

*Original Paper*

## Application of a Chelate Forming Calix[4]arene-o-vanillinthiosemicarbazone Resin to the Separation, Preconcentration and Trace Determination of Cu(II), Cd(II) and Pb(II) in Natural Water Samples

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**Abstract.** A very stable calixarene-based polymeric chelating resin has been synthesized by covalently linking calix[4]arene-o-vanillinthiosemicarbazone through its 'lower rim' to Merrifield resin. It was characterized by FT IR,  $^1\text{H}$  NMR and elemental analysis. The resin was efficiently employed to separate and preconcentrate toxic metal ions such as Cu(II), Cd(II) and Pb(II) in a column prior to their determination by UV-Vis spectrophotometry and atomic absorption spectrophotometry (GFAAS) with a relative standard deviation ranging between 1.0–1.4%. Various physico-chemical parameters such as pH, concentration of eluting agents, flow rate, total sorption capacity, metal-ligand stoichiometry, exchange kinetics, preconcentration factor, distribution coefficient, breakthrough capacity, resin stability, effect of electrolytes and associated metal ions have been studied. Uptake and stripping of these metal ions on the resin were fast, indicating better access of the metal ions to the chelating sites. Detection limits ( $3\sigma_{\text{B}}$ ) of  $4.22\ \mu\text{g L}^{-1}$ ,  $11.89\ \mu\text{g L}^{-1}$  and  $19.61\ \mu\text{g L}^{-1}$ , along with preconcentration factors of 100, 125 and 111 for Cu(II), Cd(II) and Pb(II), respectively, were achieved. The proposed method was successfully applied to the separation and

trace determination of Cu(II), Cd(II) and Pb(II) from natural water samples of Ahmedabad city.

**Key words:** Calix[4]arene; o-vanillinthiosemicarbazone; preconcentration; Merrifield resin; GFAAS.

The increase in industrial activity over the years has resulted in the contamination of ground and surface water by toxic metal ions [1]. In developing countries, water pollution generated by industrial effluents has been a serious issue [2]. In order to have ecologically sustainable growth, time-to-time analysis of metal ions such as Cr(VI), Cu(II), Cd(II), As(VI), Pb(II), Hg(II) etc. in natural water as well as in bio-fluid samples is required. In natural water and biological systems, Cu(II) is found in low levels, and it needs to be separated and enriched prior to its trace analysis [3, 4]. Cd(II) metabolism and toxicology are of great concern as it is accumulated in living organisms [5]. Cd(II) determination can be performed after preconcentration procedures, as its concentration in environmental and biological samples remains low [6]. Pb(II) content determination in food, blood and tissue samples is very important in order to diagnose Pb(II) intoxication [7, 8]. It is difficult to extract these metal ions since they are associated with a variety of complex matrix species that interfere and resist the extraction process. FAAS

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and ICP-OES techniques are not sensitive enough to detect and quantify these toxic metal ions in natural water samples since prior separation and preconcentration is required. Thus, there is a need of polymeric chelating ion exchange resins, which can act as sensitive and selective complexants. For solid phase extraction of toxic metal ions, polymeric calixarenes [9] have been significant due to their lower toxicity, affordability, ease of synthesis and operation. They are also eco-friendly unlike liquid–liquid extraction where the disposal of organic waste represents a major environmental problem.

Calixarenes, which are considered the third generation of supramolecules [10, 11], have a molecular framework with ‘upper rim’ and ‘lower rim’ that can be separately and selectively modified with different functionalities to achieve metal complexing properties and the desired solubility characteristics [12, 13]. For removal of toxic metal ions from aqueous media, immobilization of calixarenes on a polymer support has been reported in the literature [14–39]. In our earlier study we reported polymer supported calix[4]arene-semicarbazone for the separation and preconcentration of La, Ce(III), Th(IV) and U(VI) [40]. As the sulfur-containing o-vanillinthiosemicarbazone is a known complexing agent for toxic metal ions [41, 42], we contemplated incorporating this functionality into the calix[4]arene framework supported by a polymer backbone. In this article we report the synthesis of Merrifield resin supported calix[4]arene-o-vanillinthiosemicarbazone and its application to the separation, preconcentration and trace determination of Cu(II), Cd(II) and Pb(II) in natural water samples collected from Ahmedabad city.

## Experimental

### Apparatus

Precoated silica gel plates (Merck 60 F<sub>254</sub>) were used for TLC. A Systronics digital pH meter Model 335 was used for pH measurements. The flow rate in the column was adjusted with a Miclins peristaltic pump PP-10. Spectral measurements were performed on a Hitachi 3210 UV-Vis spectrophotometer using 10 mm quartz cells. FT IR spectra were recorded on a Jasco infrared spectrophotometer as KBr pellets. Elemental analysis was performed on a Heraeus Carlo Erba 1108 elemental analyzer. The <sup>1</sup>H NMR spectra were recorded on a Bruker DRX-300 operating at 300 MHz for proton in DMSO-d<sub>6</sub> with tetramethyl silane as the internal standard. Atomic absorption measurements were performed on a Perkin-Elmer model 420 atomic absorption spectrophotometer (AAS) equipped with a HGA-76 graphite furnace (GF). Pyrolytically coated graphite tubes were used throughout. Copper, cadmium and lead hollow cathode lamps operat-

ing at 10, 10 and 15 mA provided analytical wavelengths of 324.8 nm, 228.8 nm and 283.3 nm with slit widths of 0.5, 0.5 and 0.7, respectively. The measurements were based on integrated absorbance.

### Reagents

All chemicals used were of analytical grade and from E. Merck (www.merck.co.in) or Lancaster (www.lancastersynthesis.com). The solvents were purified by the method developed by Weissberger et al. [43]. Commercially available Merrifield peptide resin [chloromethylated polystyrene divinylbenzene co-polymer or CMPDB] (1% cross-linked, 1.5 mmol equivalent of Cl/g of resin, 200–400 mesh) was procured from Aldrich. All aqueous solutions were prepared with quartz distilled deionized water, which was further purified using a Millipore Milli-Q water purification system. Stock solutions (2000 µg mL<sup>-1</sup>) of Cu(II), Cd(II) and Pb(II) were prepared by dissolving the requisite amounts of CuSO<sub>4</sub>·5H<sub>2</sub>O, CdCl<sub>2</sub> and Pb(NO<sub>3</sub>)<sub>2</sub>, respectively, in water. Their final concentrations were standardized spectrophotometrically [44, 45]. pH adjustments were performed with standard buffer solutions as described elsewhere [46]. The glassware used was soaked in 10% HNO<sub>3</sub> overnight before use and cleaned repeatedly with doubly distilled deionized water. The water samples from Sabarmati river were isokinetically collected in clean polyethylene bottles from locations near a thermal power station, Ahmedabad. The ground water samples were collected from the university area and Vatva industrial zone of Ahmedabad city.

### Synthesis Routes

Synthesis of 5,11,17,23-tetratert-butyl-25,26,27,28-tetrahydroxycalix[4]arene **1** and 25, 26,27,28-tetrahydroxycalix[4]arene **2** were performed according to the method of Gutsche et al. [47, 48]. 26,28-dimethoxy-25,27-dihydroxycalix[4]arene **3** and 11,23-dinitro-26,28-dimethoxy-25,27-dihydroxycalix[4]arene **4** were synthesized as reported by Reinhoudt et al. [49].

