Poly(hydroxyethyl methacrylate)-co-N-methacryloyl-(L)-histidine methyl ester Based Cryogel for the Removal of Fe^{3+} from Human Plasma effected with Beta Thalassemia

Poli(hidroksietil metakrilat-N-metakrilo-L-Histidin metil ester) Temelli Kriyogel ile Beta talasemili İnsan Plazmasından Fe^{+3}'ün Uzaklaştırması

Research Article

Hüseyin Alkan
Dicle University, Faculty of Pharmacy, Diyarbakır, Turkey.

ABSTRACT

In this study, Fe^{3+} adsorption was carried by using supermacroporous poly(2-hydroxyethyl methacrylate-N-methacryloyl-(L)-histidine methyl ester) [poly(HEMA-MAH)] cryogel. Poly(HEMA-MAH) cryogel was characterized by swelling tests and scanning electron microscopy. The influence of flow rate, pH, initial Fe^{3+} concentration, and Fe^{3+} adsorption from human plasma effected with beta thalassemia on the adsorption efficiency of the cryogel was investigated. The equilibrium swelling degree of poly(HEMA-MAH) cryogel was found to be 7.54 g H_2O/g cryogel. Fe^{3+} adsorption capacity of poly(HEMA-MAH) cryogel from aqueous solution was estimated as 1.79 mg/g cryogel, while lower adsorption capacity was observed in human plasma (1.71 mg/g). It was also observed that Fe^{3+} could be repeatedly adsorbed and desorbed using poly(HEMA-MAH) cryogel without significant loss in its adsorption capacity.

Key Words
Fe^{3+} removal, Beta Thalassemia, Affinity Chromatography, Cryogel, poly(HEMA-MAH).

ÖZET

Bu çalışmada, Fe^{3+} adsorpsiyonu, süpermakrogözenekli poli(2-hidroksietil metakrilat-N-metakrilo-(l)-histidin metil ester) [poli(HEMA-MAH)] kriyojeli kullanılarak incelenmiştir. Poli(HEMA-MAH) kriyojeli elektron mikroskop taraması ve şişme testleriyle karakterize edilmiştir. Akış hızı, pH, Fe^{3+}'ün başlangıç derişiminin Fe^{3} adsorpsiyonuna etkileri incelenmiştir. Poli(HEMA-MAH) temelli kriyojelin denge şişme derecesi 7.54 g H_2O/g kriyojel olarak bulunmuştur. Poli(HEMA-MAH) temelli kriyojelin Fe^{3+} adsorpsiyon kapasitesi 1.71 mg/g bulunmuştur. Önemli miktarda adsorpsiyon kapasitesi kaybı olmadan Fe^{3+} iyonlarının P(HEMA-MAH) kriyojel ile adsorbe ve desorbe edildiği saptanmıştır.

Anahtar Kelimeler
Fe^{3+} uzaklaştırılması, Beta Talasemi, Afinite Kromatografisi, Kriyojel, p(HEMA-MAH).

Article History: Received: Apr 15, 2015; Revised: June 02, 2015; Accepted: Jul 20, 2015; Available Online: Oct 31, 2015.

DOI: 10.15671/HJBC.20154314240

Correspondence to: H. Alkan, Ph.D. Dicle University, Faculty of Pharmacy Department of Biochemistry, Diyarbakır, Turkey.
Tel: +90 412 248 8550 Fax: +90 412 248 8300 E-Mail: mhalkan@dicle.edu.tr

