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Displacement post-column detection reagents based on the fluorescent magnesium 8-hydroxyquinoline-5-sulfonic acid complex

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Abstract

This non-specific fluorescent reagent is based on the displacement mechanism, wherein a metal ion displaces Mg^{2+} from the $Mg(CDTA)^{2-}$ complex (CDTA = cyclohexylenedinitrilotetraacetic acid). The liberated Mg^{2+} then reacts with 8-hydroxyquinoline-5-sulfonic acid (HQS) to form an intensely fluorescent complex. For simultaneous addition of $Mg(CDTA)^{2-}$ and HQS^{2-}, the detector response is governed by the stabilities of both the CDTA and the HQS complexes of Mg^{2+} and the displacing metal. Under these conditions, alkaline earth metals respond positively, but no response is observed for transition metals such as Cu^{2+}, Ni^{2+} and Co^{2+}. For the sequential addition of $Mg(CDTA)^{2-}$ followed by HQS^{2-}, the detector response is governed by the relative stabilities of only the CDTA complexes of Mg^{2+} and displacing metal, and positive signals are observed for Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+} and Co^{2+}. The response for Mn^{2+} was linear from 25 to 2500 picomoles. The reagent pH affects the background intensity and the displacement kinetics, and to a lesser extent the fluorescence intensity.

1. Introduction

The ability to detect trace and ultra-trace concentrations of metal ions is an integral part of modern society. Ion chromatography has been used for applications as diverse as analyzing silicon wafer contamination [1], monitoring nuclear fission [2] and determining trace metals in the environment [3]. All of these applications used the non-specific 4-(2-pyridylazo) resorcinol (PAR) reagent for post-column detection of the metal ions by absorbance. Fluorescence is an intrinsically more sensitive technique than absorbance. The reagent 8-hydroxyquinoline-5-sulfonic acid (HQS) has achieved picomolar detection limits for Zn and Cd [4], Al [5] and Mg [6]. Unfortunately, most other metal–HQS complexes are either weakly fluorescent or non-fluorescent [7], thus inhibiting wider use of this sensitive reagent. No alternative metalloluminescent reagent exists which will react to form fluorescent compounds with a wide range of metals, as is desirable in ion chromatography.

Recently, Williams and Barnett [8] introduced a non-specific post-column reagent for fluorescent detection of trace metals in ion chromatography. This system used the displacement of Mg^{2+} from a Mg–EDTA (ethylenediaminetetraacetic acid) complex by other metal ions.

$$M^{n+} + Mg(EDTA)^{2-} \rightleftharpoons Mg^{2+} + M(EDTA)^{(4-n)-} \quad (1)$$

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The liberated Mg^{2+} reacts with HQS to form a strongly fluorescent complex. The intensity of the resultant fluorescent is proportional to the molar concentration of the metal ion.

$$Mg^{2+} + 2HQS^{2-} \rightleftharpoons \underset{\text{(fluorescent)}}{Mg(HQS)_2^{2-}} \quad (2)$$

Using this detection scheme, Williams and Barnett [8] achieved detection limits of 33 to 140 picomoles for a variety of metals. However, no discussion of the effect of experimental factors such as pH and order of reagent addition was given. Recent studies performed in our laboratory on the Zn–EDTA–PAR absorbance reagent have demonstrated that the kinetics and equilibrium of displacement reaction systems can be complex [9], and so are not easily optimized. This paper investigates the chemistry of HQS-based displacement post-column reagents so as to identify the key variables in the optimization of these reagents.

