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Molecular recognition based cadmium removal from human plasma

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Abstract

Molecularly imprinted polymers (MIPs) are easy to prepare, stable, inexpensive and capable of molecular recognition. MIPs can be considered as affinity separation media. Cadmium is a carcinogenic and mutagenic element. 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 ion-imprinted polymers, 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, Cd^{2+} was complexed with MAC and the Cd^{2+}-imprinted p(HEMA-MAC) beads were synthesized by suspension polymerization. After that, the template (i.e., Cd^{2+} ions) were removed using 0.1 M thiourea solution. The specific surface area of the Cd^{2+}-imprinted poly(HEMA-MAC) beads was found to be 19.4 m^2/g with a size range of 63–140 μm in diameter and the swelling ratio was 78%. According to the elemental analysis results, the beads contained 42.1 μmol MAC/g polymer. The maximum adsorption capacity was 32.5 μmol Cd^{2+}/g beads. The relative selectivity coefficients of imprinted beads for Cd^{2+}/Pb^{2+} and Cd^{2+}/Zn^{2+} were 7.8 and 1683 times greater than non-imprinted matrix, respectively. The Cd^{2+}-imprinted poly(HEMA-MAC) beads could be used many times without decreasing their adsorption capacities significantly.

Keywords: Ion-imprinting; Molecular recognition; Cadmium removal; Metal detoxification; Affinity binding

1. 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 (MIPs) are easy to prepare, stable, inexpensive and capable of molecular recognition. Therefore, MIPs can be considered as artificial affinity media. Molecular recognition-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, and (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

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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 and/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 technique for blood detoxification is extracorporeal affinity adsorption. So far, only a few affinity adsorbents were reported for metal detoxification [18–20].

In this study, ion-imprinted polymer beads were used for the selective separation of Cd^{2+} ions from human plasma. *N*-Methacryloly-(L)-cysteinemethylester (MAC) was used as the metal complexing monomer. Usually, molecularly imprinted polymers are prepared by the bulk polymerization method. The disadvantage of this method is that the obtained block polymer should be crushed, ground and sieved to produce packing materials. In this study, Cd^{2+}-imprinted poly(hydroxyethyl methacrylate-*N*-methacryloly-(L)-cysteinemethylester) beads were produced by suspension 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 [21–24]. After removal of Cd^{2+} ions, ion-imprinted beads were used for the separation of cadmium from human plasma. Cd^{2+} adsorption and selectivity studies of cadmium versus other metal ions, which are Zn^{2+} and Pb^{2+} are reported here. Finally, repeated use of the ion-imprinted beads is also discussed.

2. Experimental

2.1. Materials

Hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) 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. Benzoyl peroxide (BPO) was obtained from Fluka (Switzerland). Poly(vinyl alcohol) (PVAL; MW: 100,000, 98% hydrolyzed) was supplied from Aldrich Chem. Co. (USA). 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.

2.2. Synthesis of *N*-methacryloly-(L)-cysteinemethylester

Details of the preparation and characterization of the *N*-methacryloly-(L)-cysteinemethylester (MAC) was reported elsewhere [25]. Briefly, the following experimental procedure was applied for the synthesis of MAC monomer: 5.0 g of cysteine 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. Four milliliters 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.

2.3. Preparation of Cd^{2+}–MAC complex

In order to prepare MAC–Cd^{2+} complex, solid *N*-methacryloly-(L)-cysteinemethylester (MAC) (0.378 g, 2.0 mmol) was added slowly into 15 ml of ethanol–water mixture (50/50 v/v) and then treated with cadmium nitrate ($Cd(NO_3)_2{\cdot}4H_2O$) (0.163 g, 1.0 mmol) at room temperature with continuous stirring for 3 h. Then, the formed metal–monomer complex was filtered, washed with 99% ethanol (250 ml), and dried in a vacuum oven.

