# $Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Sr^{2+}$, and $Y^{3+}$ Binding Characteristics of 17β-Estradiol Molecularly Imprinted Polymer Particles Incorporated with Dicyclohexano-18-Crown-6 for Urine Bioassay

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**ABSTRACT:** The metal ion binding characteristics of molecularly imprinted polymer (MIP) submicron particles prepared using 17β-estradiol (E2) as a template, and incorporated with dicyclohexano-18-crown-6 (DCH18C6), were studied using differential pulse anodic stripping voltammetry. When $Sr^{2+}$ was added to DCH18C6-E2-MIP particles already occupied by $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ inside the binding sites, a displacement reaction was observed: $Cd^{2+}/Cu^{2+}/Pb^{2+}$-DCH18C6-E2-MIP + $Sr^{2+}$ = $Sr^{2+}$-DCH18C6-E2-MIP + $Cd^{2+}/Cu^{2+}/Pb^{2+}$. This demonstrated that DCH18C6 had stronger binding affinity for $Sr^{2+}$ than

$Cd^{2+}$ $Cu^{2+}$ or $Pb^{2+}$. Strong DCH18C6 binding affinity was also observed for $Y^{3+}$. Atomic emission spectrometry showed that DCH18C6-E2-MIP particles (150 mg/mL) resulted in 52% binding of $Sr^{2+}$ (2000 ppm, at pH 6.3 ± 0.1 and ionic strength of 0.1$M$ NaNO₂). © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 12–19, 2012

**Key words:** strontium; 17β-estradiol; dicyclohexano-18-crown-6; molecularly imprinted polymer; submicron particles; differential pulse anodic stripping voltammetry; atomic emission spectrometry

## INTRODUCTION

Rapid bioassay is very important for immediate and near-term consequence management, which includes identifying the contaminated individuals and providing necessary medical intervention during a radiological or nuclear emergency. Urine is the most common type of biological fluid samples used in clinical and toxicological laboratories because of its availability. A rapid bioassay method has been developed for the determination of $^{90}Sr$ in human urine sample by Sadi et al.[1] Application of this newly developed bioassay technique for $^{90}Sr$ in urine on a field deployable instrument has been reported by Li et al.[2] However, urine is inherently complex, consisting of many endogenous/exogenous substances that make direct determination of many target analytes almost not feasible at low concentrations.[3] For instance, the concentration of 17β-estradiol (E2) in human urine is normally in the range of 0.75–135 μg/mL.[4] Sample pretreatment, focusing on enhance-ment of selectivity and sensitivity, is hence required prior to analyte quantification. The most important developments have been microextraction (with a fiber, tube, or packed syringe), stir bar sorptive extraction, and solid phase extraction (with particles ranging in size from micro- to nanometers). A cartridge-based preconcentration and purification approach, intended for single use, would have the $^{90}Sr$ in a urine sample retained, followed by elution for determination of its concentration. Recently, Plionis et al. reported an automated procedure for the determination of $^{90}Sr$, by flow scintillation analysis, in aqueous samples with low levels of activity (<1000 dpm per sample).[5] They used commercially available Sr-Spec resin, consisting of 4,4′(5′)-bis(*tert*-butylcyclohexano)-18-crown-6 impregnated on polymer support beads that served as the stationary phase packed in a column. Since the impregnation solvent, octanol, dissolved into the aqueous mobile phase, the extractive ligand would bleed though the column over repetitive use.

Dicyclohexano-18-crown-6 (DCH18C6), a much less expensive chemical ligand, had previously been demonstrated to improve the isolation of strontium from environmental samples for radiometric determination of $^{90}Sr$.[6] The method was based on column extraction of strontium using DCH18C6 in tetra-chloroethane, which was held as a liquid in the

pores of styrene-divinyl benzene beads. A key step was desorption from the column, by using hot water, to recover 90% of the strontium. In conjunction with advanced radiometry, measuring $^{90}$Sr activities on the order of 0.1 Bq per sample would be feasible. It was demonstrated that condensation polymers containing subunits of crown ether carboxylic acid monomers made effective stationary phases for the chromatographic separation of Sr$^{2+}$ and Y$^{3+}$.[7] Later on, potassium-DCH18C6 complex was used as a highly efficient carrier for the transport of palladium as PdCl$_4$$^{2-}$ complex anion through a chloroform bulk liquid membrane.[30] Extraction of cadmium ions into dichloromethane solution of DCH18C6, as ion-pairs [HL$^+$][CdCl$_3$$^-$] and [HL$^+$]$_2$[CdCl$_4$$^{2-}$] (where L = DCH18C6), was reported.[8] DCH18C6 was successfully intercalated in bentonite to exploit the permanent negative charge present for more efficient extraction of strontium and cesium.[9]

