Preparation of Zr^{4+} Affinity Column by Atom Transfer Radical Polymerization for Phosphoprotein Isolation

Jung-Hyun Park, In-Ae Bae, Seong-Ho Choi

Department of Chemistry, BK 21 NanoBiosensor Research Team, Hannam University, Daejeon 305-811, Republic of Korea

Received 24 September 2008; accepted 8 March 2009
DOI 10.1002/app.30473
Published online 18 June 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This paper describes the preparation of the metallic affinity column by atom transfer radical polymerization of glycidyl methacrylate (GMA) on Wang resin surface. The metallic ions (Zr^{4+}, Fe^{3+}, and Pd^{2+}) were introduced after phosphonation of the epoxy group of the grafted poly(GMA) on Wang resin surface. The successful results on metallic ion-immobilized polymeric microsphere stationary phase were confirmed via Fourier transform infrared, scanning electron microscopy, X-ray photoelectron spectra (XPS), and inductively coupled plasma-atomic emission spectrometer. The resolution degree of the phosphonated casein and dephosphonated casein for Zr^{4+}, Fe^{3+}, and Pd^{2+} affinity column was examined. The result shows that the resolution time (min) of the phosphonated casein was higher than that of the dephosphated casein on the Zr^{4+} affinity column by liquid chromatography. However, the phosphonated casein and dephosphonated casein were not separated on the Fe^{3+} affinity column and Pd^{2+} affinity column by liquid chromatography. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 1250–1255, 2009

Key words: Zr^{4+}; affinity column; atomic transfer radical polymerization; Wang resin; phosphonated casein; resolution time

INTRODUCTION

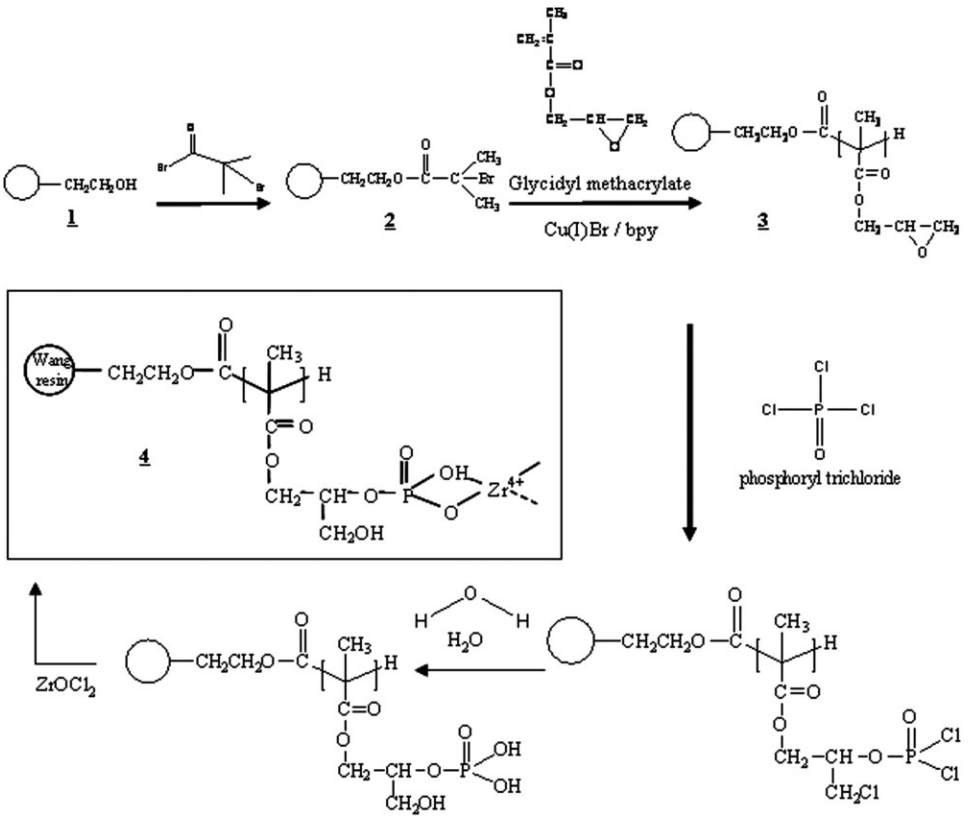
Atom transfer radical polymerization (ATRP) is a convenient and useful method to synthesize polymer with well-controlled molecular weight and molecular weight distribution.[1–4] Not only linear homopolymers but also many polymers with interesting architectures, such as block copolymers,[5,6] graft copolymers,[7–9] branched polymers,[10,11] and brush polymers,[12] have been synthesized by ATRP. Graft polymerization using ATRP is a useful method because various functional groups are easily introduced on the solid matrix with rigid properties. The rigid property of the packing materials is a very important factor in the use of high-performance liquid chromatography (HPLC) column because a packing material with soft properties cannot be packed in an HPLC column.