INTRODUCTION

Heavy metals at trace levels have important role in body functions and effects of heavy metal ions on human life vary depending on element [1,2]. Excess of iron accumulates in the body either from over repeated blood transfusions, in people having a severe form of beta thalassemia or via excess dietary iron (including iron supplements) uptake in anemia and hereditary hemocromatosis cases [3,4]. Furthermore, human body cannot excrete iron deposits efficiently. At present, desferrioxamine mesylate (desferal) is the only FDA approved clinical drug that is intravenously or intramuscular applied to chelatethe iron overload in the veins during blood transfusions in patients suffering from chronic anemia. However, desferrioxamine mesylate is known to have several short comings in its application because it causes ocular, auditory, and renal disturbances, and becomes highly toxic at higher doses or over prolonged periods. As an alternative to the adverse effects, soluble iron chelators such as desferal, and the attachment of iron chelating ligands including dye and protein molecules have been investigated [4-6]. Compared to the soluble iron chelators, iron chelating resins are believed to have some benefits in terms of stability, reusability, and minimal damage to biomolecules. Designing an efficient iron chelating system for the treatment of the patients with chronic iron overload often involves the preparation of proper and efficient adsorbents to remove the excessive iron from the body [4,7,8]

Conventional packed-bed columns have several characteristic drawbacks such as a sluggish diffusional mass transfer and a large void volume that may form among the polymeric beads packed in the column [9]. Several new stationary phases such as non-porous polymeric beads [10] and perfusion chromatography packings have been developed to overcome these problems, however, they cannot be entirely resolved yet [11]. The most obvious advancement has been made in the improvement of alternative chromatography formats such as membrane adsorbents, monoliths and cryogels [12-20]. Cryogels provide a potential solution because the presence of macropores offers lower pressure drop and negligible diffusion resistances compared to conventional column chromatography [21-23]. Cryogels enable higher flow rates ensuring the processing of larger volumes within shorter process times. Thus, whole blood may be applied to cryogels without any pre-treatment [24]. Cryogels are also inexpensive materials and they can be used as disposable materials to avoid cross-contamination between batches [25].

In the present study, the poly(HEMA-MAH) monolithic cryogel, which is a copolymer of 2-hydroxyethyl methacrylate (HEMA) and N-methacryloyl- (L)-histidine-methylester (MAH), was prepared by cryopolymerization. The poly(HEMA-MAH) cryogel was characterized and employed for selective removal of Fe^{3+} ions from aqueous solution and from human plasma.

MATERIALS AND METHOD

Materials

L-histidine methylester and methacryloyl chloride were provided from Sigma (St. Louis, USA). Hydroxyethyl methacrylate (HEMA) N,N′-methylene-bisacrylamide (MBAAm), N,N,N′,N′-tetramethylene diamine (TEMED) and ammonium persulfate (APS) were purchased from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4°C until use. All other chemicals were of reagent grade and were purchased from Merck A.G. (Darmstadt, Germany). Water used in the experiments was purified by using a Barnstead (Dubuque, IA, USA) ROpure LP® reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731), followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion-exchange packed bed system.

Synthesis of poly(HEMA-MAH) cryogels

Synthesis of N-methacryloyl-(l)-histidinemethyl ester (MAH) was described elsewhere [26]. HEMA and MAH were polymerized in free radical polymerization by using APS and TEMED as the initiator and activation pair. Water and MBAAm were placed in the polymerization recipe as the pore-former and cross-linker, respectively. Precisely HEMA (1.3 mL) and MAH (200 mg) were dissolved in 5.0 mL deionized water and MBAAm (0.283 g) was dissolved in 10 mL deionized water

sepeately. Finally, both solutions were mixed thoroughly and poly(HEMA-MAH) cryogel was produced by free radical polymerization initiated by TEMED (25 µL) and APS (20 mg). After adding APS (1% (w/v) of the total monomers), the solution was cooled in an ice bath for 2–3 min followed by the addition of TEMED (1% (w/v) of the total monomers) and stirred for 1 min. Then, the polymerization syrup was poured into a plastic syringe (5 mL, id. 0.8 cm) with closed outlet at the bottom. The polymerization solution in the syringe was frozen at -16°C for 24 h and then thawed at room temperature. NaN_3 solution (0.02%, w/v) was pumped through the monolithic cryogel to store at 4°C after use. The poly(HEMA) cryogel was produced using the same process without including MAH.

