ELSEVIER

Journal of Chromatography B, 720 (1998) 217–224

JOURNAL OF CHROMATOGRAPHY B

Short communication

Cadmium removal from human plasma by Cibacron Blue F3GA and thionein incorporated into polymeric microspheres

El-Hadi Ibrahim[a], Adil Denizli[a,*], Sema Bektaş[a], Ömer Genç[a], Erhan Pişkin[b]

[a]*Department of Chemistry, Hacettepe University, P.K. 51, Samanpazari 06242, Ankara, Turkey*
[b]*Chemical Engineering Department, Hacettepe University, Ankara, Turkey*

Received 22 June 1998; received in revised form 3 September 1998; accepted 15 September 1998

Abstract

Poly(2-hydroxyethylmethacrylate–ethyleneglycoldimethacrylate) [poly(HEMA–EGDMA)] microspheres carrying Cibacron Blue F3GA and/or thionein were prepared and used for the removal of cadmium ions Cd(II) from human plasma. The poly(HEMA–EGDMA) microspheres, in the size range of 150–200 μm in diameter, were produced by a modified suspension copolymerization of HEMA and EGDMA. The reactive triazinyl dye-ligand Cibacron Blue F3GA was then covalently incorporated into the microspheres. The maximum dye incorporation was 16.5 μmol/g. Then, thionein was bound onto the Cibacron Blue F3GA-incorporated microspheres under different conditions. The maximum amount of thionein bound was 14.3 mg/g. The maximum amounts of Cd(II) ions removed from human plasma by poly(HEMA–EGDMA)–Cibacron Blue F3GA and poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein were of 17.5 mg/g and 38.0 mg/g, respectively. Cd(II) ions could be repeatedly adsorbed and desorbed with both types of microspheres without significant loss in their adsorption capacity.

Keywords: Cadmium; Thionein

1. Introduction

One of the major uses of cadmium is in electroplating coatings. Electroplated cadmium items are used in automobile engine parts, radio and television parts, nuts and bolts. Cadmium alloys such as cadmium–nickel, cadmium–silver–copper, and cadmium–silver are the next most commonly used cadmium products. Cadmium–silver alloy and solder are used in jewellery manufacture. The third largest use of cadmium is in solders in combination with copper, lead, tin, zinc, or silver [1].

Inhalation and ingestion are two major routes of exposure to cadmium compounds. Cadmium vapor and fumes account for most inhalation exposures, but dusts of respirable size (less than 10 microns) of cadmium compounds can also be inhaled. It is estimated from animal experiments that 10% to 40% of inhaled cadmium is absorbed whereas under healthy conditions, it is estimated that 2% to 6% of ingested cadmium is absorbed. The absorption of cadmium from the gastrointestinal tract may reach 20% of a given dose. After absorption, cadmium is transported by the blood to the liver where it is bound to metallothionein, a low-molecular-weight protein synthesized there. The metallothionein–cadmium complex is transported via the blood to the

*Corresponding author. Tel. and fax: +90-312-2992-163; e-mail: denizli@eti.cc.hun.edu.tr

0378-4347/98/$19.00
PII: S0378-4347(98)00441-1

kidney where it is filtered by the glomeruli, but then reabsorbed by proximal tubular cells and subsequently degraded. The tubular cells are thought to be able to also synthesize metallothionein capable of binding cadmium, and a new complex is formed protecting the cell from the free cadmium ion. Cadmium toxicity is thought to occur when the metallothionein-producing ability of the proximal tubular cells is exceeded [2].

The highest concentrations of cadmium are found in the kidney and the liver. In the absence of significant kidney damage and loss of proximal tubular cells, the concentration of cadmium in the kidney can be fifteen-times that of the liver [2]. Cadmium is also distributed to the bone, testes, pancreas, spleen, various endocrine organs, brain, and muscle tissue [3]. Cadmium is excreted in milk [4]. The elimination of cadmium from the body is slow, with a half-life of 7 to 30 years. Thus, cadmium accumulates in the body with increasing duration of exposure [2,5].