On the other hand, glycidyl methacrylate (GMA) is one of the monomers which can be easily modified into various functional groups. After GMA is polymerized, the epoxy groups of poly(GMA) can easily be changed to alcohols,[13] amines,[14] phosphonic acid,[15] sulfonic acid,[16] etc.[17]

Correspondence to: S.-H. Choi (shchoi@hnu.kr).
Contract grant sponsor: Korean Government; contract grant number: KRF-2008-521-C00176.

In our previous paper,[18] the poly(MA) was prepared by emulsion polymerization to apply the cosmetic supporters after coating with vitamin C. The spherical poly(MA) of various sizes can be obtained by changing reaction conditions. The vitamin C coating on the surface of poly(MA) microspheres by using cyclodextrin as a binder can be achieved to 30 wt % water/methanol mixture. We also prepared a polymeric microsphere with an epoxy group by the radiation-induced polymerization of GMA and diethylene glycol dimethacrylate in reaction conditions with variations in solvents, irradiation dose, and monomer composition.[19] Lipases have been immobilized to the epoxy group of the polymeric microsphere in experimental conditions with variations in pH and epoxy group content. The activity of the lipase-immobilized polymeric microsphere (PM) was in the range of 148–342 unit/mg min. However, these polymeric microspheres cannot be used in the packing materials for the HPLC column because the polymeric microsphere has soft properties and a small diameter. Considering the desired properties for HPLC column packing materials, such as rigid properties, suitable size for using in packing materials, and having a functional group, we selected Wang resin as the packing matrix.

On the other hand, phosphonated casein can be obtained from casein by enzymatic hydrolysis during the manufacturing of milk products as well as during intestinal digestion.[20,21] The phosphonated



Scheme 1 Preparation procedure of Zr^{4+} affinity stationary phase based on Wang resin.

casein can be used in the food and drug materials as a carrying supporter for calcium ions. However, to our knowledge, there are no published reports on the isolation of phosphonated casein from casein by HPLC.

In this study, we prepared the metallic affinity column based on Wang resin microspheres with Zr^{4+}, Fe^{3+}, and Pd^{2+} ions by using ATRP of glycidyl methacrate with an epoxy group. The epoxy group was used to introduce metal ions after phosphonation. The metallic ion-immobilized Wang resin microsphere stationary phase was characterized by Fourier transform infrared (FTIR), scanning electron microscopy (SEM), XPS, and inductively coupled plasma-atomic emission spectrometer (ICP-AES). The separation efficiency for the metallic affinity column prepared by slurry packing was tested for phosphonated casein and dephosphonated casein.

EXPERIMENTAL

Chemicals

Wang resin (200–400 mesh), 2-bromoisobutyryl bromide, GMA, 2,2′-bipyridine (bpy, 99%), phosphorus oxychloride (99%), zirconyl chloride hydrate ($ZrOCl_2$, 99.9%), $FeCl_3$, $PdCl_2$, copper (I) bromide (CuBr) phosphonated casein, and dephosphated casein separated from bovine milk were purchased from Sigma-Aldrich. The other chemicals were also reagent grade.