### Synthesis of 11,23-Diamino-26,28-dimethoxy-25, 27-dihydroxycalix[4]arene **5**

A suspension of 5 g **4** (9.2 mmol), 10 mL hydrazine hydrate and a catalytic amount of Raney nickel in 50 mL methanol was refluxed for 6 hours. The reaction mixture was allowed to cool to room temperature, filtered, and the solvent was evaporated under reduced pressure. The residue was taken up in 60 mL dichloromethane, washed with water (3 × 30 mL), and dried over MgSO<sub>4</sub>. After filtration, the solvent was evaporated to give pure **5**. The yield was 3 g (68%) and the melting point was >300 °C (decomposition). FT IR data of a KBr pellets were as follows: 3180 cm<sup>-1</sup> (OH str.); 3435 cm<sup>-1</sup> (N–H str.). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 9.7 ppm (s,2,ArOH); 7.2–7.4 ppm (s,10,ArH); 3.4 ppm (br d,8,ArCH<sub>2</sub>Ar); 3.7 ppm (s,6,OCH<sub>3</sub>); 5.5 ppm (s,4,ArNH<sub>2</sub>). The elemental analysis calculated for C<sub>30</sub>H<sub>30</sub>O<sub>4</sub>N<sub>2</sub> (C = 74.65%; H = 6.27%, N = 5.81%) was found to be C = 74.39%; H = 6.08% and N = 5.61%.

### Synthesis of 11,23-bis(chlorodiazenyl)-26,28-dimethoxy-25,27-dihydroxycalix[4]arene **6**

10 g amine **5** (20.7 mmol) was diazotized in 35 mL HCl (40%) at 0 °C in an ice-bath while stirring constantly for 30 minutes using 10 g NaNO<sub>2</sub> (145 mmol) in 100 mL water-ethanol (1:1) media to yield **6**, which was then reacted in situ with o-VTSC to obtain **7**.

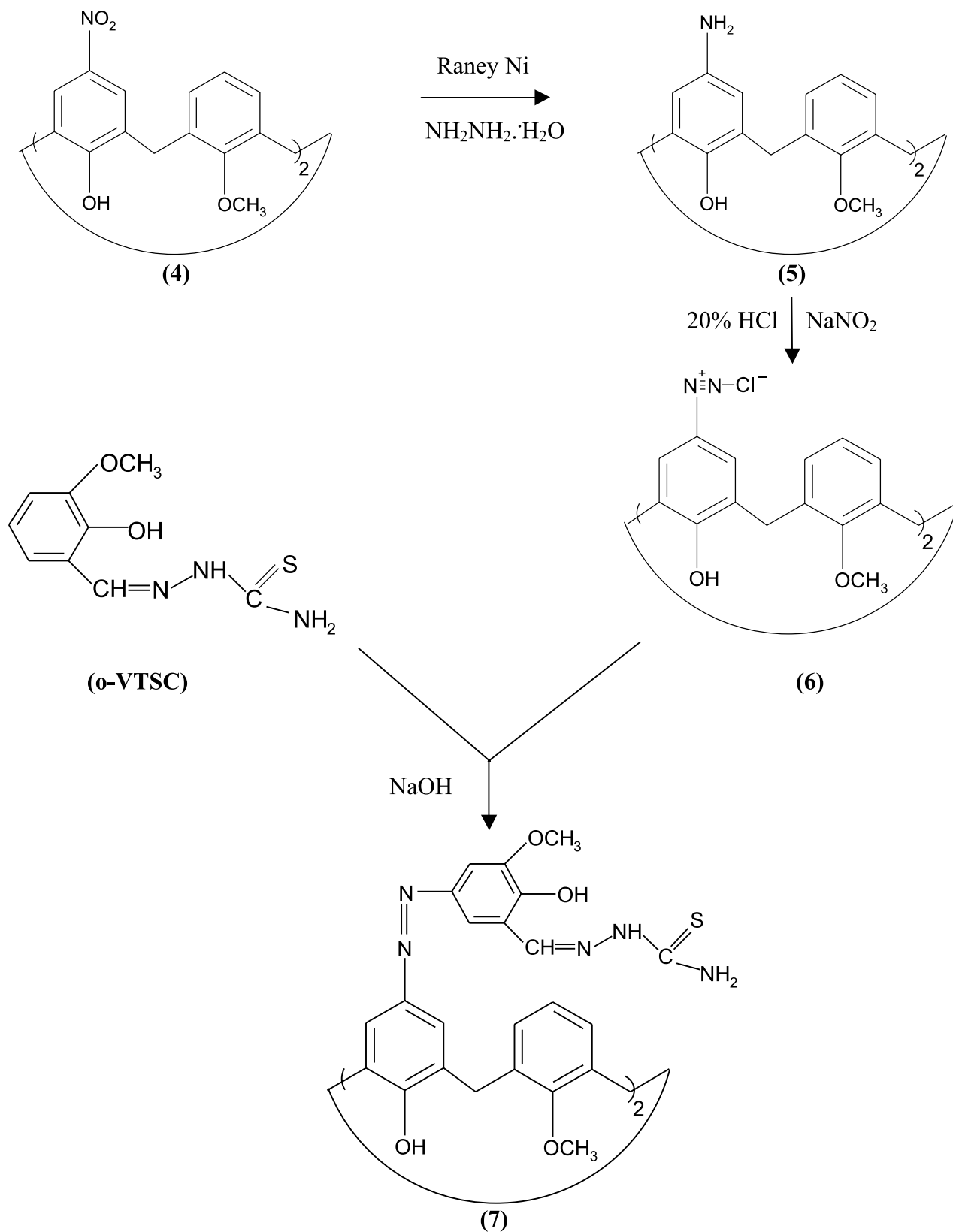
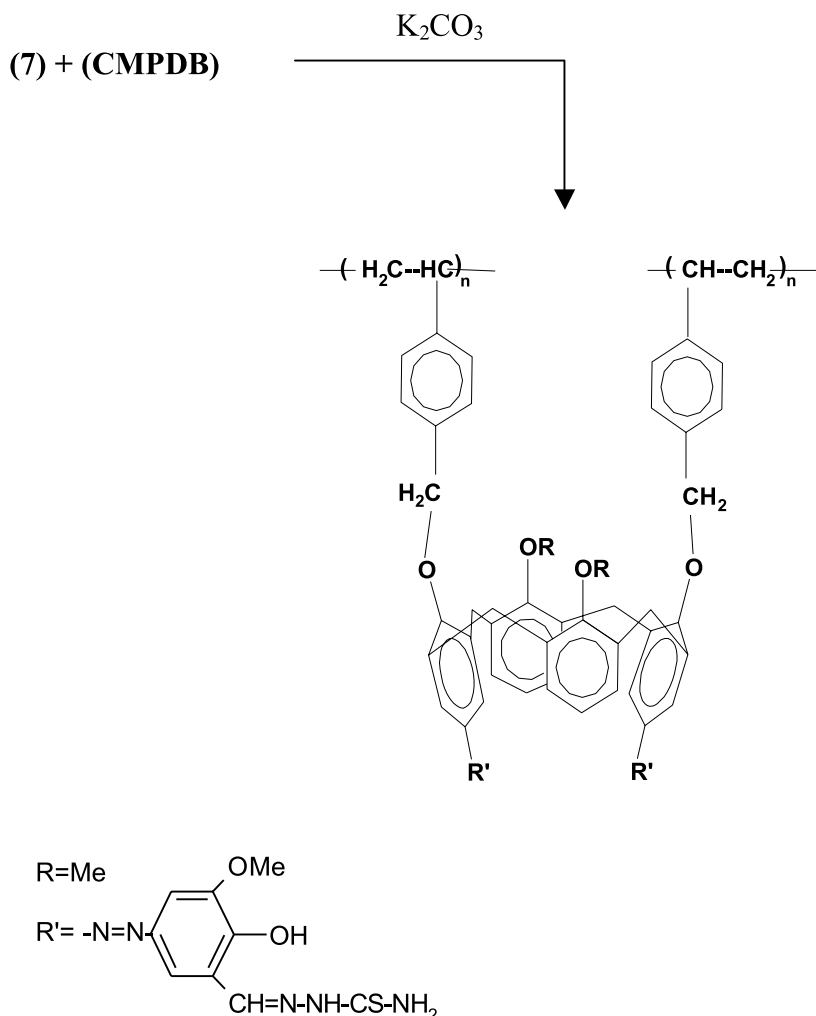


