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Synthesis of glycylcalix[6]arene and its applicability as resin for separation and preconcentration of trace transition metals

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A new glycylcalix[6]arene is synthesized by the reaction of glycine ethyl ester hydrochloride with acid chloride of *p*-carboxycalix[6]arene. Its physical properties, void volume, density, swelling, cation exchange capacity and $t_{1/2}$ have been determined. The glycylcalix[6]arene is used as resin and its metal ion exchange capacities are determined. Mn(II), Fe(II), Co(II), Cu(II), Ni(II) and Zn(II) were absorbed at 5.5, 4.5, 6.0, 4.0, 4.8 and 5.0 pH, respectively. These were eluted with NaF, H_2SO_4, acetic acid, tartrate and HCl. The chromatographic separation of Mn(II), Fe(II) Co(II), Ni(II), Cu(II) and Zn(II) from each other was studied. These metals were preconcentrated on resin and determined in blood plasma and in the environment samples.

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Calixarenes with the substituents at the lower rim have shown better complexing ability, extraction and transport properties towards a great variety of metal ions[1-5]. In recent years many calix[4]arene derivatives containing functional groups such as amides, amines, ketones and ethers have been synthesized and their ion binding properties reported[6-10]. Calix[4]arenes with glycine and histidine substitution at the lower rim have been reported and their cobalt(II)chloride complexes prepared[11]. Calixarene complexes of Cu and Zn were also reported[12-14]. However, little or very less work has been reported for the separation of Cu, Zn, Ni, Mn ions, which are of biological importance, with the calixarenes.

Some transition metals (Mn, Fe, Co, Cu, etc) play a key role in biological process, such as cell division, nitrogen fixation and photosynthesis[15]. Glycine is widely distributed in proteins and is particularly abundant in gelatin and fibrin. Glycine is also present in bile acids and contributes to detoxification process in liver[16]. Glycine forms the stable complexes with the transition metal ions *viz.* Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II)[17,18]. These metal ions are important trace metals in the biological systems and their separation is most desirable. The incorporation of glycine on the upper rim of the *p*-carboxycalix[6]arene may form more stable complexes with the transition metals. With this in view, in the present investigation a new glycylcalix[6]arene is synthesized for the selective column separation of biologically important metal ions *viz.* Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II).

Experimental Procedure

Apparatus

The pH measurements were made on a Systronics digital pH meter Model 335. Infrared spectra were recorded on a JASCO 407 FT-IR spectrophotometer as KBr pellets. Chemito Model 201 Atomic Absorption Spectrophotometer was used for the estimation of manganese, iron, cobalt, nickel, copper and zinc [Mn, 279.5; Fe, 248.3; Co, 240.7; Ni, 232.0; Cu, 324.8 and Zn, 213.9 nm]. Thermal analysis was carried out on Shematzu Models TGA-50, DSC-60 and DTA-50. Alumina was taken as reference with the heating rate of 20°C per min.

Reagents

All the chemicals used were of A. R. grade or E-Merck. Distilled water was redistilled over alkaline potassium permanganate.

Stock solutions of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) (0.01M) were prepared by dissolving 2.497 g of copper sulphate pentahydrate, 2.379 g of cobalt chloride hexahydrate, 1.690 g of

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manganese sulphate monohydrate, 2.875 g of zinc sulphate heptahydrate, 3.921 g ferrous ammonium sulphate hexahydrate and 2.371 g nickel chloride hexahydrate, respectively in double distilled water. All the solutions were standardized by complexometry[19,20] and AAS. These solutions were diluted as and when needed with double distilled water.

Standard buffer solutions were prepared as described elsewhere[19].

Synthesis of glycylcalix[6]arene

It is synthesized by reacting calix[6]arene acid chloride with glycine ethyl ester hydrochloride (Fig. 1). The calix[6]arene and its acid chloride is prepared as described elsewhere[21]. The glycine ethyl ester hydrochloride was obtained from Fluka.

In a conical flask 9.0 g (0.06 mol) glycine ethyl ester hydrochloride in 150 mL diethyl ether and 6.0 g sodium bicarbonate dissolved in 30 mL of distilled water were taken. The mixture was cooled to 5°C. Then calix[6]arene acid chloride in 100 mL of dichloromethane was added dropwise with constant stirring for 1 h. The solid glycylcalix[6]arene thus obtained was filtered, washed with distilled water, dried and recrystallized with dioxane (Yield -8.0 g, 80%).