Characterization of poly(HEMA-MAH) cryogel

The swelling degree of the cryogel (S) was determined as follows: a cryogel sample was washed with water until washing was clear. Then it was sucked dry and transferred to pre-weighed vial and weighed ($m_{wet\ gel}$). After drying to constant mass in the oven at 60°C, the mass of dried sample was determined ($m_{dry\ gel}$). The swelling degree was calculated as:

$$S = (m_{wet\ gel} - m_{dry\ gel}) / m_{dry\ gel} \quad (1)$$

The total volume of macropores in the swollen cryogel was roughly estimated by weighing the sample (msqueezed gel) after squeezing the free water from the swollen gel matrix, and then the porosity was calculated as:

$$(m_{swollen\ gel} - m_{squeezed\ gel}) / m_{swollen\ gel} \times 100 \quad (2)$$

The surface morphologies of the poly(HEMA-MAH) cryogels was examined using SEM. The sample was frozen overnight in 2.5% glutaraldehyde and dehydrated at -50°C in lyophilizer (Lyophilizer, Christ Alpha 1-2 LD plus, Germany). Finally, it was coated with gold-palladium (40:60) and examined using a scanning electron microscope (JEOL JSM 5600, Tokyo, Japan).

Adsorption studies

The cryogel was washed with 20 mL of water for 30 min and then equilibrated with 0.1 M phosphate buffer (pH 7.4) for adsorption studies. Effect of pH, flow rate, amount of Fe^{3+} ions on the adsorption capacity was investigated. To observe the effects of the Fe^{3+} ions concentration on adsorption, it was varied in the range of 5–50 mg/L. The effect of pH on the adsorption capacity was determined by changing pH of the solution between pH 2.0 and 5.0. Influence of flow rate on adsorption capacity was investigated at different flow rates in the range of 0.5–4.0 mL/min. Fe^{3+} ions captured by the cryogel column were eluted by a solution of 0.1 M EDTA. Fe^{3+} adsorption-desorption cycle was repeated for 10 times using the same cryogel column. Fe^{3+} concentration was established by using an ICP-MS 7700 x Agilent.

Fe^{3+} Removal from human plasma effected with β Thalassemia

Fe^{3+} removal tests were conducted on human plasma (obtained from a donor suffering fromβ thalassemia) via poly(HEMA-MAH) cryogel. The human blood sample was centrifuged at 500 g for 30 min at room temperature, then filtered (3 mL Saltorius filter) and frozen at -20°C. Before use, the plasma was thawed for 1 h at 37°C. Prior to application, the viscous sample was diluted with phosphate-buffered saline (PBS; pH 7.4, NaCl: 0.9%). The dilution ratios were 1/2, 1/5, 1/10 and 1/20, respectively. Then, 20 mL of the prepared human plasma was pumped through the column with a flow rate of 1 mL/min. These experiments were conducted at room temperature. The amount of Fe^{3+} adsorbed was determined by measuring the initial and final concentration of Fe^{3+} in plasma, and Fe^{3+} concentration was determined by using a ICP-MS 7700 x Agilent.

RESULTS AND DISCUSSION

Characterization

A supermacroporous cryogel was prepared by polymerization of HEMA and MAH in the frozen state iwith the assistance of APS/TEMED as initator/activator pairs. The equilibrium swelling and macroporosity of the poly(HEMA-MAH) cryogel was 7.54 g H_2O/g cryogel and 71.6%, respectively. Poly(HEMA-MAH) cryogel was opaque, sponge like, and elastic. The cryogel could be easily compressed by hand to remove water accumulated inside the pores. When the

