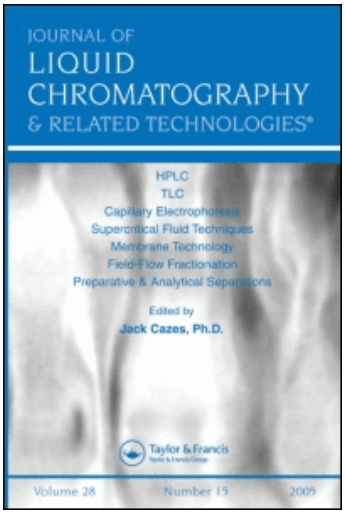

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Metal Ion Chromatography with Fluorescence Detection

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METAL ION CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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ABSTRACT

Nonpolar, agglomerated anion exchanger, and surface-sulfonated cation exchanger stationary phases have been used in conjunction with 8-hydroxyquinoline-5-sulfonic acid (HQS) in the eluent or as a postcolumn reagent for the separation and detection of a number of metals that form fluorescent HQS complexes. Several metals, notably those classified as transition metals, form nonfluorescent HQS chelates and quenches the fluorescence of other metal-HQS metal chelates. Such transition metals have been detected by introducing the fluorescent Al-HQS chelate postcolumn. Cation exchange stationary phases are the most useful for chromatographic applications involving HQS and are able to provide a variety of useful separations by tailoring elution conditions. Although not sensitive to Ba, the approach may be particularly good for the determination of the other alkaline earth metals. Fluorescence quenching resulting from Fe and Ni leaching from stainless steel chromatographic systems present a problem for trace analysis and accentuate the need for nonmetallic hardware. Subpicomole detection limits are attainable for Cd, Mg and Zn.

INTRODUCTION

Chromatographic analysis of metal ions, although not widely practiced until relatively recently, has been in the domain of active scientific endeavor for more than three decades. Reviews by Schwedt (1-3), Willeford and Veening (4), Cassidy (5), Krull (6), Nickless (7) and Dasgupta (8) adequately cover previous efforts.

When sensitivity is a consideration, electrochemical detection for electroactive compounds and fluorometric detection for fluorescent compounds are generally the modes of choice in LC; determination of metal ions is no exception. Electrochemical detection for metal species has been investigated by a number of workers (see refs. 5, 6, 8 for reviews); the most pertinent to the present investigation are the studies of Bond and Wallace (9-12) who detected several metals as their dithiocarbamate complexes either by preforming the chelate or by incorporating the ligand in the eluent. LOD's were of the order of 1 pmol (0.8 for Cr, 1.5 for Ni and Cu). The first attempt towards fluorometric detection of metal ions is due to Beckett and Nelson (13). EDTA was derivatized to form p-aminophenylEDTA; preformed chelates of this ligand with Zn, Cd and Pb were separated on a bonded anion exchanger and detected fluorometrically after postcolumn reaction with fluorescamine. While LOD's as low as 280 fmol (for Pb) could be attained, the separation required 20 min and the unreacted ligand (which produced a large off-scale

response and therefore had to be eluted before a second sample was injected) eluted at 60 min. The only other effort towards fluorescence detection, to our knowledge, is due to Shih and Carr (14). This system also involves preformed metal chelates. After separation on a reversed-phase column, metal complexes of n-butyl-2-naphthyl-methyldithiocarbamate pass through a photochemical reactor and are decomposed to form a fluorescent organic product. Detection limits in the $\sim 10^{-8}$ M range were attained for Fe, Ni, Hg and Co. Limitations are similar to the Beckett and Nelson study.

The mainstay of the current practice of metal ion chromatography is spectrophotometric detection after postcolumn reaction with a chromogenic ligand, most typically PAR or Arsenazo III, both originally introduced for such applications by Fritz and Story (15). Direct incorporation of the chromogenic ligand into the eluent has been attempted only infrequently (12, 16).

In a recent paper, we have described the fluorescence properties of metal chelates of 8-hydroxyquionoline-5-sulfonic acid, HQS (17) and indicated the potential of HQS in metal ion chromatography. In the present work, we present a detailed exploitation of these properties for chromatographic detection.

MATERIALS AND METHODS

Chromatographic experiments were conducted on a Gilson 2-pump HPLC system equipped with a pressure monitor/pulse

dampener (Gilson Medical Electronics, Middleton, WI), a low dead volume high pressure dynamic mixer (Knauer, W. Germany), a Rheodyne 7010 loop injector (Rheodyne Inc., Cotati, CA) and a variable wavelength fluorescence detector (FS970, Kratos Instruments, Ramsey, NJ). This detector uses a D_2-lamp as the excitation source, a grating monochromator on the excitation side and a high pass emission filter. All experiments were conducted with an excitation wavelength of 362 nm and a high pass filter on the emission side with the 50% cutoff point being 470 nm. Although the optimal (energy corrected) excitation wavelength for most metal-HQS complexes is around 390 nm (17), the lamp output characteristics result in greater detector response when the excitation wavelength is chosen as cited. A 50 µL sample volume was used in all work to maximize concentration sensitivity; only moderately efficient ($d_p \geq 10$ µm) columns were used in this work.