Physico-chemical properties

The physical properties *viz.* particle size, moisture content, swelling, specific weight of the resin, bulk density exchange capacity and distribution coefficient were determined as described in literature[22-26].

Sorption capacity for metal ions using batch technique

The sorption studies for Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) were made in pH range of 3.0-6.5. 1.0 g of resin was placed in a stoppered conical flask and metal solutions along with the desired buffer solutions were added. The contents were kept shaking for 24 h. The supernatant liquid was separated and the metal concentration was determined by AAS. Sorption capacity was expressed as number of mmol of metal per gram of resin.

Kinetics of sorption

The rate of uptake of metals were determined by shaking glycylcalix[6]arene with 50 mL of 0.001M solutions of Mn(II) at pH 5.5, Fe(II) at pH 4.5, Co(II) at pH 6.0, Ni(II) at pH 4.8, Cu(II) at pH 4.0, and Zn(II) at pH 5.0. Suitable aliquots of metal solution were withdrawn at the intervals of 5-80 min, and the residual concentrations of metals were determined by AAS.

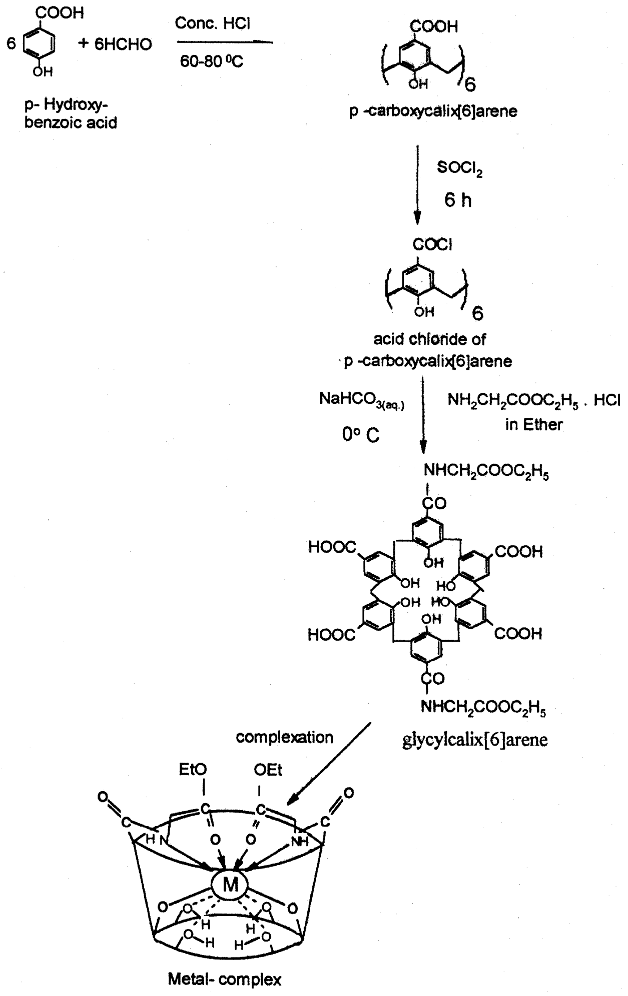


Fig. 1—Scheme for the synthesis of glycylcalix[6]arene and its metal complexes

Ion exchange column and packing

The glass column 150×4.6 mm was packed with glycylcalix[6]arene by slurry packing technique. The glycylcalix[6]arene was suspended in water and allowed to stand for 5 min. The unsettled particles were discarded and slurry was poured into column and the water was drained out slowly with constant tapping at the bottom to ensure close packing of the particles. The column was back-washed by running a stream of water through the bed at 5 mL.min^{-1} flow rate. The water was drained from the column and bed was washed with 1.0 N HCl and finally with double distilled water till the effluent was free from chloride ions.

Procedure for column operation and determination of metal ions

A glass column 0.5 mm diameter and 30 cm in length was packed with 1 g of the resin. It was treated with 25 mL of 1.0 M HCl at the flow rate of 3 mL.min^{-1} and then washed with double distilled water until the resin was freed from acid. The appropriate pH was adjusted by buffer. The solutions of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) were passed through the column after adjusting the appropriate pH at a flow rate of 0.5 mL.min^{-1} and the metal contents in the effluent were determined by AAS.

