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Süleyman Aşır[a]; Lokman Uzun[a]; Deniz Türkmen[a]; Rıdvan Say[b]; Adil Denizli[a]
[a] Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey [b] Department of Chemistry, Anadolu University, Eskisehir, Turkey

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Süleyman Aşır, Lokman Uzun, and Deniz Türkmen
Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey

Rıdvan Say
Department of Chemistry, Anadolu University, Eskisehir, Turkey

Adil Denizli
Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey

Abstract: Molecular recognition based separation systems have received much attention because of their high selectivity for target molecules. Molecular imprinting has been recognized as a promising technique for the development of affinity adsorbents. Molecularly imprinted polymers (MIP) are easy to prepare, stable, inexpensive, and capable of molecular recognition. Cadmium is a carcinogenic and mutagenic element. The limit value of cadmium in blood should be no higher than 50 pg/L when exposure to cadmium is unavoidable in industry. There is no specific treatment available for acute or chronic metal poisoning. Besides supportive therapy and hemodialysis, metal poisoning is often treated with commercially available chelating agents including EDTA and dimercaprol. However, there is histopathological evidence for increased toxicity in animals when these agents are utilized. The aim of this study is to prepare superporous ion-imprinted polymer monolith which can be used for the selective removal of Cd^{2+} ions from Cd^{2+}-overdosed human plasma. N-methacryloly-(L)-cysteinemethylester (MAC) was chosen as the complexing monomer. In the first step, MAC synthesized by using methacryloyl chloride and cysteine. Cd^{2+} was complexed with MAC monomer and the Cd^{2+}-imprinted

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Address correspondence to Adil Denizli, Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey. E-mail: denizli@hacettepe.edu.tr

poly(HEMA-MAC) monoliths were synthesized by bulk polymerization. After that, Cd^{2+} ions were removed by 0.1 M thiourea and 0.1 M HNO_3 solutions, respectively. Cd^{2+}-imprinted poly(HEMA-MAC) monoliths had a specific surface area of 226.8 m^2/g and the swelling ratio was determined to be 76%. According to the elemental analysis results, monoliths contain approximately 58.3 μmol/g of MAC. The maximum adsorption capacity for Cd^{2+} ions was 26.6 μmol/g of the dry weight of monolith. The adsorption capacity decreased significantly from 23.25 μmol/g to 3.08 μmol/g polymer with the increase of the flow-rate from 1 mL/min to 4 mL/min. The Cd^{2+}-imprinted poly(HEMA-MAC) monolith could be used many times without decreasing their adsorption capacities significantly.

Keywords: Ion imprinting, molecular recognition, cadmium removal, metal detoxification, affinity binding

INTRODUCTION

Molecular imprinting is a technology to create recognition sites in a macromolecular matrix using a molecular template (1). In other words, both the shape image of the target and alignment of the functional moieties to interact with those in the target, are memorized in the macromolecular matrix for the recognition or separation of the target during formation of the polymeric materials themselves (2). Molecularly imprinted polymers (MIP) are easy to prepare, stable, inexpensive, and capable of molecular recognition. Therefore, MIPs can be considered as artificial affinity media. Molecular recogition-based separation techniques have received much attention in various fields because of their high selectivity for target molecules. Three steps are involved in ion-imprinting process:

i. Complexation of template (i.e., metal ions) to a polymerizable ligand,
ii. Polymerization of this complex,
iii. Removal of template after polymerization. In the ion-imprinting process, the selectivity of a polymeric adsorbent is based on the specificity of the ligand, on the coordination geometry and coordination number of the ions, on their charges and sizes (3–8).

Numerous studies describing such methodology were carried out in order to adsorb metal ions (9–13) but no studies concerning metal removal from human plasma using ion-imprinting materials were reported in the literature.

Cadmium is a toxic transition heavy metal of continuing occupational and environmental concern with a wide variety of adverse effects (14). Cadmium has an extremely long biological half-life that essentially makes it a cumulative toxin. The chronic toxicity of cadmium compounds includes kidney damage with proteinuria of low-molecular-weight molecules. An epidemic of Japanese itai-itai disease also 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 (15, 16). There are several sources of human exposure to cadmium, including employment in primary metal industries and consumption of tobacco products. The average blood level of cadmium in adults without excessive or occupational exposure is about 10 mg/L, as is the amount excreted in the urine in the adult population. Blood an/or urinary cadmium excretions exceeding 50 mg/L, generally indicate excessive exposure (17). To date there are no proven effective treatments for chronic cadmium intoxication. Besides supportive therapy and hemodialysis, metal poisoning is often treated with a chelating agent. Different chelating agents that are available commercially for the treatment of cadmium poisoning are British anti lewisite and calcium disodium EDTA. These chelation agents are contraindicated for cadmium as the large concentration of cadmium brought to the kidneys may cause damage. There is histopathological evidence for increased toxicity in animals when calcium disodium EDTA is utilized (15, 16). Recently, one of the most promising techniques for blood detoxification is extracorporeal affinity adsorption. So far, only a few affinity adsorbents were reported for metal detoxification (18–22).

Conventional packed-bed columns possess some inherent limitations such as the slow diffusional mass transfer and the large void volume between the beads (23). Although some new stationary phases such as the non-porous polymeric beads (24) and perfusion chromatography packings are designed to resolve these problems, these limitations cannot be overcome in essence (25). Recently, monolith materials are considered as a novel generation of stationary phases in separation science because of their easy preparations, excellent flow properties, and high performances compared to conventional beads for the separation of biomolecules (26, 27). Porous monoliths are a very good alternative to bio-separation with many advantages. Several potential advantages of monoliths are large surface area, short diffusion path and low pressure drop for both adsorption and elution.