Four different types of stationary phases were used in this work. The first was a macroreticular (polystyrenedivinylbenzene) (PSDVB) reverse phase (PRP-1, d_p = 10 µm, Hamilton Co., Reno, NV) in 150 X 4.1 or 250 X 4.1 mm columns. The second stationary phase was an agglomerated anion exchanger (column type CS5, 250 X 4.0 mm, Dionex Corporation, Sunnyvale, CA) of capacity ~25 µeq/g, containing 13 µm surface-sulfonated core particles upon which ~0.2 µm size anion exchanger latex particles are agglomerated. This was used with the corresponding guard column (CG5, 50 x 4.0 mm). The third stationary phase was a cation

exchanger (column type CS2, 250 x 4.0 mm, Dionex Corp.) with an ion exchange capacity of 20 μeq/g, containing 15 μm surface-sulfonated packing. The Dionex Columns have no metallic contact parts. The fourth type of stationary phase was also a surface-sulfonated cation exchanger (column type HS), but of larger particle size and an ion exchange capacity of ~40 μeq/g, from Wescan Instruments (Santa Clara, CA) as short columns (50 x 4.2 mm) and also in longer custom-packed lengths (100 and 150 mm).

HQS was twice recrystallized as the monohydrate, from large volumes of hot water. Anions associated with the metal samples are as described in (17). Acetonitrile was HPLC grade and redistilled in all glass apparatus. Water used as solvent met all specifications of ASTM Type I reference Reagent Water; however, residual trace metal content was not explicitly determined. Buffering agents Tris(hydroxymethyl)aminomethane, N,N-bis(2-hydroxyethylglycine) (BICINE), 3-(N-Morpholino)-ethanesulfonic acid (MES), 3-(N-Morpholino)propane sulfonic acid (MOPS), 3-(N-Morpholino)-2-hydroxypropanesulfonic acid (MOPSO) were obtained from Serva Fine Biochemicals (Westbury, NY). All other chemicals were of reagent grade. pH was measured with an Altex PHI 71 pH meter equipped with an Orion Ross combination electrode, calibrated with aqueous buffers by the two point method. The pH cited for eluent media containing varying amounts of acetonitrile are the apparent pH values. For postcolumn

reaction studies, the pressurized porous membrane reactor described in (18) was used for reagent introduction.

RESULTS AND DISCUSSION

HQS in Eluent Mode. Incorporating HQS in the eluent obviates the need for any postcolumn reaction and permits the simplest possible setup. Major emphasis was therefore placed on studies in this mode, both to establish its capabilities and limitations.

Chromatography on PRP-1. HQS was expected to adsorb on the reverse phase column and offer differential retention of various metals, essentially functioning as an ion interaction reagent (8). From a purely aqueous eluent containing 1 mM HQS (with or without pH adjustment to ~8 with KOH), the adsorption of HQS was very large. This not only led to long equilibration times and relatively high pressure drops, but the retention of most ions of interest was much higher than desired. Incorporating 10-25% acetonitrile in this eluent reduced the retention to reasonable values and also permitted rapid ($\leq$ 1 h) equilibration times. However, both selectivity and column efficiency were poor, the latter being much worse than efficiencies obtained with the column in conventional reverse phase mode with test organic solutes. An additional problem, which is common to all of the stationary phases used in this study, is a fast eluting matrix dip/peak that is present in all chromatograms at high detector sensitivities, whenever the sample matrix is not perfectly

matched with the eluent. This is a manifestation of the adsorption equilibria that exists between HQS and the stationary phase -- the system reequilibrates after the perturbation represented by the sample injection and this shows up as a matrix peak/dip. The magnitude of the matrix peak/dip increases with increasing adsorption of HQS and the problem is worst with PRP-1, the most hydrophobic of the stationary phases examined in this study. Although the matrix-induced disturbance makes it difficult to quantitate fast eluting species, especially at trace levels, many useful separations are possible. An illustrative chromatogram for a sample prepared in the eluent is shown in Figure 1. A number of metals, including all group III A metals, elute in the same region as Al. Without HQS equalization in the sample matrix, these metals cannot be accurately quantitated at trace levels. Under the conditions listed in Figure 1, the matrix disturbance resulting from injection of pure water is ~10% of the Al-peak shown. The majority of simple divalent cations (alkaline earths, Zn, Cd, etc.), elute in the same region as Mg.

Column efficiencies appear to be dependent on specific metal ions. Although Mg, Be, Cd and Zn all elute essentially at the same time, compared to that of Mg, efficiencies for Cd and Zn are perceptibly better; whereas Be, which is known to form mixed hydroxo-HQS complexes with a tendency to polymerize (19), produces a broad tailing peak. That chemical reaction kinetics or mass transfer limitations are involved is indicated by a

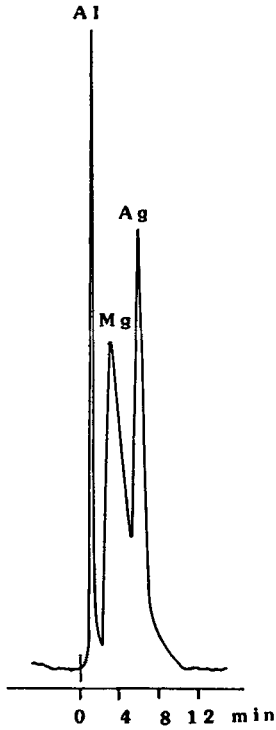

Figure 1. 5 nmole each of Al, Mg, Ag. PRP-1 column, 1 mM HQS in 10:90 $CH_3CN:H_2O$, 1 mL/min. 430 V, 1 μA FS.

general decrease of plate height for all peaks at lower flow rates. The peak due to Ag(I) is probably due to an oxidation product of HQS (20). Although no oxidation of HQS by Ag(I) has been observed to occur outside the column (17), it is possible that the stationary phase acts as a catalyst for the oxidation of HQS by Ag(I) (which is reduced to the free metal) or, more likely, Ag(I) acts as a catalyst for the oxidation of HQS by oxygen adsorbed on the stationary phase, in much the same way

Ag(I) catalyzes the oxidation of HQS by persulfate (20). The peak due to Ag(I) is also unusual in that if the flow is stopped immediately after injection and restarted minutes later, the apparent plate height for the peak decreases greatly. This is expected if the plate height observed for this peak in Fig. 1 is limited by the rate of the reaction involving Ag(I) and HQS to produce the detected product.

