Optimization of the anion-exchange separation of metal–oxalate complexes

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ABSTRACT

Anion exchangers, prepared by the sorption of cetylpyridinium chloride onto a reversed phase, have been evaluated for the separation of the transition metal ions Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}. Parameters examined included nature of complexing reagent, eluent pH, eluent concentration, speciation of the metal ion in the complexation system, and column capacity. The separations obtained with an oxalate eluent were compared to those obtained with a cation exchanger prepared by the addition of *n*-octanesulfonate to the eluent. Theoretical plate heights were in the range of 0.010 mm to 0.025 mm, and were in many instances, slightly better than those obtained with cation separation systems. Metal ion speciation in the eluent appeared to be a dominant factor determining the efficiency of the anion separation; metal ions with one dominant complexed ion generally gave more efficient separations. Compared to cation separations, analysis times were similar, but separation selectivities were considerably different.

INTRODUCTION

The separation of metal ions on modern high-performance ion exchangers has become an accepted practice in ion chromatography, and many suppliers of chromatographic equipment offer standard methods for such analyses. The majority of the methods available are based on cation-exchange separations with either bonded phases or exchangers formed from the sorption of anionic surfactants onto reversed phases [1,2]. Only a limited number of studies have been reported for the separation of anionic metal ion species [3–9]. The methods reported to date have not been widely adopted, primarily due to poor column efficiency and increased difficulty of detection relative to cation-exchange systems. However, separations based on anionic exchange offer the potential advantages of different selectivity, reduced problems from metal ion hydrol-

ysis, and application to complex sample matrices. The most successful applications reported for anion-exchange separations of metal ions have made use of stable multidentate reagents, such as ethylenediaminetetraacetic acid [4,7]. Other anionic systems [3,5,6,8,9] exhibit separation efficiencies that are much less than normally found for cation exchangers. However, these reported studies suggest that anion exchange offers potential advantages with respect to selectivity and for the analysis of complex samples [3–9].

The diversity of metal species present in the eluent of an anion separation is expected to be greater than that for a cation separation. In cation separations the formation of a small amount of a 1:1 complex with a ligand in the eluent may be sufficient to both effect elution and impart selectivity to the separation. However, for an anion separation, a greater degree of complexation is required to produce the necessary anionic complexes, and if a wider variety of complexed metal species are present, all of these

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species must be in rapid equilibrium for efficient mass transfer between the mobile and stationary phases. Consequently, there is a need to examine anionic systems in more detail in an effort to understand the factors limiting column efficiency in these systems.

In this work the anionic exchange separation of the transition metal ions Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} has been evaluated in terms of nature of complexing reagent, eluent pH, eluent concentration, speciation of the metal ion in the complexation system, and column capacity. In addition, detection with postcolumn reaction (PCR) reagents was also briefly examined. Since the complexation reactions of divalent transition metal ions with oxalate have been widely studied, oxalate was the main complexing reagent used in the eluent. Other complexing reagents briefly examined included 2,6-pyridine dicarboxylic acid (PDCA) and tartaric acid. The results obtained with the optimized anion system were then compared to standard cation systems recommended for metal ion separations. Surfactant-modified reversed phases were used in these evaluations to permit the use of the same column for the preparation of a wide variety of exchange capacities, and for application to both anion and cation-exchange separations.

EXPERIMENTAL

Apparatus

The eluent delivery system was a Waters Model 510 HPLC pump (Waters/Millipore, Milford, MA, USA). A Rheodyne Model 7161 injection port (Cotati, CA, USA) with a 20-μl sample loop was used to inject samples onto a 15 cm $\times$ 4.6 mm (I.D.) Supelcosil LC-18 (5 μm) column (Supelco, Bellefonte, PA, USA); for some studies self-packed columns with Nucleosil 100-5 C_{18} (5 μm) or 100-10 C_{18} (10 μm) (Macherey-Nagel, Düren, West Germany) were used. The detection system included a screen "T" mixer [10], a reaction coil made from 102 cm long 1/16 in. O.D. $\times$ 0.020 in. I.D. (1 in. = 2.54 cm) PTFE tubing knotted into a series of figure-eight knots, and a UV–visible detector set at 520 nm (Waters 484 Tunable Absorbance Detector, Milford, MA, USA). The PCR reagent solution