2. Experimental

2.1 Apparatus

The HPLC system consisted of a Waters metal-free solvent delivery system (Model 625, Waters Associates, Milford, MA, USA), a Rheodyne sampling valve (Model 9125, Rheodyne, Berkeley, CA, USA) fitted with a 50-μl loop and a fluorimetric detector (Model 470, Waters; excitation λ, 360 nm; emission λ, 500 nm). Chromatograms were digitized with a CHROM-1AT (Keithley MetraByte, Taunton, MA, USA) data acquisition board enhanced for faster data acquisition, and analyzed using LabCalc (Galactic, Salem NH, USA) on a 286-based microcomputer. In most experiments no column was present in the HPLC system, *i.e.*, the experiments were run in a flow injection analysis mode. When present the analytical column was a 150 mm × 3.9 mm I.D. Delta Pak C-18 (300 Å; 5 μm; Waters).

The post-column reagent was delivered by constant pressure pumping through application of nitrogen pressure (38 p.s.i.; 1 p.s.i. = 6894.76 Pa) to a multi-reagent cylinder [10,11] fitted with a six-port low-pressure switching valve (Model 5011, Rheodyne) to allow selection between up to six reagents and a three-way valve (Model 5031, Rheodyne) on an additional port on the cylinder to provide simultaneous flow of a second reagent. Low pressure tubing and fittings (Alltech) connected the post-column reagent delivery system to the effluent stream. Two configurations were used for addition of the post-column reagent. In configuration I, the carrier stream from the HPLC pump (0.5 ml/min) merged with the Mg–CDTA–HQS reagent (CDTA = cyclohexylenedinitrilotetraacetic acid) (0.4–0.5 ml/min, measured for each experiment) at the mixing tee (316 stainless steel, 90° ports), flowed through reaction coil 1, 510 cm of tightly spiraled 0.50 mm I.D. knitted PTFE (Teflon) tubing (RXN 1000 Coil, Waters; 1000 ml volume) and then directly to the detector. In configuration II, reaction coil 1 was as before and reaction coil 2 was either a 100 cm of 1.0 mm I.D. Teflon tubing or 48 cm of 0.25 mm I.D. polyether ether ketone (PEEK) tubing. Using this configuration, Mg–CDTA was added via the first mixing tee and HQS was added at the second tee.

Stopped-flow kinetic measurements were made using the instrument in configuration II. The HPLC pump provided a constant flow of $5 \cdot 10^{-5}$ *M* M^{2+} which was mixed sequentially with unbuffered 1 m*M* MgCDTA (pH ~ 6) and then 1 m*M* HQS in pH 8.0 bicine (0.6 *M*) buffer. The flow was stopped and the $Mg(HQS)_2^{2-}$ fluorescence was recorded *versus* time.

2.2 Reagents and standards

All reagent solutions were prepared using deionized water (Milli-Q Ultra Pure Water System, Millipore). Analytical grade reagents were used throughout. Post-column reagent solutions were prepared by dissolving HQS (Janssen Chimica) in the bicine (Sigma) buffer (0.3–0.6 *M*), adjusting the pH, purifying with a 3 cm × 1.5 cm I.D. column of Chelex 100 (149–297 μm sodium form, Bio-Rad), and adding an aliquot of $1.0 \cdot 10^{-3}$ *M* Mg–CDTA or Mg–EDTA stock

solution. The Mg–CDTA and Mg–EDTA stock solution were prepared by combining Mg^{2+} and the aminopolycarboxylate such that excess Mg^{2+} was present, and then passing the solution through a 3 cm × 1.5 cm I.D. column of Chelex 100 to remove the excess Mg^{2+}.

Stock metal solutions were prepared using analytical grade salts. Lower concentrations were then achieved by dilution in volumetric Nalgeneware.

3. Results and discussion

The reagent 8-hydroxyquinoline-5-sulfonic acid (HQS) and its parent compound (8-hydroxyquinoline; oxine) form stable complexes with a wide range of metals [12], but only a few of these complexes fluoresce significantly [7,13,14]. The differences in fluorescence observed for the various metals is due to the interaction of the ligand electrons involved in the fluorescence with the electron field of the metal [13]. Thus the HQS complexes that fluoresce most readily are those derived from cations with the most stable electron configurations, *i.e.*, either completely full or empty electron shells. Such metals include Cd^{2+}, Zn^{2+}, Mg^{2+} and La^{3+}. Magnesium is used in the displacement post-column reagent discussed herein, because it forms only a weak complex with aminopolycarboxylate ligands and will be displaced by the widest range of metals, thereby providing the most non-specific post-column detection.