2.4. Preparation of Cd^{2+}-imprinted poly(HEMA-MAC) beads

Suspension polymerization method was used for the preparation of spherical poly(HEMA-MAC) beads. A typical preparation procedure is described below. Continuous medium was prepared by dissolving poly(vinyl alcohol) (200 mg) in the deionized water (50 ml). For the preparation of dispersed phase, HEMA (4.0 ml), Cd^{2+}–MAC complex (500 mg), EGDMA (8.0 ml) and toluene (12 ml) were mixed and benzoyl peroxide (60 mg) was dissolved in the homogeneous organic phase. The organic phase was dispersed in the aqueous medium by stirring the mixture magnetically (600 rpm), in a sealed pyrex polymerization reactor (volume: 250 ml). The reactor content was heated to polymerization temperature (i.e., 65 °C) and the polymer-

ization was conducted for 4 h with a 600 rpm stirring rate at 65 °C. Then, temperature was increased to 90 °C and the polymerization was conducted for 2 h. Final beads were extensively washed with ethanol and water to remove any unreacted monomer or diluent and then stored in distilled water at 4 °C. Non-imprinted poly(HEMA-MAC) beads were prepared in the same way, but without addition of MAC–Cd^{2+} complex.

After the cleaning procedure, the template (i.e., Cd^{2+} ions) was removed from the polymer beads using 0.1 M acidic thiourea. The imprinted beads were added into the 0.1 M acidic thiourea solution for 48 h at room temperature. This procedure was repeated several times until the template molecule (i.e., Cd^{2+} ions) could not be detected in the filtrate with a graphite furnace atomic absorption spectrophotometer. The template free polymers were cleaned with 0.1 M HNO_3 in a magnetic stirrer for 3 h. Then the beads were filled in a fixed-bed column, and washing solutions (i.e., a dilute HCl solution, and a water–ethanol mixture) were recirculated through the system, which includes also an activated carbon column, until the beads are clean. Purity of the beads was followed by observing the change of optical densities of the samples (wavelength: 220–280 nm) taken from the liquid phase in the recirculation system, and also from the DSC thermograms of the beads obtained by using a differential scanning calorimeter microcalorimeter (Mettler, Switzerland). Heating rate was 10 °C/min. Optical density of the uncleaned beads was 1.4. But after cleaning operation this value was reduced to 0.02. In addition, when the thermogram of uncleaned beads was recorded, it has a peak around 60 °C. This peak might originate from benzoyl peroxide (it gives radicals at 60 °C). After application of this cleaning procedure, no peak between 30 and 100 °C was observed on this thermogram. When not in use, the resulting beads were kept under refrigeration in 0.02% NaN_3 solution for preventing of microbial contamination.

2.5. Characterization of beads

The average size and size distribution of the beads were determined by screen analysis performed using Standard Test Sieves (Retsch GmbH & Co., Germany). The specific surface area of the beads was determined in BET apparatus. Water uptake ratios of the beads were determined in distilled water. The experiment was conducted as follows: initially dry beads were carefully weighed before being placed in a 50 ml vial containing distilled water. The vial was put into an isothermal water bath at a fixed temperature (25 °C) for 2 h. The bead sample was taken out from the water, wiped using a filter paper, and weighed. The weight ratio of dry and wet samples was recorded. The water content of the non-imprinted and the imprinted beads were calculated using the weights of beads before and after uptake of water. The beads were examined using scanning electron microscopy (SEM). The samples were initially dried in air at 25 °C for 7 days before being analyzed. A fragment of the dried bead was mounted on a SEM sample mount and was sputter coated for 2 min. The sample was then mounted in a scanning electron microscope (Model: Raster Electron Microscopy, Leitz-AMR-1000, Germany). The surface of the sample was then scanned at the desired magnification to study the morphology of the beads. To evaluate the degree of MAC incorporation, the beads were subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932). FTIR spectra of MAC and the imprinted beads were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan). The beads (about 0.1 g) were thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a pellet and FTIR spectrum was then recorded.

2.6. Adsorption of Cd^{2+} ions from human plasma

The batchwise adsorption tests of Cd^{2+} ions from human plasma were studied for the imprinted and non-imprinted polymer beads. 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. After the predetermined adsorption time, 10 ml of the plasma freshly separated from the human blood was spiked with 2 ml of Cd^{2+} solution containing different amounts of Cd^{2+} to obtain different cadmium concentrations. Then, the cadmium-spiked human plasma was incubated with a 100 mg of the beads at 20 °C for 3 h. The concentration of the Cd^{2+} ions in the aqueous phase, after the desired treatment periods was measured using a graphite furnace atomic absorption spectrophotometer (GFAAS, 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 beads was evaluated by using the mass balance.