Results and Discussion

The method adopted for the synthesis of glycylcalix[6]arene is simple which gives pure product (99.5%) with good yield (80%). The stability of the glycylcalix[6]arene was studied by the thermal analysis (Thermogravimetry, TG, Differential Thermal Analysis, DTA and Differential Scanning Calorimetry, DSC). The TG thermogram shows the weight loss at 300 and 500°C. The first weight loss at 300°C is 42% due to the loss of glycineethyl ester moiety and finally, the compound gets decomposed at 500°C. Similar observations are recorded in the DTA and DSC curves. The DTA shows two exotherms at 263°C and another around 400°C while the DSC shows the three peaks at 133, 423 and 489°C. The endotherm at 133°C with a heat –31J/g is due to the phase transition while the exotherm at 423°C and the endotherm at 489°C show the decomposition with the ΔH 193.73 and -32.46 J/g, respectively. It has been characterized by FTIR, ^{1}H NMR, ^{13}C NMR spectral techniques. FTIR ν_{max} (cm^{-1}): 3420 (N-H and O-H stretching superimposed), 1734 (C=O stretching of ester), 2900 (C-H stretching of CH_3), 1609, 1488 ($\mathrm{C}\overset{\cdots}{-}\mathrm{C}$ ring stretching), 1166 (C-O stretching vibration). ^{1}H NMR δ_H: 1.15 (t, 6H, J = 7.2 Hz, OCH_2CH_3), 3.43 (q, 4H, J = 6.9 Hz, OCH_2CH_3), 3.752, 3.733 (AB, 12H, J = 7.2 Hz, $ArCH_2Ar$), 3.8 (d, 4H, J = 4.8, NCH_2), 6.97 (s, 4H, ArH), 7.00 (s, 8H, ArH), 7.99 (t, 2H, J = 2.1, $NHCH_2$). ^{13}C NMR δ_C: 16.28 (CH_3 attached to CH_2), 39.80 (CH_2 attached to two aromatic rings), 66.59 (CH_2 attached to CH_3), 115.26, 117.45, 128.55, 128.84, 131.50, 132.6 (C_8, C_{11}, C_6, C_{13}, C_{12} and C_7 of aromatic ring). FAB-MS: The mass spectra of the glycylcalix[6]arene shows a molecular ion peak at 1070, which on fragmentation losses one of the glycine ethyl ester groups ($NHCH_2COOC_2H_5$ group) and three protons of the outer center group resulting the m/e at 965. Then, further loss of C_2H_5 group and all the protons of OH, COOH and NH resulting m/e at 925. Then, it losses N and CH_2 groups to give m/e at 897. On further fragmentation it gives the following m/e: 859, 836, 798, 759, 733, 656, 635, 614, 540, 505, 460, 413, 355, 329, 254, 176.

The IR spectra of the metal complexes show that the peaks due to the stretching vibration of O-H and N-H become less intense and more broad. The carbonyl peaks also shift to lower frequencies (1640 cm^{-1}). This may be due to complexation in cavity to form the sandwich complexes.

Physicochemical properties

The physical properties of glycylcalix[6]arene are given in Table 1. The moisture content of the resin is 2%. The dry and wet densities of the resins are 0.857 and 0.782 g.cm^{-3}, respectively. The difference between dry and wet densities of resin is due to the swelling and solvation of resin in the solvent. The value for apparent density (column density) is

Table 1—Physical properties of glycylcalix[6]arene

State	Solid	Particle size	100-200 mesh
Nature	Non-hygroscopic	Solid (%)	98.000
Colour	White	Moisture content (%)	2.000
Stability	Stable against air, light and water, thermally stable upto 250°C	Swelling (%)	0.080
TLC	n-Butanol-25% acetic acid (9 : 1)	Bulk density (dry) (g.cm^{-3})	0.857
IR spectra (cm^{-1})	3420,1733, 1609, 1488, 1166, 979, 760	Bulk density (wet) (g.cm^{-3})	0.782
Melting point	250°C d	Apparent density (wet) (g.cm^{-3})	0.821
Yield (%)	80	Tapped down density (wet) (g.cm^{-3})	1.003
Mol. wt.(g.mol^{-1})	1070	True density (wet) (g.cm^{-3})	1.478
C, H, N (%)	62.80, 4.67, 2.62	Void	0.440
		Cation exchange capacity (mmol.g^{-1})	4.301
		Concentration of ionogenic groups (mmol.cm^{-3})	4.228