In this study, ion-imprinted polymer monolith was used for the selective removal of Cd^{2+} ions from human plasma. N-methacryloly-(L)-cysteinemethylester (MAC) was used as the metal complexing monomer. Cd^{2+}-imprinted poly(hydroxyethyl methacrylate-N-methacryloly-(L)-cysteinemethylester) monoliths were produced by bulk polymerization. Poly(2-hydroxyethyl methacrylate) (PHEMA) was selected as the basic matrix by considering properties which make it useful for possible extracorporeal therapy, including hydrophilic character, good blood-compatibility, minimal non-specific protein interactions, high chemical and mechanical stability, and resistance toward microbial and enzymatic attacks [28–32]. After removal of template (i.e., Cd^{2+} ions), the ion-imprinted monolith was used for the removal of Cd^{2+} ions from human plasma. Cd^{2+} adsorption and selectivity studies of Cd^{2+} versus other metal ions which are Zn^{2+} and Pb^{2+} are reported here. Finally, repeated use of the ion-imprinted monoliths is also discussed.

EXPERIMENTAL

Materials

Hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) were obtained from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4°C until use. Cysteinemethylester dihydrochloride and methacryloyl chloride were supplied by Sigma (St Louis, USA) and used as received. Potassium persulphate (KPS) was obtained from Surechem Products (England). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany). All water used in the adsorption experiments was purified using a Barnstead (Dubuque, IA) 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 MAC

For the synthesis of N-methacryloly-(L)-cysteinemethylester (MAC) monomer, the following experimental procedure was applied: 5.0 g of cysteinemethylester dihydrochloride and 0.2 g of $NaNO_2$ were dissolved in 30 mL of K_2CO_3 aqueous solution (5%, v/v). This solution was cooled to 0°C. 4.0 mL of methacryloyl chloride was poured slowly into this solution under nitrogen atmosphere and then this solution was stirred magnetically at room temperature for 2 h. At the end of this period, the pH of this solution was adjusted to 7.0 and then was extracted with ethylacetate. The aqueous phase was evaporated in a rotary evaporator. The residue (i.e., MAC) was crystallized in ethanol and ethylacetate.

Preparation of Cd^{2+}-imprinted Poly(HEMA-MAC) Monolith

In the first part, MAC-Cd^{2+} complex was prepared. In order to prepare MAC-Cd^{2+} complex, MAC (0.378 g, 2.0 mmol) in ethanol and treated with cadmium nitrate ($Cd(NO_3)_2 \cdot 4H_2O$) (0.163 g, 1.0 mmol) at room temperature with continuous stirring for 24 h. Then the formed metal-comonomer complex used in the polymerization procedure. Poly(HEMA-MAC)] monolith was prepared by an in-situ polymerization within a glass tube using potassium persulfate as the initiator. Toluene was included in the polymerization recipe as the diluent (as a pore former). Initiator potassium persulphate (KPS) (100 mg) was dissolved in the mixture of monomer (HEMA: 2.0 ml, EDMA: 1.0 ml, MAC: 1.0 g, hydroxyethylpiperazine ethanesulfonic acid (HEPES): 2.0 ml and porogenic diluent (toluene: 1.0 ml). The monomer mixture was sonicated to obtain a

clear solution and was then purged with nitrogen for 15 min. The glass tube (20×10 mm inside diameter) was filled with the above mixture and then sealed. The polymerization was allowed to proceed at 75°C for 45 min. The tube was then attached to a chromatographic system. Ethyl alcohol (50 ml) and water (50 ml) were pumped through the column at a flow rate of 1.0 ml/min to remove the unreacted monomers and porogenic diluents present in the monolith after the polymerization was completed. From the resulting polymer, Cd^{2+} ions were removed by treatment with 0.1 M acidic thiourea. The imprinted monolith was washed with acidic thiourea solution for 48 h at room temperature. The template free polymers were cleaned again with 0.1 M HNO_3. The monolith was stored in buffer containing 0.02% sodium azide at 4°C until use.

Characterization of Monoliths

The surface area of the poly(HEMA-MAC) monoliths was measured by Brunauer-Emmett-Teller (BET) model using single point analysis and a Flowsorb II 2300 from Micromeritics Instrument Corporation, Norcross, USA. 0.5 g of polymer monolith was placed in a sample holder and degassed in a N_2-gas stream at 150°C for 1 h. Adsorption of the gas mixture (30% N_2/70% He) was performed at −210°C and desorption was performed at room temperature. Values from the desorption step was used for the specific surface area calculations.

Water Uptake Ratio of the Cd^{2+}-imprinted Poly(HEMA-MAC) Monolith was Determined in Distilled Water

The experiment was conducted as follows: initially dry monolith was carefully weighed before being placed in a 50 mL vial containing distilled water. The vial was put into an isothermal water bath with a fixed temperature (25 ± 0.5°C) for 2 h. The monolith sample was taken out from the water, wiped using a filter paper, and weighed. The weight ratio of the dry and the wet sample was recorded. The water content of the Cd^{2+}-imprinted poly(HEMA-MAC) monolith was calculated by using the following expression:

$$\text{Water uptake ratio } \% = [(W_s - W_o)/W_o] \times 100 \quad (1)$$

where W_o and W_s are the weights of monolith before and after uptake of water, respectively.

The surface morphology of the monolith was examined using optical and scanning electron microscopy (SEM). The samples were initially dried in air at 25°C for seven days before being analyzed. A fragment of the dried

monolith was mounted on a SEM sample mount and was sputter coated for 2 minutes. The sample was then mounted in a scanning electron microscope (Model: Raster Electronen Microscopy, Leitz-AMR-1000, Germany). The surface of the sample was then scanned at the desired magnification to study the morphology of the Cd^{2+}-imprinted poly(HEMA-MAC) monolith.