The chronic toxicity of cadmium compounds includes kidney damage with proteinuria of low-molecular-weight molecules. An epidemic of Japanese itai-itai (ouch, ouch) disease is believed to be the result of chronic ingestion of Cd(II) (via environmental pollution), with altered renal tubular function, impaired regulation of calcium and phosphorus, manifesting bone demineralization, osteomalacia, and pathological fractures [1,4,6].

No specific treatments for acute or chronic cadmium poisoning are available. However, in addition to supportive therapy and hemodialysis, heavy metal poisoning is often treated with a chelating agent. Different chelating agents that are available commercially for the treatment of cadmium poisoning are British antilewisite and calcium disodium EDTA but there is histopathological evidence of the toxicity in animals when calcium disodium EDTA is utilized [1,6,7].

We have recently developed poly(2-hydroxyethylmethacrylate – ethyleneglycoldimethacrylate) [poly(HEMA–EGDMA)] based microspheres carrying different bio-ligands (i.e., Protein A, DNA, heparin) and chelating dyes (Cibacron Blue F3GA, Congo Red, Alkali Blue 6B) for the removal of toxic substances (pathogenic antibodies, cholesterol and bilirubin) from human plasma and of metal ions (i.e., aluminum and iron) from both human plasma and aqueous media [8–13]. In this study, we have attempted to use these new chelate-forming microspheres for Cd(II) poisoning. Reactive dye-ligand Cibacron Blue F3GA was covalently attached onto the poly(HEMA–EGDMA) microspheres. Then a metallopeptide (i.e., thionein) was bound to the Cibacron Blue F3GA-incorporated microspheres. They were used for cadmium removal from human plasma contaminated with Cd(II). This communication presents data on the cadmium adsorption capacities and rates of these new chelate-forming polymer microspheres carrying Cibacron Blue F3GA and thionein.

2. Experimental

2.1. Preparation of Cibacron Blue F3GA-incorporated microspheres

HEMA was purchased from Sigma (St. Louis, MO, USA) and purified by passing through active alumina. EGDMA supplied from Rohm and Haas (Darmstadt, Germany) was purified by the same procedure. Benzoyl peroxide (BPO) and polyvinylalcohol (PVAL) (number-average molecular weight: 100 000, 98% hydrolyzed; Aldrich, Rockford, IL, USA) were utilized as the initiator and the stabilizer, respectively, and were used without further purification. Toluene (Merck, Darmstadt, Germany) was selected as the diluent and used as received. Dispersion medium was distilled water.

Poly(EGDMA–HEMA) copolymer microspheres were produced by a modified suspension polymerization. The reactor was flushed by bubbling nitrogen and then was sealed. The EGDMA/HEMA ratio, the amounts of BPO, PVAL, toluene and the agitation speed were 8 ml/4 ml, 0.06 g, 0.2 g, 12 ml and 600 rpm, respectively. Polymerizations were carried out at 65°C for 4 h and at 90°C for 2 h. In order to remove the diluent (i.e., toluene), unreacted monomers and the physically adsorbed PVAL (i.e., stabilizer) molecules, an extensive cleaning procedure was applied as follows: the microspheres were separated from the aqueous phase by filtration of the polymerization mixture on 5 μm filter papers. The microspheres were first washed with water, and the

polymeric aggregates were removed by filtration (sieving). The microspheres were dispersed in water, and sonicated for 30 min in an ultrasonic bath (200 W, Bransonic 200, USA). The water phase was removed, the microspheres were resuspended in ethyl alcohol and sonicated for 1 h. Ethyl alcohol was removed and the microspheres were transferred into toluene, and were stirred about 30 min. Toluene was removed then microspheres were left within ethyl alcohol for about 30 min. Ethyl alcohol was removed, and the microspheres were washed with distilled water once again, then they were filtered and dried in a vacuum oven at 60°C for 48 h. Of note about 90% of the microspheres obtained with the recipe and conditions given above were in the size range of 150–200 μm (i.e., the swollen size).