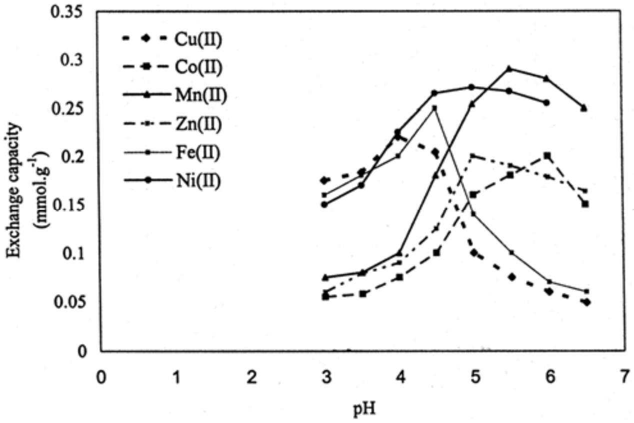


Fig.2—Exchange capacity ofglycylcalix[6]arene for Mn(II), Fe(II), Co(II),Ni(II), Cu(II) and Zn(II)

0.821 g.cm^{-3}. The void volume was evaluated from density data and found to be 0.44.

The swelling of resin has high impact on the design and operation of the column, life of particle and selectivity. Highly swellable resins are not encountered in the column operations, as the expanded particles become more elastic, which requires close packing in column and thus hindered the flow of solution through the bed. The resistance to flow depends on the degree of swelling. The frequent volume changes may be in between regeneration and operation cycles, ruptures or develops cracks in original structure of particles and hence reduces the life. In the present study, the degree of swelling is 0.08% and it is observed, that, there is no change in the bed volume on washing with acid.

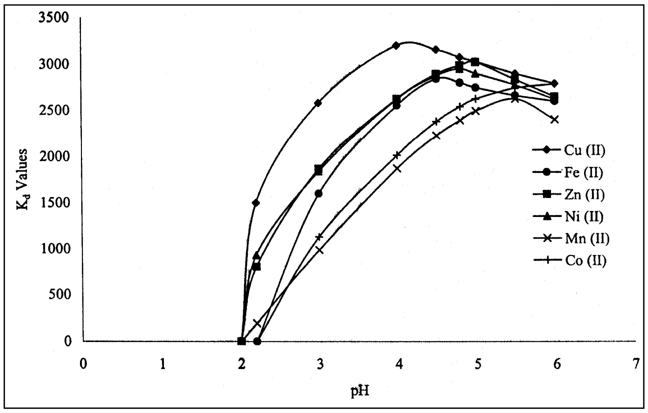


Fig. 3—Variation of K_d with pH for glycylcalix[6]arene

Table 2—Sorption capacities at optimum pH of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) on glycylcalix[6]arene

Metal ion	Optimum pH for sorption	Sorption capacity mmol.g^{-1}
Mn(II)	5.5	0.29
Fe(II)	4.5	0.25
Co(II)	6.0	0.20
Ni(II)	4.8	0.27
Cu(II)	4.0	0.22
Zn(II)	5.0	0.20

Table 3—Time required for the 50% of maximum sorption of the metal ions on glycylcalix[6]arene

Metal ion	Mn(II)	Fe(II)	Co(II)	Ni(II)	Cu (II)	Zn (II)
Time $t_{1/2}$ (min)	2.7	2.5	2.4	2.2	1.8	3.0