To evaluate the degree of MAC incorporation the Cd^{2+}-imprinted poly(HEMA-MAC) monolith was subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932).

FTIR spectra of MAC and the Cd^{2+}-imprinted poly(HEMA-MAC) monolith were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan). The dry monolith (about 0.1 g) was thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a pellet and the FTIR spectrum was then recorded.

Chromatographic Procedure

Adsorption of Cd^{2+}ions from human plasma on the Cd^{2+}-imprinted poly(HEMA-MAC) monolith was carried out in a recirculating system equipped with a water jacket for temperature control. Fresh human plasma was used in all experiments and obtained from a healthy donor. Blood samples were centrifuged at 500 g for 30 min at room temperature. Nitrate salt was used as the source of Cd^{2+} ions. The monolith was washed with 30 ml of water and then equilibrated with 25 mM phosphate buffer containing 0.1 M NaCl (pH 7.4). 20 mL of the plasma solution was overloaded with Cd^{2+} solution containing different amounts of Cd^{2+} to obtain different initial cadmium concentrations. Then, the cadmium-overloaded human plasma was passed through the column containing Cd^{2+}-imprinted poly(HEMA-MAC) monolith at 20°C for 2 h. Also, the flow rate of the aqueous solution (i.e., 20 mL of the solution with a Cd^{2+} content of 100 ppm) was changed between 1.0–4.0 mL/min. The concentration of the Cd^{2+} ions in the aqueous phase, after the desired treatment periods was measured by using a graphite furnace atomic absorption spectrophotometer (Analyst 800/Perkin Elmer, USA). Deuterium background correction was used and the spectral slit width was 0.7 nm. A hollow cathode cadmium lamp was used. The working current/wavelength were 8.0 mA/228.8 nm. The instrument response was periodically checked with known Cd^{2+} solution standards. The experiments were performed in replicates of three and the samples were analyzed in replicates of three as well. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error. The amount of Cd^{2+} adsorption per unit mass of the monolith was evaluated by using the mass balance.

Selectivity Experiments

In order to show Cd^{2+} specificity of the Cd^{2+}-imprinted poly(HEMA-MAC) monolith, competitive adsorption (i.e., Pb^{2+}; MW: 207.2 g/mol, ionic radius: 133 pm and Zn^{2+}; MW: 65.39 g/mol, ionic radius: 88 pm) was also studied. 20 mL of fresh human plasma solution was overloaded with 100 ppm lead and zinc ions by the same procedure. The Cd^{2+}-imprinted poly(HEMA-MAC) monolith was treated with this competitive ions. After adsorption equilibrium, the concentration of Pb^{2+} and Zn^{2+} ions in the remaining solution was measured by GFAAS.

Distribution and selectivity coefficients of Pb^{2+} and Zn^{2+} with respect to Cd^{2+} were calculated as explained by the following (Eq. (2).

$$K_d = [(C_i - C_f)/C_f] \times V/m \tag{2}$$

Here, K_d represents the distribution coefficient; C_i and C_f are initial and final concentrations of metal ions, respectively. V is the volume of the solution (mL) and m is the mass of monolith used (g).

The selectivity coefficient for the binding of a metal ion in the presence of competitor species (Eq. (4) can be obtained from equilibrium binding data according to (Eq. (4).

$$M_{1(solution)} + M_{2(sorbent)} \Leftrightarrow M_{2(solution)} + M_{1(sorbent)} \tag{3}$$

$$k = ([M_2]_{solution}[M_1]_{sorbent}/([M_1]_{solution}[M_2]_{sorbent})$$

$$k = K_d(Cd^{2+})/K_d(X^{2+}) \tag{4}$$

where k is the selectivity coefficient and X^{2+} represents Pb^{2+} and Zn^{2+} ions. A comparison of the k values of the imprinted rods with those metal ions allows an estimation of the effect of imprinting on selectivity.

A relative selectivity coefficient k′ (Eq. (5) can be defined as

$$k' = k_{imprinted}/k_{control} \tag{5}$$

Desorption and Repeated Use

Desorption of Cd^{2+} ions were studied with two different desorption agents; 0.1 M acidic thiourea (NH_2CSNH_2) solution and 0.1 M nitric acid (HNO_3) solution, respectively. The Cd^{2+}-imprinted poly(HEMA-MAC) monolith was washed with this desorption medium and for 1 h at room temperature. The final Cd^{2+} ion concentration in the desorption medium was measured by atomic adsorption spectrometer. The desorption ratio was calculated from the amount of Cd^{2+} ions adsorbed on the monolith and the final Cd^{2+} ions concentration in the desorption medium.

In order to test the reusability of the Cd^{2+}-imprinted poly(HEMA-MAC) monolith, Cd^{2+} ions adsorption-desorption procedure was repeated five times by using the same polymeric sorbent. In order to regenerate and sterilize, after desorption; the monolith was washed with 50 mM NaOH solution.

RESULTS AND DISCUSSION

Characterization Studies

In a previous study, we prepared Cd^{2+} imprinted beads (in the size range of 63–140 μm in diameter) by suspension polymerization (33). Adsorption chromatography with a packed column of beads as a support material is not easy to scale up because the pressure drop in packed columns is high, leading to compaction of the bed under pressure and low flow rates (34). Rigid porous beads overcome the compressibility problem, but the bead size employed to prevent high operating pressures may lead to diffusional limitations which degrade performance. Packed-bed columns usually require long elution times. The development of alternative chromatography support is therefore of considerable interest. Monolithic materials have the advantages of large surface area, short diffusion path, and low pressure drop. As a result of the convective flow of the solution through the pores, the mass transfer resistance is tremendously reduced and the binding kinetics dominates the adsorption process. This results in a rapid processing, which greatly improves the adsorption, washing, desorption and regeneration steps and decreases the probability of inactivation of biomolecules.