Fig. 1. Reaction flowchart



### (8) : CMPDB supported calix[4]arene-o-vanillinthiosemicarbazone

Fig. 1 (continued)

Synthesis of Calix[4]arene-11,23-bis(o-vanillinthiosemicarbazone) **7**

The solution containing **6** was placed in an ice-bath. A solution containing 10 g o-VTSC (44.4 mmol) in 40 mL NaOH (15 g, 375 mmol) in water was placed in a dropping funnel and slowly added while constantly stirring for 1 hour at 0 °C. The brownish-red coloured product **7** was solidified by evaporation of the solvent. Purification was performed by crystallization in iso-amyl acetate. The yield was 16 g (81%) and the melting point >300 °C (decomposition). FT IR data of KBr pellets were as follows: 3464 cm<sup>-1</sup> & 3227 cm<sup>-1</sup> (OH str.); 3440 cm<sup>-1</sup> (N-H str.); 1591 cm<sup>-1</sup> (CH=N str.). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 3.3 ppm (s,8,ArCH<sub>2</sub>Ar); 3.6–3.8 ppm (s,12,OCH<sub>3</sub>); 7.1–8.1 ppm (s,14,ArH); 10.1 ppm (s,4,ArOH). The elemental analysis calculated for C<sub>48</sub>H<sub>46</sub>O<sub>8</sub>N<sub>10</sub>S<sub>2</sub> (C = 60.34%; H = 4.86%; N = 14.67%; S = 6.72%) were found to be C = 59.96%; H = 4.65%; N = 14.76% and S = 6.45%.

Synthesis of **8** by Loading of **7** on CMPDB

A mixture containing 10 g **7** (10.5 mmol), 10 g K<sub>2</sub>CO<sub>3</sub> (72 mmol), and 10 g of CMPDB in 150 mL of dimethylformamide:tetrahydrofuran

(2:1) was heated at 75 °C for 8 hours under N<sub>2</sub> gas atmosphere. Thereafter the polymeric beads of resin **8** were separated through a porous filter at the pump. Repeated washing with the solvents dimethylformamide and tetrahydrofuran followed by water were carried out to ensure complete removal of unreacted **7**, and the weight of the dried resin **8** was found to be 12.34 g. The amount of **7** loaded on CMPDB was evaluated based on the difference of mass taken before and after coupling for the dried resin and found to be 23.4%. Elemental analysis of nitrogen and sulphur in **8** yielded values of 2.78% and 1.26%, respectively, which further confirmed the amount of loading to be 23–24%. This result showed that only 0.4 mmol, i.e. 26.5% of the total available Cl (1.5 mmol per g of CMPDB), reacted with 0.2 mmol **7**.

*Procedure for Column Concentration and Determination of Cu(II), Cd(II) and Pb(II)*

A glass column (200 × 8.0 mm) was prepared by adding a slurry of 1 g polymeric chelating resin beads in CH<sub>3</sub>OH:H<sub>2</sub>O (1:1). The height of the column section containing the resin (bed height) was 15 mm. It was conditioned with different buffer solutions for the samples containing Cu(II), Cd(II) and Pb(II) prior to their elution

**Table 1.** Parameters optimized for sorption and desorption of Cu(II), Cd(II) and Pb(II) on the resin

No.	Parameters	Metal ions		
		Cu(II)	Cd(II)	Pb(II)
1	pH range	3.0–4.0	6.5–7.5	7.0–8.0
2	Flow rate (mL min <sup>-1</sup> )	1.5	2.0	1.5
3	Concentration of acid for desorption	3.0 N HCl	1.5 N HCl	0.1 N HNO <sub>3</sub>
4	Total sorption capacity (mg g <sup>-1</sup> of resin)	13.21	21.14	36.92
5	Distribution coefficient (K <sub>d</sub> )	2600	2850	3690
6	Preconcentration factor (PF)	100	125	111
7	Breakthrough capacity (mg g <sup>-1</sup> of resin)	2.44	3.70	5.91
8	R. S. D. (%) <sup>*</sup>	1.0–1.4	1.0–1.1	1.1–1.3
9	Average recovery (%)	97–98	98	97
10	t <sub>1/2</sub> for exchange (minutes)	4.5	7.0	11.0

<sup>\*</sup>Average of 10 determinations.

from the column. A suitable aliquot of the solution containing 0.010–5.64 µg mL<sup>-1</sup> Cu(II), 0.006–5.12 µg mL<sup>-1</sup> Cd(II) and 0.006–5.12 µg mL<sup>-1</sup> Pb(II) was passed through the column after adjusting to the appropriate pH at an optimum flow rate. Stripping of the metal ions from the resin bed was carried out by suitable eluting agents such as HCl or HNO<sub>3</sub>. The eluants were collected in a 25 mL volumetric flask, made to volume with doubly distilled water and determined spectrophotometrically as well as by GFAAS (Table 1).

## Results and Discussion

### Spectral Interpretation of Synthesized Compounds

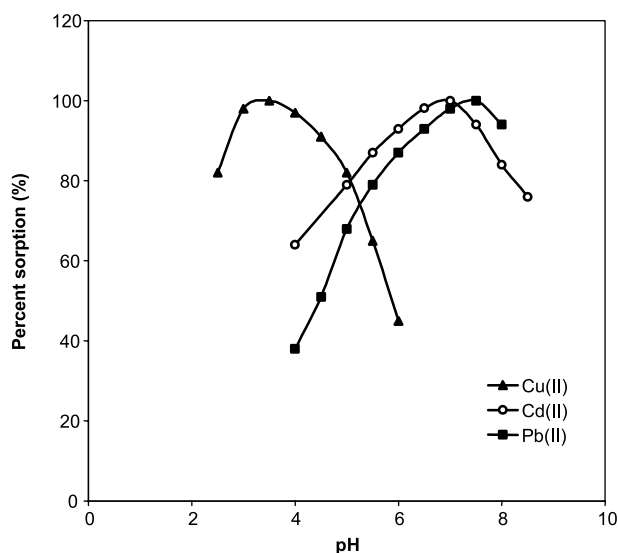
The FT IR and <sup>1</sup>H NMR spectra of compounds **1**, **2**, **3** and **4** were in good agreement with the reported values [47–49]. The reduction of **4** to yield corresponding **5** is done with Raney Ni (W-2) and hydrazine hydrate. In **5**, the IR band at 3435 cm<sup>-1</sup> due to N–H stretching and absence of any peak due to N–O showed complete reduction of **4**. The IR band at 3227 cm<sup>-1</sup> and 3464 cm<sup>-1</sup> represented the phenolic –OH stretching at the ‘lower rim’ of calix[4]arene [10] and o-VTSC, respectively, in **7**. In **8**, disappearance of the band at 3227 cm<sup>-1</sup> suggested the binding of CMPDB at the ‘lower rim’ of calix[4]arene. A sharp IR peak at 1591 cm<sup>-1</sup> in **7** and **8** indicated the presence of CH=N stretching. In <sup>1</sup>H NMR spectra of **7**, the peak at 10.1 ppm corresponded to the phenolic –OH groups.