If a largely noncomplexing buffering agent, e.g., BICINE, MES, MOPS (21), or Tris is used to buffer the eluent at an apparent pH of 7-9 (depending on the buffering agent), the sensitivities for the alkaline earths are increased while that for Zn, Cd, Al, etc. are decreased. This behavior is expected from the pH dependence of HQS-complexation and fluorescence of the respective metals (17).

Reduction of the HQS concentration and addition of an anionic ion interaction reagent (8), sodium dodecyl sulfate, with or without added electrolytes (up to 0.1 M KNO_3) did not improve either column efficiency, selectivity or limits of detection.

Chromatography on Agglomerated Anion Exchange Columns. The original impetus behind experimentation with this column was that eluent compositions have already been developed by the manufacturer for the separation of several metal ions (22). We envisioned incorporating a small concentration of HQS in the eluent (the pH of these eluents are too low for HQS to exert significant chelating action and thus change retention

characteristics) and changing the pH postcolumn by base introduction through a passive membrane reactor (23, 24); or leaving the chromatographic conditions completely unaltered and introducing HQS postcolumn instead of PAR or other chromogenic ligands. However, eluents containing substantial concentrations (10-100 mM) of pyridinedicarboxylate (PDCA), oxalate, or citrate as recommended by the manufacturer for use with its nonmetallic chromatographic hardware were found to be completely incompatible with our more conventional, stainless steel hardware in that large amounts of iron (and presumably Ni and Cr as well) were leached from the system even after the dissolved oxygen content of the eluent was reduced by degassing. The magnitude of the problem decreased in the order PDCA>oxalate>>citrate. In several instances, the green color of the Fe-HQS chelate was readily evident in the effluent from the system. Even with low concentrations of citrate, which appeared to be the least corrosive, the signals obtained from a given metal ion, e.g., Cd, were substantially smaller than signals obtained with the same sample in the PRP-1 based chromatographic system at comparable retention times, peak efficiencies and under identical detector settings. Because this decrease was much larger than can be accounted for by competitive binding with citrate, we attributed it to quenching by iron (25) and sought alternative eluent systems. The basic eluent compositions chosen was 1 mM HQS buffered with 10 mM MOPS (adjusted to pH ~7 with NaOH). It was

not possible to elute most metal ions of interest within a reasonable period of time without adding electrolytes (principally $NaClO_4$; or Na_2SO_4/NMe_4ClO_4 in some experiments) to the basic eluent composition. A typical chromatogram with 250 mM $NaClO_4$ in the eluent is shown in Figure 2. Al elutes partially superimposed on the water dip, the Zn peak is unusually broad and the Mg peak shows the expected efficiency of the column. Experimentation with the type of electrolyte added to the eluent reveals some interesting behavior. If some of the $NaClO_4$ in the eluent is replaced by an equimolar amount of the NMe_4ClO_4, the retention of Al is unaffected, the retention of Zn, and especially Mg, is decreased. If some of the $NaClO_4$ in the eluent is replaced by half the equimolar amount of Na_2SO_4, the retention of Zn and especially Al, increases, the retention of Mg is essentially unaffected. Based on the data available on metal-HQS or metal-HQ complexation constants (see citations in ref. 17), we conclude that under the operating conditons, Al is present dominantly as the anionic HQS chelate while Mg is present dominantly as the cation. It is well known that both cation and anion exchange sites are accessible in this type of packing (26). We conclude therefore that Al is retained by anion exchange and exhibits a relatively small retention because of the considerable eluting power of the perchlorate ion. Both mechanisms are operative for Zn (Cd behaves in the same fashion), leading to a broadening of the peak.

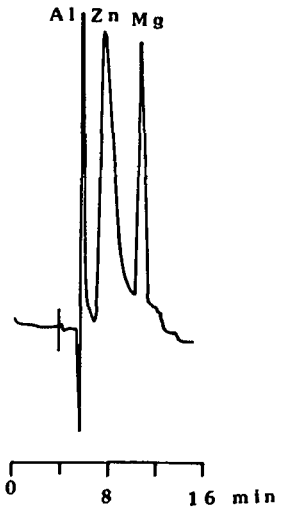

Figure 2. 400 pmole each Al, Mg, 1.2 nmole Zn. Dionex CG5 + CS5, 1 mM HQS, 10 mM MOPS-Na, pH 7.0, 250 mM $NaClO_4$, 1 mL/min. 630 V, 500 nA FS.