2.7. Selectivity experiments

In order to show Cd^{2+} specificity of the imprinted beads, competitive adsorptions of Pb^{2+} and Zn^{2+} were also studied. Ten milliliters of fresh human plasma was spiked with 1 ml of 40 mg/L lead and zinc ions by the same procedure. The Cd^{2+}-imprinted poly(HEMA-MAC) beads (total mass: 100 mg) were treated with these competitive ions. After adsorption equilibrium was reached, 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 by Eq. (1)

$$K_d = \left(\frac{C_i - C_f}{C_f}\right)\frac{V}{m} \quad (1)$$

where K_d represents the distribution coefficient (ml/g); C_i and C_f are initial and final concentrations of metal ions (μmol/ml), respectively. V is the volume of the solution (ml) and m is the mass of beads (g).

The selectivity coefficient for the binding of a metal ion in the presence of competitor species can be obtained from equilibrium binding data according to Eq. (2) [26].

$$k = \frac{K_{\text{template metal}}}{K_{\text{interferent metal}}} \quad (2)$$

A comparison of the k values of the imprinted beads with those metal ions allows an estimation of the effect of imprinting on selectivity. A relative selectivity coefficient k' can be defined as

$$k' = \frac{k_{\text{imprinted}}}{k_{\text{control}}} \quad (3)$$

Blood protein adsorption (i.e., albumin, fibrinogen, and g-globulin) was also monitored. The ion-imprinted beads were incubated with a human plasma containing albumin (38.7 mg/ml), fibrinogen (2.2 mg/ml) and g-globulin (16.1 mg/ml) at room temperature for 2 h. Total protein concentration was measured by using the total protein reagent (Ciba Corning Diagnostics Ltd, Halstead, Essex, England; Catalog Ref. No: 712076) at 540 nm which is based on the Biuret reaction [27]. Chronometric determination of fibrinogen according to the Clauss method on plasma was performed by using Fibrinogene-Kit (Ref. No: 68452 and 68582, bioMerieux Laboratory Reagents and Instruments, Marcy-l'Etoile, France) [28]. Human serum albumin concentration was determined by using Ciba Corning Albumin Reagent (Ciba Corning Diagnostics Ltd, Halstead, Essex, England; Catalog Ref. No: 229241) which based on bromocresol green (BCG) dye method [27]. g-Globulin concentration was determined from the difference.

2.8. Desorption and repeated use

Desorption of Cd^{2+} ions was performed using 0.1 M acidic thiourea solution (pH 4.5). The Cd^{2+}-imprinted poly(HEMA-MAC) beads were placed in this desorption medium and stirred continuously (at a stirring rate of 400 rpm) for 1 h at room temperature. The final Cd^{2+} ions concentration in the desorption medium was measured by GFAAS. The desorption ratio was calculated from the amount of Cd^{2+} ions adsorbed on the imprinted beads 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) beads, the Cd^{2+} ion adsorption–desorption cycle was repeated five times using the same imprinted beads. In order to regenerate and sterilize the imprinted beads they were washed with 50 mM NaOH solution after each desorption procedure.

3. Results and discussion

3.1. Characterization of Cd^{2+}-imprinted beads

Cross-linked imprinted and non-imprinted beads were spherical in shape with a size range of 63–140 μm in diameter. The specific surface areas were found to be 18.9 m^2/g for non-imprinted and 19.4 m^2/g for imprinted. The equilibrium swelling ratios of the non-imprinted and imprinted beads are 65% and 78%, respectively. Compared with poly(HEMA-MAC), the water uptake ratio of the Cd^{2+}-imprinted beads increased. Formation of metal ion-imprinted cavities in the polymer structure introduced more hydrodynamic volume into the polymer chains, which can result uptake in the more water molecules by polymer matrix.

The surface morphology and internal structure of Cd^{2+}-imprinted poly(HEMA-MAC) beads are exemplified by the electron micrographs in Fig. 1. As clearly seen here, the polymeric beads have a spherical form and rough surface due to the pores which formed during the polymerization procedure. The photograph in Fig. 1B was taken with broken beads to observe the internal part of the polymeric structure. The presence of pores within the bead interior is clearly seen in this photograph. These images show that the Cd^{2+}-imprinted poly(HEMA-MAC) beads have a microporous interior surrounded by a reasonably rough surface, in the dry state.

The incorporation of the MAC was found to be 42.1 μmol/g polymer by using nitrogen stoichiometry. Note that HEMA and other polymerization ingredients do 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 monomer 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 are shown in Fig. 2. FTIR spectrum of MAC has the characteristic stretching vibration amide I and amide II absorption bands at 1651 and 1558 cm^{-1}, carbonyl band at 1724 cm^{-1}. For the characteristic determination of complex, due to linear coordinate covalent complex formation, the characteristic strong S–H stretching vibration bands at 1130 and 970 cm^{-1} slips to the higher frequency field at 950 and 750 cm^{-1}, as a result of decreasing the electron density of sulfhydryl group of MAC monomer.