was introduced with helium gas pressure (*ca.* 25 p.s.i.; $1.8 \cdot 10^4$ kg$\cdot$m^{-2}) at 0.57 ml$\cdot$min^{-1} with a Matheson Model No. 3473 gas regulator. A small amount of citric acid was placed in a tube between the reagent reservoir and the regulator to prevent diffusion of ammonia into the gas regulator. A Waters BASELINE 810 Chromatography Workstation software (Waters/Millipore) was used to record and analyze the data. Values of height equivalent to a theoretical plate (HETP) were calculated from peak widths measured by the tangent method. Extra-column contribution to band broadening was determined by plots of peak variance as a function of sample volume in the absence of a column. These values of variance were used to correct the values of peak widths used for the calculation of the number of theoretical plates, N, from the common equation, $N = 16(t_R/t_w)^2$ (t_w is peak width).

Chemicals and supplies

Aqueous solutions were prepared with distilled deionized water, and all chemicals were standard analytical grade. Cetylpyridinium chloride (CPCl) (Sigma, St. Louis, MO, USA) in acetonitrile–water (20:80, v/v) solution was used to "permanently" coat reversed-phase columns with CPCl, and thus convert them into anion-exchange columns. Sodium octanesulfonate (SO) (Aldrich, Milwaukee, WI, USA) was used for separations based on ion-interaction exchange mechanisms. Metal ion samples for anion and cation separations were made by dissolving metal salts in the desired eluent, normally at a concentration of 5.0 μg$\cdot$ml^{-1}. The postcolumn reagents, 4-(2-pyridylazo)resorcinol (PAR) (Aldrich, Milwaukee, WI, USA), and eriochrome black T (EBT) (Aldrich), were used as PCR reagents at a concentration of $2.0 \cdot 10^{-4}$ mol$\cdot$l^{-1} in a 3.0 mol$\cdot$l^{-1} NH_3 and 1.0 mol$\cdot$l^{-1} acetic acid pH 10 buffer. Eluent pH was adjusted with sodium hydroxide. All eluents were filtered through a 0.2-μm filter prior to use.

Column preparation

Self-packed columns were packed with an isooctane–chloroform (1:3, v/v) slurry as described elsewhere [11]. Anion-exchange columns
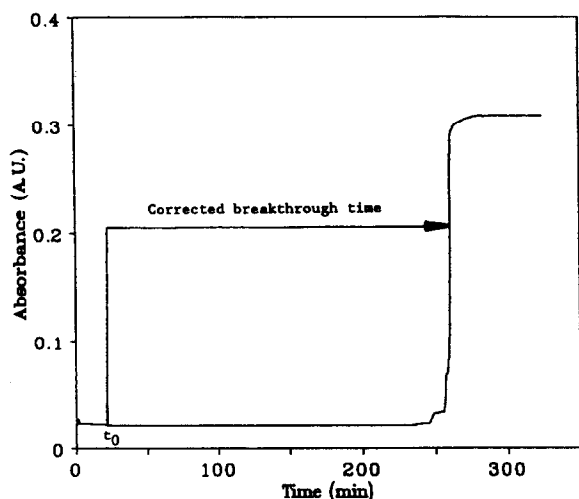
Fig. 1. Breakthrough curve for CPCl adsorption onto a reversed-phase column. Experimental conditions: $1.0 \cdot 10^{-4}$ mol$\cdot$l^{-1} CPCl in acetonitrile–water (20:80, v/v); 15 cm $\times$ 4.6 mm I.D. Supelcosil LC-18 (5 μm) column; CPCl solution flow started at $t = 5.0$ min; the UV absorbance of the eluent was monitored at 254 nm.

were prepared by equilibration of reversed-phase columns with 1-l solutions of CPCl in acetonitrile–water (20:80, v/v) as described elsewhere [2]; concentrations of CPCl in the range of $1.00 \cdot 10^{-2}$ to $1.00 \cdot 10^{-4}$ mol$\cdot$l^{-1} were used to produce a wide range of exchange capacities. The coating solutions were filtered through 0.2-μm nylon filters and eluted through the reversed-phase
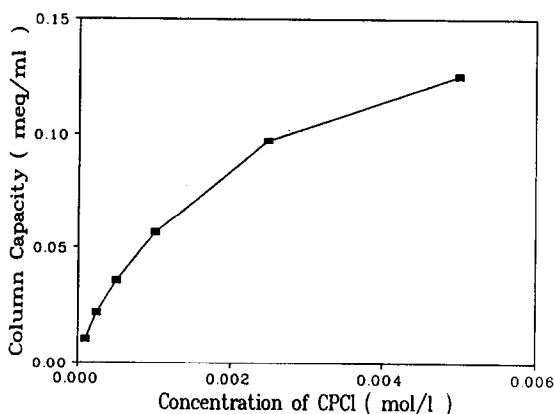