Cibacron Blue F3GA was obtained from Sigma and used as received. It was covalently coupled to the poly(HEMA–EGDMA) microspheres via the nucleophilic reaction between the chloride of its triazine ring and the hydroxyl groups of the HEMA molecules under alkaline conditions. Briefly, 3 g of poly(HEMA–EGDMA) microspheres were magnetically stirred (at 400 rpm) in a sealed reactor at a constant temperature of 80°C for 4 h with 100 ml of the Cibacron Blue F3GA aqueous solution containing 4.0 g NaOH. Under these conditions, a chemical reaction takes place between the group of the Cibacron Blue F3GA having chloride and the hydroxyl group of the HEMA, with the elimination of NaCl, resulting in the coupling of Cibacron Blue F3GA to the poly(HEMA–EGDMA) microspheres. The initial concentration of the Cibacron Blue F3GA in the medium was 3.0 mg/ml. After incubation the solution was cooled down to room temperature and the Cibacron Blue F3GA-incorporated microspheres were first filtered, and then washed with distilled water and methanol several times until all the physically adsorbed Cibacron Blue F3GA molecules were removed.

The amount of Cibacron Blue F3GA coupled onto microspheres was evaluated by using an elemental analysis instrument (Leco, CHNS-932, USA), by measuring the nitrogen and sulfur stoichiometry. The leakage of the Cibacron Blue F3GA from the microspheres was followed by treating the microspheres with fresh human plasma samples for 24 h at room temperature. Cibacron Blue F3GA released after this treatment was measured in the liquid phase spectrophotometrically at 630 nm. The modified microspheres were stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

2.2. Thionein adsorption studies

Adsorption of thionein (acetate salt, Lys–Cys–Thr–Cys–Cys–Ala, molecular mass: 627.8; Sigma) on the Cibacron Blue F3GA-incorporated poly(HEMA–EGDMA) microspheres was studied in a batch system. The pH of the adsorption medium was adjusted to 7.4 by using 0.1 *M* phosphate buffer (K_2HPO_4–KH_2PO_4). The initial concentration of thionein in the aqueous phase was varied between 0.1–1.0 mg/ml. In a typical adsorption experiment, thionein was dissolved in 10 ml of buffer solution containing microspheres (100 mg). The adsorption experiments were carried out for 1 h (i.e., equilibrium time) at 25°C at a stirring rate of 50 rpm. At the end of the equilibrium period, the microspheres were separated from the solution. The thionein adsorption capacity was determined by measuring the difference in the initial and the final concentrations of thionein within the adsorption medium spectrophotometrically (λ: 280 nm).

2.3. Cd(II) removal from human plasma

Cd(II) removal from human plasma by Cibacron Blue F3GA and thionein carrying microspheres was also studied batchwise. The blood samples were obtained from a healthy donor at the University Hospital (Hacettepe, Ankara, Turkey). Blood samples were centrifuged at 500 *g* for 30 min at room temperature to separate plasma. A 10 ml volume of plasma was overloaded with 2 ml of cadmium solution containing different amounts of Cd(II) to obtain different initial Cd(II) concentrations. Then, Cd(II)-overloaded human plasma was incubated with 100 mg of the microspheres at 25°C for 1 h. The amounts of Cd(II) removed (i.e., final plasma Cd(II) concentration) were determined by using a graphite furnace atomic absorption spectrophotometer (GBC 932 AA, Australia). All instrumental conditions were optimized for maximum sensitivity as described by the manufacturer. For each sample, the mean of ten atomic absorption spectrophotometer measurements

was recorded. The amount of adsorption per unit mass of the microspheres were calculated by using the following expression.

$$Q = [(C_0 - C) \cdot V]/m \quad (1)$$

where, Q is the amount of Cd(II) ions adsorbed onto unit mass of the microspheres (mg/g); C_0 and C are the concentrations of the Cd(II) ions in the initial and final in the plasma for certain period of time, respectively (mg/ml); V is the volume of the plasma (ml); and m is the amount of poly(HEMA–EGDMA) microspheres used (g).

2.4. Desorption and repeated use

Desorption of Cd(II) ions was studied in buffer solutions containing 25 m*M* EDTA. The microspheres loaded with Cd(II) ions were placed in this desorption medium and stirred (at a stirring rate of 600 rpm) for 2 h at room temperature. The final Cd(II) concentration in the aqueous phase was determined by an atomic absorption spectrophotometer. The desorption ratio was calculated from the amount of Cd(II) ions adsorbed on the microspheres and the final Cd(II) concentration in the desorption medium.