Then, MAC–Cd^{2+} complex was polymerized with HEMA comonomer by suspension polymerization technique. The FTIR spectrum of Cd^{2+}-imprinted poly(HEMA-MAC) beads has the characteristic stretching vibration band of hydrogen bonded alcohol, O–H, around 3586 cm^{-1}, carbonyl

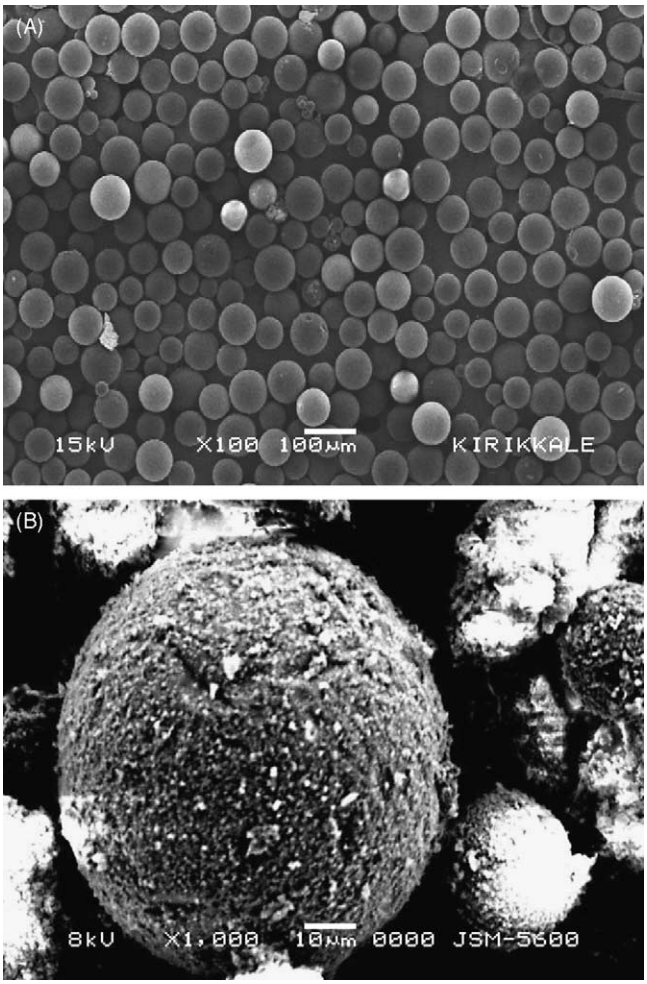

Fig. 1. SEM photographs of Cd^{2+}-imprinted poly(HEMA-MAC) beads: (A) surface and (B) internal structure.

at 1645 cm^{-1} amide II absorption bands at 1516 cm^{-1}, respectively.

3.2. Adsorption of Cd^{2+} from human plasma

3.2.1. Effect of time

Fig. 3 shows the time dependence of the adsorption values of Cd^{2+} ions on Cd^{2+} imprinted poly(HEMA-MAC) beads.

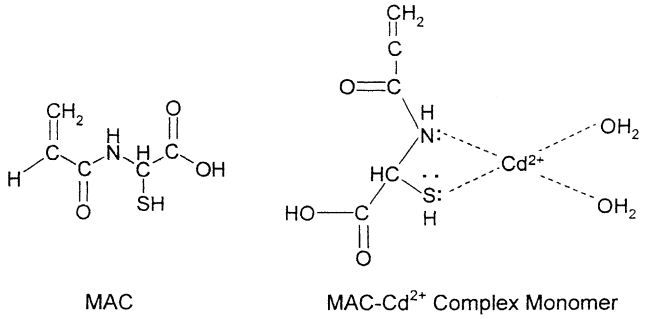

Fig. 2. Molecular formula of MAC and MAC–Cd^{2+} complex monomer.