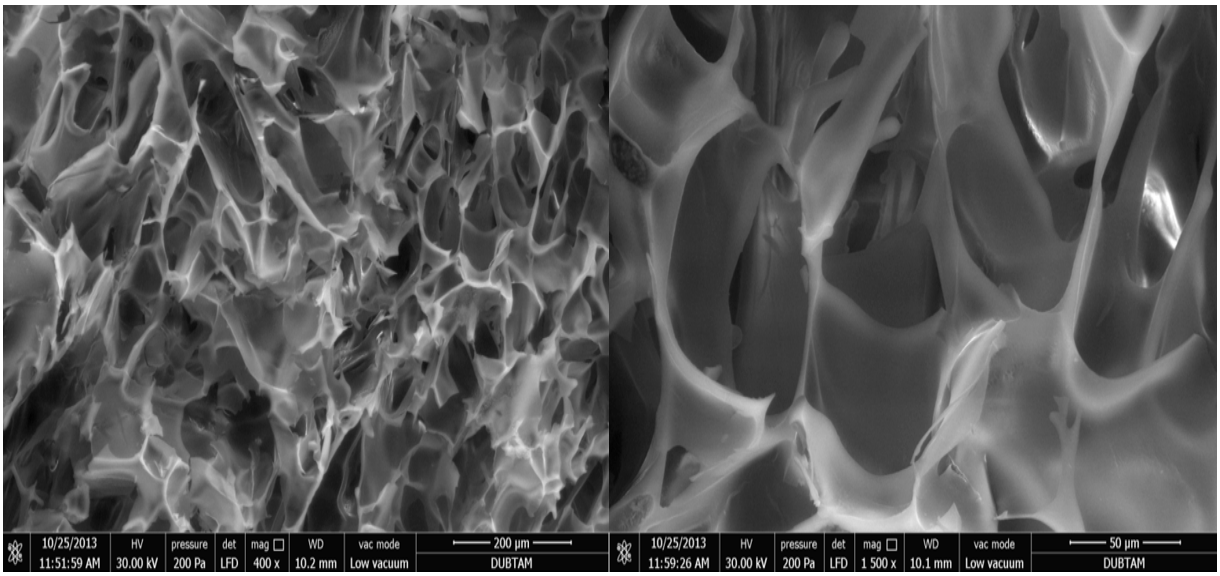

Figure 1. SEM images of p(HEMA-MAH) cryogel.

compressed piece of cryogel was submerged in water, it soaked in water and within 1 to 2 s restored its original size and shape.

The scanning electron photographs of internal structure of the cryogel are shown in Figure 1. poly(HEMA-MAH) cryogel possess large continuous interconnected pores (50 to 100 µm in diameter) providing channels for the mobile phase to flow through. Pore size of the matrix is larger than size of metal ions, allowing them to pass easily through the pores. Because of the connective flow of the solution through the pores, the mass transfer resistance is practically negligible.

Effect of the flow rate

The adsorption amounts at different flow rates are given in Figure 2, and the results indicates that the metal ion adsorption capacity onto the poly(HEMA-MAH) cryogel decreased when the flow rate was increased thorough the column. The adsorption capacity reduced from 0.58 to 0.15 mg/g of polymer with the increase in flow rate from 0.5 to 4.0 mL/min. An increase in the flow rate reduces volume of the efficiently treated solution until breakthrough point and therefore decreases the service time of cryogel column, which may be due to the decrease in contact time between the metal ions and the poly(HEMA-MAH)

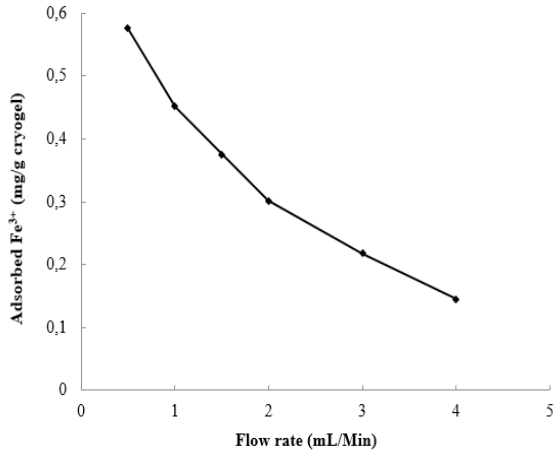

Figure 2. Effect of flow rate on Fe^{3+} adsorption. Fe^{3+} concentration: 10 mg/L.