In this work, we focused on the preparation of polymeric submicron particles from methacrylic acid (MAA) to exploit its negative charge. A molecularly imprinted polymer (MIP) of E2 was tested for better incorporation of DCH18C6 to minimize bleeding. The metal ion binding characteristics of these novel particles were studied systematically using both differential pulse anodic stripping voltammetry (DPASV) and atomic emission spectrometry (AES). Our ultimate goal would be to develop the most efficient and selective particles for solid phase extraction of Sr$^{2+}$ from urine samples for emergency bioassay. DPASV had proven to be an efficient tool for rapid diagnosis of metal poisoning and for evaluating the progress of chelation therapy.[10] This technique was able to determine zinc, cadmium, lead, bismuth, copper, and thallium in urine, blood, hair, teeth, feces, and bone for medical management and postmortem studies. DPASV was also used to study the complexation of Pb$^{2+}$ and Cu$^{2+}$ with two polymers synthesized from maleic anhydride.[11] Both polymers contained carboxylic and phenolic groups as complexing agents. DPASV could distinguish between metal adsorption to particulate matter and metal complexation by soluble ligands adhered to particles.[12] DPASV methods were developed to determine cadmium, lead, zinc and copper in urban aerosol samples,[13] as well as Cu and As in utility poles treated with chromated copper arsenate.[14]

## EXPERIMENTAL

### Materials

E2, DCH18C6, MAA, ethylene glycol dimethacrylate (EGDMA), and 2,2′-azobis(2-isobutyronitrile) (AIBN) were all purchased from ς-Aldrich (St. Louis, MO, U.S.).

Acetonitrile (HPLC grade) and acetone (spectro grade) were obtained from Caledon.

### Preparation of MIP particles

The method for synthesizing E2- and DCH18C6-MIP particles was adopted from a previous report by Wei et al.[4] A molar ratio of 1:8:4 was used, by dissolving E2 (0.272 g) or DCH18C6 as the template, MAA (0.343 mL) as the functional monomer, EGDMA (0.668 mL) as the crosslinker, and AIBN (0.0234 g) as the initiator in acetone/acetonitrile (1:3 v/v) as the porogen. The mixture was deoxygenated with nitrogen for 5 min, and then polymerized at 60°C for 24 h in a water bath. The E2-MIP particles were then washed 3 times with acetonitrile to remove any remaining chemicals, collected by centrifugation, and dried at 70°C in an oven. The average particle size was measured by dynamic light scattering (DLS) using a nano-DLS analyzer (Brookhaven Instruments, Holtsville, NY, U.S.).
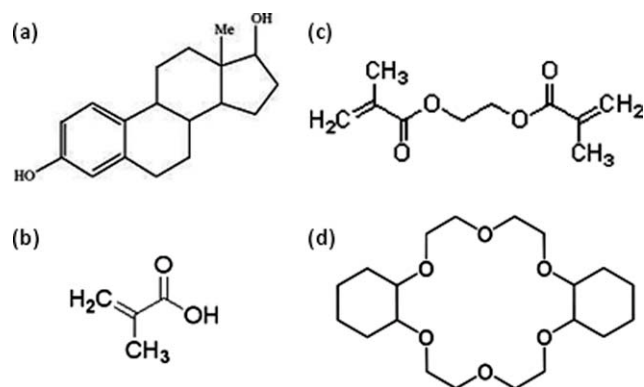
### DCH18C6 as binding ligands in E2-MIP particles

Different concentrations (86–500 ppm) of DCH18C6 were added, in 5-mL aliquots, to 75 mg of E2-MIP particles in each microtube. After incubation for 1 h, the microtubes were centrifuged at 3400 ± 100 rpm for 50 min. The supernatant was withdrawn for high-performance liquid chromatography (HPLC) analysis with UV detection to determine the remaining DCH18C6 concentration, and hence the quantity of DCH18C6 incorporated by the E2-MIP particles. The DCH18C6-E2-MIP particles were washed, collected and dried for binding tests. 75 mg of DCH18C6-E2-MIP particles were added into 0.5 mL of standard metal solution (containing 10$^{-5}$ M of Cd$^{2+}$, Cu$^{2+}$, Pb$^{2+}$, Sr$^{2+}$, and/or Y$^{3+}$). After incubation for 1 h, the mixture was centrifuged for 50 min to collect all particles on the bottom. Upon transferring 0.1 mL of the supernatant into a voltammetric cell containing 10.5 mL of supporting electrolyte solution, DPASV analysis was performed to determine the remaining Cd$^{2+}$, Cu$^{2+}$, and Pb$^{2+}$ concentrations.

### HPLC analysis and DPASV analysis

HPLC analysis was performed on a Perkin–Elmer LC240 instrument. The mobile phase was composed of acetonitrile/methanol/distilled deionized water (1:1:2 v/v) at a flow rate of 0.5 mL/min, and the UV detector was set at a wavelength of 278 nm.

DPASV was used to determine the binding efficiency of E2-MIP and DCH18C6-E2-MIP particles with trace concentrations of Cd$^{2+}$, Cu$^{2+}$, and Pb$^{2+}$ at room temperature. All analyses were performed on a Metrohm model 797 VA instrument, with an Ag/

**Figure 1** Molecular structures of (a) 17β-estradiol (E2), (b) MAA, (c) ethylene glycol dimethacrylate (EGDMA), and (d) dicyclohexano-18-crown-6 (DCH18C6).