Fig. 2. Anion-exchange capacity as a function of concentration of CPCl in the coating solution. Experimental conditions: 15 cm $\times$ 4.6 mm I.D. Supelcosil LC-18 (5 μm) column; CPCl solution, 1 l, was in acetonitrile–water (20:80, v/v).

separation column and a pre-column at a flow-rate of 1.0 ml$\cdot$min^{-1}. Breakthrough curves [12] were used to determine column capacity, and an example of one of these curves is shown in Fig. 1. The relationship between the concentration of the surfactant in the coating solution and the final effective exchange capacity (per ml of column volume) is shown in Fig. 2.

RESULTS AND DISCUSSION

Detection

Simple metal ions can not be detected with good sensitivity by direct UV–visible absorption. Detection as anionic complexes formed with complexing reagents in the eluent is possible if the complexes exhibit large molar absorptivities at wavelengths different from the free ligand, but this was not the case for any of the eluents used in these studies. Consequently, detection after a PCR was investigated. The main PCR reagents studied were PAR and EBT. PAR is commonly used for the PCR detection of metal ions, and EBT was selected since its conditional stability constants are higher for some metal ions. At pH 10 both PAR and EBT can react with several metal ions, and to maintain a pH value of 10, a 3 mol$\cdot$l^{-1} ammonia and 1 mol$\cdot$l^{-1} acetic acid buffer was used in the PCR reaction. Although ammonia can complex metal ions, it did not have an important effect on the formation of PAR or EBT complexes. In spite of the larger conditional formation constants for EBT complexes, experimental results showed that sensitivities with EBT were much worse than those with PAR, possibly due to a slower ligand exchange between metal complexes formed in the eluent and EBT.

Except for Ni^{2+}, good sensitivities were obtained when PAR was used with oxalate eluents. Detection sensitivity for Ni^{2+} decreased with an increase in the concentration of free oxalate in the eluent. Since Ni^{2+} is known to exhibit slow ligand exchange (see below) relative to the other metal ions studied [13], the poor sensitivity with Ni^{2+} was likely a result of a slow reaction with PAR. This conclusion is supported by the fact that with a reaction-delay coil Ni^{2+} was detected with a sensitivity comparable to that for the

other metal ions. With 0.100 mol·l^{-1} PDCA (pH = 5.4) as the eluent, only Co^{2+} and Cu^{2+} were detected without a reaction coil. When the reaction coil was used all metal ions were detected, and detection sensitivities were increased by up to 5-fold for Co^{2+} and Cu^{2+}. However, relative to oxalate eluents, detection limits with PDCA were lower by 2- to 50-fold than those observed for the highest concentration (0.20 mol·l^{-1}) of oxalic acid used as an eluent. The poorer detection limits for PDCA eluents may be a result of the larger conditional formation constants for PDCA complexes (calculated from data in ref. 14). Tartaric acid was also considered as a possible eluent because its conditional formation constants are smaller than those of complexes of oxalate and PDCA [14]. However, detection sensitivities were much lower than with 0.20 mol·l^{-1} oxalic acid as eluent, possibly as a result of a slower reaction between PAR and the tartrate complexes, or the formation of ternary PAR–tartrate–metal complexes, which had smaller molar absorbances, and thus lower detection sensitivity. Consequently, all further studies reported here were obtained with oxalate eluents.