In order to verify the reusability of the dye-incorporated poly(HEMA–EGDMA) microspheres, the Cd(II) adsorption–desorption procedure was repeated five times by using the same sample of polymeric sorbent. It should be also noted that, after desorption of Cd(II) ions with EDTA, adsorbed protein molecules from human plasma (i.e., especially albumin via Cibacron Blue F3GA) were eluted with 0.1 *M* KSCN (pH 8.0).

3. Results and discussion

In this study, we have prepared the poly(HEMA–EGDMA)–Cibacron Blue F3GA and poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein microspheres as specific sorbent for Cd(II) removal from human plasma. The poly(HEMA–EGDMA) microspheres were selected as the polymer matrix which carries functional hydroxyl groups for further modification. Cibacron Blue F3GA was used as the dye-ligand for specific binding of thionein molecules and Cd(II) ions. Dye leakage is a serious problem in dye-ligand chromatography. However, it is reported that Cibacron Blue F3GA has no adverse effect on biochemical systems [14]. On the other hand, all commercial reactive dyes (including Cibacron Blue F3GA) contain various impurities which may affect their biochemical and related use [14–16]. Reactive dyes have been purified by a number of chromatographic procedures such as thin-layer chromatography, high-performance liquid chromatography and column chromatography on Silica gel or Sephadex [17]. However, it is suggested that purification of reactive dyes is necessary only when free dyes are used [15]. In cases where immobilized dyes are used, purification of the dye before its immobilization is not likely to be necessary because few of the contaminants will be covalently attached to the support matrix, and the proper washing of the matrix should remove them [15].

Cibacron Blue F3GA molecules were covalently attached to the polymeric microspheres. It is accepted that ether linkages are formed between the reactive triazine ring of the dye and the hydroxyl groups of the sorbent. Elemental analysis of the unmodified and Cibacron Blue F3GA-attached poly(HEMA–EGDMA) microspheres was performed, and the attachment of the dye was found as 16.5 μmol/g from the nitrogen stoichiometry. Cibacron Blue F3GA leakage was also investigated in the presence of human plasma. Cibacron Blue F3GA leakage was not observed from any of the dye-attached microspheres, even after a long period of time (more than 2 months). Preparation and characterization details of both the unmodified and Cibacron Blue F3GA-attached poly(HEMA–EGDMA) microspheres are given elsewhere [18,19].

3.1. Thionein adsorption

Metallothioneins are low-molecular-mass, cysteine-rich proteins that bind metals such as Zn(II), Cd(II) and Hg(II). Thionein is a small metallopeptide (molecular mass: 627.8) composed of six amino acids, three of them being cysteine residues. The sulfhydryl groups in the thionein permit it to form metal clusters including Cd(II), Zn(II), and some other divalent metal ions [20]. This makes it an

attractive ligand for the reactions of various metal ions and electrophilic agents, including platinum complexes for different medical applications [21].

Fig. 1 shows adsorption of thionein molecules onto the plain polymer and poly(HEMA–EGDMA)–Cibacron Blue F3GA microspheres. As seen in Fig. 1, a negligible amount of thionein was adsorbed nonspecifically on the plain poly(HEMA–EGDMA) microspheres, which was 0.19 mg/g, while Cibacron Blue F3GA attachment significantly increased the thionein adsorption capacity of the microspheres (up to 14.3 mg/g). This increase in the thionein adsorption capacity may have resulted from a cooperative effect of different interaction mechanisms such as hydrophobic, electrostatic and hydrogen bonding caused by the acidic groups and aromatic structures on the Cibacron Blue F3GA and by the side chains of amino acids on the thionein molecules. It should be mentioned that Cibacron Blue F3GA is not very hydrophobic overall, but it has planar aromatic surfaces that prefer to interact with hydrophobic groups in the thionein structure (e.g., methyl groups in both threonine and alanine structures and methylene groups in the lysine structure).