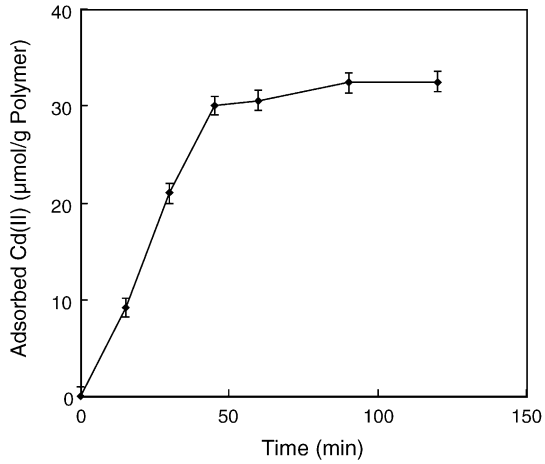

Fig. 3. Effect of time on Cd^{2+} adsorption; Cd^{2+} concentration: 40 mg/L.

As seen here, cadmium adsorption increases with the time during the first 60 min and then levels off as equilibrium is reached. This fast adsorption equilibrium is most probably due to high complexation and geometric shape affinity (or memory) between Cd^{2+} ions and Cd^{2+} cavities in the beads structure. The maximum adsorption capacity for Cd^{2+} ions was 32.5 µmol/g dry weight of imprinted beads.

3.2.2. Effect of Cd^{2+} ions concentration

Fig. 4 shows the dependence of the equilibrium concentration on the adsorbed amount of the Cd^{2+} onto the Cd^{2+}-imprinted poly(HEMA-MAC) beads. The adsorption values increased with increasing concentration of Cd^{2+} ions, and a saturation value is achieved at cadmium ion concentration of 40 mg/L, which represents saturation of the active binding cavities on the Cd^{2+}-imprinted poly(HEMA-MAC) beads. Maximum adsorption capacity was 32.5 µmol/g.

An adsorption isotherm is used to characterize the interactions of each molecule with the adsorbents. This provides a relationship between the concentration of the molecules in the solution and the amount of ion adsorbed on the solid

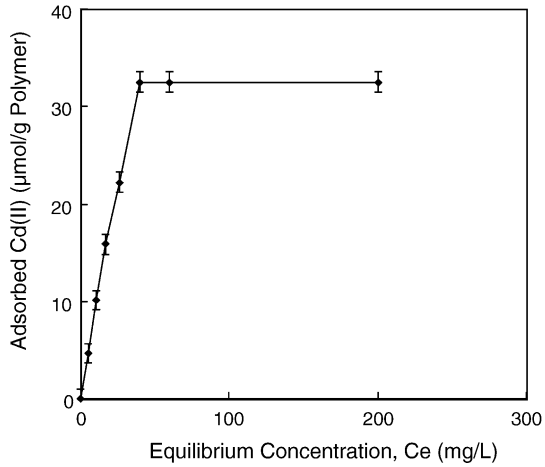

Fig. 4. Adsorption isotherm of Cd^{2+}-imprinted poly(HEMA-MAC) beads.

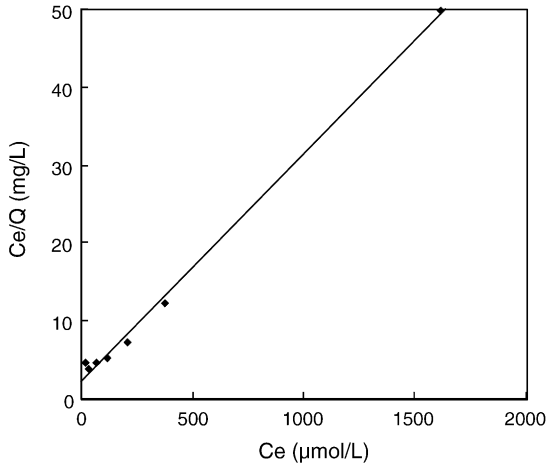

Fig. 5. Langmuir adsorption isotherm of Cd^{2+}-imprinted poly(HEMA-MAC) beads; Cd^{2+} concentration: 40 mg/L.

phase when the two phases are at equilibrium. The Langmuir adsorption model assumes that the molecules are adsorbed at a fixed number of well-defined sites, each of which is capable of holding only one molecule. These sites are also assumed to be energetically equivalent, and distant from each other so that there are no interactions between molecules adsorbed on adjacent sites.