Effect of metal ion speciation on retention

Due to its d^9 structure, Cu^{2+} tends to form primarily 2:1 square planar complexes in the presence of weak-field bidentate ligands [15]. For Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}, the difference in crystal-field stabilization energy between the square planar and the octahedral complexes is considerably smaller, and six-coordinated octahedral complexes can form with weak field ligands [15]. Among these cations, Ni^{2+} and Zn^{2+} have been reported to form 1:3 six-coordinated octahedral complexes with oxalate (Ox^{2-}) [14,15]. The fraction of free Ox^{2-} changes from 0.06 to 0.99 in the pH range of 3 to 6, and since the pH value of the eluent can have a pronounced effect on the speciation of the metal ion–oxalate complexes, this pH range was selected for further studies. Values of pH less than 3 were not used because retention times decreased with time due to slow loss of the surfactant from the coated columns.

When the test metal ions were separated on a higher capacity column ($ca.$ 0.14 meq·ml^{-1}) at a constant oxalate concentration, significant differences in the pattern of retention order as a function of pH were observed, as shown in Fig. 3. The observed increases in retention can be related to an increase in the anionic character of the metal complexes with increasing pH. Calculations from stability constants [14] showed that the fractions of MOx_2^{2-} for Mn^{2+} and Co^{2+}, and the sum of the fractions of MOx_2^{2-} and MOx_3^{4-} for Ni^{2+} and Zn^{2+} increased over the pH range, and thus retention is expected to increase. The fraction for $CuOx_2^{2-}$ remained almost constant at 1.0 throughout this pH range, and thus the increasing concentration of Ox^{2-} in the eluent caused a decrease in the retention time of Cu^{2+}. This increase in eluent strength with pH would also offset some of the increase in retention time expected for the other metal ions.

Since Ni^{2+} and Zn^{2+} can form small amounts of MOx_3^{4-}, it is not unexpected that these two metal ions are retained longer than Co^{2+} and Mn^{2+} (see Fig. 3). The calculated fraction of $NiOx_3^{4-}$ was larger than that for $ZnOx_3^{4-}$, and this is consistent with the longer retention for Ni^{2+}. It was found that, for any one metal ion, the effect of pH on retention decreased as the exchange capacity of the column was decreased. For example, with an exchange capacity of $ca.$ 0.06 meq·ml^{-1} and a 0.1 mol·l^{-1} oxalate eluent
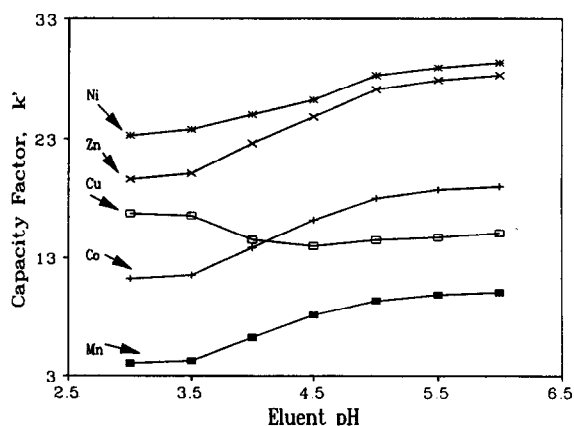

Fig. 3. Variation of capacity factor with eluent pH. Experimental conditions: 0.10 mol·l^{-1} oxalate eluent; 15 cm × 4.6 mm I.D. Supelcosil LC-18 (5 μm) column coated with $1.0 \cdot 10^{-2}$ mol·l^{-1} CPCl solution ($ca.$ 0.13 meq·ml^{-1}); sample, 20 μl of 5 μg·ml^{-1} test metal ion.

it was found that the retention times of the metal ions remained relatively constant as a function of pH, except for Cu^{2+} which showed a slight decrease with an increase in eluent pH. The different retention patterns on the lower capacity columns can be attributed to a weaker interaction between the anionic complexes and the lower capacity ion exchanger, resulting in a greater relative effect from the increase in elution strength with pH. With a 0.200 $mol \cdot l^{-1}$ oxalic eluent, a slight increase in the retention time of Cu^{2+} was observed with increased eluent pH. The reason for this is not clear, but may be a result of possible formation of $CuOx_3^{4-}$ at the higher oxalate concentrations.

At pH 4.00, the metal ions could be separated within a relatively short time with good column efficiency (see below). In addition, this pH value is close to $pK_{a,2} = 4.19$ for oxalic acid [14], and thus oxalate is an effective buffer at this pH. Consequently, a pH of 4.00 was selected for further studies.