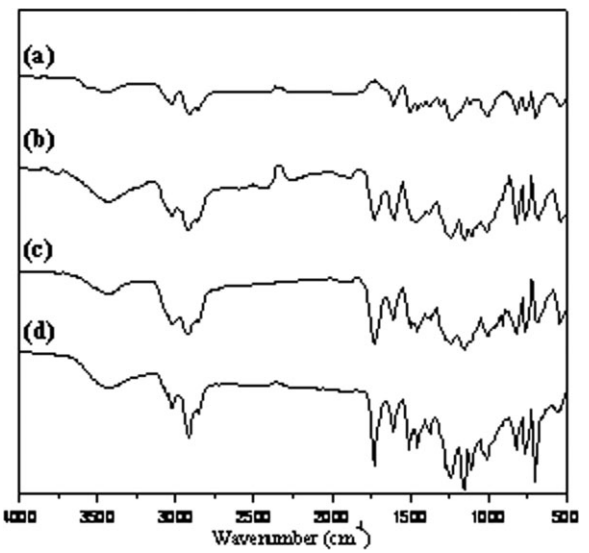


Figure 1 FTIR spectra of Zr^{4+} affinity stationary phase based on Wang resin. (a) Wang resin (1), (b) brominated Wang resin (2), (c) epoxy group-modified Wang resin (3), and (d) Zr^{4+}-modified Wang resin (4 in Scheme 1).

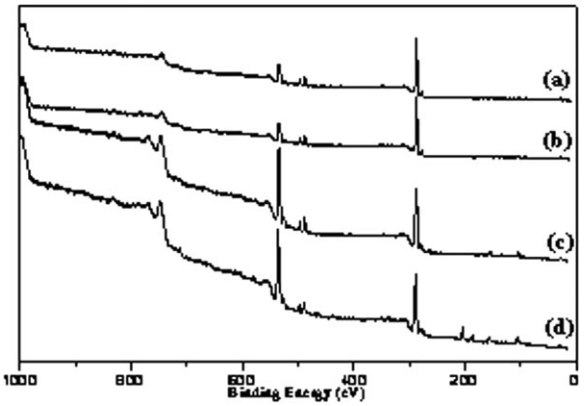


Figure 2 XPS survey scan spectra of Zr^{4+} affinity stationary phase based on Wang resin. (a) Wang resin (1), (b) brominated Wang resin (2), (c) epoxy group-modified Wang resin (3), and (d) Zr^{4+}-modified Wang resin (4 in Scheme 1).

Synthesis of metallic affinity stationary phase based on Wang resin microspheres

Scheme 1 shows the preparation procedure of the Zr^{4+} affinity stationary phase based on Wang resin with hydroxy groups. The Wang resin with hydroxyl group (**1**) was used as packing material supports. Wang resin introduced Zr^{4+} ions as follows: The initiator (**2**) was obtained by the reaction of 2-bromoisobutyryl bromide (3.6 mmol) and Wang resin (5.0 g) in toluene (70 mL) at room temperature for 3 h under nitrogen atmosphere. Next, the epoxy group-containing polymer stationary phase (**3**) was obtained by ATRP of GMA in the presence of CuBr (1.67 mmol), **2** compound as initiator, and bpy (3.07 mmol) as ligand at room temperature for 3 h. The epoxy group of 3 polymeric microspheres was reacted with $POCl_3$ (600 mg, 1.00 mol) in acetonitrile (5.00 mL) in the presence of pyridine (0.93 g, 3.00 mol) under nitrogen for 12 h. The white powder was obtained using filter paper and then dried in a vacuum oven at 50°C for 8 h. The powder was again dispersed in acetonitrile (5.00 mL). Subsequently, the deionized water (0.60 mL) was added under nitrogen at room temperature for 12 h. To immobilize Zr^{4+} to the phosphoric acid group, $ZrOCl_2$ (500 mg) was added under nitrogen at room temperature and was reacted for 48 h. Finally, the obtained powder was washed with acetonitrile and dried in a vacuum oven at 50°C for 8 h. The obtained Zr^{4+} affinity stationary phase was suspended in a hexane/2-propanol (90/10) mixture and packed in a stainless steel column (100 × 2.1 mm I.D.) by a slurry packing method for use in HPLC.