Sorption capacity

Sorption capacities on glycylcalix[6]arene for Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) were studied between pH 3.0-6.5. Fig. 2 shows that glycylcalix[6]arene forms the chelates with these metal ions in the pH range between 4.0 and 6.0. Since the availability of the chelating anion increases with the increase of pH of the medium, the sorption capacities also increase, however, with further increase in the pH the metal starts getting hydrolysed and then there is a decrease in sorption capacity. The metal ions maximum sorption and capacity at the respective pH are given in Table 2. The time(t) for the maximum sorption of the metal ions is between 0 and 5 min. The complete change takes place within 18-20 h. The time required ($t_{1/2}$) for the sorption to reach 50% of its maximum value, which is the measure of the uptake of various metal ions is around 2.5 min (Table 3). The $t_{1/2}$ for Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) is 2.7, 2.5, 2.4, 2.2, 1.8 and 3.0 min, respectively. The variation of K_d with pH for Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) is shown in Fig. 3. Copper has maximum K_d value, while zinc and nickel have nearly the same K_d values, and the lowest K_d value is obtained for manganese. The order of K_d values is: Cu(II) > Ni(II) ~ Zn(II) > Co(II) > Fe(II) > Mn(II).

Breakthrough capacity

The breakthrough capacities are more significant for chromatographic applications of the resin. The breakthrough curves for Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) at the pH of the maximum sorption at the flow rate of 0.5 mL.min^{-1} are shown in Fig. 4. All the curves are steep at the breakthrough points. The metal ions reach their breakthrough points at different pH indicating the possibility of separating these metal ions from their mixtures.

Table 4—Percentage recovery of metal ions from glycylcalix[6]arene with various eluants

Metal ion	Recovery %													
	Acetic acid			HCl			H_2SO_4				NaF		Tartrate	
	0.05 M	0.5 M	1.0 M	0.05 M	0.5 M	1.0 M	4.0 M	0.1 M	0.5 M	1.0 M	0.01 M	0.05 M	0.01 M	0.05 M
Mn(II)	25.4	59.0	61.0	1.0	30.0	30.0	35.0	5.0	25.0	50.0	99.0	100.0	-	-
Fe(II)	-	-	-	-	-	5.0	100.0	15.0	35.0	40.0	-	-	-	-
Co(II)	90.0	98.0	100.0	81.0	99.0	100.0	100.0	-	-	-	-	-	-	-
Ni(II)	-	-	-	-	-	-	-	-	-	-	-	-	95.0	100.0
Cu(II)	-	-	-	80.0	99.0	100.0	100.0	70.0	90.0	100.0	-	-	-	-
Zn(II)	-	-	-	39.0	89.0	100.0	100.0	67.0	88.0	99.0	-	-	-	-

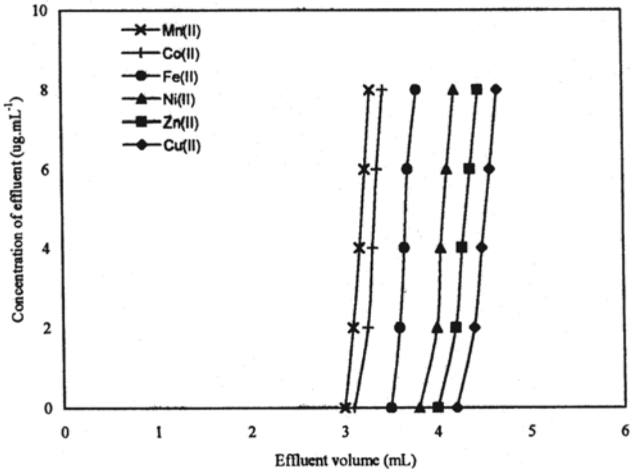
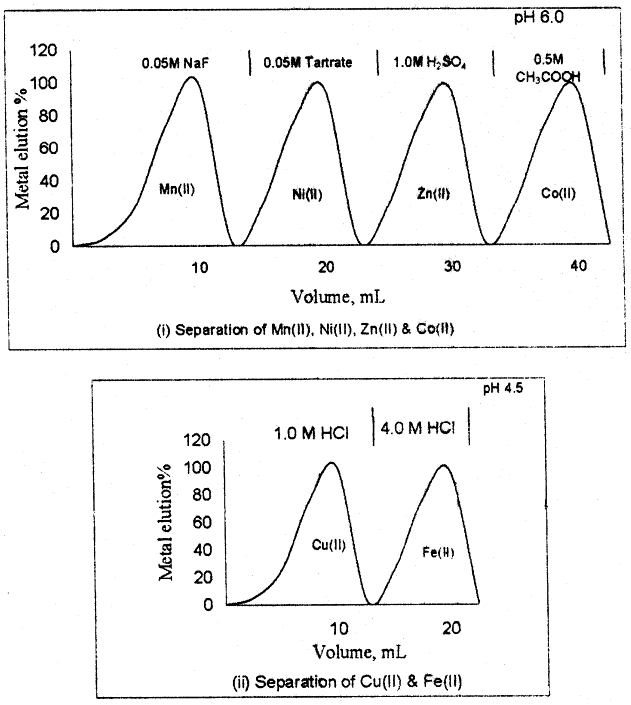