During the batch experiments, adsorption isotherms were used to evaluate adsorption properties. For the systems considered, the Langmuir model was found to be applicable in interpreting cadmium adsorption by imprinted beads. The Langmuir adsorption isotherm is expressed by Eq. (5). Langmuir adsorption model assumes that the molecules are adsorbed at a fixed number of well-defined sites, each of which can only hold one molecule. These sites are also assumed to be energetically equivalent, and distant to each other so that there are no interactions between molecules adsorbed to adjacent sites [29]. The corresponding transformations of the

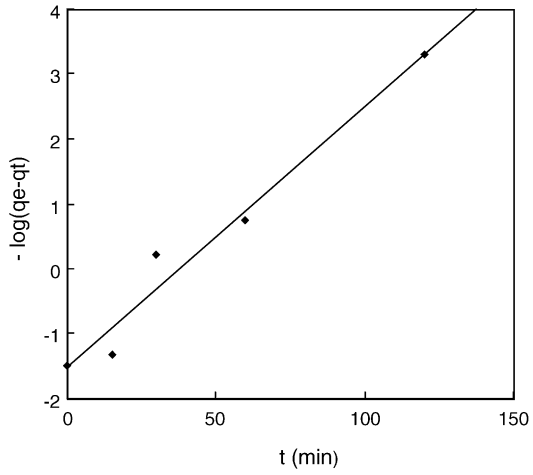

Fig. 6. Pseudo-first-order kinetic of the experimental data for the Cd^{2+}-imprinted poly(HEMA-MAC) beads.

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

$$Q = Q_{\max} b \frac{C_e}{1 + bC_e} \quad (4)$$

where Q is the concentration of bound Cd^{2+} ions in the adsorbent (μmol/g), C_e is the equilibrium Cd^{2+} ions concentration in solution (μmol/L), b is the Langmuir constant (g/μmol) and, $Q_{\max}$ is the adsorption capacity (μmol/g). This equation can be linearized.

$$\frac{1}{Q} = \left(\frac{1}{Q_{\max} b}\right)\left(\frac{1}{C_e}\right) + \left(\frac{1}{Q_{\max}}\right) \quad (5)$$

The plot of $1/C_e$ versus $1/Q$ was employed to generate the intercept of $1/Q_{\max}$ and the slope of $1/Q_{\max} b$ (Fig. 5).

The maximum adsorption capacity ($Q_{\max}$) data for the adsorption of Cd^{2+} ions was obtained from the experimental data. The correlation coefficients (R^2) was 0.9965 at pH 7.4. The Langmuir adsorption model can be applied in this affinity adsorbent system. It should be also noted that the maximum adsorption capacity ($Q_{\max}$) and the Langmuir constant were found to be 34.3 μmol/g (pH 7.4) and 1.28×10^{-2} 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 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 [30].

A comparison of the experimental adsorption capacity and the theoretical values which obtained from Figs. 6 and 7 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 against t for the pseudo-second-order equation was lower than 0.90. The theoretical q_e value was slightly more different from the

Table 1
Kinetic constants for the Cd^{2+}-imprinted poly(HEMA-MAC) beads

Initial concentration (mg/L)	40
Experimental	
q_e (μmol/g)	30.1 ± 0.28
k_1 (l/min)	0.093 ± 0.007
First-order kinetic	
q_e (μmol/g)	33.3 ± 0.30
R^2	0.96
Second-order kinetic	
q_e (μmol/g)	34.6 ± 0.35
k_2 (g/μmol min)	$2.4 \times 10^3 \pm 95$
R^2	0.89

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

Metal ion*	Non-imprinted beads		Imprinted beads		k'
	K_d	k	K_d	k	
Cd^{2+}	4.00 ± 0.18	–	0.25 ± 0.01	–	
Pb^{2+}	4.48 ± 0.22	0.89 ± 0.06	$3.6 \times 10^{-2} \pm 5 \times 10^{-3}$	6.94±0.15	7.8 ± 0.22
Zn^{2+}	8.52 ± 0.29	0.45 ± 0.04	$3.3 \times 10^{-4} \pm 1 \times 10^{-5}$	757.6±5.6	1683.5 ± 9.78

* Metal ion concentration: 40 mg/L for all metal ions.

experimental value. These values showed that this adsorbent system was not so well described by the pseudo-second-order kinetic model.

3.2.3. Selectivity experiments

Competitive adsorption of Pb^{2+}/Cd^{2+} and Zn^{2+}/Cd^{2+} from their mixtures were also studied in a batch 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) 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 7.8 and 1683 times greater than non-imprinted matrix, respectively (Table 2).