According to mercury porosimetry data, the average pore size of monolith was 780 nm. Specific surface area of the monolith was found to be 226.8 m^2/g by BET method. The specific surface area was found to be 19.4 m^2/g for the ion-imprinted poly(HEMA-MAC) beads (33). By comparing these values, it may be concluded that the monolithic material is a superporous. The total pore volume was 3.6 ml/g and represented a porosity over 68%. This indicated that the monolith contained mainly micropores. This pore diameter range is available for diffusion of the cadmium ions. The Cd^{2+}-imprinted poly(HEMA-MAC) monolith is crosslinked hydrophilic matrices. The equilibrium swelling ratios of the non-imprinted poly(HEMA-MAC) and Cd^{2+}-imprinted poly(HEMA-MAC) monoliths used in this study, were %64 and %76 respectively.

The cross-sectional structure of poly(HEMA-MAC) monolith is exemplified in Fig. 1. It can be clearly seen that poly(HEMA-MAC) monolith is composed of much smaller particles. The particles are irregular. There are also many pores on the bulk structure of the particles. This also provides metal ions adsorption capacity. The back pressure drop was about 4.7 mPa at the flow-rate of 1.0 ml/min. So the poly(HEMA-MAC) monolith has good flow properties.

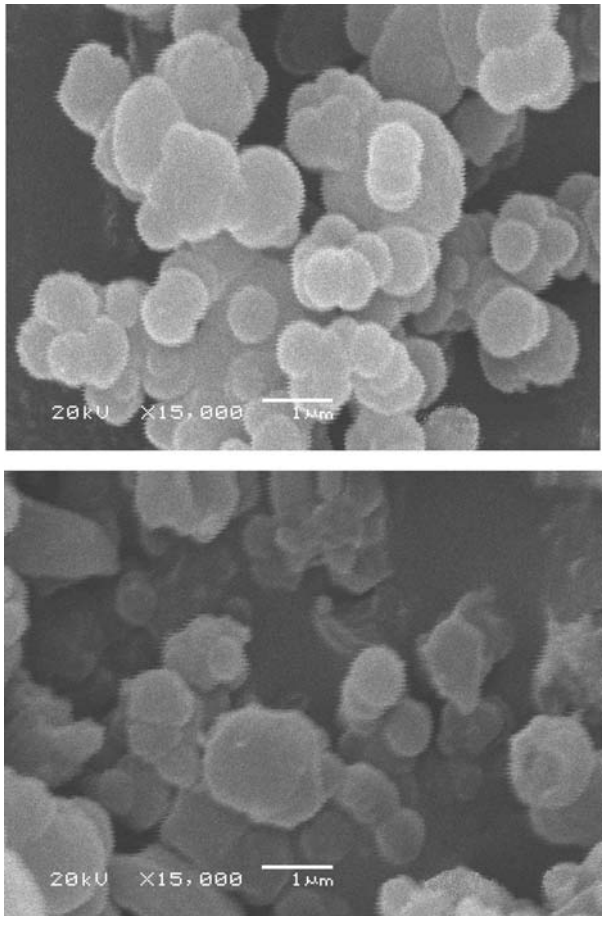

Figure 1. SEM micrographs of poly(HEMA-MAC) monolith.

The incorporation of the MAC was found to be 58.3 μmol/g polymer by using nitrogen stoichiometry. Note that HEMA and other polymerization ingredients does not contain nitrogen. This nitrogen amount determined by elemental analysis comes from only incorporated MAC groups into the polymeric structure.

N-methacryloly-(L)-cysteinemethylester (MAC) was selected as the comonomer and ion-imprinted ligand for the selective separation of Cd^{2+} ions from human plasma. In the first step, MAC was synthesized from cysteine and methacryloyl chloride and complexed with Cd^{2+} ions. The molecular formula of synthesized MAC comonomer and MAC-Cd^{2+} complex is shown in Fig. 2. FTIR spectrum of MAC has the characteristic stretching vibration amide I and amide II absorption bands at 1651 cm^{-1}

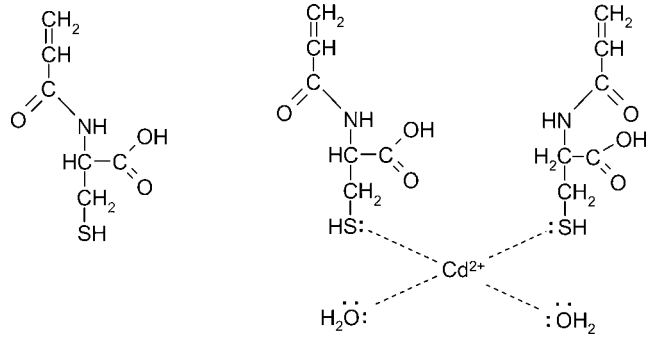

Figure 2. The molecular formula of MAC and MAC-Cd^{2+} complex.

and 1558 cm^{-1}, an carbonyl band at 1724 cm^{-1} (Figure 3A). For the characteristic determination of complex, due to linear coordinate covalent complex formation, the characteristic strong S–H stretching vibration bands at 1130 cm^{-1} and 970 cm^{-1} slips to the higher frequency field at 950 cm^{-1} and 750 cm^{-1}, as a result of decreasing the electron density of sulfhydryl group of MAC monomer (Figure 3B). The FTIR spectrum of Cd^{2+}-imprinted poly(HEMA-MAC) monolith has the characteristic stretching vibration band of hydrogen bonded alcohol, O-H, around 3586 cm^{-1}, carbonyl at 1645 cm^{-1} amide II absorption bands at 1516 cm^{-1} (Figure 4).