AgCl reference electrode and a hanging mercury drop working electrode. A total of 10.5 mL of supporting electrolyte solution (containing 0.5 mL of $0.1M$ acetate buffer), 0.1 mL of $3M$ KCl, and 0.1 mL of supernatant were added in the voltammetric cell. The solution was deoxygenated with high-purity nitrogen, and then analyzed by setting the initial potential to $-1.2$ V, the final potential to $-0.1$ V, the deposition time to 60 s, the step potential to 2 mV, and the frequency to 50 Hz. Determination of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ concentrations was achieved by the standard addition method. Data acquisition and

**TABLE I**
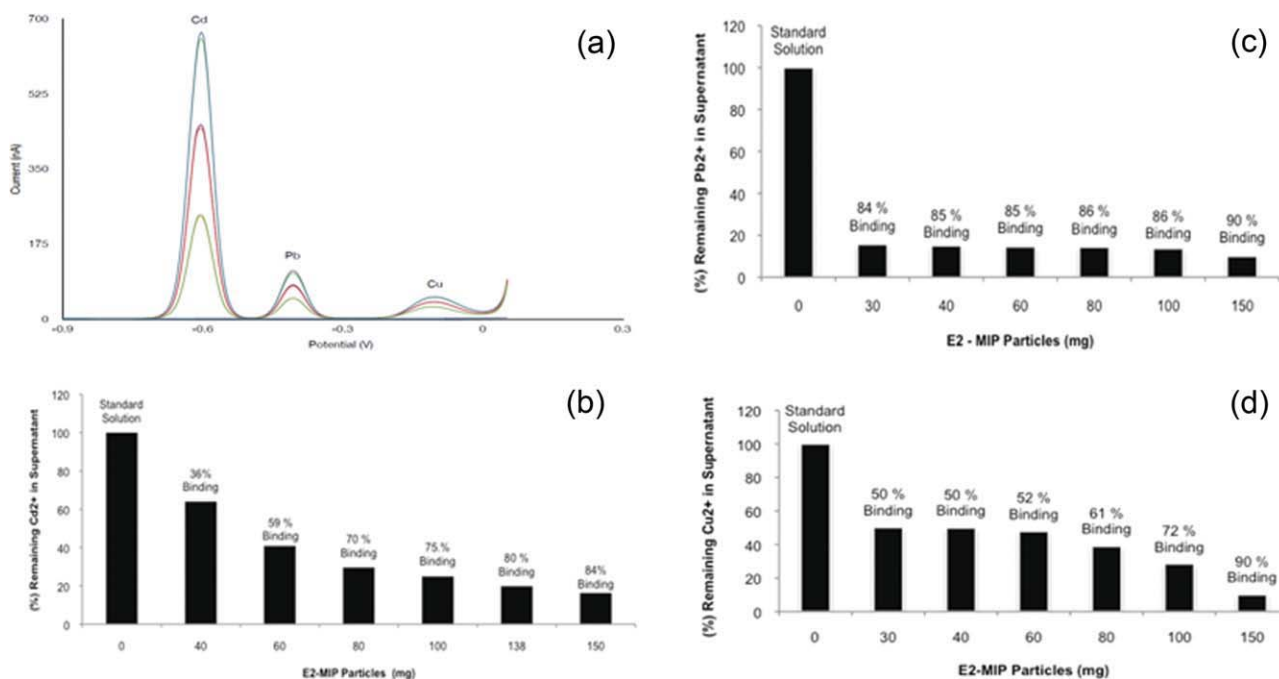**Ionic Radii of Metal Ions**

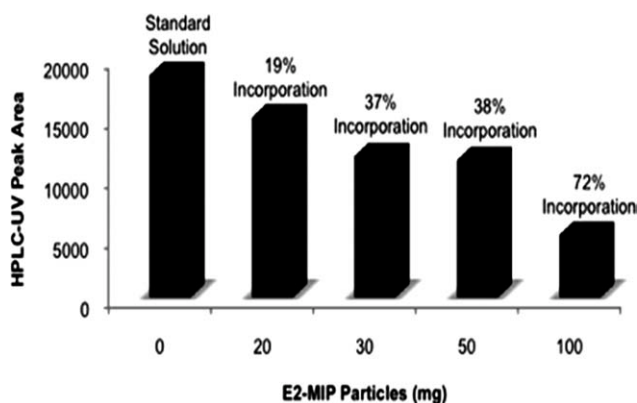| Metal | Ionic radius (pm) |
|---|---|
| $Cd^{2+}$ | 109 |
| $Cu^{2+}$ | 87 |
| $K^+$ | 152 |
| $Pb^{2+}$ | 133 |
| $Sr^{2+}$ | 132 |
| $Y^{3+}$ | 104 |

analysis were controlled through a PC computer connected to the potentiostat.