Instrumentation

The surface morphology of the samples was determined by using SEM (S-3000N; Hitachi Science Sys-

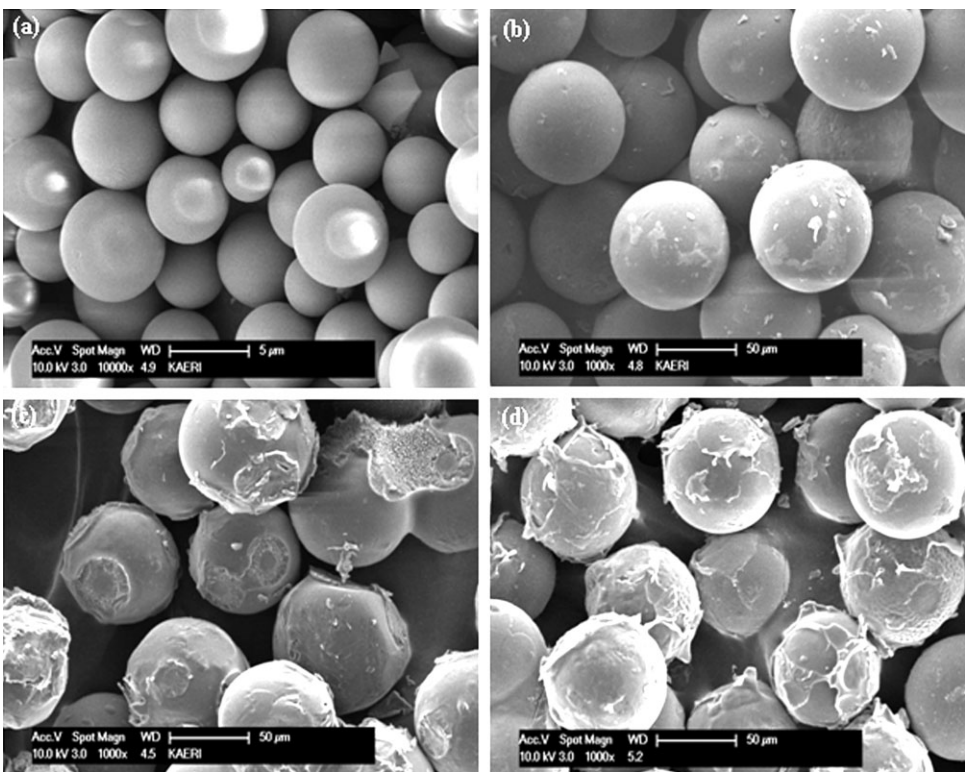


Figure 3 SEM images of Zr^{4+} affinity stationary phase based on Wang resin. (a) Wang resin (1), (b) brominated Wang resin (2), (c) epoxy group-modified Wang resin (3), and (d) Zr^{4+}-modified Wang resin (4 in Scheme 1).

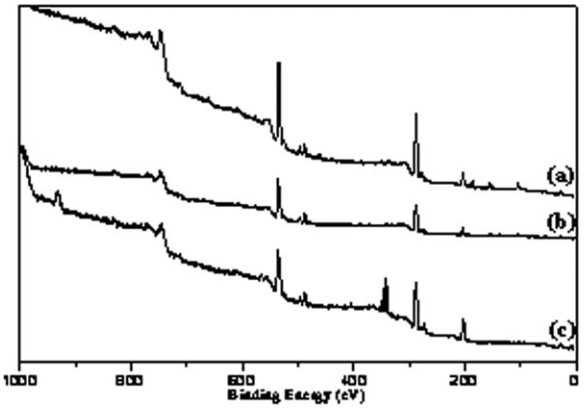


Figure 4 XPS survey scan spectra of metallic affinity stationary phase based on Wang resin with Zr^{4+} (a), Fe^{3+} (b), and Pd^{2+} (c). Content of Zr = 0.24%, Fe = 0.08%, and Pd = 8.00% by ICP-AES.