### Parameters Optimized for Separation and Preconcentration of Cu(II), Cd(II) and Pb(II)

#### Effect of pH

A glass column was packed with 1 g resin. 100 mL solutions containing 5, 5 and 12 µg mL<sup>-1</sup> Cu(II), Cd(II) and Pb(II), respectively, were passed through

the column at their optimum flow rate and at varying pH values. The percentage of metal sorption was measured by desorbing the resin with a suitable eluting agent and then determined by both spectrophotometry and GFAAS. The pH curves showed a marked change in the selective sorption of Cu(II) as compared to Cd(II) and Pb(II) in the column. This may be due to the difference in pH of hydrolysis of Cu(II) which is in the range of pH 4–5. The optimum pH for Cu(II), Cd(II) and Pb(II) was found to be 3.5, 7.0 and 7.5, respectively (Table 1, Fig. 2). Their selectivity in pH of sorption suggests the possibility of separation of these metal ions in the presence of each other in the column.



**Fig. 2.** Effect of pH on sorption of Cu(II), Cd(II) and Pb(II) on the resin. Amount of resin in the column: 1 g; Cu(II): 100 mL, 5 µg mL<sup>-1</sup>, Elution by: 3.0 N HCl; Cd(II): 100 mL, 5 µg mL<sup>-1</sup>, Elution by: 1.5 N HCl; Pb(II): 100 mL, 12 µg mL<sup>-1</sup>, Elution by: 0.1 N HNO<sub>3</sub>

### Effect of Flow Rate

The effect of the flow rate on the extent of metal ion sorbed on the resin was studied in the column packed with 1 g resin at 30 °C. Feed solutions containing 5, 5 and 12  $\mu\text{g mL}^{-1}$  Cu(II), Cd(II) and Pb(II), respectively, were passed at different flow rates (0.5, 1.0, 1.5, 2.0 etc.  $\text{mL min}^{-1}$ ) maintained by the peristaltic pump. The flow rate of the liquid during chromatographic separation should be such that the working conditions are close to the equilibrium state, since the flow rate depends on the particle size and the temperature. The optimum flow rate may be defined as the rate of flow of the effluent through the column at which more than 98% sorption takes place. The optimum flow rates obtained were 1.5, 2.0 and 1.5  $\text{mL min}^{-1}$  for Cu(II), Cd(II) and Pb(II), respectively. An increase in the flow rate resulted in a decrease in sorption due to insufficient contact time between the resin and the metal ion solution (Table 1).

### Effect of Concentration of Eluting Agents

The effect of eluants such as HCl, HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> was studied by taking their different normal concentrations. 1 g resin in the column was conditioned at the pH of maximum sorption and then fed with 100 mL solutions containing 5, 5 and 12  $\mu\text{g mL}^{-1}$  Cu(II), Cd(II) and Pb(II), respectively. The metal ions were desorbed with different eluting agents and then determined by spectrophotometry and GFAAS (Tables 1 and 2). It was observed that quantitative elution was possible with 20 mL 3.0 N HCl, 1.5 N HCl and 0.1 N HNO<sub>3</sub> for Cu(II), Cd(II) and Pb(II), respectively.

### Total Sorption Capacity

Total sorption capacity studies were carried out using the batch techniques for Cu(II), Cd(II) and Pb(II). 1 g

resin was equilibrated with 500 mL of solution containing 100  $\mu\text{g mL}^{-1}$  metal ions at their optimum pH for 2 hours at 30 °C. The amount of metal ions sorbed on the resin was calculated from the difference in the metal ion concentration before and after desorption (Table 1). The total sorption capacities of the resin were found to be 13.21, 21.14 and 36.92  $\text{mg g}^{-1}$  for Cu(II), Cd(II) and Pb(II), respectively.

### Equilibrium Distribution Coefficient $K_d$

Exchange equilibria are very often expressed in terms of the distribution coefficient. This quantity is given by the ratio of the equilibrium concentrations of the same metal ion in the resin phase and in the solution. The distribution coefficient  $K_d$  of the metal ions between the resin and aqueous phase was determined by a batch experiment. 1 g resin was equilibrated with 25 mL solution containing not more than 529, 846 and 1477  $\mu\text{g mL}^{-1}$  Cu(II), Cd(II) and Pb(II), respectively, for 2 hours at 30 °C. The solution was filtered to remove the resin, and the filtrate was subjected to spectrophotometric and GFAAS determination of the metal ion content. The concentration of the eluted metal ion solution was also measured (Table 1).  $K_d$  for Cu(II), Cd(II) and Pb(II) were found to be 2600, 2850 and 3690, respectively. The distribution coefficient  $K_d$  or efficiency of the resin was measured using the equation

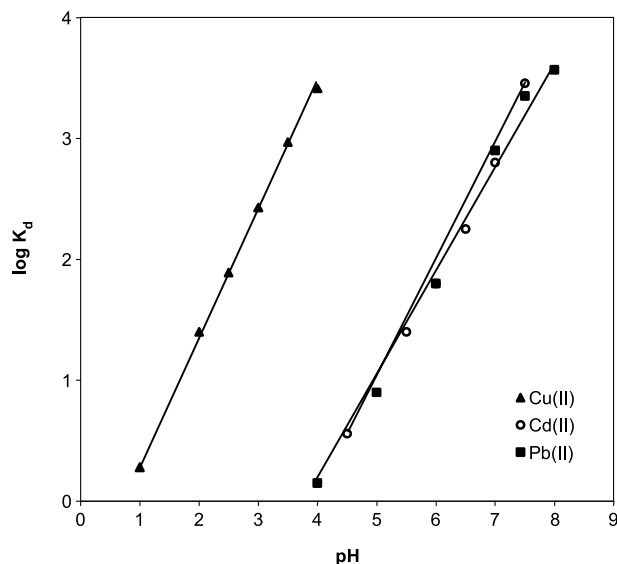
$$K_d = \frac{\text{Amount of the metal ion taken up by the resin}}{\text{Amount of the metal ion remaining in the solution}} \times \frac{\text{Volume of the solution}}{\text{Amount of resin taken}}$$

The plots of  $\log K_d$  against pH (Fig. 3) were straight lines with the slopes nearly equal to unity, indicating 1:1 stoichiometry between the metal ions and the chelating resin [50].