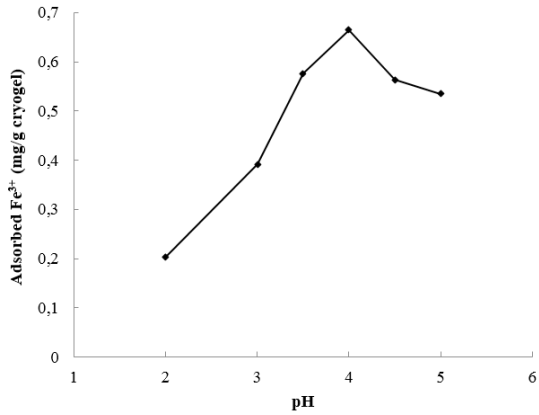

Figure 3. Effect of pH on Fe^{3+} adsorption. Fe^{3+} concentration: 10 mg/L. Flow rate: 0.5 mL/min.

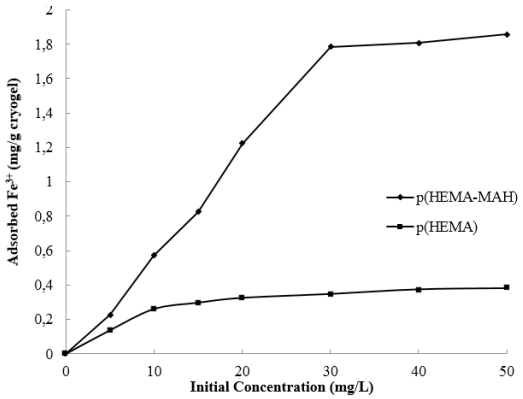

Figure 4. Effect of Fe^{3+} concentration on Fe^{3+} adsorption onto p(HEMA-MAH) cryogel. Flow rate: 0.5 mL/min, pH: 7.4.

cryogel at higher flow rates. These results are also in accordance with the literature [16,27,28]. When flow rate decreases the contact timebetween the metal ions and the poly(HEMA-MAH) cryogel increases. Thus, Fe^{3+} ions have more time to diffuse to the porouse walls of the cryogel and to bind to the ligand, hence a better adsorption capacity is obtained. Additionally, for column operation the cryogel is continuously in contact with a fresh Fe^{3+} solution. Therefore, the concentration in the solution in contact with a given layer of the cryogel in a column is relatively constant.

Effect of pH

The metal ion complexation of polymeric ligands and speciation are highly dependent on the equilibrium pH of the medium. In the present study, the pH was optimized in the range of 2.0 to 5.0. The effect of pH on the Fe^{3+} binding of the poly(HEMA-MAH) cryogel was demonstrated in Figure 3. As seen here, binding of Fe^{3+} ions increased with increasing pH and then reached the maximum at pH 4.0. The increasing pH of the solution favors complexation between the nitrogen groups of MAH in the ion cavities and Fe^{3+} ions. Thus the specific adsorption of Fe^{3+} ions via MAH was pH dependent. Fe^{3+} binding at around pH 2.0 was lower, which may be due to protonation of the functional groups on the MAH structure. High binding at higher pH values indicates that Fe^{3+} ions interact with MAH groups by chelating and ion-exchange.

Adsorption isotherm

Figure 4 displays the effect of equilibrium concentration of Fe^{3+} on the adsorption capacity poly(HEMA-MAH) and p(HEMA) cryogel. The adsorption isotherm of Fe^{3+} on p(HEMA-MAH) cryogel indicates that the adsorption capacity increased when the equilibrium concentration of Fe^{3+} increased. Maximum Fe^{3+} adsorption on poly(HEMA) cryogel was low (about 0.34 mg/g), although Fe^{3+} adsorption on poly(HEMA-MAH) was significant (up to 1.79 mg/g Fe^{3+}).

Two important physico-chemical aspects for evaluation of the adsorption process as a unit operation are the kinetics and the equilibrium of adsorption. Equilibrium modeling of data has been done using the Langmuir and Freundlich isotherms. The Langmuir and Freundlich isotherms are represented by Equatons. (3) and (4), respectively as follows:

$$1/qe = (C_e/q_{max}) + (1/b.q_{max})\,(1/C_e) \quad (3)$$

$$\ln\beta qe = \ln K_F\,\beta + (1/n)\,\ln C_e \quad (4)$$

where b is the Langmuir isotherm constant, K_F is the Freundlich constant, and n is the Freundlich exponent. 1/n is a measure of the surface heterogeneity ranging between 0 and 1, becoming more heterogeneous as its value gets closer to zero. The ratio of qe gives the theoretical monolayer saturation capacity of polymer. The Langmuir adsorption capacity, q_{max} was obtained as 47.39 mg/g from the experimental data. The adsorption isotherm was obtained from batch experiments and the results are given in Table 1.