## RESULTS AND DISCUSSION

### Binding of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ onto E2-MIP particles

The molecular structures of E2,[15] MAA,[16] EGDMA,[17] and DCH18C6[18] are illustrated in Figure 1. Note that for E2 the O—O interatomic distance is 1096 pm,[19] which is significantly greater than the ionic radii of $Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Sr^{2+}$, and $Y^{3+}$ listed in Table I.[20] The synthesis of E2-MIP particles was simple, involving a one-step suspension polymerization procedure. Their submicron size (153 ± 1 nm as measured by DLS) facilitated uniform dispersion in water for up to 17 days.[21] Capillary electrophoresis provided an estimation of their negative surface charges from the —COOH group of MAA.[22]



**Figure 2** (a) DPASV voltammogram typically exhibited three characteristic peaks for sensitive determination of $Cd^{2+}$, $Pb^{2+}$, and $Cu^{2+}$ by standard additions. % Remaining of (b) $Cd^{2+}$, (c) $Pb^{2+}$, and (d) $Cu^{2+}$ in supernatant after 1 mL of $10^{-5}$ M standard solution was treated with different weights of E2-MIP particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Figure 3** HPLC-UV determination of remaining DCH18C6 in the supernatant after addition of 1 mL of 86 ppm DCH18C6 onto different weights of E2-MIP particles, followed by incubation for 1 h.
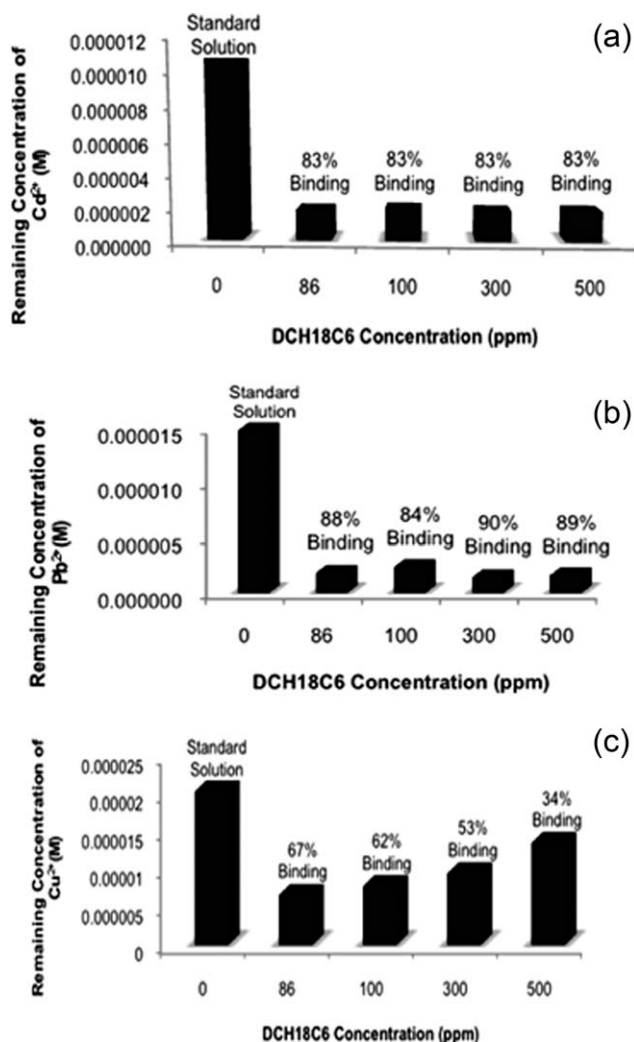
The DPASV technique lent itself to sensitive determination of small changes in metal ion concentrations, while its low-detection limits allowed the use of sample solutions containing as little as $10^{-7}$ M of metal ions for binding tests. Figure 2(a) shows a DPASV voltammogram that exhibited three characteristic peaks for $Cu^{2+}$ (at $-0.11$ V), $Pb^{2+}$ (at $-0.40$ V), and $Cd^{2+}$ (at $-0.60$ V) simultaneously. As shown in Figure 2(b–d), up to $84 \pm 1\%$ of $Cd^{2+}$, $90 \pm 1\%$ of $Pb^{2+}$, and $90 \pm 1\%$ of $Cu^{2+}$ bound with 150 mg of E2-MIP particles in 10.7 mL of aqueous solution. Apparently competitive binding of $Cd^{2+}$, $Pb^{2+}$, and $Cu^{2+}$ with the E2-MIP particles occurred, and differences in % binding could be attributed to the equilibrium constant between each metal ion and —COOH/—COO— groups from MAA/EGDMA. A previous study by Kesenci et al. had used poly(ethylene glycol dimethacrylate-co-acrylamide) beads for the separation of $Pb^{2+}$ and $Cd^{2+}$ ions in aqueous solution.[23] Their beads showed selectivity towards $Pb^{2+}$ in a mixture with $Cd^{2+}$.