The attainable detection limits for this particular system depend on the background fluorescence of the eluent which is controlled by the purity of the $NaClO_4$ used. We have found the background fluorescence to vary by as much as a factor of five, when $NaClO_4$ from different suppliers, or even from different lots from the same supplier, are used to make up the eluent. While recrystallizing the $NaClO_4$ does reduce the background fluorescence to a degree, a more effective method is to slowly filter the prepared eluent through a sintered glass crucible containing a 2-3 cm bed of Chelex-100. The status of exhaustion of the Chelex-resin bed is easily monitored by a long wavelength UV-lamp, the spent layer (from the top) fluoresces blue. The

resin is regenerated with alkaline EDTA and then repeatedly washed. The pH of the eluent increases upon this treatment (the Chelex is in sodium form) and is readjusted back to 7 before use. The limits of detection attainable for a number of metals after such treatment are worth the purification effort. The signal from a 2.5 μg/L (10^{-7} M) Mg standard is shown in Figure 3. This would appear more respectable when we consider that at levels lower than 10^{-6} M, the signals for all the metals decreases in a greater than linear fashion. We ascribe this to the quenching effect exerted by iron which is probably still leached from the system in trace amounts. The greater than linear decrease in concentration is not a property of the fluorescence system, when we use a persitaltic pump to deliver the same eluent as carrier into the detector operated at the same sensitivity in a flow injection arrangement (27), linearity of response to the injected metal ion is observed essentially all the way to the LOD.

In spite of the broadening effects caused by the dual retention mechanism, a number of separations are possible with this column and the HQS-MOPS-$NaClO_4$ eluent system. Trivalent Al, Ga, In and Tl can be separated with 200 mM $NaClO_4$ in the eluent, albeit not with baseline resolution and elute in that order. Unlike the preceeding three, Tl(III) does not form a fluorescent HQS chelate but acts as a quencher. Consequently, it shows up as a dip (which is obviously more visible if the eluent has a perceptible fluorescence background (i.e., not Chelex-filtered).

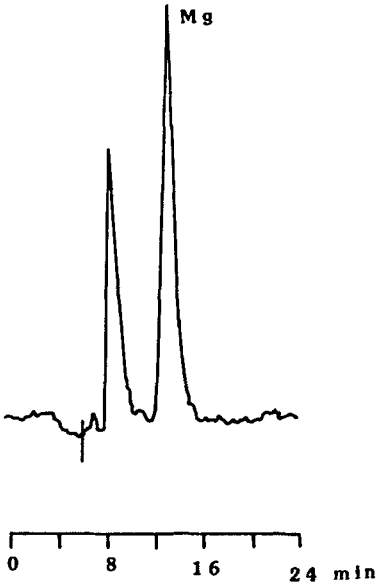

Figure 3. 5 pmole Mg. Conditions as in Figure 2, eluent filtered through Chelex-100. The first peak is the matrix peak. 695 V, 50 nA FS.

Figure 4 shows log k' vs. log [$NaClO_4$] in eluent for the HQS-MOPS-$NaClO_4$ eluent system for a nunmber of metal ions and the matrix peak. (Residence time of an unretained solute in the system was estimated by optical detection of injected iodate with 250 mM $NaClO_4$ as eluent). Note the parallel slopes among Al/Ga/In and between Cd/Zn, and the difference in slope between the two sets. The difference in k' values among Mg, Ca and Sr increases in the order cited for any given eluent composition, reinforcing the hypothesis that cation exchange is the operative retention mechanism for these metals. The differences in k'

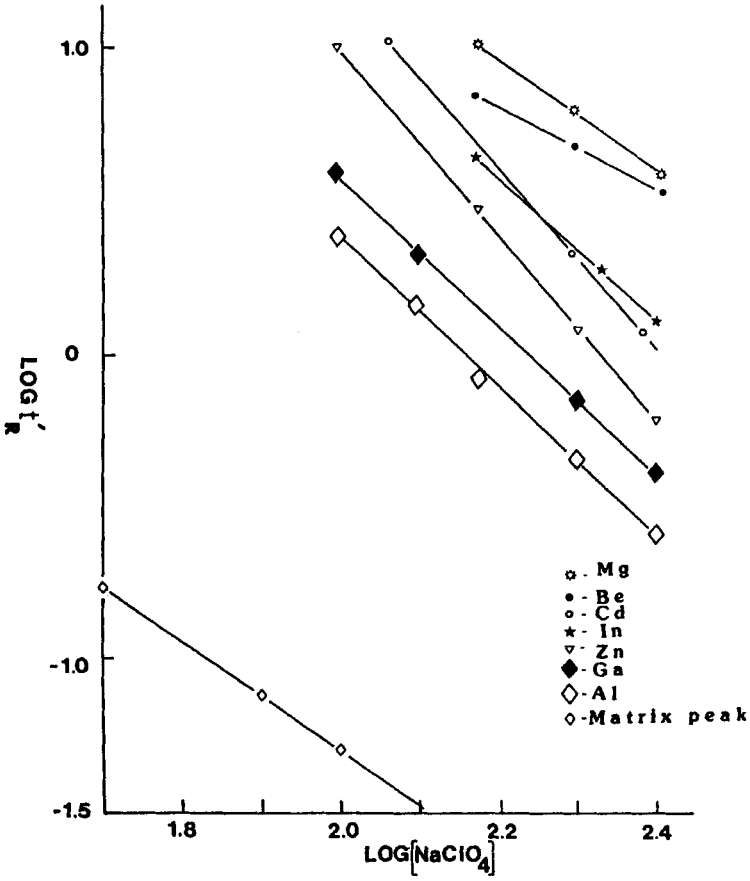

Figure 4. Chromatographic retention behavior of several metals in the column-eluent system of Figure 2 as a function of $NaClO_4$ concentration (mM).