tem, Japan). FTIR spectra were recorded in the range of 400–4000 cm^{-1} with a 4 cm^{-1} resolution from KBr pellets on a Perkin-Elmer Spectrum 1000 system (Perkin-Elmer Life and Analytical Sciences). The X-ray photoelectron spectra of the samples were obtained using MultiLab ESCA2000 (Thermo Fisher Scientific). The Zr contents were analyzed by an ICP-AES (Ultima-C; Jobin-Yvon). Also, the separation efficiency of the prepared column was determined by HPLC (1100 Series; Agilent) with a UV detector.

RESULTS AND DISCUSSION

Chromatography based on silica ball supports has numerous qualities, such as high mechanical stability, resistance to swelling, and excellent efficiency. However, affinity columns based on silica ball cannot be used in the isolation of phosphonated casein from casein because the mobile phase (buffer solution) is not passed on the affinity column based on the silica ball. To pass the buffer solutions onto the column, the column supports should have hydrophobic properties. Therefore we selected Wang resin to prepare packing material for liquid chromatography for separation of phosphorylated protein. For introduction of metal ions, the surface modification was performed by phosphonation of the epoxy group after ATRP of GMA on Wang resin surface. Subsequently, we introduced Zr^{4+}, Fe^{3+}, and Pd^{2+} on the phosphoric acid-modified Wang resin.

Figure 1 shows FTIR spectra of the Wang resin (**1**), brominated Wang resin (**2**), epoxy group-modified Wang resin (**3**), and Zr^{4+}-modified Wang resin (**4**). In Figure 1(b), the characteristic absorption peak at 1730 cm^{-1} appeared due to >C=O (carbonyl group) of 2-methylpropanoyl bromide (see **2** in Scheme 1). After ATRP of GMA, the epoxy group appeared at 910 cm^{-1} due to the epoxy group of poly(GMA) and a characteristic peak at 1730 cm^{-1} due to the carbonyl group of the poly(GMA). As a result, the epoxy group was successfully introduced on the surface of Wang resin by ATRP (see **3** in Scheme 1). In Figure 1(d), the epoxy group at 930 cm^{-1} disappeared because of phosphonation of the epoxy group of the grafted poly(GMA) as described in Scheme 1. This means that the Zr^{4+} ion was successfully introduced on Wang resin surface.