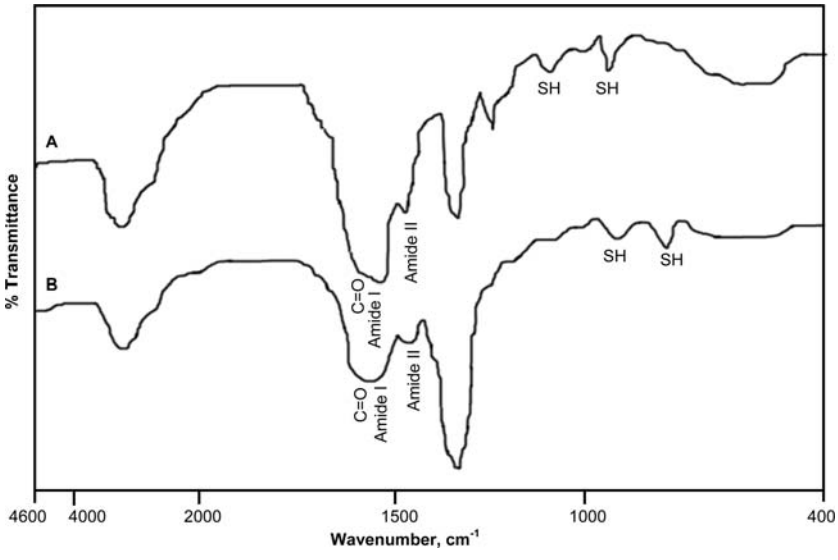

Figure 3. FTIR spectra of MAC and MAC-Cd^{2+} complex.

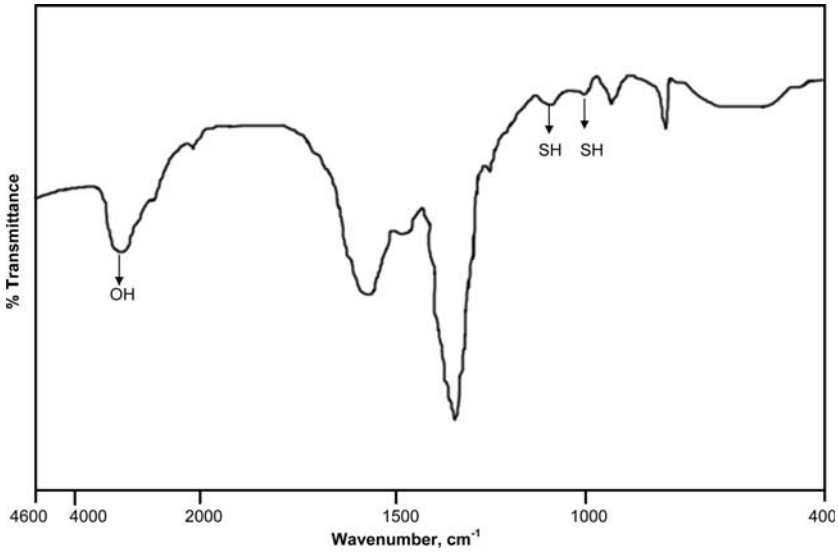

Figure 4. FTIR spectrum of Cd^{2+} imprinted poly(HEMA-MAC) monolith.

ADSORPTION OF Cd^{2+} FROM HUMAN PLASMA

Adsorption Isotherm and Langmuir Adsorption Model

Figure 5 shows the adsorption isotherms. The adsorption values increased with increasing concentration of Cd^{2+} ions, and reached plateau value which represents saturation of the active adsorption cavities (which are available and accessible for Cd^{2+} ions) on the monolith. Maximum adsorption capacities were 26.6 μmol/g for Cd^{2+}-imprinted poly(HEMA-MAC) monolith and 4.9 μmol/g for non-imprinted poly(HEMA-MAC) monolith. Molecular ion-imprinting significantly increased the Cd(II) adsorption capacity of the monolith, because of the specific recognition between Cd(II) ions and formed cadmium cavities in the polymer structure, in keeping with the objective of this study.

The Langmuir adsorption isotherm is expressed by Eq. (6.) The corresponding transformations of the equilibrium data for Cd^{2+} ions gave rise to a linear plot, indicating that the Langmuir model could be applied in these systems and described by the equation:

$$Q = Q_{max} \cdot b \cdot C_{eq}/(1 + bC_{eq}) \tag{6}$$

where Q is the concentration of bound Cd^{2+} ions in the adsorbent (μmol/g), Ceq is the equilibrium Cd^{2+} ions concentration in solution (μmol/L), b is the

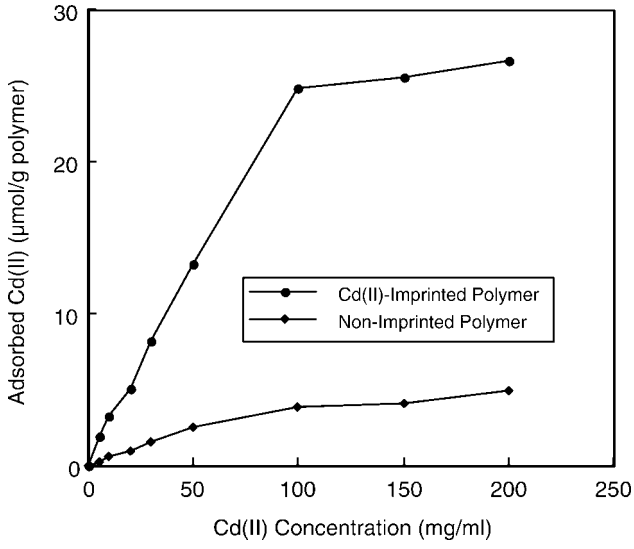

Figure 5. Effect of equilibrium Cd^{2+} concentration on Cd^{2+} adsorption; Flow rate: 1.0 ml/min; pH 7.4; T: 25°C.