Metal speciation and column efficiency

In the oxalate eluent system MOx, MOx_2^{2-} and MOx_3^{4-} complexes can be formed, and these different anionic complexes must be in rapid equilibria with one another to maintain fast mass transfer and high separation efficiency. The calculated fractions of the different metal species present at pH 4 as a function of oxalate concentration are shown in Fig. 4. To evaluate if speciation of metal ion and kinetics of ligand
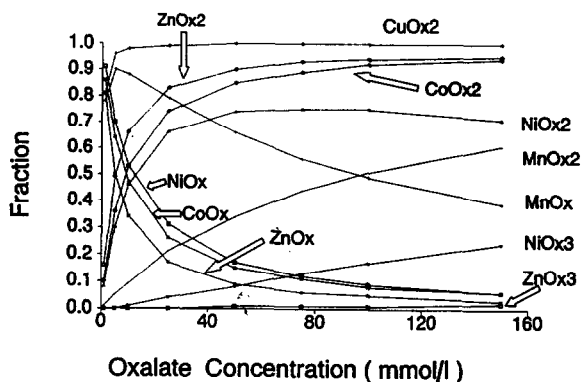
exchange influenced column efficiency, a concentration range of $1.50 \cdot 10^{-1}$ to $1.00 \cdot 10^{-3}$ $mol \cdot l^{-1}$ oxalic acid (at pH = 4.00) was evaluated for seven different column capacities over the range of 0.01 to 0.12 $meq \cdot ml^{-1}$. At a constant eluent pH and with increasing concentration of oxalate from 0.001 to 0.15 $mol \cdot l^{-1}$, all metal ions showed a decrease in retention time, and the order of elution was normally $Mn^{2+} < Co^{2+} < Zn^{2+} < Ni^{2+} < Cu^{2+}$. On columns with capacities > 0.1 $meq \cdot ml^{-1}$ and with high oxalate concentrations in the eluent, the order of Cu^{2+} and Ni^{2+} was reversed, possibly due to the ability of Ni^{2+} to form octahedral complexes such as $NiOx_3^{4-}$ (see Fig. 4). The retention order normally observed for the other metal ions was $Ni^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+}$. This order corresponds to what would be expected based on the speciation in Fig. 4. Nickel has the greatest percentage of MOx_3^{4-} species, and the order for the concentration of MOx_2^{2-} species is $Zn^{2+} > Co^{2+} > Mn^{2+}$.

For almost all of the exchange capacities evaluated the HETP values for each metal ion did not show any obvious trend as the concentration of oxalic acid was decreased from $1.50 \cdot 10^{-1}$ to $2.50 \cdot 10^{-2}$ $mol \cdot l^{-1}$. Fig. 5 shows typical results for one of the experimental conditions used in this study. For each metal ion HETP values were generally in the range of 0.010 to 0.025 mm. However, at lower oxalate concen-
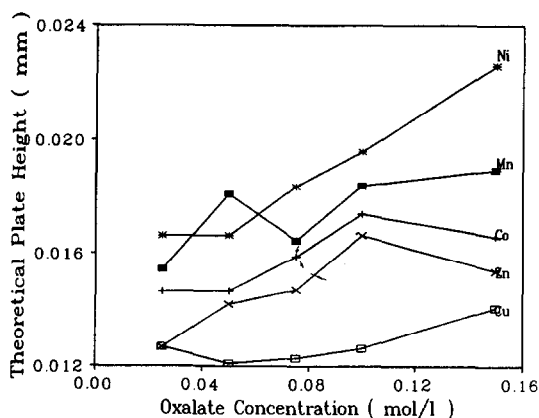

Fig. 4. Fractions of metal oxalate complexes as a function of oxalate concentration at eluent pH of 4.0. Data calculated from constants in ref. 14.

Fig. 5. Column efficiency as a function of oxalate concentration. Experimental conditions: pH 4.0; concentration of CPCl coating solution, $8.0 \cdot 10^{-4}$ $mol \cdot l^{-1}$; 15 cm × 4.6 mm I.D. Supelcosil LC-18 (5 μm) column.

trations with lower capacities columns (coated with $1.00 \cdot 10^{-4}$ and $2.00 \cdot 10^{-4}$ mol $\cdot$ l^{-1} CPCl) the HETP values for Ni^{2+} and Mn^{2+} increased significantly, and the Mn^{2+} peak which had the shortest retention time, began to show obvious tailing. The behaviour of Mn^{2+} may be related to slow exchange between the different manganese species: the fraction of the 2:1 complex, MnOx$_2^{2-}$ was always smaller than that of any of the other metal ions. The behaviour of Ni^{2+} may be related to an inherently slow rate of ligand exchange for Ni^{2+} complexes, as discussed below.