**Table 2.** Effect of concentration of eluting agents for desorption of Cu(II), Cd(II) and Pb(II) from the resin

Conc. (N)	HCl			HNO <sub>3</sub>			H <sub>2</sub> SO <sub>4</sub>		
	Cu(II)(%)	Cd(II)(%)	Pb(II)(%)	Cu(II)(%)	Cd(II)(%)	Pb(II)(%)	Cu(II)(%)	Cd(II)(%)	Pb(II)(%)
0.01	10.5	22.0	3.0	2.3	7.3	64.5	6.6	5.8	4.3
0.1	26.5	56.5	8.7	13.5	16.3	100	14.6	11.5	9.4
1.0	43.1	83.6	12.1	37.7	29.5	–	27.5	15.8	13.1
1.5	52.6	100	17.5	42.5	41.6	–	41.5	22.9	18.6
2.0	79.5	–	22.3	54.5	57.5	–	50.3	34.5	23.5
3.0	100	–	43.0	70.1	66.3	–	68.5	43.2	29.1

Experimental condition: Resin: 1 g, Volume of solution passed: 100 mL, Cu(II): pH 3.5; 5  $\mu\text{g mL}^{-1}$ , Cd(II): pH 7.0; 5  $\mu\text{g mL}^{-1}$ , Pb(II): pH 7.5; 12  $\mu\text{g mL}^{-1}$ .



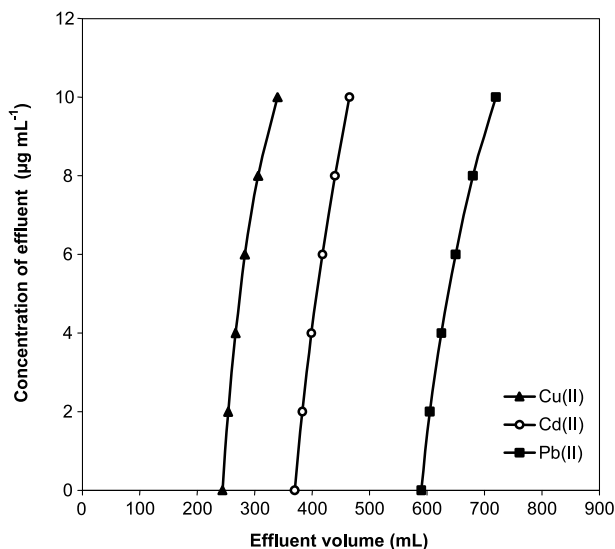
**Fig. 3.** Plot of  $\log K_d$  against pH for sorption of Cu(II), Cd(II) and Pb(II) on the resin. Amount of resin: 1 g; volume of the solution: 25 mL; Cu(II):  $529 \mu\text{g mL}^{-1}$ ; Cd(II):  $846 \mu\text{g mL}^{-1}$ ; Pb(II):  $1477 \mu\text{g mL}^{-1}$

#### Exchange Kinetics

Batch experiments were carried out to determine the rate of loading of Cu(II), Cd(II) and Pb(II) on the resin: 1 g resin was stirred with 100 mL feed solution containing 132, 212 and  $370 \mu\text{g mL}^{-1}$  Cu(II), Cd(II) and Pb(II), respectively, at  $30^\circ\text{C}$ . 5.0 mL of the feed solution was withdrawn at predetermined intervals and analyzed. The concentration of metal ions in aqueous phase was determined by spectrophotometry and GFAAS. The amount of metal ions loaded on the resin phase was calculated by measuring the concentration of metal ions in the feed solution (Table 1). The time needed for sorption of 50% of the metal ions ( $t_{1/2}$ ) was found to be 4.5, 7.0 and 11.0 minutes for Cu(II), Cd(II) and Pb(II), respectively, which indicated very good access of these metal ions to the chelating sites.

#### Breakthrough Studies

Breakthrough capacities are more significant and useful than total sorption capacities in ion exchange chromatographic applications, since they give the actual working capacity of the resin in the column. The breakthrough capacity or the effective capacity may be defined as the capacity at the moment when the adsorbed component has appeared in the effluent with increased concentration. It is one of the most critical parameters when working under dynamic conditions. Breakthrough studies were carried out with 1 g resin



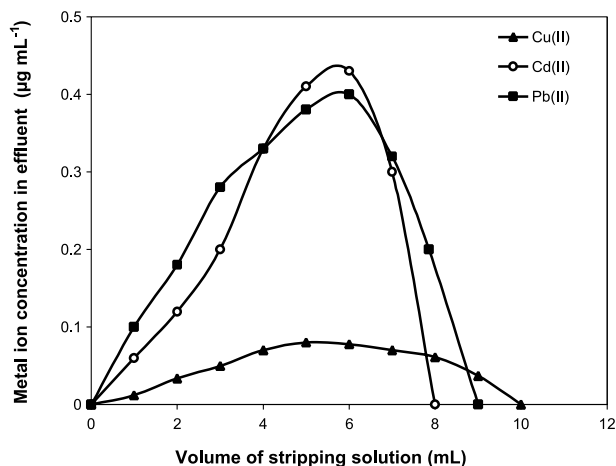
**Fig. 4.** Breakthrough curves for Cu(II), Cd(II) and Pb(II) on the resin. Amount of resin in the column: 1 g; Cu(II):  $10 \mu\text{g mL}^{-1}$ ; Cd(II):  $10 \mu\text{g mL}^{-1}$ ; Pb(II):  $10 \mu\text{g mL}^{-1}$

in a column of 200 mm in length and an 8.0 mm internal diameter with a bed height of 15 mm and passing  $10 \mu\text{g mL}^{-1}$  of metal ions [Cu(II), Cd(II) or Pb(II)] at their optimum pH and flow rate. An aliquot of 2.0 mL eluant was collected each time and analyzed by spectrophotometry and GFAAS for the metal ion content (Fig. 4). The breakthrough capacities of the resin for Cu(II), Cd(II) and Pb(II) were found to be 2.44, 3.70 and  $5.91 \text{ mg g}^{-1}$ , respectively (Table 1). All the curves were steep at their breakthrough points at different times, indicating the possibility of separating these metal ions from their mixtures.

#### Preconcentration of Cu(II), Cd(II) and Pb(II) on Resin

The concentration of trace elements in natural water is too low for direct determination. Therefore, a preconcentration or enrichment step is unavoidable even with a sensitive detection method. The resin was studied for the column concentration of Cu(II), Cd(II) and Pb(II) in terms of their preconcentration factor (PF).

1000 mL solutions containing 5, 15,  $20 \mu\text{g L}^{-1}$  Cu(II), Cd(II) and Pb(II) at pH 3.5, 7.0, 7.5, respectively, were passed through the column containing 1 g resin. The preconcentrating ability of the resin was assessed from the elution profile of the metal ions by plotting the concentration of effluents as a function of the volume of the stripping solutions: 10 mL, 3.0 N HCl for Cu(II); 8 mL, 1.5 N HCl for Cd(II); 9 mL, 0.1 N HNO<sub>3</sub> for Pb(II), respectively. The preconcentration



**Fig. 5.** Elution profile of Cu(II), Cd(II) and Pb(II) on the resin. Amount of resin in the column: 1 g; Volume of the solution passed: 1000 mL; Cu(II): 5 µg L<sup>-1</sup>; Cd(II): 15 µg L<sup>-1</sup>; Pb(II): 20 µg L<sup>-1</sup>

factors were found to be 100, 125 and 111 for Cu(II), Cd(II) and Pb(II), respectively, with a 98–99% recovery (Table 1, Fig. 5).