Langmuir constant (g/μmol) and, Q_{max} is the adsorption capacity (μmol/g). This equation can be linearized so that;

$$1/Q = [1/(Q_{max} \cdot b)][1/C_{eq}] + [1/(Q_{max}.] \quad (7)$$

The plot of $1/C_{eq}$ versus $1/Q$ was employed to generate the intercept of $1/Q_{max}$ and the slope of $1/Q_{max}$.b (Figure 6).

The maximum adsorption capacity (Q_{max}) data for the adsorption of Cd^{2+} ions was obtained from the experimental data. The correlation coefficient (R^2) was 0.992 at pH 7.4. The Langmuir adsorption model can be applied in this monolithic MIP affinity adsorbent system. It should be also noted that the maximum adsorption capacities (Q_{max}) and the Langmuir constants were found to be 30.2 μmol/g (pH 7.4) and 1.78×10^{-3} g/μmol, respectively.

In order to examine the controlling mechanism of adsorption process such as mass transfer and chemical reaction, kinetic models were used to test experimental data. The kinetic models (Pseudo-first and Pseudo-second order equations) can be used in this case assuming that the measured concentrations are equal to adsorbent surface concentrations. The first-order rate equation of Lagergren is one of the most widely used for the adsorption of solute from a liquid solution. It may be represented as follows:

$$dq_t/dt = k_1(q_{eq} - q_t) \quad (8)$$

where k_1 is the rate constant of pseudo-first order adsorption (min^{-1}) and q_{eq} and q_t denote the amounts of adsorbed metal ion at equilibrium and at time t

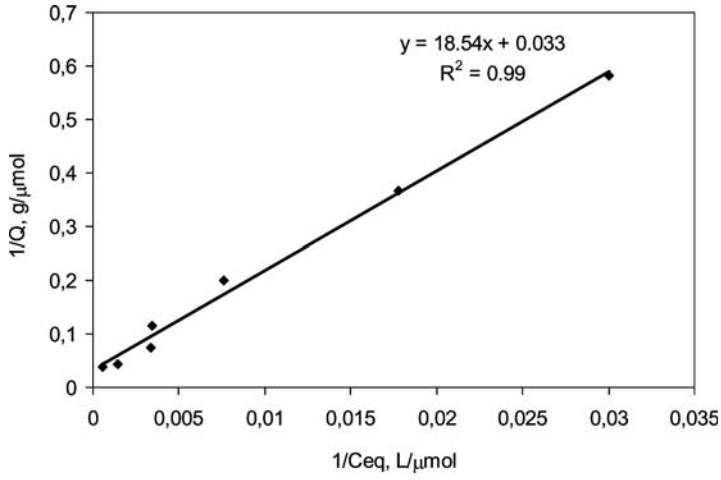

Figure 6. Langmuir adsorption isotherm of Cd^{2+}-imprinted poly(HEMA-MAC) monolith.

(mg/g), respectively. After integration by applying boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, gives;

$$\log[q_{eq}/(q_{eq} - q_t)] = (k_1 t)/2.303 \tag{9}$$

Equation 9 can be rearranged to obtain a linear form;

$$\log(q_{eq} - q_t) = \log(q_{eq}) - (k_1 t)/2.303 \tag{10}$$

a plot of $\log(q_{eq})$ versus t should give a straight line to confirm the applicability of the kinetic model. In a true first-order process $\log q_{eq}$ should be equal to the interception point of a plot of $\log(q_{eq} - q_t)$ via t.

In addition, a pseudo-second order equation based on adsorption equilibrium capacity may be expressed in the form;

$$dq_t/dt = k_2(q_{eq} - q_t)^2 \tag{11}$$

where k_2 (g $mg^{-1} \cdot min^{-1}$) is the rate constant of pseudo-first order adsorption process. Integrating Eq. (11), q and applying the boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, leads to;

$$1/(q_{eq} - q_t)] = (1/q_{eq}) + k_2 t \tag{12}$$

or equivalently for linear form;

$$(t/q_t) = (1/k_2 q_{eq}^2) + (1/q_{eq})t \tag{13}$$

a plot of t/q_t versus t should give a linear relationship for the applicability of the second-order kinetics. The rate constant (k_2) and adsorption at equilibrium (q_{eq}) can be obtained from the intercept and slope, respectively.

A comparison of the experimental adsorption capacity and the theoretical values which obtained from Figs. 7 and 8 are presented in Table 1. The theoretical q_e value estimated from pseudo-first order kinetic model was very close to the experimental value and the correlation coefficient was high. Results indicate that this ion-imprinted adsorbent system was described by the first-order kinetic model.

The correlation coefficient for the linear plot of t/q_t againts t for the pseudo-second order equation was lower than 0.90. The theoretical q_e value was slightly more different from the experimental value. These values showed that this adsorbent system was not so well described by the pseudo-second order kinetic model.