3.1 Background signal from reagent

Only the 1:2 metal–HQS complex fluoresces [15]. The free reagent and the 1:1 complex do not contribute to the observed fluorescence. Thus the reagent background results solely from the residual $Mg(HQS)_2^{2-}$ present in the reagent solution. The background is then governed by the relative stability of the aminopolycarboxylate (L) and HQS complexes with Mg^{2+}:

$$Mg(HQS)_2^{2-} + L^{4-} \rightleftharpoons 2HQS^{2-} + MgL^{2-} \qquad (3)$$

The equilibrium constant governing the intensity of the background fluorescence, is thus:

$$K'_{background} = \frac{\alpha_{Mg^{2+}}\alpha_{L^{4-}}K_{MgL^{2-}}}{\alpha_{Mg^{2+}}(\alpha_{HQS^{2-}})^2\beta_{2,Mg(HQS)_2^{2-}}} = \frac{\alpha_{L^{4-}}K_{MgL^{2-}}}{(\alpha_{HQS^{2-}})^2\beta_{2,Mg(HQS)_2^{2-}}} \qquad (4)$$

That is, the intensity of the background will be a function of the stability constants of the magnesium–aminopolycarboxylate complex and the magnesium–HQS complex, the fractional composition of the ligands at the reagent pH, and the total concentration of HQS.

In their work, Williams and Barnett used EDTA as the complexing agent [8]. CDTA is an analog of EDTA, which, due to its greater structural rigidity, forms metal ion complexes that are ten to a thousand times more stable than those of EDTA [16]. Table 1 shows the relative background fluorescence observed for $Mg(EDTA)^{2-}$ and $Mg(CDTA)^{2-}$, and indicates that a lower background is achieved using CDTA, as expected based on literature stability constants [12,17] CDTA is therefore used in all further studies.

3.2 Signal from reagent

Table 2 shows that optimum sensitivity for $Mg(HQS)_2^{2-}$ is achieved at a pH from 8 to 11. This is in agreement with the results of Bishop [15], who observed that $Mg(HQS)_2^{2-}$ fluorescence efficiency increased to a maximum by pH 7 and decreased at higher pH values presumably due to the formation of either hydroxyl complexes or the hydroxide. Soroka *et al.* [7] also observed such behavior, although they reported a much narrower optimum pH range than evident in Table 2. A pH of 8.0 was used in all further experiments except those explicitly studying the effect of pH.

3.3 Simultaneous addition of Mg–CDTA and HQS

The maximum sensitivity that is achievable for any given metal with the Mg–CDTA–HQS re-

Table 1
Comparison of the background intensity for Mg–CDTA–HQS and Mg-EDTA-HQS post-column fluorescent reagents

pH	Background intensity[a]		Relative background Mg–CDTA/Mg–EDTA
	Mg–CDTA	Mg–EDTA	
8.0	0.201	0.530	0.38
9.1	0.127	0.352	0.36
10.0	0.070	0.196	0.36
10.75	0.043	0.149	0.29
12.1	0.025	0.178	0.14
12.8	0.024	0.246	0.098

Conditions: mode, flow injection analysis in configuration I; reaction coil 1, 510 cm of 0.5 mm I.D. tubing; carrier, 0.5 ml/min distilled water; reagent, 0.4 ml/min of 1 m*M* HQS with 0.3 *M* bicine adjusted to the appropriate pH; injection, 50 μl of 1 m*M* Mg–CDTA or Mg–EDTA.
[a] Background intensity is defined as the peak area observed for duplicate injections of Mg–CDTA and Mg–EDTA.

agent is that observed for Mg^{2+}. That is, maximum sensitivity is achieved when each metal ion displaces one Mg^{2+} from its CDTA complex. Calibration curves were determined for the injection of 500 to 5000 picomoles of a variety of metals. All of these plots were linear with correlation coefficients (r^2) greater than 0.99. Table 3 presents the results observed for a number of metal ions using simultaneous addition of $Mg(CDTA)^{2-}$ and HQS^{2-}. The sensitivity observed for magnesium differs from that in Table 2 due to variation in the reagent flow.