#### Resin Stability Tests

The stability of the synthesized polymeric chelating resin was tested to evaluate its sustainability for repeated cycles of sorption and desorption with different mineral acids. It was observed that there was no detectable change in the physical or chemical properties of the resin in concentrated acidic solutions of 5.0 N HCl, 3.0 N HNO<sub>3</sub> and 3.5 N H<sub>2</sub>SO<sub>4</sub>. To check the regenerating capacity of the synthesized resin, it was subjected to repeated sorption of Cu(II), Cd(II) and Pb(II) at their optimum conditions in the column. 1 g resin was placed into the column, and 500 mL solution containing 100 µg mL<sup>-1</sup> metal ions was passed through it at 30 °C. The resin was then stripped of metal ions with 25 mL of suitable eluant. The resin showed very good stability up to 15 repeated cycles of sorption and desorption, after which it showed a

2–3% decrease in efficiency, which may be due to hydrolysis of the resin when treated with mineral acids.

#### Effect of Electrolytes and Associated Cations

The limit of tolerance of interference of anions and cations concerning the sorption of Cu(II), Cd(II) and Pb(II) is defined as that which causes an error of 2–3% in the recovery of these metal ions. The effect of commonly associated cations in natural water samples, such as Fe(II), Fe(III), Co(II), Ni(II), Mn(II), alkali [Li(I), Na(I), K(I), Rb(I), Cs(I)] and alkaline earth metals [Mg(II), Ca(II), Sr(II) and Ba(II)], was studied under optimum conditions of metal ion sorption. Mn(II) did not affect the sorption of Cu(II), Cd(II) or Pb(II) up to 6 mg, however, for other cations the tolerance limits were much higher. Alkali and alkaline earth metals do not interfere (Table 3). The effect of anions and their limit of tolerance on the sorption of Cu(II), Cd(II) and Pb(II) by the resin was studied by taking different concentrations of some electrolytes including NaCl, NaF, NaBr, NaNO<sub>2</sub>, NaNO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, Na<sub>3</sub>PO<sub>4</sub> and CH<sub>3</sub>COONa. The results (Table 4) showed that except for Na<sub>3</sub>PO<sub>4</sub>, none of the electrolytes interfered in a concentration range of between 1.5 and 3.5 M, which further augments the potential application of the resin for the analysis of real samples.

**Table 3.** Effect of commonly associated cations in water samples on the sorption of Cu(II), Cd(II) and Pb(II) on the resin

Metal ions (100 µg each)	Interfering ions (mg)				
	Fe(II)	Fe(III)	Co(II)	Ni(II)	Mn(II)
Cu(II)	19	20	23	25	6
Cd(II)	16	14	21	21	4
Pb(II)	7	10	12	11	6

Experimental condition: Resin: 1 g, Volume of solution passed: 100 mL, Cu(II): pH 3.5, Cd(II): pH 7.0, Pb(II): pH 7.5.

**Table 4.** Tolerance limits of electrolytes on the sorption of Cu(II), Cd(II) and Pb(II) on the resin

Metal ions (2.5 µg mL <sup>-1</sup> )	Concentration of electrolytes: mol L <sup>-1</sup> Volume of electrolytes: 100 mL							
	NaF	NaCl	NaBr	NaNO <sub>2</sub>	NaNO <sub>3</sub>	Na <sub>2</sub> SO <sub>4</sub>	Na <sub>3</sub> PO <sub>4</sub>	CH <sub>3</sub> COONa
Cu(II)	1.8	2.5	2.0	3.0	3.5	1.5	0.2	2.5
Cd(II)	2.2	3.0	2.2	3.0	3.0	1.5	0.2	2.0
Pb(II)	1.4	2.0	1.5	3.2	3.2	1.5	0.2	2.0

Experimental condition: Resin: 1 g, Volume of solution passed: 100 mL, Cu(II): pH 3.5, Cd(II): pH 7.0, Pb(II): pH 7.5.



### Separation of Cu(II), Zn(II), Cd(II) and Pb(II)

The resin can be useful for separating copper, zinc, cadmium and lead from the mixtures due to favourable kinetics and different breakthrough capacities in the column. The synthesized chelating polymeric resin was tested for simultaneous separation of their binary and ternary mixtures. Hence, the following mixtures (each 100  $\mu\text{g}$  in 25 mL of buffer solution) were passed through the column at the pH of maximum sorption and optimum flow rate. Separation was

carried out for binary mixtures of (A) copper and lead, and from their ternary mixtures (B) copper, zinc and lead and (C) copper, cadmium and lead. The column effluents were analyzed for the metal ions by spectrophotometry and GFAAS. For the separation of a ternary mixture (C), 100  $\mu\text{g}$  each of Cu(II), Cd(II) and Pb(II) was passed through the column and maintained at pH 7.5 at a flow rate of 2.0 mL min<sup>-1</sup>. At this pH, Cu(II) came out with the effluent, while Cd(II) and Pb(II) were retained in the column. Cd(II) and Pb(II) were then separated on the basis of selective elution