In order to test the dye attachment conditions on thionein adsorption, poly(HEMA–EGDMA) microspheres exposed to dye-attachment conditions without Cibacron Blue F3GA, were also used for thionein adsorption. The thionein adsorption observed was in the same amount (0.19 mg/g) as that detected for plain microspheres. It can be said that the cross-linked poly(HEMA–EGDMA) microspheres are impervious to the harsh alkaline conditions used for Cibacron Blue F3GA-attachment. No carboxylic moieties are likely to be formed by hydrolysis of the ester linkage of HEMA molecules in the polymer chain and hence they don't contribute to thionein adsorption. Therefore, it can be concluded that thionein molecules were adsorbed specifically on the microspheres via attached Cibacron Blue F3GA molecules.

3.2. Cd(II) removal from human plasma

Fig. 2 illustrates the adsorption of Cd(II) ions onto the plain poly(HEMA–EGDMA), poly(HEMA–EGDMA)–Cibacron Blue F3GA and poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein microspheres. As seen in Fig. 2, Cd(II) adsorption increased with an increase in the initial concentration of Cd(II) ions within the aqueous phase. Cibacron Blue F3GA attachment significantly increased the Cd(II) adsorption capacity of the microspheres (up to 17.5 mg/g) as reported earlier [18,22].

Thionein incorporation resulted in further increases in the Cd(II) adsorption capacity of the microspheres (up to 38.0 mg/g polymer). Although

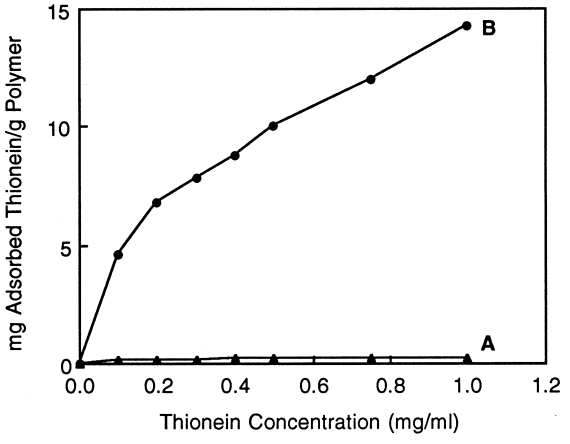

Fig. 1. Effect of initial thionein concentration on thionein adsorption: (A) poly(HEMA–EGDMA); (B) poly(HEMA–EGDMA)–Cibacron Blue F3GA; Cibacron Blue F3GA loading = 16.5 μmol/g; pH = 7.4; T = 25°C.

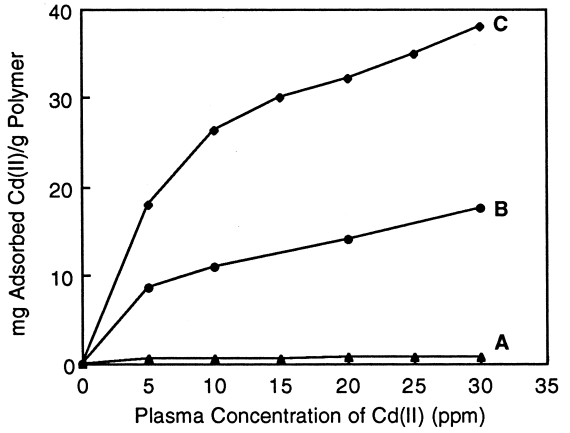

Fig. 2. Cd(II) removal from human plasma: (A) poly(HEMA–EGDMA); (B) poly(HEMA–EGDMA)–Cibacron Blue F3GA; (C) Poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein; Cibacron Blue F3GA loading = 16.5 μmol/g; thionein loading = 14.3 mg/g; T = 25°C.

different interaction mechanisms of metal ions with proteins and polypeptides have been proposed [23], the macromolecular recognition of proteins with metal ions remains unclear. In one proposed mechanism, the formation of a coordination complex between immobilized protein and metal ions is considered to be the major binding mode. However, more than one type of interaction mechanism is operational [24]. The major functional groups on protein contributing toward the interaction with metal ions consist of the histidine residues and the occurrence of sulfydryl groups. While maintaining a free cysteine residue in a natural protein is rare [25], the exposed histidine residues are dominant binding residues in protein–metal ion adsorption [26]. In the present case, thionein contains three cysteines and these amino acids are dominant in the Cd(II) adsorption.