Table 1. Adsorption constants of Langmuir and Freundlich isoterms.

Langmuir Adsorbsion Isoterm	Freundlich Adsorbsion System
Q_{max} = 47.39 mg/g	K_F = 48.08
b = 301.43 ml/mg	n = 4.16
R^2 = 0.998	R^2 = 0.951

The correlation coefficient (R^2) for Langmuir isotherm model is much higher than that of Freundlich isotherm. Therefore, we can say that this system is more fitted to the Langmuir model follows and monolayer adsorption .

Table 2. Fe^{3+} absorption amount from human plasma.

Sample	Adsorption Capacity (mg/g)
Non-diluted plasma	1.17
Diluted Plasma (1:2,PBS; pH 7.4, NaCl: 0.9%	0.66
Diluted Plasma (1:5,PBS; pH 7.4, NaCl: 0.9%	0.47
Diluted Plasma (1:10,PBS; pH 7.4, NaCl: 0.9%	0.22
Diluted Plasma (1:20,PBS; pH 7.4, NaCl: 0.9%	0.11

Fe^{3+} adsorption from human plasma

Table 2 shows Fe^{3+} adsorption data from human plasma. At this particular instance, lower adsorption capacity of Fe^{3+} was obtained for human plasma diluted with PBS buffer. However, there was a significant adsorption of Fe^{3+} (1.7 mg/g) on the poly(HEMA-MAH) cryogel for non-diluted blood.

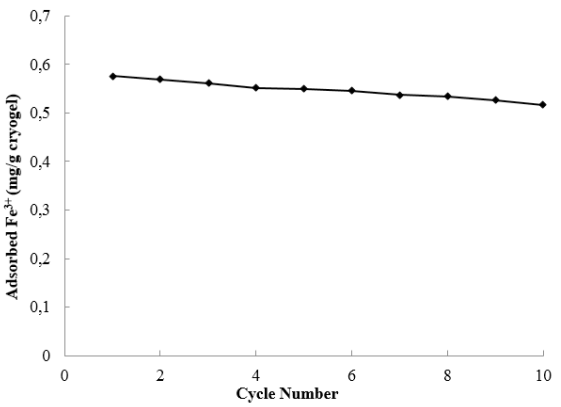

Figure 5. Reusability of the poly(HEMA-MAH) cryogel. Fe^{3+} concentration : 10 mg/L. Flow rate: 0.5 mL/min.

Reusability of Cryogel

Reusability is very important for effectivenes of adsorbent, which means that in designing a test system, attention must be paid to the chemical stability of the affinity adsorbent [29-31]. To show the reusability of the poly(HEMA-MAH) cryogel, adsorption-desorption cycle of Fe^{3+} was repeated 10 times with the same cryogel. As shown in Figure 5, the adsorption capacities for the cryogel did not change noticeably during the adsorption-desorption operations

ACKNOWLEDGEMENTS

This work was supported by of Dicle University Research Fund (DUBAP, Project no:09-FF-76).