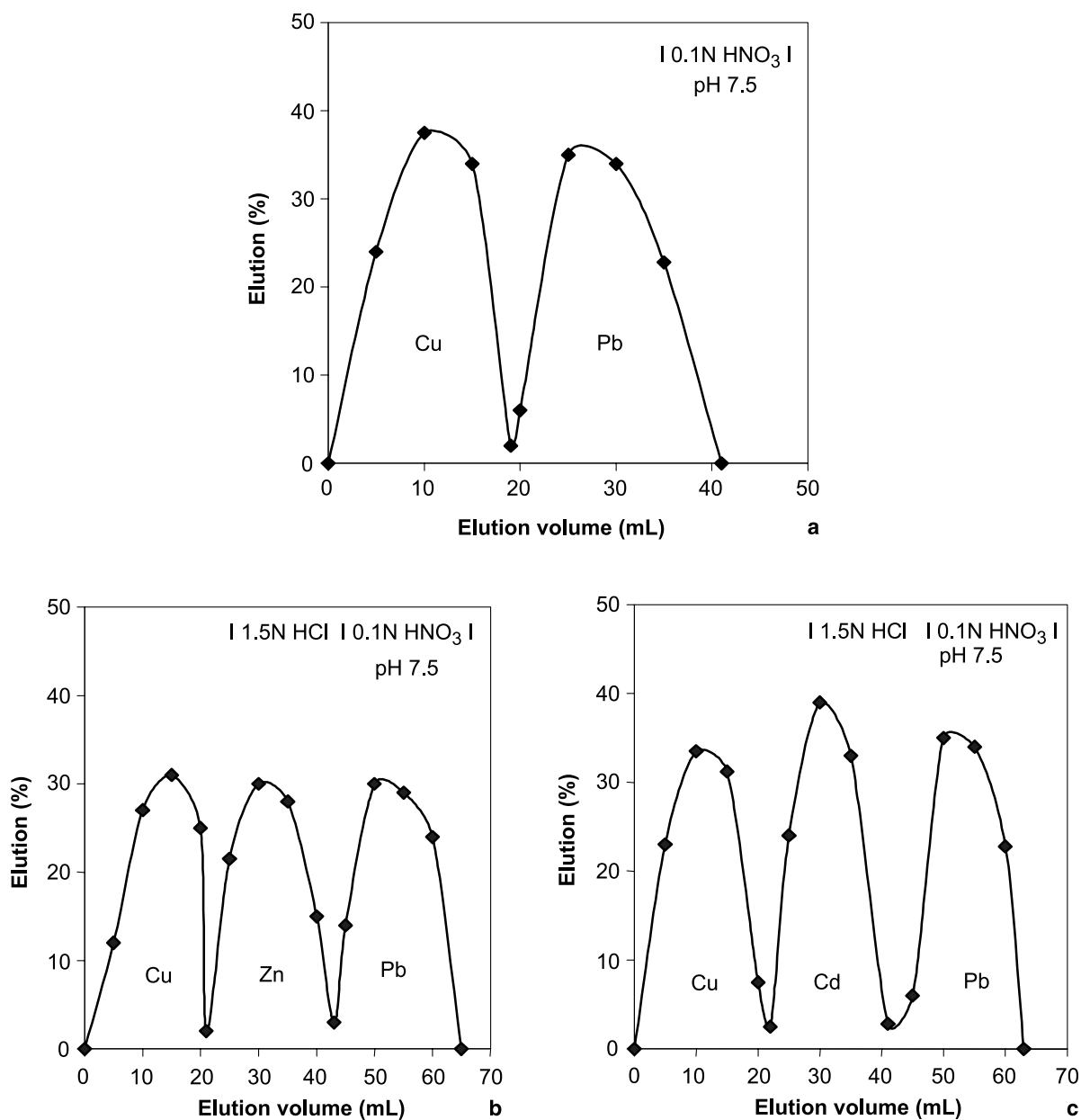


Fig. 6. Separation of (a) Cu and Pb; (b) Cu, Zn and Pb and (c) Cu, Cd and Pb on the resin

**Table 5.** Regression analysis for the calibration graph, linear range, detection limit and precision

Metal ions	Linear range ( $\mu\text{g mL}^{-1}$ )	Regression equation	Correlation coefficient	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )	R. S. D.* (%)
Cu(II)	0.010–5.64	$A = C \times 0.2161 - 0.0003$	1.0000	4.22	14.0	1.4
Cd(II)	0.006–5.12	$A = C \times 0.3316 - 0.0013$	0.9999	11.89	39.60	1.1
Pb(II)	0.050–12.12	$A = C \times 0.0906 + 0.0006$	1.0000	19.61	65.36	1.3

A Absorbance, C Concentration ( $\mu\text{g mL}^{-1}$ ).

\*Average of 10 determinations.

**Table 6.** Determination of Cu(II), Cd(II) and Pb(II) in natural water samples on the resin

Sample	Method	Cu(II)		Cd(II)		Pb(II)	
		Amount ( $\mu\text{g L}^{-1}$ )	R. S. D.* (%)	Amount ( $\mu\text{g L}^{-1}$ )	R. S. D.* (%)	Amount ( $\mu\text{g L}^{-1}$ )	R. S. D.* (%)
Sabarmati river, near thermal power station, Ahmedabad	present method	41	1.0	13	1.1	30	1.1
	standard addition	43	1.1	14	1.2	32	1.3
Ground water, university area, Ahmedabad	present method	10	1.4	14	1.0	29	1.3
	standard addition	13	1.2	15	1.2	26	1.1
Ground water, Vatva industrial zone, Ahmedabad	present method	54	1.2	37	1.0	60	1.2
	standard addition	56	1.3	39	1.1	62	1.2

Experimental condition: Resin: 1 g, Sample volume: 1000 mL.

\*Average of 10 determinations.

with different eluting agents. Cd(II) was eluted first with 25 mL of 1.5 N HCl followed by Pb(II) with 25 mL of 0.1 N HNO<sub>3</sub>. Quantitative separation was achieved in all the three mixtures as shown in their separation pattern (Fig. 6).

### Analytical Performance (Calibration Graph, Detection Limit and Precision)

For preparation of the calibration standards for Cu(II), Cd(II) and Pb(II), the standard stock solutions of these elements were used after appropriate dilution. The precision of the proposed method was investigated based on the optimum condition for pre-concentration in the column and expressed as R. S. D. (%), i.e. 1.4% for Cu(II), 1.1% for Cd(II) and 1.3% for Pb(II). The limit of detection (LOD) was defined as the analyte concentration giving a signal equal to three times the standard deviation of the blank signal [51]. The limit of quantification (LOQ) was then set as ten times the standard deviation of the blank signal (Table 5).

### Application to Analysis of Water Samples

The application of the proposed method was checked by subjecting the resin to natural water samples. The

results obtained by the proposed method were compared with those of the standard addition method. River water and ground water samples were isokinetically collected in polyethylene bottles and then passed through the column as described in the general procedure for column concentration. The amount of metal ions present in these samples and their R. S. D. values are given in Table 6. Higher efficiency of separation curves with lower values of R. S. D. (1.0–1.4%) suggests the potential of the resin for various analytical applications in the determination of Cu(II), Cd(II) and Pb(II).

### Conclusion

Polymeric calixarenes have demonstrated potential ionophoric capabilities that can be exploited for various analytical applications. The newly synthesized resin shows very good potential for trace enrichment of Cu(II), Cd(II) and Pb(II) as is evident from the column studies. The separation curves demonstrate the efficiency of the resin in comparison to resins reported in the literature [8] and the references cited therein for the simultaneous separation of Cu(II), Cd(II) and Pb(II) in the presence of each other with minimum interference from alkali and alkaline earth metal ions. As is evident from the data given in Table 7, the high-

**Table 7.** Comparable methods for preconcentration and determination of Cu(II), Cd(II) and Pb(II)

Ligand	Sorption capacity (mg g <sup>-1</sup> )			Preconcentration factor		
	Cu <sup>2+</sup>	Cd <sup>2+</sup>	Pb <sup>2+</sup>	Cu <sup>2+</sup>	Cd <sup>2+</sup>	Pb <sup>2+</sup>
Amberlite XAD-2 1-(2-pyridylazo)-2-naphthol [8]	6.810	2.550	1.350	50	50	50
Amberlite XAD-2 o-vanillinthio-semicarbazone [42]	0.850		0.200	90		100
Amberlite XAD-2 Pyrocatechol violet [52]		1.254	1.358		50	23
Amberlite XAD-2 Chromotropic acid [55]	8.429	9.316		100	100	
Amberlite XAD-2 Camalgite [54]	0.100			50		
Amberlite XAD-7 Xylenol Orange [53]	1.586	1.992		50	50	
Amberlite XAD-16 1-(2-pyridylazo)-2-naphthol [56]				150		
Amberlite XAD-16 Silicagel Salicyldioxime [57]	5.04			40		
C18-bonded silica 1-(2-thiazolylazo)-2-naphthol [58]				33		
1-(2-thiazolylazo)-2-naphthol [59]				64	58	56

light of the proposed method are the high preconcentration factors and total sorption capacities for Cu(II), Cd(II) and Pb(II). The resin shows excellent selectivity and preconcentrating abilities for these metal ions. The resin can be successfully regenerated for 15 operating cycles in the column. The results obtained from the analysis of natural water samples prove the reliability of the proposed method for application to various samples for determination by GFAAS.