Fig. 4—Breakthrough curves for Mn(II), Fe(II), Co(II),Ni(II), Cu(II) and Zn(II) with glycylcalix[6]arene

Effect of eluants

Various acids and salts eluants have been used for the elution of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) from the loaded glycylcalix[6]arene resin in the columns. It was observed that HCl, H_2SO_4, CH_3COOH and NaF and tartrate are the good eluents, however, electrolytes NaCl, KCl etc swell to a larger extent and could not be used as eluent. The studies show that Cu(II) can be easily eluted quantitatively with HCl, H_2SO_4, however, it cannot be eluted with CH_3COOH. Similarly, Co(II) and Zn(II) can be eluted with CH_3COOH and HCl. Ni(II) is eluted with tartrate. However, all the attempts have been failed to elute Ni(II) with HCl, H_2SO_4 and CH_3COOH. Mn(II) is eluted with NaF.

The percentage elution of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) from column by various eluants is given in Table 4. On the basis of sorption and elution studies at various pH, the mixture of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) was separated using the column.



(i) Separation of Mn(II), Ni(II), Zn(II) & Co(II)

(ii) Separation of Cu(II) & Fe(II)

Fig. 5—Separation of mixture of Mn(II), Fe(II), Co(II),Ni(II), Cu(II) and Zn(II)

Separation of the mixture of metal ions

Separation of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) was achieved on glycylcalix[6]arene column by passing the mixture of the metal ions at pH 6.0 in the column (Fig. 5 and scheme 1). Cu(II) and Fe(II) were not retained in the column and got eluted out, while Mn(II), Co(II), Ni(II) and Zn(II) were retained in the column. Mn(II) was separated by eluting it with 0.05 M NaF. Then Ni(II) and Zn(II) were eluted out with 0.05 M tartrate and 1.0 M H_2SO_4, respectively. Finally, Co(II) was eluted out with 0.5 M CH_3COOH. The column was washed with buffer solution of pH 4.5 and the eluant containing Cu(II) and Fe(II) was again passed through the column. Cu(II) was separated by eluting it with 1.0 M

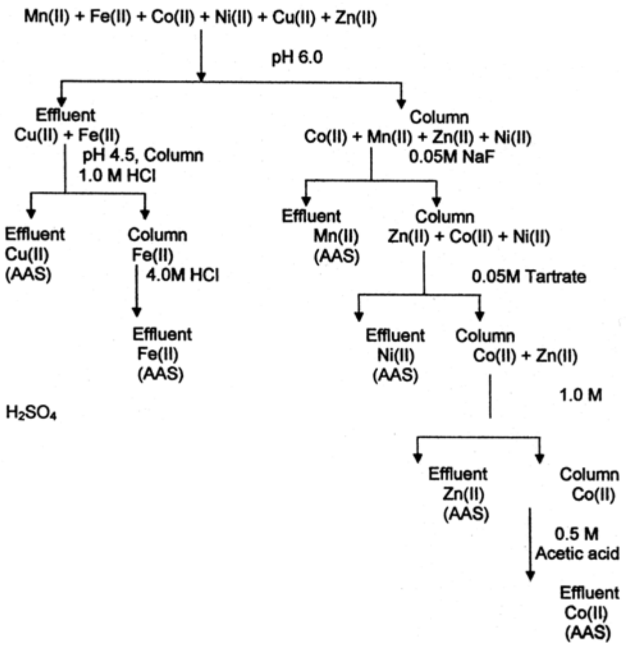
HCl, while Fe(II) was eluted with 4.0 M HCl. The concentrations of the metal ions in the eluants were determined by AAS.