## Binding of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ onto DCH18C6-MIP particles

DCH18C6-MIP particles were next tested for binding with $10^{-5}$ M standard solution of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$. As determined from DPASV data not shown here, up to $59 \pm 1\%$ of $Cd^{2+}$, $86 \pm 1\%$ of $Pb^{2+}$, and $23 \pm 1\%$ $Cu^{2+}$ bound with 150 mg of DCH18C6-MIP particles in 10.7 mL of aqueous solution. Intuitively, DCH18C6 did not bind efficiently with $Cd^{2+}$ and $Cu^{2+}$ due to their small ionic radii (see Table I above). By incorporating DCH18C6 within the MIP particles, the size match between the cavity radius of crown ether and the radius of cation is an important parameter governing metal extraction from aqueous solutions.[24] Apparently, $Cd^{2+}$ was a bad match and $Cu^{2+}$ was even worse.
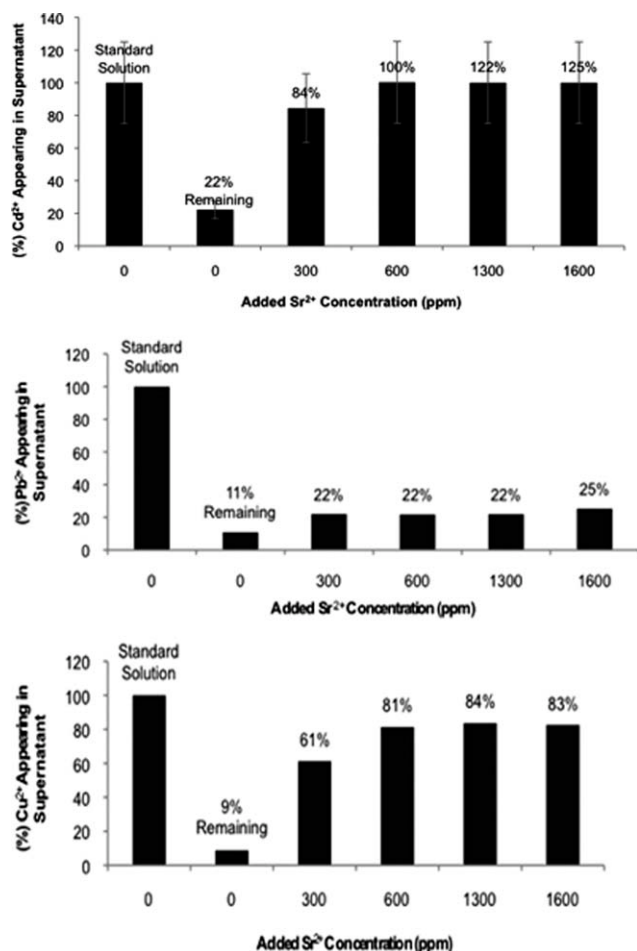
## Binding of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ onto DCH18C6-E2-MIP particles

As evidenced by the results presented in Figure 3, DCH18C6 was successfully incorporated with E2-MIP particles, up to 72%. Batches of E2-MIP particles had up to 500 ppm of DCH18C6 incorporated, for binding tests with the $10^{-5}$ M standard solution of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$. As shown in Figure 4(a), up to $83 \pm 1\%$ of $Cd^{2+}$ bound onto 75 mg of DCH18C6-E2-MIP particles, which was almost the same efficiency (or % binding) as obtained in Figure 2(b) above using 75 mg of E2-MIP particles (without DCH18C6). Note that the % binding remained almost constant with varying concentrations of DCH18C6 incorporated. This could be explained by a low-binding efficiency between DCH18C6 and







**Figure 4** Binding of (a) $Cd^{2+}$, (b) $Pb^{2+}$, and (c) $Cu^{2+}$ onto DCH18C6-E2-MIP particles. A total of 0.5 mL of $10^{-5}$ M standard solution was added to 75 mg of E2-MIP particles incorporated with different concentrations of DCH18C6. After incubation for 1 h and centrifugation, 0.1 mL of the supernatant was withdrawn for DPASV analysis.

**Figure 5** Displacement of (a) $Cd^{2+}$, (b) $Pb^{2+}$, and (c) $Cu^{2+}$ from 75 mg of E2-MIP particles (already bound with $10^{-5}$ M standard solution of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$) by different concentrations of $Sr^{2+}$, as determined by DPASV analysis of 0.1 mL supernatant after incubation for 1 h and centrifugation.

$Cd^{2+}$, considering how the crown ether and E2-MIP may share the 83% of $Cd^{2+}$ bound. Next, binding of $Pb^{2+}$ onto E2-MIP particles (without DCH18C6) was

86 ± 2% in Figure 2(b) above. After 86–500 ppm DCH18C6 was incorporated to form DCH18C6 E2-MIP particles, $Pb^{2+}$ binding increased to 90 ± 3% as shown in Figure 4(b). One plausible explanation is that the ionic radius for $Pb^{2+}$ is 133 pm (see Table I above) and the cavity radius of 18-crown-6 is 130–160 pm. Thus, incorporation of DCH18C6 increased the binding efficiency for $Pb^{2+}$ by a significant 4%.

## Reversibility of binding by $Sr^{2+}$ competition

E2-MIP particles were used next to investigate the selectivity in binding (for $Sr^{2+}$ against $Cd^{2+}$, $Cu^{2+}$ and $Pb^{2+}$). When different concentrations of $Sr^{2+}$ were added to these particles already occupied by $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$, their concentrations in the supernatant increased as determined by DPASV analysis. The results in Figure 5 suggest that $Sr^{2+}$ successfully competed for some binding sites according to the following ion exchange reaction:

$$M^{2+} - E2 - MIP + Sr^{2+} = Sr^{2+} - E2 - MIP + M^{2+}$$

(1)
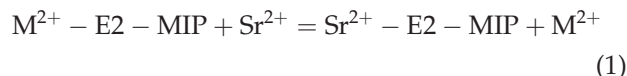
where $M^{2+}$ could be $Cd^{2+}$, $Pb^{2+}$, or $Cu^{2+}$. The effect was prominent for both $Cd^{2+}$ and $Cu^{2+}$, but only moderate for $Pb^{2+}$. One plausible explanation is the very similar ionic radii of $Sr^{2+}$ (132 pm) and $Pb^{2+}$ (133 pm), which render the ion exchange less favorable.

