorbed the same amount of BSA which was about 1 g BSA/g metal ion-chelated PVIm gel.

Table 1. Swelling % values and amount of adsorbed BSA on PVIm and PVIm-metal ion complexes in solutions with different ionic strength (pH=5.3).

Gel type	Swelling % value		Adsorbed g BSA / g dry gel	
	I=0.0	I=0.1	I=0.0	I=0.1
PVIm	3000	1730	0.55	0.28
PVIm-Cu(II)	13	13	1.00	0.53
PVIm-Co(II)	46	37	1.06	0.32

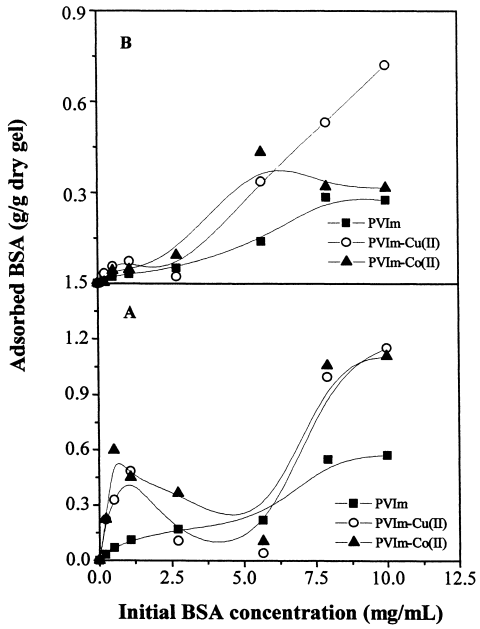


Figure 3. Effects of BSA initial concentration on BSA adsorption for the PVIm, Cu(II) and Co(II)-chelated gels at pH:5.3; (A) Ionic strength: 0.02; (B) Ionic strength: 0.1.

To clarify the metal ion effect on the PVIm for BSA adsorption, BSA adsorbed PVIm gel immersed into the 1000 ppm Cu(II) solution and the BSA released into the solution measured. We observed that the amount of released BSA was very low at pH=5.3 and the colour on the gel was corresponding the BSA-Cu(II) chelated form. Note that a variety of sorbents with a wide range of adsorption capacities were reported in literature for albumin adsorption[15,16]. The maximum BSA adsorption that we achieved with the sorbent system developed in this study was maximum 0.55 g BSA/g dry gel that was quite high as compared to the literature values. Here swellable gel form is very high open structure and there is not any restriction for the BSA to get inside the polymeric material.

Desorption of BSA

Figure 4 and 5 give the desorption of BSA from PVIm and Cu(II)-chelated PVIm gels, respectively. For PVIm gel, desorption of BSA with 0.1 M KSCN at pH=7.0 is 71% and the released BSA in 0.02 M phosphate buffer solution is about 15% representing non-specific BSA adsorption. If 0.5 M KSCN at pH=7.0 was used, more than 95% BSA could be desorbed from gel sorbent (data not shown). If the interaction is in the electrostatic nature, high salt concentration in desorption media is decreased the electrostatic interaction force and protein could be released from the sorbent into the desorption medium[17]. This is called "salt in" effect. So that, desorption ratio of BSA increases with increasing concentration of KSCN in the desorption medium. The BSA desorption effect of KSCN shows the similar effect with NaCl behaviour. Desorption of BSA from Cu(II) ion-chelated gel was investigated by using 0.02 M phosphate buffer at pH=7.0, 0.1 M KSCN at pH=7.0, and 50mM EDTA at pH=4.7. These results are shown in Figure 5. All of adsorbed BSA was desorbed from Cu(II) ion-chelated gel by using 50 mM EDTA solution. This is the case of competitive complexation of EDTA with Cu(II) and transfering the metal ion from gel surface into the solution. 0.1 M of KSCN at pH=7.0 was desorbed 45% of BSA. When concentration of KSCN was further increased, no more BSA desorption could be observed. This shows that a high percent BSA was adsorbed on Cu(II) ion-chelated gel via metal ion. The non-specific BSA adsorption which was released by 0.02 M phosphate buffer solution is about 32%. Also, 0.4 M NaCl solution could be used for BSA desorption from both PVIm and Cu(II) ion-chelated PVIm gels. In all cases, desorption of BSA was completed in the first 0-6 h. The same desorption experiments were repeated with Co(II) ion-chelated PVIm gel. It was observed that high desorption percent could be obtained by same desorption agents which were used in the case of Cu(II) ion-chelated PVIm (data not shown).