References

1. D. Purves, Trace Element Contamination of the Environment, Elsevier Science Publishers BV, Amsterdam, 1985.
2. B. Tang, L. Zhang, J. Zhang, Z. Z. Chen, Y.Wang, Spectrochim. Acta, 60 (2004) 2425.
3. M.C. Rouan, F. Marfil, P. Mangoni, R. Sechaud, H.Humbert, G. Maurer,. J. Chromatogr., B, 755 (2001) 203.
4. S. Ozkara, R. Say, C. Andac, Ind. Eng. Chem. Res., 47 (2008) 7849-7856
5. A. Denizli, B. Salih, E. Piskin, J. Biomater. Sci. Polym. Ed., 9 (1998) 175-187.
6. H. Yavuz, Y. Arica, A. Denizli, J. Appl.Polym. Sci., 82 (2001) 186-194.
7. M. Andac, E. Ozyapi, S. Senel, R. Say, A. Denizli, Ind. Eng.Chem. Res., 45 (2006) 1780-1786.
8. G. Baydemir, M. Andac, N. Bereli, R. Say, A. Denizli, Ind. Eng. Chem. Res., 46 (2007) 2843-2852.
9. M. McCoy, K. Kalghatgi, F.E. Regnier, N. Afeyan, J. Chromatogr. A, 743 (1996) 221–229.
10. E.B. Altıntas, A. Denizli, J. Chromatogr. B, 832 (2006) 216–223.
11. L. Uzun, R. Say, A. Denizli, React. Funct. Polym., 64 (2005) 93–102.
12. K. Thommes, M.R. Kula, Biotechnol. Prog., 11 (1995) 357–367.
13. T.B. Tennikova, B.J. Reusch, J. Chromatogr. A, 1065 (2005) 13–17.
14. N. Bereli, M. Andacβ , G. Baydemir, R. Say, I.Y. Galaev, A. Denizli, J. Chromatogr. A, 1190 (2008) 18–26.
15. F. Yılmaz, N. Bereli, H. Yavuz, A. Denizli, Biochem. Eng. J., 43 (2009) 272–279.
16. H. Alkan, N. Bereli, Z. Baysal, A. Denizli, Biochem. Eng. J., 45 (2009) 201–208.
17. G. Baydemir, N. Bereli, M. Andacβ , R. Say, I.Y. Galaev, A. Denizli, React. Funct. Polym., 69 (2009) 36–42.
18. G. Baydemir, N. Bereli, M. Andacβ , R. Say, I.Y. Galaev, A. Denizli, Colloid Surf. B, 68 (2009) 33–38.
19. L. Uzun, H. Yavuz, R. Say, A. Ersoz, A. Denizli, Ind. Eng. Chem. Res., 43 (2004) 6507–6513.
20. A. Cavus, Z. Baysal, H. Alkan, Colloids and Surfaces B: Biointerfaces, 107 (2013) 84–89
21. V.I. Lozinsky, I.Y. Galaev, F.M. Plieva, I.N. Savina, H. Jungvid, B. Mattiasson, Trends Biotechnol., 21 (2003) 445–451.
22. P. Arvidsson, F.M. Plieva, V.I. Lozinsky, I.Y. Galaev, B. Mattiasson, J. Chromatogr., A 986 (2003) 275–290.
23. V.I. Lozinsky, F.M. Plieva, I.Y. Galaev, B. Mattiasson, Bioseparation 10 (2002) 163–188.

24. W. Noppe, F.M. Plieva, K. Vanhoorelbeke, H. Deckmey, M. Tuncel, A.Tuncel, I.Y. Galaev, B. Mattiasson, J. Biotechnol., 131 (2007) 293–299.
25. P. Arvidsson, F.M. Plieva, I.N. Savina, V.I. Lozinsky, S. Fexby, L. Bulow, I.Y. Galaev, B. Mattiasson, J. Chromatogr. A, 977 (2002) 27–38.
26. B. Garipcan, A. Denizli, Macromol. Biosci., 2 (2002) 135-144.
27. E. Valdman, L. Erijman, F.L.P. Pessoa, S.G.F. Leite, Process Biochem., 36 (2001) 869-873.
28. M. Odabaşı, G. Baydemir, M. Karataş, A. Derazshamshir, J. Appl. Polym. Sci., 116 (2010) 1306-1312.
29. P. Girot, E. Averty, I. Flayeuz, E. Boschetti, J. Chromatogr. B., 808 (2004) 25-33.
30. Z.P. Liang, Y.Q. Feng, Z.Y. Liang, S.X. Mang, Biochemical Eng. Journal., 24 (2005) 65-72.
31. H. Sun, B. Ge, S. Liu, H. Chen, J. Sep. Sci., 31 (2008) 1201.