In order to show the ion-imprinted beads specificity, protein adsorption was also monitored. It is worth noting that adsorption of plasma proteins on the ion-imprinted beads are 1.24 mg/g for albumin, 0.86 mg/g for fibrinogen, and 0.75 mg/g for γ-globulin.

3.2.4. Desorption and repeated use

The regeneration of the adsorbent is likely to be a key factor in improving process economics. Desorption of the Cd^{2+} ions from the Cd^{2+}-imprinted poly(HEMA-MAC) beads was performed in a batch experimental set-up. Various factors are probably involved in determining rates of Cd^{2+} 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 85%). In order to obtain the reusability of the Cd^{2+}-imprinted poly(HEMA-MAC) beads, adsorption–desorption cycles were repeated five times by using the same imprinted beads. The adsorption capacity of the recycled Cd^{2+}-imprinted poly(HEMA-MAC) beads can still be maintained at 90% level at the 5th cycle (Fig. 8). It can be concluded that the Cd^{2+}-imprinted poly(HEMA-MAC) beads can be used many times without decreasing their adsorption capacities significantly.

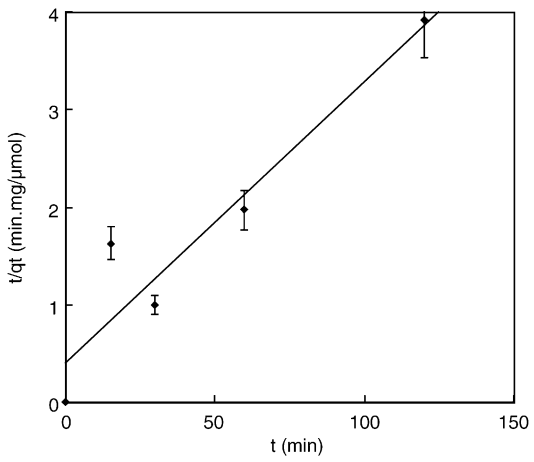

Fig. 7. Pseudo-second-order kinetic of the experimental data for the Cd^{2+}-imprinted poly(HEMA-MAC) beads.

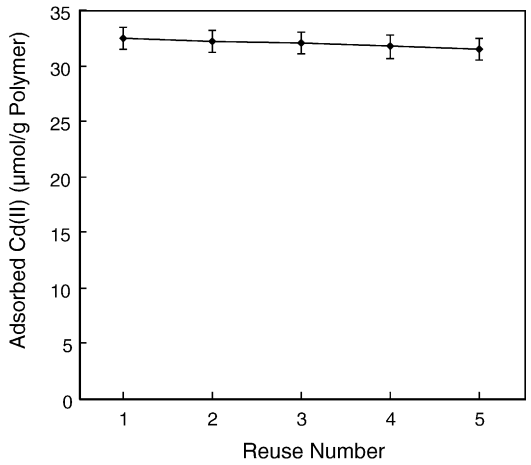

Fig. 8. Adsorption–desorption cycle of Cd^{2+}-imprinted poly(HEMA-MAC) beads. Adsorption conditions: Cd^{2+} concentration: 40 mg/L; incubation time: 60 min; T: 25 °C.

4. Conclusions

Molecularly imprinted materials have been demonstrated to possess a very high degree of selectivity towards targeted substances [1–13]. Up to date, molecular imprinted polymers have been produced by bulk polymerization. These bulk polymers have to be ground and sieved to obtain particles of the desired dimensions for further use [31,32]. This process is not only wasteful and time consuming, but also produces irregularly shaped particles which are unfavorable for applications, and therefore, spherical molecularly imprinted

polymers are more desirable [33–37]. Molecularly imprinted beads spherical in shape were prepared by suspension polymerization. The average size of the beads was controlled to be between 63 and 140 μm in diameter. The adsorption was relatively fast and the time required to reach equilibrium conditions was about 60 min. The maximum adsorption capacity for Cd^{2+} ions was 32.5 μmol/g dry weight of beads. This fast adsorption equilibrium is most probably due to high complexation and geometric affinity between Cd^{2+} ions and Cd^{2+} cavities in the beads 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) beads. 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 beads for Cd^{2+}/Pb^{2+} and Cd^{2+}/Zn^{2+} were 7.8 and 1683 times greater than non-imprinted matrix, respectively. The desorption time was found to be 30 min. The Cd^{2+}-imprinted poly(HEMA-MAC) beads can be used many times without decreasing their adsorption capacities significantly.

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