among these metals are however, too small to allow efficient separations. The behavior of Be is quite different and detection limits for Ba are very poor. The k' values for other metal ions of potential interest for a HQS-MOPS-$NaClO_4$ (1 mM - 1 mM - 250 mM) eluent are as follows: Hf (IV), 3.7; La (III), 3.5; W (IV), Nb (V), Ta (V), 3.4; Zr (IV), Sc (III), Pr (III), 3.2; Ir (IV), 2.9; Lu (III), 1.4. Efficiencies obtained for the majority of the ions tested in this mode were mediocre to poor and were occasionally complicated by multiple peaks from a single sample. We have not fully pursued the elucidation of the occurrence of these multiple peaks; however, they are probably related to multiple retention mechanisms. It is worthwhile to note that the occurrence of such multiple peaks was found to be much more commonplace when older glass columns from Dionex, containing similar, albeit less efficient, agglomerated anion exchanger packing were used.

Anion exchange may well be a viable mode to perform these types of separations if a purely anion exchanger stationary phase is used and multiple retention mechanisms are thus avoided.

Chromatography on Surface-sulfonalted Cation Exchangers. For both types of surface-sulfonated columns, the typical eluent composition chosen was HQS (1 mM), buffered with 10 mM MOPS or Tris (at pH 7.0 and 8.0, respectively) with varying amounts of KNO_3. The latter was chosen as the pushing electrolyte because it is available in excellent purity at low cost and because it

does not corrode the metallic hardware. HQS eluents containing up to 200 mM recrystallized KNO_3 displayed essentially the same background fluorescence as without the added salt.

It became clear from the initial results with the two column types that retention behavior cannot be predicted simply on the basis of exchange capacity. Consider the retention behavior of the alkaline earth metals for the CS2 column (250 X 4.0 mm, ~20 μeq/g): Mg - 6.7, Ca - 9.5, and Sr - 15.6 min are the net retention times, respectively, and for the Wescan HS column (50 X 4.2 mm - 40 μeq/g: Mg - 0.75, Ca - 3.0 and Sr - 5.1 min respectively with the same eluent system (1 mM HQS, 10 mM Tris, 100 mM KNO_3, pH 8.0, flow rate 1 mL/min). Even after one accounts for the column lengths and slight differences in flow velocities, ~10%), it is clear that retention does not increase with increasing column exchange capacity. The exchange capacities of the columns were rechecked and manufacturer's specifications were found to be quite close to our determined values. We hypothesize therefore that the retention behavior for these metals (hard cations: strong ion exchange affinity, relatively weak HQS complexation) may be controlled, at least in part, by HQS adsorption on the stationary phase and the behavior of the adsorbed anionic ligand as an ion interaction reagent (8). It is our further belief that adsorption of anionic HQS decreases with increasing ion exchange capacity of the stationary phase, i.e., with increasing number of negatively charged sulfonate

groups bonded to the matrix. This phenomenon complicates the reliability of simplistic prediction of retention orders based on the column exchange capacities. The net retention times of Mg, Ca and Sr for the Wescan HS column were also determined with a 25 cm column and found, predictably, to be 5 times those obtained with the 5 cm column. When such data are compared with the corresponding data for the CS2 column, it is clear that Mg is retained more strongly on the CS2 column whereas Ca is retained more strongly on the HS column and Sr even more so. The noteworthy point is that unlike a conventional IIR eluent system (e.g., an alkylsulfonate solution), the use of HQS as eluent not only leads to the number of virtual cation exchange sites increasing with increasing HQS concentration, HQS is also a complexing eluent and helps elute the metal by forming a complex, presumably anionic, that transfers to the mobile phase. The relative retention differences of Mg, Ca and Sr between the two columns are explicable if one considers that the complexation constant and thus the eluting power of HQS increases along the series Mg, Ca and Sr. To elucidate the nature of these phenomena further, we carried out experiments with a constant KNO_3 and buffer concentration (and pH) while varying HQS concentration. It was found that for any given column, the retention of Ca and especially Sr are affected to a greater degree than is that of Mg. A parallel set of experiments were conducted at constant HQS and buffer concentration with varying KNO_3 content. The

retention of Mg was found to be most dependent, that of Ca less so and that of Sr lesser still on the amount of the added KNO_3. These observations are in general accord with our hypothesis regarding the general behavior of such a system. Operating the column at an elevated temperature also indicates that the retention-elution process here is not a simple ion exchange process. For the alkaline earth metals, column efficiency does not markedly increase at 40°C relative to ambient, while retention markedly increases, especially along the series. Further, it appears clear that the eluting ability of HQS overrides whatever extra retention capacity it imparts to the column via ion interaction effects. This appears to be true not only for alkaline earths but for virtually all other metals as well. Taking Zn and Cd as examples, the net retention times (flow rate 1 mL/min) on the CS2 column for an eluent system containing a constant buffer composition (10 mM MOPS, pH 7.0) and varying concentrations of HQS are: 1.6, >12 (0.5mM HQS); 0.33, 7.6 (0.7 mM HQS); and 0.15, 1.4 (1.0 mM HQS) minutes, respectively, for Zn and Cd. It is also interesting to note that metals such as Al which are very strongly complexed by HQS not only elute in virtually the void volume (≤ 0.1 min. net retention), they also exhibit retention times that are independent of HQS concentration in the HQS concentration range 0.5-5 mM. The retention order in the aluminum family (trivalent ions) increases with atomic weight: Tl>>In>Ga≈Al, for the CS-2

column; Tl produces a negative peak (quenching effect, see ref. 17).