The order of HETP values for the oxalate system was normally in the order, Ni $\geqslant$ Mn > Co $\approx$ Zn > Cu. Since mass transfer should be related to the rate of ligand exchange between the different complex species for any given metal ion, one might expect a relationship between ligand-exchange rates and column efficiency. Exchange rates for all of the metal oxalate systems were not available in the literature, but it is not unreasonable to expect that the general order of rates of exchange for the different metal ions should be related to the exchange rates for coordinated water. This order of water exchange is: Cu $(1 \cdot 10^9) >$ Zn $(5 \cdot 10^7) \approx$ Mn $(2.7 \cdot 10^7) >$ Co $(2 \cdot 10^6) >$ Ni $(3 \cdot 10^4)$ [13]. Except for Ni^{2+} the order of HETP values does not agree with this pattern. An examination of the speciation in Fig. 4 shows that, for any given metal ion, the number and concentration of species other than MOx$_2^{2-}$ increased in the order Mn > Co $\approx$ Zn > Cu, which is the same as the order of HETP values. It can be expected that the overall rate of mass transfer would increase with an increase in the number of species in equilibrium with each other. Consequently, although there may be a relationship between the rate of ligand exchange (water exchange rates) and HETP values for the metal ions when the differences between rates are large, the order of the HETP values appears to be primarily determined by the diversity of the speciation.

Comparison of cation and anion separation systems

The performance of the anion separations were compared with separations on a cation

exchanger, which was based on an ion-interaction system that used n-octanesulfonate to modify the reversed phase. The same column was used for both systems, and this eliminated the influence of variations in the packed bed on the relative performance of the two systems. Fig. 6 shows a comparison of the column efficiencies obtained with a recommended cation procedure [16] and the results obtained with the oxalate anion-exchange separation; also included in Fig. 6 is a cation separation performed with an oxalate eluent. These results show that the anion-exchange system can provide column efficiencies that compare well to that for cation systems. To achieve these efficient anion separations it is necessary to minimize the number of species in equilibrium with one another. Consequently, surfactant-modified reversed phases offer advantages relative to bonded phases because both the capacity and eluent composition can be varied to optimize the speciation of the metal ions. As expected, the order of separation on the anion-exchange system was considerably different from that observed with cation exchange, which could be attractive for certain analytical problems. Both systems were also comparable in terms of total analysis time. Total analysis time for isocratic separations with the anion systems was actually less than that for
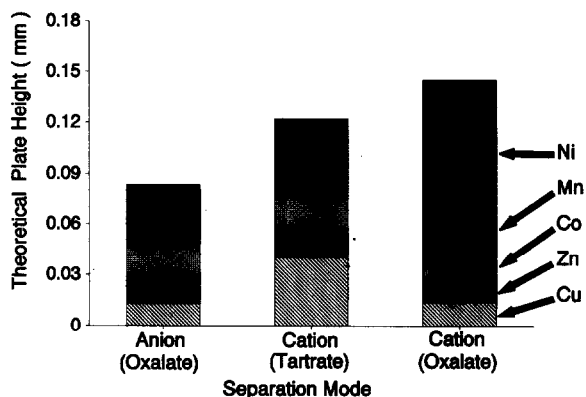

Fig. 6. Comparison of column efficiency for anion- and cation-exchange systems. Experimental conditions: anion system as for Fig. 5; cation systems; 0.0020 mol $\cdot$ l^{-1} sodium octanesulfonate, pH 3.4, and 2% (v/v) acetonitrile in eluent; tartrate concentration, 0.0020 mol $\cdot$ l^{-1}; oxalate concentration, 0.010 mol $\cdot$ l^{-1}; 15 cm × 4.6 mm I.D. Supelcosil LC-18 (5 μm) column.

the cation systems, but this advantage would be reduced if gradient separation conditions were used. Sensitivities, which were also comparable, were generally in the range of 0.3 absorbance units per μg of injected metal ion.

ACKNOWLEDGEMENTS

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