Notice that the amount of Cd(II) ions adsorbed from aqueous solutions (45.2 mg/g) onto the poly-(HEMA–EGDMA)–Cibacron Blue F3GA–thionein microspheres was higher than the amounts adsorbed from the human plasma, which was 38.0 mg/g. This may be due to the competitive adsorption by plasma constituents such as proteins, especially albumin.

Fig. 3 gives adsorption rates of Cd(II) ions onto the poly(HEMA–EGDMA)–Cibacron Blue F3GA and poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein microspheres from human plasma. High adsorption rates were observed at the beginning of adsorption process and then plateau values were achieved. Cd(II) adsorption onto Cibacron Blue F3GA–thionein microspheres was much faster than to Cibacron Blue F3GA-incorporated microspheres, due to immobilized thionein molecules. Equilibrium was achieved in 10 min for thionein carrying microspheres.

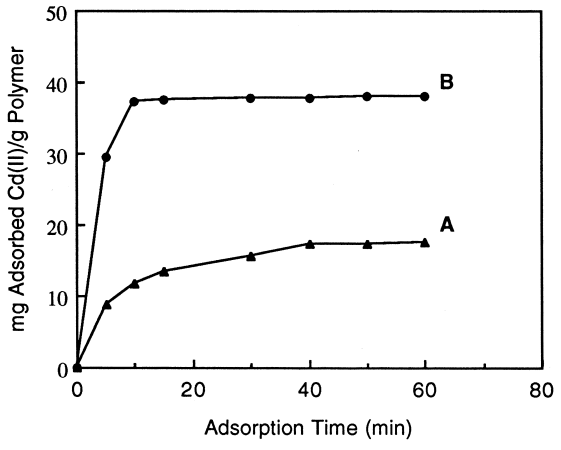

Fig. 3. Cd(II) removal rate from human plasma: (A) poly-(HEMA–EGDMA)–Cibacron Blue F3GA; (B) poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein; Cibacron Blue F3GA loading = 16.5 μmol/g; thionein loading = 14.3 mg/g; Cd(II) initial concentration = 30 ppm; $T = 25$°C.

Hitherto, different affinity sorbents with a wide range of adsorption capacities for cadmium ions have been reported. Shreedhara-Murthy and Ryan found 3.9–14.4 mg cadmium/g removal by cellulose–dithiocarbamate resins [27]. Roozemond et al. reported 40 mg cadmium/g with pyrazole-containing poly(styrene-divinyl-benzene) sorbents [28]. Konishi et al. reported 4.5 mg cadmium/g with alginic acid gels [29]. Denizli et al. used Alkali Blue 6B-attached poly(EGDMA–HEMA) sorbents, in which the maximum adsorption capacity was 5.5 mg cadmium/g [18]. Dev and Rao reported 15.5 mg cadmium/g adsorption capacity for polystyrene-vinylbenzene macroreticular resin functionalized with bis-(*N*,*N*′-salicylidene)1,3-propane-diamine [30]. Liu et al. used *N*-(hydroxymethyl)thioamide chelating resin and they reported approximately 22 mg per gram polymer adsorption capacity for cadmium ions [31]. We conclude that the poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein microspheres are promising for the removal of Cd(II) ions from human plasma.

3.3. Desorption of Cd(II) ions

Desorption of Cd(II) ions from the poly(HEMA–EGDMA)–Cibacron Blue F3GA and poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein microspheres was also studied in a batch experimental set-up. The microspheres loaded with different amounts of Cd(II) ions were placed in the desorption medium containing 25 m*M* EDTA, at pH 4.9, and the amount of Cd(II) desorbed in 1 h was determined. The desorption ratio was calculated by using the following expression:

$$\text{Desorption ratio (\%)} = \frac{\text{amount of Cd(II) released}}{\text{amount of Cd(II) adsorbed}} \times 100 \quad (2)$$

Table 1
Desorption of Cd(II) ions

Cd(II) concentration (ppm)	Desorption ratio (%)	
	I[a]	II[b]
5.0	98.7	95.6
10.0	96.9	97.6
15.0	99.3	98.5
20.0	99.2	99.2
25.0	98.7	99.4
30.0	98.5	99.3

[a] Poly(HEMA–EGDMA)–Cibacron Blue F3GA.
[b] Poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein.