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## References

- [1] Clayton GD, Clayton FE (1994) Patty's industrial hygiene and toxicology, 4<sup>th</sup> ed. John Wiley & Sons, New York
- [2] Agency for Toxic Substances and Disease Registry (2000) U. S. Public Health Service, Chapman and Hall, New York
- [3] Sorenson JRJ (1982) Inflammatory diseases and copper. Humana Press, New Jersey
- [4] Skinner CD, Salin ED (2003) J Anal At Spectrom 18: 495–500
- [5] Nordberg GF (1992) Cadmium in the Human environment: toxicity and carcinogenicity. IARC Scientific Pub. No. 118
- [6] Olajire AA, Ayodele ET, Oyedirdan GO, Oluyemi EA (2003) Environ Monit Assess 85: 135–155
- [7] Singhal RL, Thomas JA (1980) Lead toxicity. Urban & Schwarzenberg, Baltimore
- [8] Bermejo-Barrera P, Nancy MA, Cristina DL, Adela BB (2003) Microchim Acta (in press)
- [9] Yilmaz M (1997) Solution state metal complexes of calixarenes and polymeric calixarenes, handbook of engineering polymeric materials. In: Cheremisinoff NP (ed) Marcel Dekker Inc., New York
- [10] Gutsche CD (1998) Calixarenes revisited, monographs in supramolecular chemistry. In: Stoddart JF (ed) Royal Society of Chemistry, Cambridge
- [11] Asfari Z, Bohmer V, Harrowfield J, Vicens J (2001) Calixarenes 2001. Kluwer Academic Press, Dordrecht
- [12] Ikeda A, Shinkai S (1997) Chem Rev 97: 1713–1734
- [13] Mandolini L, Ungaro R (2000) Calixarenes in action. Imperial College Press, London
- [14] Parzuchowski P, Rokicki G (2000) Phenol-formaldehyde macrocycles in polymer systems, part I. Preparation methods involving chain polyreactions and immobilization on polymer matrix polimery, 45: 381–392
- [15] Roundhill DM (1995) Progr Inorg Chem 43: 533–592
- [16] Harris SJ, Barrett G, McKervey MA (1991) J Chem Soc Chem Commun 1224–1225
- [17] Kneafsey B, Rooney JM, Harris SJ (1990) Polymerization catalysts and polymerization process using such catalysts. U. S. Patent, 4,912,183
- [18] Georgiev EM, Koljo T, Roundhill DM (1993) Supramol Chem 2: 61–64
- [19] Yilmaz M, Deligoz H (1994) Macromol Rep 31: 137–145
- [20] Deligoz H, Tavasli M, Yilmaz M (1994) J Polym Sci, Part A, Polym Chem 32: 2961–2964
- [21] Deligoz H, Yilmaz M (1995) J Polym Sci, Part A, Polym Chem 33: 2851–2853
- [22] Deligoz H, Yilmaz M (1995) Solv Extn Ion Exch 13: 19–26
- [23] Deligoz H, Yilmaz M (1996) Synth React Inorg Met Org Chem 2: 285–294
- [24] Deligoz H, Yilmaz M (1996) React Funct Polym 31: 81–88
- [25] Shinkai S, Manabe O, Kondo Y, Yamamoto T (1986) Uranium adsorbent (Kanebo Ltd.) Jpn. Kokai Tokkyo Koho, JP 62,136,242
- [26] Kondo Y, Yamamoto T, Manabe O, Shinkai S (1987) Uranium adsorbent. Jpn. Kokai Tokkyo Koho, JP 62,210,055
- [27] Shinkai S, Kawaguchi H, Manabe O (1988) J Polym Sci, Polym Lett 26: 391–396
- [28] Munakata M, Wu LP, Kuroda-Sowa T, Maekawa M, Suenaga Y, Sugimoto K, Ino I (1999) J Chem Soc, Dalton Trans 3: 373–378
- [29] Castellano RK, Rebek J (1998) J Am Chem Soc 120: 3657–3663

- [30] Dieleman CB, Matt D, Neda I, Schmutzler R, Thoennesen H, Jones PG, Harriman A (1998) *J Chem Soc, Dalton Trans*, 13: 2115–2122
- [31] Dieleman CB, Matt D, Harriman A (2000) *Eur J Inorg Chem* 831–834
- [32] Alexandratos SD, Natesan S (2001) *Macromol* 34: 206–210
- [33] Shinohara T, Wakisaka S, Ohto K, Inoue K (2000) *Chem Lett* 640–641
- [34] Uysal G, Memon S, Yilmaz M (2001) *React Funct Polym* 50: 77–84
- [35] Memon S, Uysal G, Yilmaz M (2001) *React Funct Polym* 47: 165–174
- [36] Memon S, Yilmaz A, Yilmaz M (2000) *J Macromol Sci, Pure Appl Chem A* 37: 865–879
- [37] Hall CW, Cockayne JS, Kan MJ, Nicholson GP (2001) *Green Chem* 3: 114–122
- [38] Ohto K, Inoue S, Eguchi N, Shinohara T, Inoue K (2002) *Sep Sci Technol* 37: 1943–1958
- [39] Plutnar J, Rohovec J, Kotek J, Zak Z, Lukes I (2002) *Inorg Chim Acta* 27–35
- [40] Jain VK, Handa A, Pandya R, Shrivastav P, Agrawal YK (2002) *React Funct Polym* 51: 101–110
- [41] Garg BS, Jain VK (1988) *Microchem J* 38: 144–169
- [42] Jain VK, Sait SS, Shrivastav P, Agrawal YK (1997) *Talanta* 45: 397–404
- [43] Weissberger A, Proshauer ES, Riddick JA, Toops EE Jr (1955) *Techniques of organic chemistry*, vol. VII. Interscience, New York
- [44] Marzenko Z (1976) *Spectrophotometric determination of elements*. Ellis-Horwood Ltd., Chichester
- [45] Jeffery GH, Bassett J, Mendham J, Denny RC (1989) *Vogel's textbook of chemical analysis*, 5<sup>th</sup> ed. Wiley, New York
- [46] Dean JA (1999) *Lange's handbook of chemistry*, 15<sup>th</sup> ed. McGraw-Hill, New York
- [47] Gutsche CD, Iqbal M (1989) *Org Synth* 68: 234–237
- [48] Gutsche CD, Lin L (1986) *Tetrahedron* 42: 1633–1640
- [49] Van Loon JD, Arduini A, Coppi L, Verboom W, Pochini A, Ungaro R, Harkema S, Reinhoudt DN (1990) *J Org Chem* 55: 5639–5646
- [50] Gidwani MS, Menon SK, Agrawal YK (2002) *React Funct Polym* 53: 143–156
- [51] Lajunen LHJ (1992) *Spectrochemical analysis by atomic absorption and emission*. Royal Society of Chemistry, Cambridge, p 9
- [52] Saxena R, Singh AK (1997) *Anal Chim Acta* 340: 285–290
- [53] Sandell EB, Onishi H (1978) *Photometric determination of traces of metals. General Aspects*, 4<sup>th</sup> ed. John Wiley & Sons, p 478
- [54] Ferreira SLC, Ferreira JR, Dantas AF, Lemos VA, Araujo NML, Costa ACS (2000) *Talanta* 50: 1253–1259
- [55] Tewari PK, Singh AK (1999) *Analyst* 124: 1847–1851
- [56] Yebra MC, Garcia A, Carro N, Moreno A, Puig L (2002) *Talanta* 56: 777–785
- [57] Kumar M, Rathore DPS, Singh AK (2000) *Analyst* 125: 1221–1226
- [58] Queiroza ZF, Rochab FRP, Knapp G, Krug FJ (2002) *Anal Chim Acta* 463: 275–282
- [59] Chen J, Teo KC (2001) *Anal Chim Acta* 450: 215–222