Preconcentration of metal ions

Each litre solution containing 25 μg of the metal ion was passed through the column after adjusting the appropriate pH at the rate of 5 mL min^{-1}. The Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) were eluted with 0.05 M NaF, 4.0 M HCl, 0.5 M CH_3COOH, 0.05 M tartrate, 1.0 M HCl and 1.0 M H_2SO_4, respectively and the concentrations were determined by AAS. The elution profile is shown in Table 5, Fig. 6. The column concentration behaviour for all the metal ions was evaluated in terms of the preconcentration factor. The profiles were constructed by plotting the preconcentration factor as a function of volume of stripping solution. Quantitative collection of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) was possible from the solution having metal ion concentration of the order of 10-50 μg.mL^{-1} with the recovery upto 97-99%. The preconcentration factors for Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) were 98, 99, 94, 88, 99 and 82, respectively.

Determination of metal ions in environmental and biological samples

The present chromatographic method is applied for the separation and the determination of metal ions in environmental and biological samples. The soil, solid waste and blood plasma samples were collected and digested with concentrated HCl and concentrated HNO_3 (2:1) and diluted to 100 mL with double distilled water. An appropriate aliquot was transferred



Scheme 1—For the separation of the mixture of Mn(II), Fe(II), Co(II),Ni(II), Cu(II) and Zn(II)

Table 5—Preconcentration factor for Mn(II), Fe(II), Co(II), Cu(II), Ni(II) and Zn(II) on glycylcalix[6]arene

Metal ion	Feed solution (mL)	Concentration (ppb)	Stripping solution (mL)	Recovery (%)	Preconcentration factor
Mn(II)	1000	25	10.0	98	98
Fe(II)	1000	25	9.8	97	99
Co(II)	1000	25	10.5	99	94
Ni(II)	1000	25	11.0	97	88
Cu(II)	1000	25	10.0	99	99
Zn(II)	1000	25	12.0	98	82

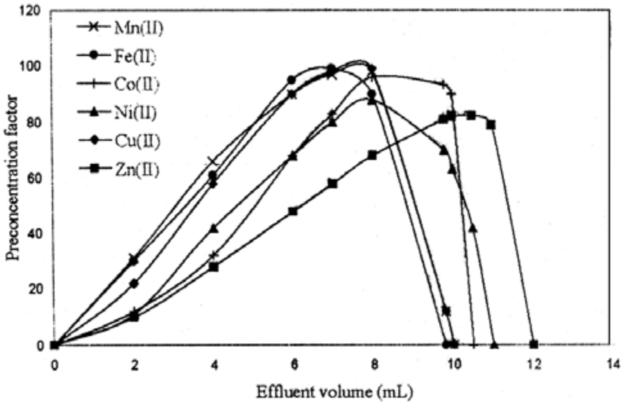


Fig.6—Elution profile for Mn(II),Fe(II), Co(II), Ni(II), Cu(II) and Zn(II)

Table 6—Concentration of the metal ions in the environmental and biological samples

Sample		Mn(II)	Fe(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
		ppm					
Soil	a.	0.50	175.00	1.20	1.00	135.00	4.20
	b.	0.30	250.00	0.90	0.80	55.00	11.00
	c.	0.40	210.00	0.80	0.70	20.00	7.60
Water	d.	10.00	13.00	1.00	12.00	21.00	37.00
	e.	25.00	15.00	0.80	16.00	25.00	48.0
	f.	2.10	10.00	0.10	1.80	3.20	10.00
Blood Plasma		0.30	1.13	0.10	0.02	1.20	1.50

(a) Soil samples from pigment manufacturing area of Vatva, Ahmedabad
(b) Soil samples from dyes manufacturing area of Vatva, Ahmedabad
(c) Soil samples from chemical industries
(d) Water samples from Sabarmati river (Upstream)
(e) Water samples from Sabarmati river (Downstream)
(f) Water samples from Sea
Note: The values are the average of the 20 sample estimations.

and optimum pH was adjusted and passed through the column. Mn(II), Fe(II), Co(II), Cu(II), Ni(II) and Zn(II) were eluted by judicious choice of eluants and pH, and determined by AAS. The data are given in Table 6.

The water samples were also collected from different industrial areas and filtered through the Whatman filter paper No. 541 to remove any suspended particles and after adjustment of pH, were passed through the column. The metal ions were eluted by appropriate eluants and the concentrations of the metal ions were determined by AAS. The data are given in Table 6.

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