Table 1 gives the desorption data of Cd(II) ions. Up to 99.4% of the adsorbed Cd(II) ions was desorbed. Note that there was no thionein release in this case which shows that thionein molecules are attached to Cibacron Blue F3GA molecules on the microspheres by strong ionic and hydrophobic interactions. This means that EDTA scavenges Cd(II) ions from its chelates with Cibacron Blue F3GA–thionein molecules. In addition, when EDTA was used as a desorption agent, the coordination spheres of chelated metal ions is disrupted and subsequently the thionein molecule changes its conformation and releases the bound Cd(II) ions. With the desorption data given above we concluded that EDTA is a suitable desorption agent, and allows repeated use of the affinity sorbents used in this study.

In order to verify the reusability of the poly-(HEMA–EGDMA)–Cibacron Blue F3GA and poly-(HEMA–EGDMA)–Cibacron Blue F3GA–thionein microspheres, the adsorption–desorption cycle was repeated five times using the same polymeric microspheres. As seen in Fig. 4, there was no significant loss in the adsorption capacity of both types of microspheres. The cadmium adsorption capacity decreased only 10% after five cycles.

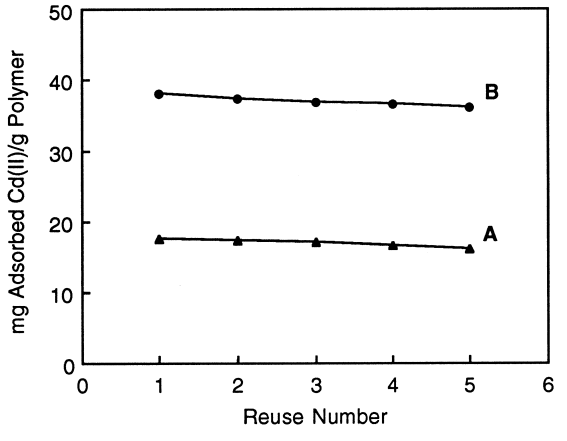

Fig. 4. Repeated use of microspheres: (A) poly(HEMA–EGDMA)–Cibacron Blue F3GA; (B) poly(HEMA–EGDMA)–Cibacron Blue F3GA–thionein; Cibacron Blue F3GA loading = 16.5 μmol/g; thionein loading = 14.3 mg/g; Cd(II) initial concentration = 30 ppm; and $T = 25°C$.

4. Conclusions

The poly(HEMA–EGDMA) microspheres, in the size range of 150–200 μm, were prepared by a modified suspension copolymerization of HEMA and EGDMA. A reactive dye-ligand, Cibacron Blue F3GA, was then successfully incorporated into these microspheres to reach a load up to 16.5 μmol Cibacron Blue F3GA per gram of the microspheres, which resulted in Cd(II) ions adsorption of 17.5 mg/g. Further modification by incorporation of thionein molecules to these dye-loaded microspheres caused a significant increase in the Cd(II) adsorption capacity (up to 38.0 mg/g). Adsorption of Cd(II) ions onto the microspheres carrying both Cibacron Blue F3GA and thionein from human plasma was less than that adsorbed from the aqueous solutions, but it was still quite significant. Successful desorption ratios (more than 95% of the adsorbed Cd(II) ions) were achieved by using 25 m*M* EDTA. It was possible to reuse these novel metalloprotein affinity sorbents without significant losses in their adsorption capacities.

References

[1] H.E. Stokinger, in: G.D. Clayton, F.E. Clayton (Eds.), Patty's Industrial Hygiene and Toxicology, Wiley, New York, 1981, p. 1563.
[2] L. Friberg and C.G. Elinder (Eds.), Encyclopedia of Occupational Health and Safety, 3rd ed, International Labor Organization, Geneva, Switzerland, 1985.
[3] J.S. Lee and K.L. White (Eds.), Environmental and Occupational Medicine, Little Brown, Boston, 1983, p. 1563.