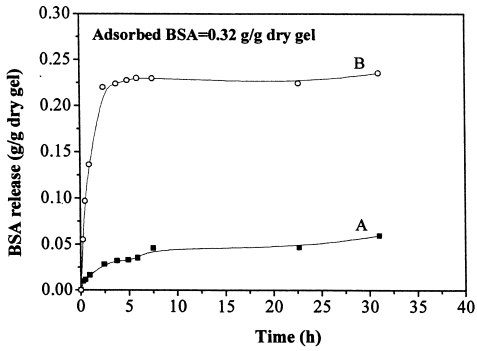


Figure 4. Desorption of BSA from PVIm gel in (A) acetate buffer at pH=5.3 and (B) 0.1 M KSCN solutions at 25°C at pH=7.0.

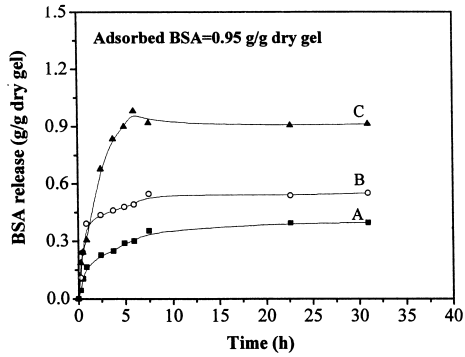
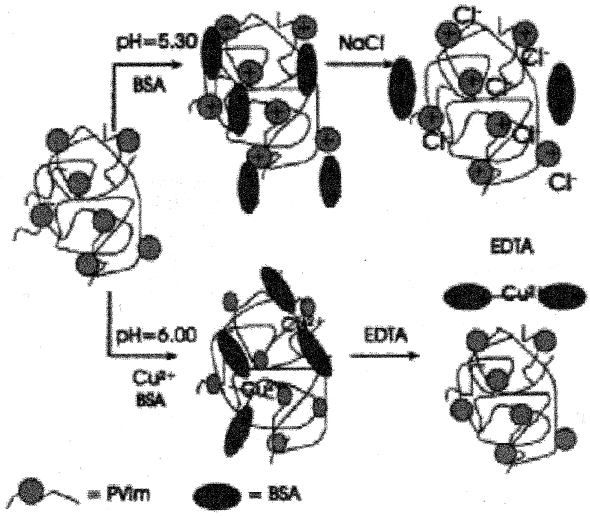


Figure 5. Desorption of BSA from Cu(II)-chelated PVIm gel in (A) acetate bufferat pH=5.3, (B) 0.1 M KSCN at pH=7.0 and (C) 50 mM EDTA solutions at 25°C and at pH=7.0.

Conclusions

Poly (N-vinylimidazole) hydrogels were synthesized by γ-irradiating binary mixtures of N-vinylimidazole-water in a ^{60}Co-γ source reaching 3000% swelling ratio in acetate buffer at pH=5.3. This new hydrogel and its metal ion-chelated form were used in adsorption-desorption of model protein BSA. Maximum metal ion complexation capacities of the hydrogel is 0.12 g Cu(II) and 0.04 g Co(II) /g dry gel, respectively. The maximum BSA adsorption capacities are found as 0.55, 1.00, and 1.06 g/g dry gel for PVIm, PVIm-Cu(II), and PVIm-Co(II), respectively. These capacities are significantly high for the protein adsorption applications comparing to the literature values. Metal ion-chelated form of PVIm caused in increase in the BSA adsorption capacity about two folds and the rigidity of the chelated form of PVIm is very high. Consequently, chelated form of PVIm can be used easily for chromatographic applications. More than 95% desorption ratios could be performed with 0.5 M KSCN, 0.4 M NaCl, and 50mM EDTA solutions for PVIm and metal ion-chelated PVIm gels. It was possible to reuse these hydrogel sorbents without significant losses in the adsorption capacities. As a result, BSA adsorption and desorption mechanisms for PVIm, Cu(II)-PVIm and Co(II)-PVIm was summarized in Scheme 1.



Scheme 1. Schematic representation of adsorption and desorption mechanisms of BSA on PVIm, Cu(II)-PVIm and Co(II)-PVIm gels.

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