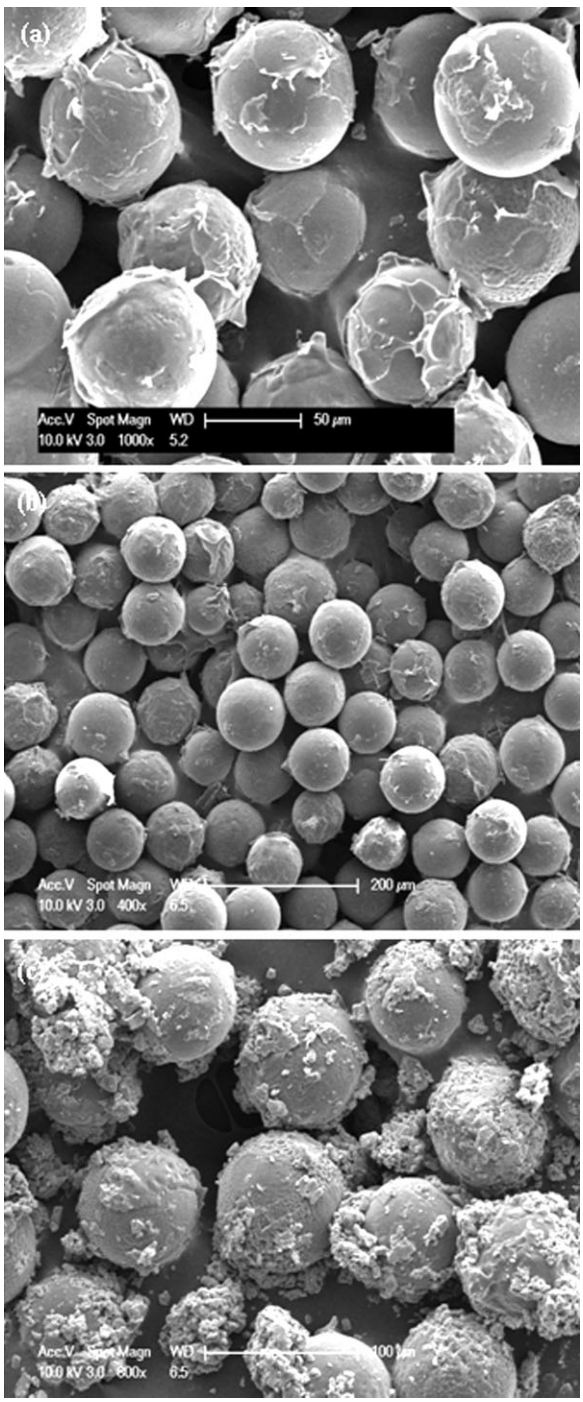


Figure 5 SEM images of metallic affinity stationary phase based on Wang resin with Zr^{4+} (a), Fe^{3+} (b), and Pd^{2+} (c).

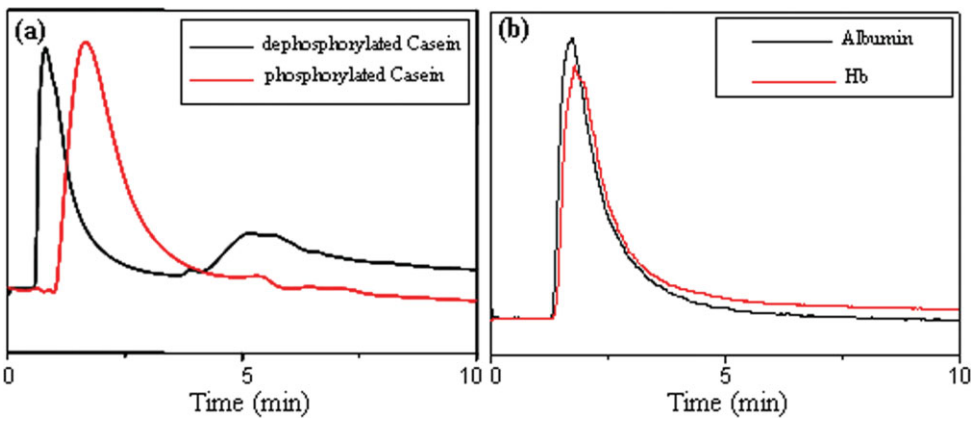


Figure 6 Resolution of phosphorylated casein/dephosphorylated casein (a) and albumin/Hb (b) by use of Zr^{4+} affinity column (phosphonate buffer pH = 7.0; flow rate, 0.1 mL/min; detector, UV 254 nm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 2 reveals the XPS survey scan spectra of the Zr^{4+} affinity stationary phase based on Wang resin microsphere: Wang resin (**1**), brominated Wang resin (**2**), epoxy group-modified Wang resin (**3**), and Zr^{4+}-modified Wang resin (**4**). In the Wang resin [Fig. 2(a)], the C 1s peak at 285 eV was high in intensity compared with that of O 1s peak at 532 eV. There are no large-change XPS results after surface modification of 2-methylpropanoyl bromide [Fig. 2(b)]. However, after ATRP of GMA [Fig. 2(c)], the O 1s peak intensity was relatively increased compared with that of C 1s peak. This means that the poly(GMA) was successfully introduced on the surface of Wang resin. In XPS spectra of Figure 2(c,d), the copper element used in the initiator appeared despite washing the polymer. After the complexation of Zr^{4+} ion to the phosphoric acid group (see the structure **4** in Scheme 1), the P 2s peak around 200 eV and Zr 3d peaks around 200 eV appeared as shown in Figure 2(d). This means that the Zr^{4+} affinity stationary phase was successfully prepared.