For the alkaline earth metals, the sensitivity of the Mg–CDTA–HQS reagent decreased with increasing atomic mass. Large alkaline earth metal ions do not form stable HQS complexes. As a result the overall equilibrium and associ-

Table 2
Effect of pH on the fluorescence intensity of the Mg-HQS complex

pH	Sensitivity [a]	Intercept
8.0	1.947 ± 0.001	−0.0042 ± 0.0005
9.1	2.00 ± 0.01	0.0038 ± 0.0027
10.0	1.893 ± 0.001	−0.0056 ± 0.0001
10.75	1.851 ± 0.002	−0.007 ± 0.001
12.1	1.335 ± 0.004	−0.005 ± 0.001
12.8	0.59 ± 0.02	+0.002 ± 0.005
13.1	0.24 ± 0.03	+0.008 ± 0.009

Conditions: mode, flow injection analysis in configuration I; reaction coil 1, 510 cm of 0.5 mm I.D. tubing; carrier, 0.5 ml/min distilled water; reagent, 0.5 ml/min of 1 m*M* HQS in 0.3 *M* bicine adjusted to the indicated pH; injection, 50 μl of four Mg^{2+} standards ranging from 10–100 μ*M* (500–5000 picomoles).
[a] Sensitivity is defined as the slope of the plot of peak area *versus* the picomoles of Mg^{2+} injected. The values have been multiplied by $1 \cdot 10^4$.

Table 3
Sensitivity of metal ions upon simultaneous addition of Mg-CDTA-HQS

Metal ion	Sensitivity[a]	Intercept	Relative sensitivity	$K'_{detection}$
Mg^{2+}	1.25	−0.0406	1.00	$10^{6.0}$
Ca^{2+}	0.75	+0.0124	0.60	$10^{5.6}$
Sr^{2+}	0.36	+0.0076	0.29	$10^{6.2}$
Ba^{2+}	0.02	+0.00048	0.01_9	$10^{4.0}$
Mn^{2+}	0.80	+0.00834	0.64	$10^{5.3}$
Hg^{2+}	0.15	+0.0059	0.12	–
Co^{2+}	nd [b]	–	–	$10^{1.1}$
Ni^{2+}	nd	–	–	$10^{-2.2}$
Cu^{2+}	nd	–	–	$10^{-3.4}$

Conditions: mode, flow injection analysis in configuration I; reaction coil 1, 510 cm of 0.5 mm I.D. tubing; carrier flow, 0.5 ml/min distilled water; reagent flow, 0.4 ml/min of 1 m*M* Mg–CDTA–HQS in 0.6 *M* bicine (pH 8.0).
[a] Sensitivity is defined as the slope of the plot of peak area versus the picomoles of Mg^{2+} injected. The values have been multiplied by 1×10^4.
[b] nd = Not detected.

ated constant for detection of alkaline earth metals are:

$$M^{2+} + 2HQS^{2-} + Mg(CDTA)^{2-} \rightleftharpoons Mg(HQS)_2^{2-} + M(CDTA)^{2-} \quad (5)$$

$$K'_{detection} = \frac{\beta'_{2,Mg(HQS)_2^{2-}} K'_{f,M(CDTA)^{2-}}}{K'_{f,Mg(CDTA)^{2-}}} = \alpha_M \alpha^2_{HQS^{2-}} \frac{\beta_{2,Mg(HQS)_2^{2-}} K_{f,M(CDTA)^{2-}}}{K_{f,Mg(CDTA)^{2-}}} \quad (6)$$