A variety of metals produce useful chromatographic retention and reasonable responses with cation exchange columns and HQS bearing eluents. Although for any given metal, the best response is obtained at a specific pH (see ref. 17), HQS is essentially completely ionized at pH 8.8. We have therefore determined the retention behavior at a constant pH of 8.8 and a constant HQS concentration of 1 mM. In one series of experiments, the eluent medium was also altered from purely aqueous to 25:75 acetonitrile:water to minimize HQS adsorption effects, with and without the incorporation of 100 mM KNO_3 in the eluent. These data for the 5 cm Wescan HS column are shown in Table I.

There are a few noteworthy points regarding the data in Table I. First, while incorporation of acetonitrile in the eluent reduces HQS adsorption on the stationary phase and is thereby expected to lead to decreased retention times, the organic solvent also depresses the ionization of HQS, making it a weaker eluent. The net result is that there is no uniform trend among the various metals as to the effect of incorporation of acetonitrile in the eluent. Predictably, the use of methanol leads to similar results. The second factor concerns the retention time of the matrix peak (which is easily identified when any sample, containing a concentration of HQS different from that in the eluent, is injected). This peak/dip essentially

reflects the retention of HQS itself in the system and is predictably lower with acetonitrile incorporated in the eluent. The matrix peak moves to increasingly higher retention times with increasing electrolyte concentration in the eluent, whether the eluent media is purely aqueous or hydroorganic. Increased adsorption of HQS is indeed expected with increasing electrolyte concentration; this has been demonstrated in exemplary fashion by Cantwell and Puon (28) experimentally and was also shown by them to be consistent with the Stern-Guoy-Chapman theory.

We have also carried out a series of retention experiments with a few metals with varied KNO_3 concentrations, both with the purely aqueous and the hydroorganic eluent media. Unlike the case for the anion exchange column (see Figure 4), no linear relationship was found between log t_R' and log [KNO_3].

When the chromatographic system (pump, injector, detector) is freshly passivated by pumping 0.5 M HNO_3 and column equilibration/chromatography is carried out on the same day, detector linearity, especially in terms of peak area, is excellent with a column containing no metallic parts, even at trace levels. This is demonstrated for Ga(III) in the concentration range 1-100 μM in Figure 5. It is important to emphasize however that such results can be obtained only if meticulous care is taken to minimize iron contamination. Figure 6 shows a separation of Zn and Cd at the 10^{-6} M level on the CS2 Column, the second chromatogram is that of the same sample when

Table I.

Capacity Factors (k') for Various Metals on a Surface-sulfonated Cation Exchange Column-Sulfoxine Eluent system.[a]

Metal Eluent	A:1 mM HQS (aq) pH 8.8	B:1 mM HQS (25:75 $CH_3CN:H_2O$) apparent pH 8.8	C:B+100 mM KNO_3
Ag(I)	4.1	10.1	1.1
Al(III)	0.3	0.8	0.6
Be(II)	5.9	1.5	3.5
Ca(II)	vh[b]	>50	9.5
Cd(II)	0.5	1.4	0.5
Ce(IV)	0.2	0.5	1.7
Ga(III)	0.4	1.1	0.6
Gd(III)	4.2	3.7	2.7
Hf(IV)	nd[c]	3.4	3.0
Ho(III)	nd	4.9	3.1
La(III)	nd	nd	1.3
Lu(III)	8.6	7.1	3.1
Mg(II)	28.6	>40	8.3
Nd(III)	4.3	3.1	3.0
Pt(II)	nd	nd	1.3
Sm(III)	nd	4.8	2.5
Ta(IV)	nd	nd	8.3
Ti(IV)	nd	5.9	4.0
Tl(I)	nd	1.7	1.7
W(VI)	vh	vh	vh
Y(III)	nd	nd	17
Zn(II)	0.2	0.65	0.7
Zr(IV)	nd	1.2	2.0
Matrix peak	0.3	0.25	0.4

a. Wescan HS column 50 x 4.2 mm, Cation exchange capacity 40 μeq/g. Eluent flow rate 1 mL/min.

b. Very high, not eluted in a reasonable time

c. not determined.

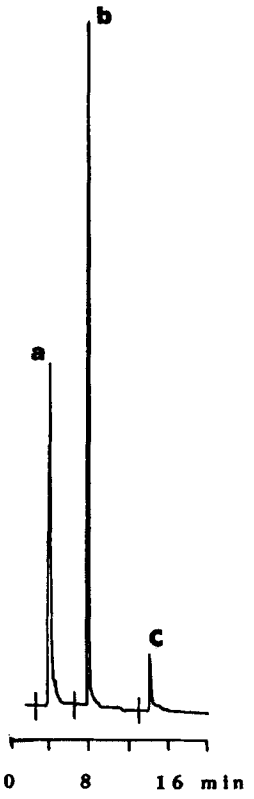

Figure 5. Response linearity in the absence of significant iron contamination. Dionex CS2, 1 mM HQS, 10 mM MOPS, pH 7.0, 10 mM KNO_3, 1 mL/min. PMT 450 V; (a) 5.0 nmole Ga, 1.0 μA FS (b) 500 pmol Ga, 50 nA FS (c) 50 pmol Ga, 50 nA FS.

both the sample and the eluent have been allowed to remain stationary in the stainless steel system for 20 min before re-injection. Not surprisingly, at trace levels, day-to-day response reproducibility with the other cation exchanger, contained in metallic hardware, was distinctly worse.