Figure 3 shows the SEM images of Wang resin (**1**), brominated Wang resin (**2**), epoxy group-modified Wang resin (**3**), and Zr^{4+}-modified Wang resin (**4**). After the surface modification of the 2-methylpropanoyl bromide on the surface of Wang resin, the surface morphology of Wang resin was changed from a smooth surface [Fig. 3(a)] to a rough surface [Fig. 3(b)] due to immobilization of 2-methylpropanoyl bromide of the Wang resin surface. After the ATRP of GMA, the surface morphology of Wang resin appeared like grape skin, as shown in Figure 3(c). This means the poly(GMA) was successfully introduced on the surface of Wang resin via ATRP. The surface morphology of the Zr^{4+} affinity stationary phase showed a pattern similar to that in Figure 3(c). To prepare various plus charge metallic affinity stationary phases, we introduced the Fe^{3+} and Pd^{2+} on the surface of the phosphonated Wang resin as described in Scheme 1.

Figure 4 reveals the XPS survey scan spectra of the metallic affinity stationary phase based on Wang resin with Zr^{4+}, Fe^{3+}, and Pd^{2+}. There was no Fe peak in XPS spectra on the Fe^{3+} affinity stationary phase [Fig. 4(b)] because of the low Fe content. In determination results of ICP-AES, the content of the Zr, Fe, and Pd was 0.24%, 0.08%, and 8.00%, respectively. In Figure 4(c), the Pd 3d peaks around 360 eV appeared. From these results, the metallic affinity stationary was successfully prepared using the ATRP method.

Figure 5 also shows the SEM images of the metallic affinity stationary phase based on Wang resin with Zr^{4+}, Fe^{3+}, and Pd^{2+}. In Figure 5(b), the surface morphology of Wang resin shows the grape skin form similar to that shown in Figure 5(a). However, the morphology of the Pd^{2+} affinity stationary phase in Figure 5(c) looks like the aggregation form because of immobilization of large amounts of Pd^{2+} on the surface of Wang resin.

To use an affinity column to isolate phosphonated protein from proteins, the prepared metallic affinity stationary phase was packed in stainless steel columns. Subsequently, the resolution of phosphonated casein and dephosphonated casein as the model protein was performed using HPLC. The resolution conditions were as follows: Column, 100 × 2.1 mm I.D.; triple-distilled water; flow rate, 0.1 mL/min; room temperature; detector, UV 254 nm. In the case of Fe^{3+} and Pd^{2+} affinity column, phosphonated casein and dephosphonated casein were not separated. However, in the Zr^{4+} affinity column, phosphonated casein and dephosphated casein were separated, as shown in Figure 6(a). In our previous paper,[22] the separation of bovine serum albumin (BSA) and chicken egg albumin (CEA) on the polymeric stationary phase with various amines was investigated.

The affinity degree of BSA was higher than that of CEA for the EDA, HEDA, and DETA columns, whereas the affinity degree of CEA was higher than that of BSA for the TETA column. However, hemoglobin and albumin could not be separated on the Zr^{4+} affinity column, as shown in Figure 6(b). In Figure 6(a), the resolution time (min) of the phosphonated casein and dephosphonated casein was at 2.5 and 1.9 min, respectively. This means that the phosphonated casein strongly interacted with the Zr^{4+} ion of the affinity column.

