Preparation and Characterization of Immobilized Metal Affinity Media Based on Monodisperse Crosslinked Poly (glycidyl methacrylate-ethyleneglycol dimethacrylate) Microspheres

Zhiya Ma[1], Yueping Guan[1], Junguo Liu[1,2], Huizhou Liu[1]

1. Laboratory of Separation Science and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, P.O. Box 353, Beijing 100080, P. R. China.

2. Department of Bioscience and Bioengineering, Hebei University of Science and Technology, Shijiazhuang, Hebei Province, 050018, P. R. China

Abstract: Monodisperse polyglycidyl methacrylate (PGMA) microspheres crosslinked with ethyleneglycol dimethacrylate (EGDMA) were prepared by a single one-step dispersion polymerization in ethanol/water medium. The microspheres had average size of 2.2 μm and abundant functional epoxy groups distributed on the surface. Immobilized metal affinity media were prepared by reaction between the epoxy groups on the microspheres and iminodiacetic acid (IDA). When charged with metal ion (Cu^{2+}), the microspheres were capable of binding proteins which displayed metal affinity. A model protein, bovine hemoglobin (BHb), was used to characterize the protein adsorption capacity of these microspheres, which showed as high as 120 mg protein per g of microspheres. The microspheres were investigated by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). They are very useful in biomolecules separation or purification.

Key words: Poly (glycidyl methacrylate-ethyleneglycol dimethacrylate), Dispersion polymerization, Immobilized metal affinity adsorption

1. INTRODUCTION

The rapid development of biotechnology and biomedicine requires more reliable and efficient separation techniques for isolation and purification of the biomolecules such as proteins, enzymes, and nucleic acids. Immobilized metal ion affinity chromatography (IMAC) is an efficient method for the purification of biomolecules in both analytical and large-scale modes. It is based on the selective interaction of immobilized metal ions with proteins through their surface-exposed amino acid residues such as histidine, cysteine, and tryptophan (Porath et al., 1975). Immobilized metal ions offer many advantages. They are small, robust, inexpensive, chemically and physically stable, and can be easily coupled to matrices at high density resulting in high capacity adsorbents (O'Brien, et al., 1996). At present, most of the matrices used in IMAC are macroporous bead-shaped particles such as agarose, cross-linked dextran and silica (Luo, et al., 2001). The inherent disadvantages of macroporous matrices are slow diffusion of solutes within the pores of the bead matrices. In recent years, micron-sized non-porous particles, such as silica and polystyrene based particles, have gained much interest as the support for biomolecules separation. The micron-sized non-porous particles take advantage of being higher resistance to fouling, better mass transfer and no diffuse resistance (Lee, 1997). However, polystyrene based particles are hydrophobic and exhibit pronounced nonspecific protein adsorption. Micron-sized polymer particles can be prepared by dispersion polymerization, which is a simple and efficient method for preparing monodisperse spherical particles in a single step. Polyglycidyl methacrylate (PGMA) has been utilized in biotechnology in recent years for the hydrophilic characteristics and the easily transformable epoxy groups. Nevertheless, preparation of monodisperse crosslinked PGMA microspheres is still problematic and such particles are not readily available.

In this study, monodisperse crosslinked PGMA microspheres were prepared with dispersion polymerization. By introduction of iminodiacetic acid chelator to the microspheres and then charged with Cu^{2+}, a new immobilized metal affinity matrix was achieved. Bovine hemoglobin was selected as a model protein to investigate its adsorption characteristics in a batch system.

2. EXPERIMENTAL

2.1 Materials

Unless stated otherwise, all the chemicals were purchased from Beijing Chemical Reagent Company. GMA obtained from Aldrich was distilled under vacuum. Ethylene glycol dimethacrylate (EGDMA) was used as crosslinker. 2, 2'-Azobisisobutyronitrile (AIBN) and polyvinylpyrrolidone (PVP K-30, Mw=40,000) were used as initiator and stabilizer respectively. Iminodiacetic acid (IDA) and bovine hemoglobin (BHb) were obtained from Sigma. All other chemicals were of analytical grade and used as received.

2.2 Preparation of monodisperse crosslinked PGMA microspheres by dispersion polymerization

Monodisperse crosslinked PGMA microspheres were prepared by dispersion polymerization using the recipe and reaction conditions listed in Table 1.

Table 1 The standard recipe for the dispersion polymerization of GMA and EGDMA. (70 □; 24 h; nitrogen gas; 120 rpm)

Ingredient	Weight (g)
GMA	9.9
EGDMA	0.1
PVP K-30	3
AIBN	0.2
Ethanol	81
H_2O	9

2.3 Preparation of Cu^{2+} chelated PGMA microspheres

0.2 g PGMA microspheres were mixed with the 2 g IDA solution in 50 ml H_2O and 2 M Na_2CO_3 were added. The mixture was stirred at 60 □ for 12 h. The resulting PGMA-IDA microspheres were centrifuged and washed with water. Then PGMA-IDA microspheres, were mixed with $CuSO_4$ solution (30 mg/ml) under shaking at room temperature for 4 h. The PGMA-IDA-Cu^{2+} microspheres were separated by centrifugation and thoroughly washed with water to remove the excess unbound Cu^{2+}.

2.4 Protein adsorption on PGMA-IDA-Cu^{2+} microspheres

Approximately 15 mg amount of PGMA-IDA-Cu^{2+} microspheres were mixed with 4 ml of various concentration of BHb solution (0.05-1.0 mg/ml) in a binding buffer (20 mM sodium phosphate, pH 8.0, 1.0 M NaCl). The mixture was shaken at 25 □ for 2 h. Then the mixture was centrifuged and the supernatant was assayed for protein concentration which was determined by a spectrophotometer at 406 nm. The amount of adsorbed protein was calculated by mass balance.