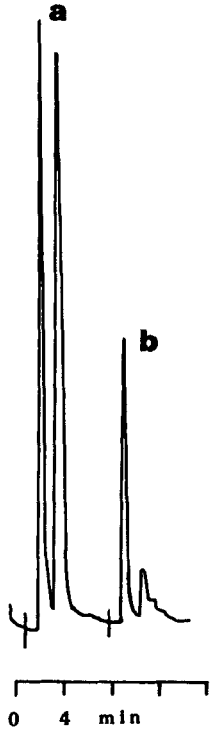

Figure 6. Drastic effects of iron leaching from system components. Dionex CS2, 1 mM HQS, 10 mM MOPS, pH 7.0, 1 mL/min. 700 V, 0.1 μA FS. 50 pmole each Zn^{2+} and Cd^{2+} in eluent. Chromatogram (b) was obtained after allowing the same sample and eluent to remain stationary in the system for 10 min.

Separation and Detection of Alkaline Earth Metals. Because the alkaline metals as a group are retained more strongly than virtually any other metal in the eluent systems we have studied with the cation exchange columns, the approach can clearly be of particular merit in their separation and determination. A special effort was therefore made toward this end. First, it is useful to note that if the column eluent system is configured to

provide fast elution of Mg, this metal can be detected at levels below 10^{-8} M(17).

The response for Ca is much lower than that of Mg. The response to Sr is lower still and Ba cannot normally be detected. While the drastic decrease in sensitivity along the series is unfortunate, the reverse, i.e., a poor sensitivity for Mg compared to Ca will be worse in that most real samples of interest contain much more Ca than Mg. Simultaneous determination of Mg and Ca in potable water supplies is facile, Figure 7 shows a typical chromatogram of a 10 fold diluted sample of local tap water; the chromatographically determined values agree within 10% of those determined by atomic absorption spectroscopy. Similarly, the Ca and Mg content of analytical reagent grade $NaNO_3$ and $NaClO_4$ were determined and found to contain 1.4 and 3.0 ppm Ca and 0.2 and 0.2 ppm Mg respectively, by weight. Motor oil, to which Ba-stearate is typically added, was digested in Ultrex HNO_3, and analysis of the neutral aqueous extract indicated the presence of 0.3 ppm Ca and 10 ppb Mg by weight.

The fact that the fluoresence of dilute solutions of Sr- or Ba-HQS chelates increase upon addition of a small amount of EDTA (17) led us to explore the fluorescence quenching of the HQS complexes of these metals in greater detail. It was found that among divalent cobalt, copper, manganese, and nickel and trivalent iron and chromium, Ni(II) and especially Fe(III), are

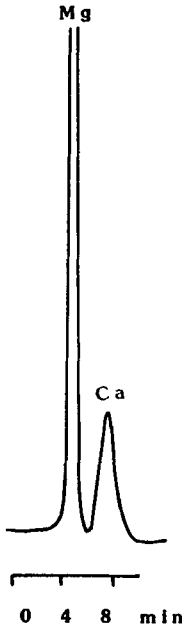

Figure 7. Mg and Ca in 10 fold diluted water. Wescan HS column (100 X 4.2 mm) 2 mm HQS, 10 mM Tris, pH 8.0, 150 mM KNO_3, 1 mL/min, 550 V, 1.0 μA FS.

by far the most powerful quenchers, their quenching ability is easily detectable at 0.1 μM concentration. Furthermore the susceptibility to quenching decreases in the order Ba>Sr>>Ca>Mg, making sensitive detection of the heavier metals difficult.

Because EDTA is effective in (at least partial) removal of the quencher ions, experiments were conducted with a small concentration of EDTA incorporated in the eluent. While the presence of EDTA is expected to increase iron leaching from the

system, we reasoned that this is not likely to be stoichiometric and an overall beneficial effect might be obtained. This proved to be the case. Signals for Ca and Sr were significantly enhanced, as shown in Figure 8. However, it was not possible to detect Ba in less than 1 μmol amounts of any of the above systems.

Postcolumn Reaction Mode. Despite the additional complexity introduced into the system by incorporating a postcolumn reactor, the separation scheme itself is obviously facilitated because one does not have to deal with the adsorption of HQS on the column. Further, the occurrence of matrix dip/peaks are greatly reduced as well. Since a large variety of techniques to accomplish the separation of metal ions on a number of different stationary phases have already been developed (see for example citations in ref. 8), we demonstrate here two principal types of applications of HQS as a postcolumn reagent. For metals that form fluorescent HQS-chelates (see ref. 17 for a complete list) the obvious detection mode is to introduce a buffered solution of HQS postcolumn. Since most complexation reactions are virtually instanteous, the present case being no exception, no additional delay line (other than the transit tube from the postcolumn reactor (PCR) to the detector) is needed. Because of the quenching problems involved with iron leaching from stainless steel components, non-metallic PCR components need to be used; the pressurized porous membrane reactor developed in this