CONCLUSION

Polymeric stationary phases with Zr^{4+}, Fe^{3+}, and Pd^{2+} were prepared by ATRP of GMA with an epoxy group onto the Wang resin surface and with subsequent complexation of metallic ions. The separation of phosphonated casein and dephosphonated casein using polymeric stationary phases with metallic ions was investigated. From the results, the conclusions were as follows:

1. Polymeric stationary phases with Zr^{4+}, Fe^{3+}, and Pd^{2+} were successfully prepared by ATRP of GMA with epoxy group onto the Wang resin surface and with subsequent complexation of metallic ions.
2. The phosphonated casein and dephosphonated casein was not separated for the Fe^{3+} affinity column and the Pd^{2+} affinity column.
3. Resolution time of the phosphonated casein was higher than that of dephosphonated casein on the Zr^{4+} affinity column.
4. Hemoglobin and albumin were not separated on Zr^{4+} affinity column.

References

1. Gao, C.; Li, W. W.; Morimoto, H.; Nagaoka, Y.; Maekawa, T. J Phys Chem B 2006, 110, 7213.
2. Bai, Y. P.; Teng, B.; Chen, S. Z.; Chang, Y.; Li, Z. L. Macromol Rapid Commun 2006, 27, 2107.
3. Zhou, M.; Yuan, J. Y.; Yuan, W. Z.; Yin, Y. W.; Hong, X. Y. Nanotechnology 2007, 18, 405704.
4. Guo, Z. P.; Chen, Y. W.; Zhou, W. H.; Huang, Z. F.; Hu, Y. H.; Wan, M. X.; Bai, F. L. Mater Lett 2008, 62, 4542.
5. Zheng, G.; Stöiver, H. D. H. Macromolecules 2003, 36, 1808.
6. Siegwart, D. J.; Wu, W.; Mandalaywala, M.; Tamir, M.; Sarbu, T.; Silverstein, M. S.; Kowalewski, T.; Hollinger, J. O.; Matyjaszewski, K. Polymer 2007, 48, 7279.
7. Fan, Q.-L.; Neoh, K.-G.; Kang, E.-T.; Shuter, B.; Wang, S.-C. Biomaterials 2007, 28, 5426.
8. Zhou, L.; Yuan, W.; Yuan, J.; Hong, X. Mater Lett 2008, 62, 1372.
9. Yamamoto, K.; Tanaka, H.; Sakaguchi, M.; Shimada, S. Polymer 2003, 44, 7661.
10. Wang, A. R.; Zhu, S. J Polym Sci Part A: Polym Chem 2005, 43, 5710.
11. Chang, C.; Pugh, C. Macromolecules 2001, 34, 2027.
12. Ishizu, K.; Satoh, J.; Sogabe, A. J Colloid Interface Sci 2004, 274, 472.
13. Saito, K.; Kaga, T.; Yamagishi, H.; Hurusaki, S. J Membr Sci 1989, 43, 131.
14. Choi, S.-H.; Lee, K. P.; Lee, J. G.; Nho, Y. C. Microchem J 2001, 68, 473.
15. Choi, S.-H.; Nho, Y. C. Kor J Chem Eng 1999, 16, 725.
16. Choi, S.-H.; Nho, Y. C. J Appl Polym Sci 1999, 71, 2227.
17. Malmsten, M.; Larsson, A. Colloid Surf B Biointer 2000, 18, 277.
18. Kim, K.-H.; Choi, S.-H. Anal Sci Tech 2006, 19, 468.
19. Choi, S.-H.; Lee, K. P.; Kang, H. D. J Appl Polym Sci 2003, 88, 1153.
20. Plank, J.; Andres, P. R.; Krause, I.; Winter, C. Protein Expr Purif 2008, 60, 176.
21. Kumosinski, T. F.; King, G.; Farrell, H. M., Jr., J Protein Chem 1994, 13, 681.
22. Choi, S.-H.; Hwang, Y. M.; Lee, K. P. J Chromatogr A 2003, 987, 323.