This situation is analogous to that observed for the determination of alkaline earth metals using the Zn–EDTA–PAR displacement reaction [9]. The overall detection efficiency for alkaline earth metals will be dependent on the fraction of the alkaline earth metal in the free form and on the fraction of HQS that is fully ionized. However, when these variables are maintained constant as in Table 3, $K'_{detection}$, and thus the detection sensitivity, decreases as the stability of the alkaline earth–CDTA complex decreases down the column of the periodic table.

For the divalent transition metals a different behavior was observed. Metals such as Mn^{2+} and Hg^{2+} responded to the Mg–CDTA–HQS reagent, whereas many other metals which form stronger CDTA complexes than Mg^{2+} showed no positive response. This difference in behavior results because the transition metals form stable complexes with HQS [12]. Under these conditions the free metal, M, is rapidly complexed by HQS, and thus the detection equilibrium is:

$$M(HQS)_2^{2-} + Mg(CDTA)^{2-} \rightleftharpoons Mg(HQS)_2^{2-} + M(CDTA)^{2-} \quad (7)$$

The equilibrium constant governing the detection of divalent transition metals is thus:

$$K'_{detection} = \frac{K_{f,M(CDTA)^{2-}} \beta_{2,Mg(HQS)_2^{2-}}}{K_{f,Mg(CDTA)^{2-}} \beta_{2,M(HQS)_2^{2-}}} \quad (8)$$

The fraction composition (α) of each ligand and metal appears in both the numerator and denominator, and so cancels out. Thus pH will not affect the degree to which a metal ion displaces Mg^{2+}, and so should have no thermodynamic effect on the observed detection sensitivities.

For divalent transition metals, the differing detection equilibrium (eqn. 7) results in a lower $K'_{detection}$ being required for detection than is the case for the alkaline earth metals. Mn^{2+} forms a HQS complex of greater stability than that of Mg^{2+}, but is nevertheless detected by the Mg–CDTA–HQS reagent as a result of the driving force provided by the stability of the $Mn(CDTA)^{2-}$ complex. Mercury responds weakly to the reagent. Unfortunately no stability constant data is available for comparison. Co^{2+} was predicted to respond weakly to the reagent ($K'_{detection} = 10^{1.1}$), whereas no signal was detectable. No response was observed for Ni^{2+} and Cu^{2+}, as was predicted (*i.e.*, $K'_{detection} < 1$) using the literature stability constants. Thus overall the agreement between the response predicted by eqn. 8 and that observed (Table 3) indicates that eqns. 7 and 8 govern detection for transition metal ions using simultaneous addition of $Mg(CDTA)^{2-}$ and HQS^{2-}.

The fluorescence intensity of the $Mg(HQS)_2^{2-}$ complex and the $K'_{detection}$ for divalent transition metals are independent of pH (Table 2 and eqn. 8, respectively). Therefore the sensitivity of the reagent for divalent transition metals should be independent of pH. Nevertheless, the sensitivity for Mn^{2+} was much greater at pH 8.0 [$(7.8 \pm 0.4) \cdot 10^{-5}$] than at pH 10.0 [$(1.27 \pm 0.04) \cdot 10^{-5}$]. This behavior results from the slow displacement of Mg^{2+} from its CDTA complex. The increased sensitivity upon lowering the pH results from acid catalyzed dissociation of the aminopolycarboxylate from the metal ion. This behavior is discussed further below. At pH 8.0, the response of the Mg–CDTA–HQS reagent to Mn^{2+} was linear from 25 to 2500 picomoles ($r^2 = 0.996$) with a slope of $(1.07 \pm 0.03) \cdot 10^{-4}$ and an intercept of 0.003 ± 0.007. Below 25 picomoles, standards were equivalent to the blank.