[4] J.K. Dunnick, B.A. Fowler, in: H.G. Seiler, H. Siger (Eds.), Handbook on Toxicity of Inorganic Compounds, Marcel Dekker, New York, 1988, p. 155.
[5] L.R. Goldfrank, N.E. Flomenbaum, N.A. Lewin, R.S. Weisman and M.A. Howland (Eds.), Goldfrank's Toxicologic Emergencies, 4th ed, Appleton and Lange, Norwalk, 1990.
[6] L. Friberg, G.F. Nordberg and V.B. Vouk (Eds.), Handbook of the Toxicology of Metals, Elsevier, North-Holland, 1979, p. 355.
[7] L. Friberg, M. Piscator, G.F. Nordberg, T. Kjellstrom, Cadmium in the Environment, 2nd ed, CRC Press, Cleveland, 1974.
[8] A. Denizli, E. Pişkin, J. Chromatogr. B 666 (1995) 215.
[9] A. Denizli, A.Y. Rad, E. Pişkin, J. Chromatogr. B 668 (1995) 13.
[10] A. Denizli, E. Pişkin, J. Chromatogr. B 670 (1995) 157.
[11] A. Denizli, B. Salih, C. Kavakli, E. Pişkin, J. Chromatogr. B 698 (1997) 89.
[12] A. Denizli, M. Kocakulak, E. Pişkin, J. Chromatogr. B 707 (1998) 25.
[13] A. Denizli, B. Salih, E. Pişkin, J. Biomater. Sci. Polym. Ed. 9 (1998) 175.
[14] B. Weber, K. Willeford, J. Moe, D. Piskiewicz, Biochem. Biophys. Res. Commun. 86 (1979) 252.
[15] C.V. Stead, in: Y.D. Clonis, A. Atkinson, C.J. Bruton, C.R. Lowe (Eds.), Reactive Dyes in Protein and Enzyme Technology, Macmillan, Basingstoke, 1987, p.13.
[16] P.M. Boyer, J.T. Hsu, Adv. Biochem. Eng. 49 (1993) 1.
[17] C.R. Lowe, in: A. Wiseman (Ed.), Topics in Enzyme and Fermentation Technology, Ellis Horwood, Chichester, 1984, p. 78.
[18] A. Denizli, B. Salih, E. Pişkin, React. Functl. Polym. 29 (1996) 11.
[19] A. Denizli, M. Kocakulak, E. Pişkin, J. Appl. Polym. Sci. 68 (1998) 373.
[20] A.H. Robbins, D.E. McRee, M. Williamson, S.A. Collect, N.H. Xuong, W.F. Purey, B.C. Wang, C.D. Stout, J. Mol. Biol. 211 (1991) 1269.
[21] K.B. Nielson, C.L. Atkin, D.R. Winge, J. Biol. Chem. 260 (1985) 5342.
[22] A. Denizli, D. Tanyolaç, B. Salih, A. Özdural, J. Chromatogr. A 793 (1998) 47.
[23] E. Sulkowski, Trends Biotechnol. 3 (1985) 1.
[24] E.S. Hemdan, J. Porath, J. Chromatogr. 323 (1985) 255.
[25] F.H. Arnold, Biotechnology 9 (1991) 151.
[26] W.Y. Chen, C.F. Wu, C.C. Liu, J. Colloid Interface Sci. 180 (1996) 135.
[27] R.S. Shreedhara-Murty, D.E. Ryan, Anal. Chim. Acta 140 (1982) 163.
[28] D.A. Roozemond, F.D. Hond, J.B.J. Veldhuis, H. Strassdeit, W.L. Driessen, Eur. Polym. J. 24 (1988) 867.
[29] Y. Konishi, S. Asai, Y. Midoh, M. Oku, Sep. Sci. Technol. 228 (1993) 1691.
[30] K. Dev, G.N. Rao, Talanta 43 (1996) 451.
[31] C.Y. Liu, H.T. Chang, C.C. Hu, Inorg. Chim. Acta 172 (1990) 151.