Selectivity Experiments

Competitive adsorption of Pb^{2+}/Cd^{2+} and Zn^{2+}/Cd^{2+} from their mixtures were also studied in a continuous system. Pb^{2+} and Zn^{2+} were chosen as competitive metal ions. The ionic radius of Pb^{2+} is larger (120 pm) and the ionic radius of Zn^{2+} is smaller (88 pm) than Cd^{2+} ions (114 pm). Table 2 summarizes K_d, k and k′ values of Pb^{2+} and Zn^{2+} with respect to Cd^{2+}. A comparison of the K_d values for the Cd^{2+} imprinted poly(HEMA-MAC)

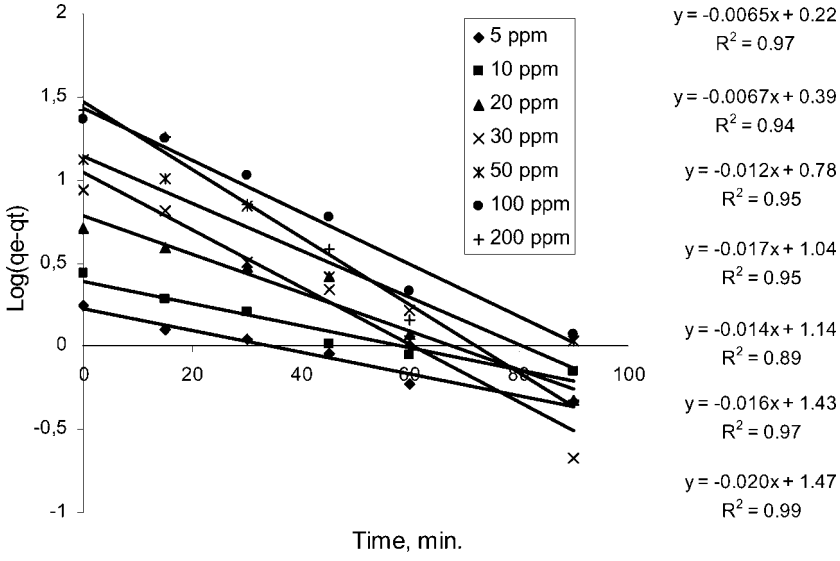

Figure 7. Pseudo-first order kinetic of the experimental data for the Cd^{2+}-imprinted poly(HEMA-MAC) monolith.

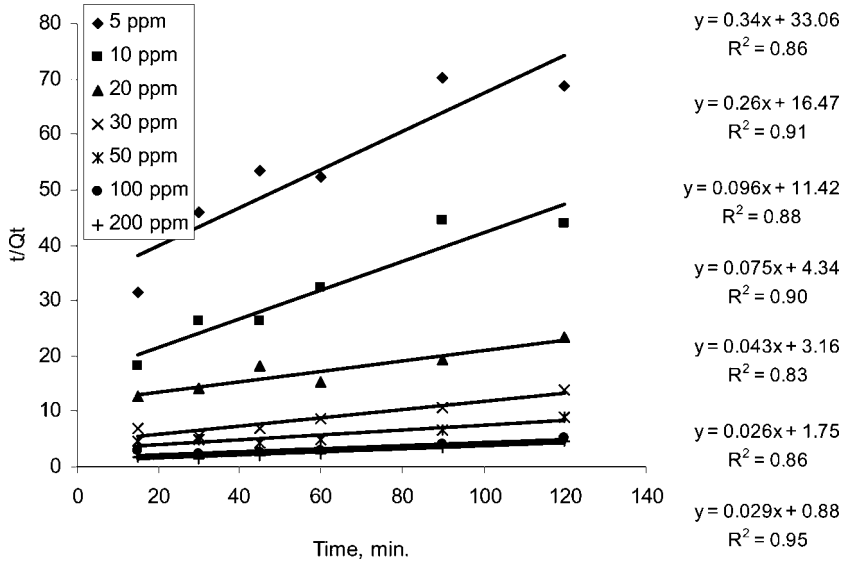

Figure 8. Pseudo-second order kinetic of the experimental data for the Cd^{2+}-imprinted poly(HEMA-MAC) monolith.

samples with the control samples shows an increase in K_d for Cd^{2+} while K_d decrease for Pb^{2+} and Zn^{2+}. The relative selectivity coefficient is an indicator to express an adsorption affinity of recognition sites to the imprinted Cd^{2+} ions. These results showed that the relative selectivity coefficients of imprinted beads for Cd^{2+}/Pb^{2+} and Cd^{2+}/Zn^{2+} were 9.9 and 8.9 times greater than non-imprinted matrix, respectively (Table 2).

Table 1. The first- and second order kinetic constants for p(HEMA-MAC) monolith

Initial conc. (mg/ml)	Exp q_e (μmol/g)	First-order kinetic			Second-order kinetic		
		k_1 (1/min)	q_e (μmol/g)	R^2	$k_2 \times 10^{-4}$ (g/μmolmin)	q_e (μmol/g)	R^2
5	1.75	1.50	1.67	0.974	35.84	2.90	0.861
10	2.73	1.54	2.45	0.940	39.99	3.89	0.911
20	5.10	2.65	6.08	0.951	8.05	10.43	0.879
30	8.61	3.96	11.07	0.947	12.80	13.42	0.903
50	13.28	3.32	14.03	0.904	5.75	23.47	0.825
100	23.24	3.59	26.82	0.968	3.83	38.61	0.864
200	26.56	4.69	29.72	0.990	9.33	34.84	0.945

Table 2. K_d, k and k, values of Pb^{2+} and Zn^{2+} with respect to Cd^{2+}

	Non-imprinted monolith		Imprinted monolith		
Metal ion	K_d	k	K_d	k	k′
Cd^{2+}	24.3	—	25.7	—	
Pb^{2+}	28.2	0.86	2.14	12.06	14.03
Zn^{2+}	40.3	0.60	4.11	6.25	10.41

*Metal ion concentration: 100 ppm for all metal ions.