2.5 Characterization of PGMA microspheres

The size, size distribution and surface morphology of the PGMA microspheres were examined with scanning electron microscopy (SEM, JEOL, JSM-6700F). Fourier transform infrared spectra of the particles were recorded with a Fourier transform infrared spectrophotometer (FTIR, Bruker, Vector 22).

3. RESULTS AND DISCUSSION

Fig. 1 shows the SEM photograph of the PGMA microspheres. As clearly seen, the microspheres are spherical in shape and exhibit high monodispersity. The average size is 2.2 μm. It is well known that it is hard to prepare crosslinked polymer particles directly by dispersion polymerization. However, in this study, due to the co-stabilizing effect of surface active groups in PGMA, the crosslinked PGMA microspheres were obtained without any aggregation up to 1wt% of crosslinker to monomer added in the reaction medium.

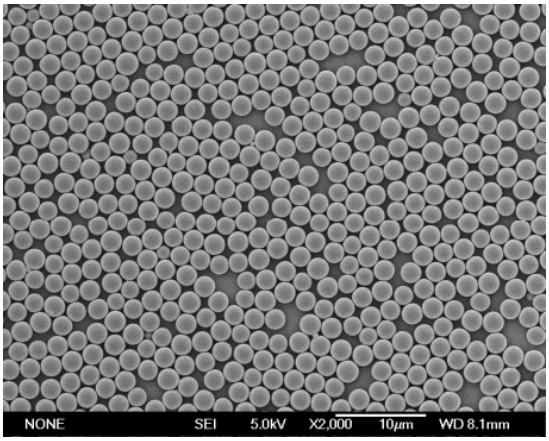

Fig. 1. SEM photograph of PGMA microspheres

Fig. 2 shows the FTIR spectra of PGMA (a) and IDA-modified PGMA (b) particles. Spectra of PGMA showed the characteristic absorption bands at 1726 cm^{-1} (carbonyl groups) and 850 and 910 cm^{-1} (epoxy groups). The adsorption bands at 1574 cm^{-1} (carboxylate groups) indicated the IDA was successfully introduced onto the PGMA particles.

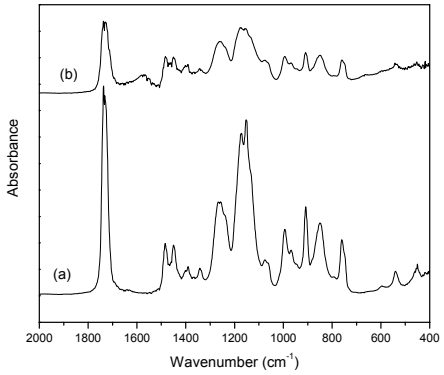

Fig. 2. FTIR spectra of PGMA (a) and PGMA-IDA (b) microspheres

The adsorption isotherms of BHb on PGMA-IDA-Cu^{2+} particles are shown in Fig. 3. The adsorption capacity was found to be 120 mg/g.

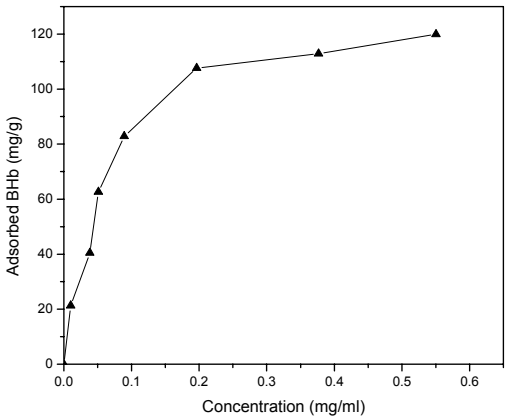

Fig. 3. Adsorption isotherm of BHb on PGMA-IDA-Cu^{2+} microspheres

4. CONCLUSIONS

Nonporous monodisperse crosslinked PGMA microspheres with average size of 2.2 μm were prepared by dispersion polymerization of GMA and EGDMA. IDA as a metal chelating group was covalently coupled to the particles and after charging with Cu^{2+}, the microspheres were applied for affinity adsorption of BHb. The results showed that the Cu^{2+} charged microspheres had high protein adsorption capacity. With the advantage of low cost, easy regeneration, and better mass transfer, the PGMA microspheres can be used efficiently in affinity separation of proteins exhibiting metal affinity.

ACKNOWLEDGEMENTS

This work was financially supported by the National High Technology Research and Development Program of China (No.2002AA302211), the National Natural Science Foundation of China (No. 20206032).

REFERENCES

1. Lee, W.C. (1997) Protein separation using non-porous sorbents. *J. Chromotogr. B*, **699**, 29-45.

2. Luo, Q.Z., Zou, H.F., Xiao, X.Z., Guo, Z., Kong, L., Mao, X.Q. (2001) Chromatographic separation of proteins on metal immobilized iminodiacetic acid-bound molded monolithic rods of macroporous poly (glycidyl methacrylate-co-ethylene dimethacrylate). *J. Chromotogr. A,* **926**, 255-264.

3. O'Brien, S.M., Thomas, O.R.T., Dunnill, P. (1996) Nonporous magnetic chelator supports for protein recovery by immobilized metal affinity adsorption. *J. Biotechnol*. **50**, 13-26.

4. Porath J., Carlson J., Olsson I., Belfrage G. (1975) Metal chelate affinity chromatography: A new approach to protein fractionation. *Nature*, **258**, 598-599.