The reversibility investigation was repeated using DCH18C6-E2-MIP particles already occupied by $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$. These particles had just incorporated 86–500 ppm DCH18C6. As expected, $Sr^{2+}$ successfully competed for some binding sites in a displacement reaction similar to eq. (1) above. The displacement effect was again prominent for both $Cd^{2+}$ and $Cu^{2+}$, but only moderate for $Pb^{2+}$.

**TABLE II**
Summary of $Cd^{2+}$, $Pb^{2+}$, and $Cu^{2+}$ Binding Results for E2-MIP and DCH18C6-E2-MIP Particles, Followed by Displacement Using $Sr^{2+}$
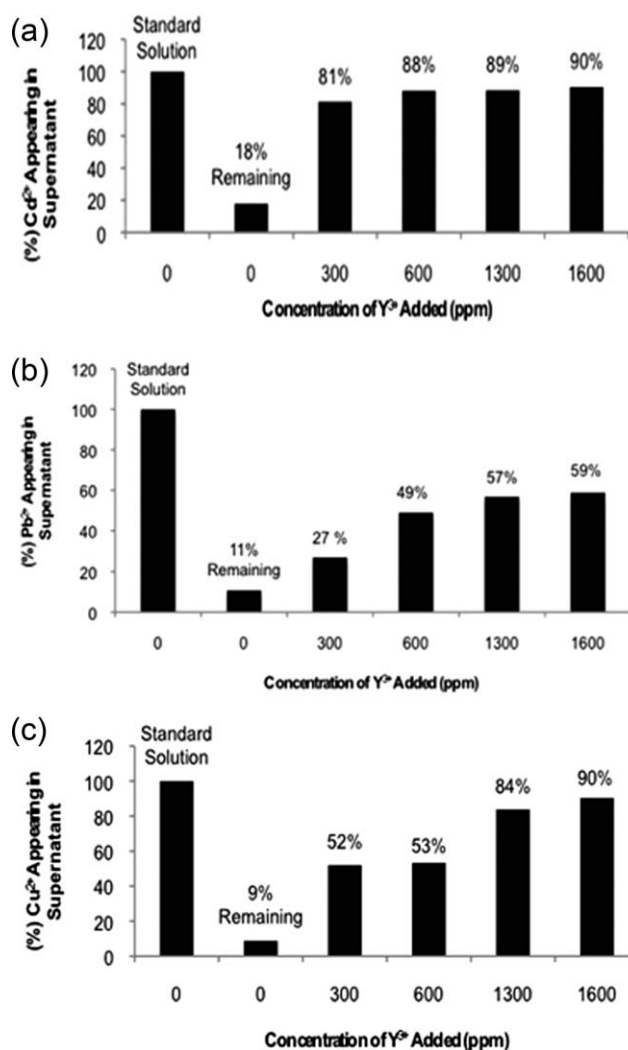
|  | Particles | % Binding[a] | % Remaining in supernatant before addition of 1300 ppm $Sr^{2+}$ | % Appearing in supernatant after addition of 1300 ppm $Sr^{2+}$ |
|---|---|---|---|---|
| $Cd^{2+}$ | E2-MIP | 84 ± 4 | 16 ± 1 | 100 ± 5 |
|  | DCH18C6-E2-MIP | 83 ± 4 | 17 ± 1 | 93 ± 4 |
|  | DCH18C6-MIP | 59 ± 3 |  |  |
| $Pb^{2+}$ | E2-MIP | 89 ± 4 | 11 ± 0.6 | 22 ± 1 |
|  | DCH18C6-E2-MIP | 90 ± 5 | 10 ± 0.6 | 21 ± 1 |
|  | DCH18C6-MIP | 87 ± 4 |  |  |
| $Cu^{2+}$ | E2-MIP | 98 ± 3 | 2 ± 0.2 | 84 ± 4 |
|  | DCH18C6-E2-MIP | 96 ± 3 | 4 ± 0.2 | 94 ± 5 |
|  | DCH18C6-MIP | 23 ± 1 |  |  |

% Binding results for DCH18C6-MIP particles are included for quick reference.
[a] % binding = 100% − % remaining in supernatant.