Effect of Flow-rate

The adsorption capacity at different flow-rates are given in Figure 9. As seen in figure the adsorption of Cd^{2+} ion is decreased with increasing flow rate, as expected. An increase in the flow rate reduces the plasma volume treated efficiently and therefore decreases the service time of monolithic column. This is due to decrease in contact time between the Cd^{2+} ions and the poly(HEMA-MAC) monolith at higher flow rates. Therefore, the adsorption capacity decreased significantly from 23.25 μmol/g to 3.08 μmol/g polymer with the increase of the flow-rate from 1 mL/min to 4 mL/min.

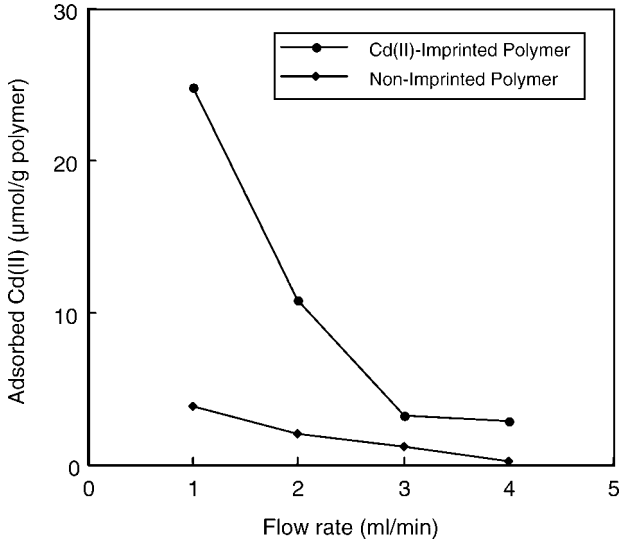

Figure 9. Effect of flow-rate on Cd^{2+} adsorption: Cd^{2+} concentration: 100 ppm, pH: 7.4; T: 25°C, adsorption time:120 min.

Desorption and Repeated Use

The regeneration of the adsorbent is likely to be a key factor in improving process economics. Desorption of the Cd ions from the Cd-imprinted poly(HEMA-MAC) monolith was performed in continuous column experimental set-up. Various factors are probably involved in determining rates of Cd desorption, such as the extent of hydration of the metal ions and polymer microstructure. However, an important factor appears to be binding strength. In this study, the desorption time was found to be 30 min. Desorption ratios are high (up to 95%). In order to obtain the reusability of the Cd^{2+}-imprinted poly(HEMA-MAC) monolith, adsorption-desorption cycles were repeated 10 times by using the same imprinted monolith. The adsorption capacity of the recyled Cd^{2+}-imprinted poly(HEMA-MAC) monolith can still be maintained at 97% level at the 10^{th} cycle (Fig. 10). It can be concluded that the Cd^{2+}-imprinted poly(HEMA-MAC) monolith can be used many times without decreasing their adsorption capacities significantly.

CONCLUSIONS

Molecularly imprinted materials have been demonstrated to possess a very high degree of selectivity towards targeted substances. Molecularly imprinted monoliths were prepared by bulk polymerization. The adsorption

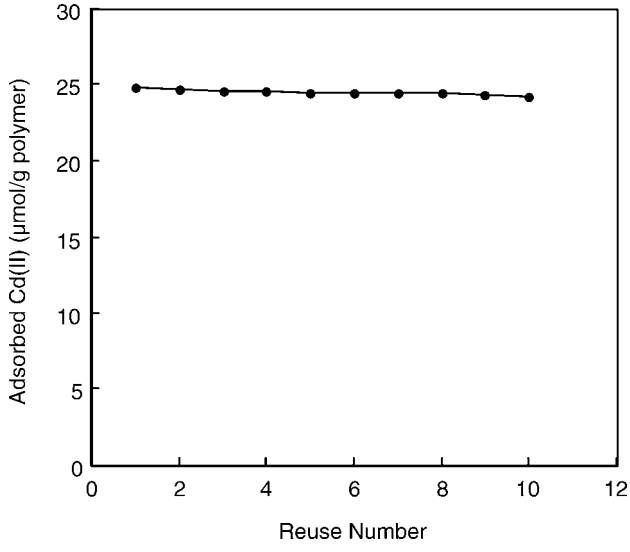

Figure 10. Adsorption-desorption cycle of Cd^{2+}-imprinted poly(HEMA-MAC) monolith.

was relatively fast and the time required to reach equilibrium conditions was about 60 min. The maximum adsorption capacity for Cd^{2+} ions was 26.7 μmol per gram dry weight of monolith. This fast adsorption equilibrium is most probably due to high complexation and geometric affinity between Cd^{2+} ions and Cd^{2+} cavities in the monolith structure. The adsorption values increased with increasing concentration of Cd^{2+} ions, and a saturation value is achieved at ion concentration of 40 mg/L, which represents saturation of the active binding cavities on the Cd^{2+}-imprinted poly(HEMA-MAC) monoliths. The relative selectivity coefficient is an indicator to express an adsorption affinity of recognition sites to the imprinted Cd^{2+} ions. The results showed that the imprinted monoliths for Cd^{2+}/Pb^{2+} and Cd^{2+}/Zn^{2+} were 9.9 and 8.9 times greater than non-imprinted matrix, respectively. The desorption time was found to be 30 min. The Cd^{2+}-imprinted poly(HEMA-MAC) monoliths can be used many times without decreasing their adsorption capacities significantly.

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