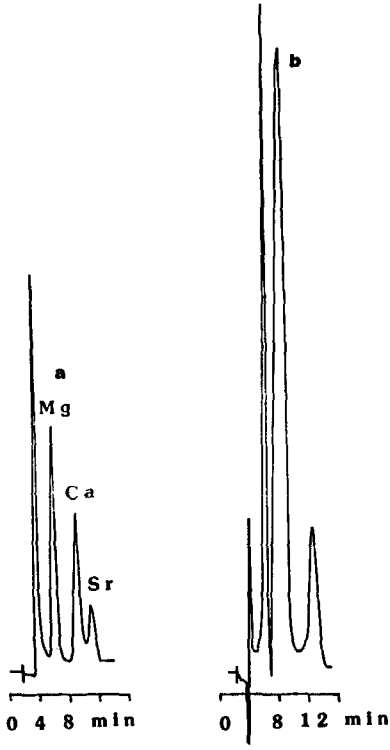

Figure 8. 40 pmole Mg, 1 nmole Ca, 10 nmole Sr. All other metals present as impurities elute as the first peak. Wescan HS column (150 X 4.2 mm). PMT 535 V. (a) 1 mM MOPS., pH 7.0, 300 mM KNO_3, 0.5 mL/min, 10 nA FS (b) as in a except 275 mM KNO_3, 3 μM Na_2EDTA, 20 nA FS.

laboratory (18) provided an ideal solution. Other caveats that are applicable to the present system are: (a) buffering of the HQS reagent in the PCR must be done with noncomplexing agents and the reagent pH chosen such that the PCR effluent pH permits optimal fluorescence of the metal-HQS chelates of interest (see ref. 17 for the optimal pH values for the various metals); (b) If a complexing ligand is used in the eluent, its chelating ability must be modest compared to that of HQS. In many cases, we have found citrate-based eluents to be particularly useful; iron leaching from system components with deoxygenated solutions of citrate is also minimal.

Excellent sensitivities can be obtained in this mode as shown in Figure 9. The first peak is associated with the sample pH being different from that of the eluent and is observed only at high sensitivities. Based on a S/N of 3, 75 fmole of Zn could be detected.

A second application of PCR based detection involving HQS pertains to the detection of metals that quench the fluorescence of other metal-HQS chelates (for a complete listing, see ref. 17). Since we have found the Al-HQS chelate to be particularly susceptible to such quenching, especially when sensitized by the surfactant cation hexadecyltrimethylammonium, HTA^+ (25), a suitable postcolumn reagent for such applications is an appropriately buffered solution of the Al-HQS chelate sensitized by HTA-chloride micelles. An example chromatogram is shown in Figure 10.

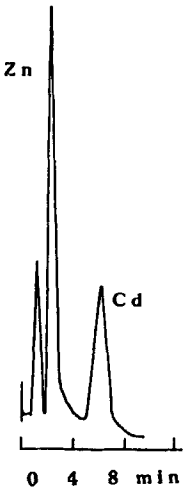

Figure 9. 1 pmole Zn, 2 pmole Cd, Wescan HS column (100 X 4.2 mm) 0.03 M K-citrate, pH 3.4, 0.5 mL/min. PCR: 1 mM HQS buffered in 0.3 M BICINE, pH 9.2, 0.25 mL/min; effluent pH ~ 8. 675 V, 100 nA FS.

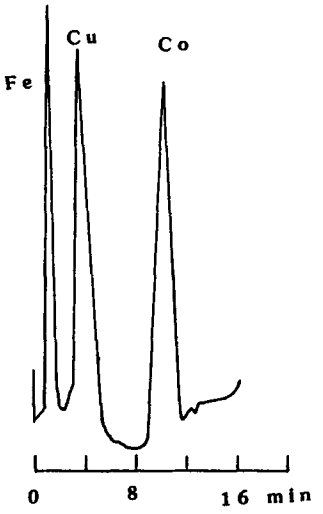

Figure 10. 4 nmole Fe, 2 nmole Cu, 1 nmole Co by quenching detection. Wescan HS column (150 X 4.2 nm), 0.2 M K-citrate 0.5 mL/min. PCR: 100 µM Al, 300 µM HQS, 500 µM HTA-chloride, 0.3 M MOPSO pH 7.9; effluent pH ~ 7. 610 V, 100 nA FS.

Micellar sensitization may also be performed with HQS-bearing eluents, by introducing a HTA-chloride solution through the PCR. Net gains in the analyte signals were observed for alkaline earth separations despite the dilution caused by the PCR; however, the gain in S/N was marginal.

CONCLUSIONS

The direct use of fluorogenic complexing ligands such as 8-hydroxyquinoline-5-sulfonic acid in the eluent opens a new avenue for trace and ultratrace determinations, especially with cation exchanger stationary phases. Subpicomole detection limits are attainable for a number of metals, including Cd, Mg and Zn. With the exception of Ba which responds very poorly, the approach may be of particular value for the separation and trace determination of alkaline earth metals.

The great ability of Fe and Ni, two principal components of stainless steel, to quench the fluorescence of other metal-HQS complexes presents a significant problem in obtaining reliable and reproducible responses at trace levels, because of inevitable leaching of low levels of ferrous metals from stainless steel chromatographic hardware. This study thus greatly accentuates the need for more extensive availability of nonmetallic chromatographic hardware and columns. Metal-leaching problems, however, can be minimized in PCR based detection systems with appropriately chosen eluents.

ACKNOWLEDGEMENTS

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