The % $Cd^{2+}$ appearing in the supernatant increased to 93%, which can be compared with 122% when E2-MIP particles (without DCH18C6) were investigated in Figure 5(a) above. Also, the % $Cu^{2+}$ appearing in the supernatant increased to 94%. However, the % $Pb^{2+}$ appearing in the supernatant increased to only 20%. Although the ionic radii of $Sr^{2+}$ and $Pb^{2+}$ are very similar (132 and 133 pm, respectively), $Sr^{2+}$ is a hard cation and $Pb^{2+}$ is an intermediate cation. Knowing that DCH18C6 is considered as a hard ligand, $Sr^{2+}$ would replace $Pb^{2+}$ to increase its concentration in the supernatant (up to 20% while 80% of $Pb^{2+}$ remained bound to DCH18C6-E2-MIP particles even in the presence of $Sr^{2+}$). Rounaghi and Mofazzeli had previously studied complex formation between DCH18C6 and four alkaline earth metal cations using a conductometric method.[25] Their results showed that DCH18C6 formed a 1:1 complex (DCH18C6-$Sr^{2+}$) with $Sr^{2+}$, and the order of selectivity was $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$.

Our major findings are now summarized in Table II for easy comparison. It appears that the increased $Cd^{2+}$, $Pb^{2+}$, and $Cu^{2+}$ contents in the supernatant both before and after the inclusion of DCH18C6 were primarily due to the E2-MIP rather than the crown ether. Note that the cavity of DCH18C6 is much larger than the ionic radius of $Cu^{2+}$ (87 pm).[26] Moreover, $Cu^{2+}$ is an intermediate cation and DCH18C6 is considered to be a hard ligand. Both factors would explain why there was better binding of $Cu^{2+}$ with E2-MIP particles than with DCH18C6-E2-MIP particles (as evidenced by a higher % appearing in the supernatant after addition of 1300 ppm $Sr^{2+}$). When the concentration of DCH18C6 increased, a decrease in the % binding of $Cu^{2+}$ was actually observed in Figure 4(c) above.



**Figure 6** Displacement of (a) $Cd^{2+}$, (b) $Pb^{2+}$, and (c) $Cu^{2+}$ from 75 mg of E2-MIP particles (already bound with $10^{-5}$ M standard solution of $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$) by different concentrations of $Y^{3+}$, as determined by DPASV analysis of 0.1 mL supernatant after incubation for 1 h and centrifugation.

**TABLE III**
**Summary of $Cd^{2+}$, $Pb^{2+}$, and $Cu^{2+}$ Binding Results for E2-MIP and DCH18C6-E2-MIP Particles, Followed by Displacement Using $Y^{3+}$**

| | Particle's description | % Binding[a] | % Remaining in supernatant before addition of 1300 ppm $Y^{3+}$ | % Remaining in supernatant after addition of 1300 ppm $Y^{3+}$ |
|---|---|---|---|---|
| $Cd^{2+}$ | E2-MIP | 84 ± 4 | 16 ± 1 | 88[b] ± 5 (cf. 100 for $Sr^{2+}$)[c] |
| | DCH18C6-E2-MIP | 83 ± 4 | 17 ± 1 | 99 ± 6 (cf. 93 for $Sr^{2+}$) |
| | DCH18C6-MIP | 59 ± 3 | | |
| $Pb^{2+}$ | E2-MIP | 89 ± 5 | 11 ± 0.6 | 57 ± 3 (cf. 22 for $Sr^{2+}$) |
| | DCH18C6-E2-MIP | 90 ± 5 | 10 ± 0.5 | 27 ± 2 (cf. 21 for $Sr^{2+}$) |
| | DCH18C6-MIP | 87 ± 4 | | |
| $Cu^{2+}$ | E2-MIP | 98 ± 5 | 2 ± 0.1 | 84[b] ± 4 (cf. 84 for $Sr^{2+}$) |
| | DCH18C6-E2-MIP | 96 ± 5 | 4 ± 0.2 | 98 ± 5 (cf. 94 for $Sr^{2+}$) |
| | DCH18C6-MIP | 23 ± 1 | | |

% Binding results for DCH18C6-MIP particles are included for quick reference.
[a] % Binding = 100% − % remaining in supernatant.
[b] Increased to 90% when $Y^{3+}$ was added up to 1600 ppm.
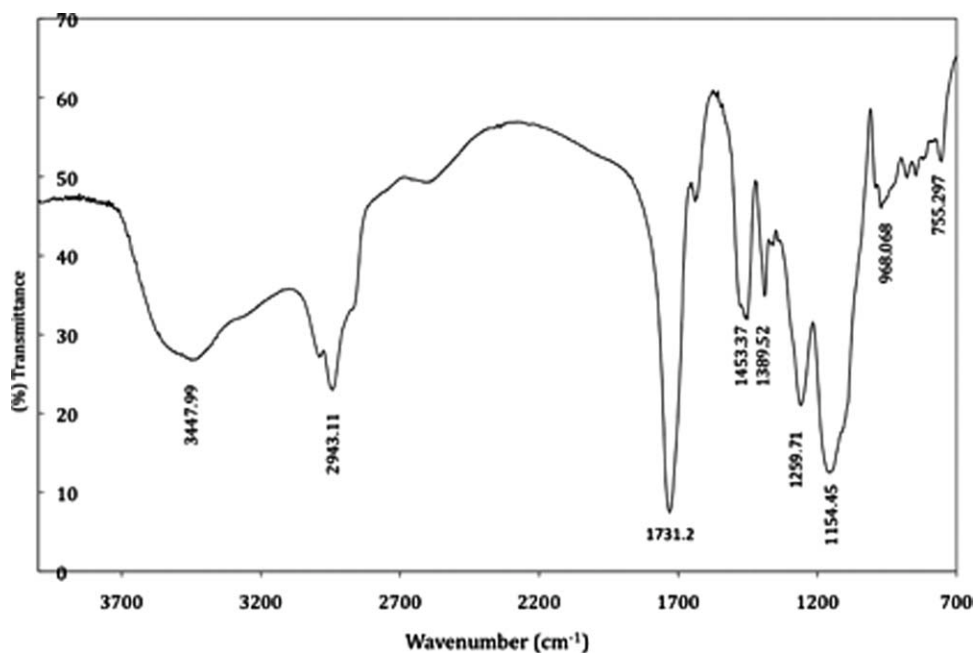[c] Confer (compare with).

**Figure 7** The FTIR spectra of E2-MIP, DCH18C6-MIP, and DCH18C6-E2-MIP particles.

### Reversibility of binding by $Y^{3+}$ competition

Furthermore, $Y^{3+}$ was added to compete for the binding sites already occupied by $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ inside E2-MIP particles. As shown in Figure 6, the concentrations of all three metal ions in the supernatant increased with the addition of more $Y^{3+}$. $Cd^{2+}$ was displaced easily from the E2-MIP particles by $Y^{3+}$, $Cu^{2+}$ was displaced with reluctance, while $Pb^{2+}$ was hardly displaced. By comparison, Figure 5 above demonstrated that $Sr^{2+}$ was able to displace $Cd^{2+}$ more easily and $Cu^{2+}$ much more readily. It is logical to deduce that the E2-MIP particles are selective in binding for $Sr^{2+}$ over $Y^{3+}$. However, $Y^{3+}$ seemed to be better able to displace $Pb^{2+}$ than $Sr^{2+}$ probably due to its higher ionic charge.

The reversibility investigation was repeated using DCH18C6-E2-MIP particles already occupied by $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$ inside the binding sites. As expected, $Y^{3+}$ successfully competed for some binding sites in a displacement reaction. Again, $Cd^{2+}$ was displaced easily from the DCH18C6-E2-MIP particles by $Y^{3+}$, $Cu^{2+}$ was displaced with reluctance, while $Pb^{2+}$ was hardly displaced. This behavior is similar to that of $Sr^{2+}$ discussed above. As summarized in Table III, all results for % remaining in the supernatant after addition of 1300 ppm $Y^{3+}$ suggest that DCH18C6 is a ligand with stronger binding affinity for $Y^{3+}$ (over and above those for $Cd^{2+}$, $Cu^{2+}$, and $Pb^{2+}$) just like $Sr^{2+}$. Note that $Y^{3+}$ is a trivalent cation that bound to E2-MIP more strongly than $Pb^{2+}$. Hence, 57% of $Pb^{2+}$ appeared in the supernatant (compared to only 22% of $Sr^{2+}$).

### Binding of $Sr^{2+}$ onto DCH18C6-E2-MIP particles (as determined by AES)

Since DPASV cannot be used directly to determine $Sr^{2+}$, binding of $Sr^{2+}$ onto DCH18C6-E2-MIP particles was studied further by AES at 407.7 nm or 421.5 nm. 150 mg of DCH18C6-E2-MIP particles showed 52% binding, as compared with 19% binding for DCH18C6-MIP particles, under pH 6.3 $\pm$ 0.1 and ionic strength of $0.1M$ $NaNO_2$. It is interesting to note that pH 6.3 yielded better binding results than pH 5.5 and pH 9.0. If the sample pH was lower than 3, the MIP particles would become positively charged and therefore $Sr^{2+}$ would not bind onto them. Experimental results also showed dependency of % binding on increasing ionic strength. Apparently, $Sr^{2+}$ binding onto the DCH18C6-E2-MIP particles may involve outer-sphere complex formation.[27]

### FTIR spectra of E2-MIP, DCH18C6-MIP, and DCH18C6-E2-MIP particles

The three types of MIP particles have similar IR spectra, indicating commonality in their backbone structures, as shown in Figure 7. Absorption peaks due to carboxyl OH stretch ($\sim 3500$ cm$^{-1}$), C=O stretch ($\sim 1730$ cm$^{-1}$), C—O stretch ($\sim 1260$ cm$^{-1}$), and C—H vibrations ($\sim 756$, $\sim 1390$, $\sim 1460$, and $\sim 2956$ cm$^{-1}$) were observed as expected.[28]

### CONCLUSIONS

Three new kinds of polymeric submicron particles were prepared by molecular imprinting of E2 and

incorporation of DCH18C6. Their metal ion binding properties in aqueous samples have been successfully characterized by DPASV and AES. DPASV can readily be used to determine metal ions in a sample solution containing particles, without the need to have the particles removed (by centrifugation or filtration) prior to analysis. In the presence of DCH18C6, binding is favorable for Sr$^{2+}$ and Y$^{3+}$ over Cd$^{2+}$, Pb$^{2+}$, or Cu$^{2+}$. These particles are field-deployable for urine bioassays in population monitoring after a radiological or nuclear emergency. Sample throughput can potentially be improved by putting a superparamagnetic core inside the particles,[29] as will be detailed in a separate report.

## References

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