3.4 Sequential addition of Mg–CDTA and HQS

As discussed above, simultaneous addition of Mg–CDTA and HQS did not yield a positive

response for many of the metals that are of interest in ion chromatography. Williams and Barnett [8] added Mg–CDTA to the column eluent, allowed the mixture to react in a 50 cm × 0.8 mm I.D. reaction coil, and only then added the HQS. Using this order of reagent addition they observed a response for a much wider range of metals than was observed in Table 3.

Sequential addition of $Mg(CDTA)^{2-}$ and then HQS^{2-} performed herein also resulted in positive responses from a wide range of metals, as shown in Table 4. For the sequential addition of $Mg(CDTA)^{2-}$ and HQS^{2-}, the equilibrium established in the first reaction coil (Rxn Coil 1) is:

$$M^{2+} + Mg(CDTA)^{2-} \rightleftharpoons M(CDTA)^{2-} + Mg^{2+} \quad (9)$$

$$K'_{\text{detection}} = \frac{K_{f,M(CDTA)^{2-}}}{K_{f,Mg(CDTA)^{2-}}} \quad (10)$$

Metal ions displace Mg^{2+} based on the relative complex stability of the CDTA complexes. Thus as almost all metal ions form stronger CDTA complexes, Mg^{2+} is displaced by most metals. Upon the addition of HQS^{2-}, the Mg^{2+} is rapidly complexed to form the fluorescent $Mg(HQS)_2^{2-}$. Experiments performed herein indicate that even at the shortest reaction time studied (1.6 s) the reaction between Mg^{2+} and HQS^{2-} had proceeded to completion. Indeed, reaction half-lives on the order of a few milliseconds have been observed for the reaction between Mg^{2+} and 8-hydroxyquinoline (oxine) using relaxation kinetics techniques [18].

Thus, upon the addition of HQS^{2-} the operative equilibrium is that given by eqn. 7. For metals such as Cu^{2+}, Ni^{2+} and Co^{2+} this equilibrium lies far to the left [*i.e.*, $K'_{\text{detection}} < 1$ (eqn. 8)], and so thermodynamically no response would be expected, as had been observed above for the simultaneous addition of reagent. However, the $M(CDTA)^{2-}$ complex must dissociate in order for this equilibrium to be achieved. Given the high stability of this complex and the steric rigidity of CDTA, this process would be expected to be slow. Stopped-flow kinetic measurements of mixtures of $M(CDTA)^{2-}$ and $Mg(HQS)_2^{2-}$ revealed that the $Mg(HQS)_2^{2-}$ fluorescence decreases exponentially ($r > 0.99$) at a very slow rate; half-lives of 530, 16 000 and 24 000 s for Cu^{2+}, Co^{2+} and Ni^{2+}, respectively. Thus the use of a short reaction coil does not allow sufficient time for the mixed reagent to relax, and so the observed response will obey

Table 4
Response of metal ions upon sequential addition of Mg–CDTA and HQS

Metal ion	Sensitivity[a]	Intercept	Relative sensitivity	$K_{f,M\text{-}CDTA}/K_{f,Mg\text{-}CDTA}$
Mg^{2+}	0.95	−0.0167	1.00	$10^{0.0}$
Ca^{2+}	0.79	−0.0146	0.83	$10^{-0.1}$
Sr^{2+}	0.55	+0.0117	0.58	$10^{-0.5}$
Ba^{2+}	0.089	+0.00056	0.09	$10^{-2.5}$
Mn^{2+}	1.06	−0.0196	0.64	$10^{6.4}$
Hg^{2+}	nd[b]	–	0.00	$10^{13.7}$
Cu^{2+}	0.70	−0.0172	0.73	$10^{10.8}$
Ni^{2+}	0.75	−0.0154	0.79	$10^{9.1}$
Co^{2+}	0.78	−0.0151	0.82	$10^{8.5}$

Conditions: mode, flow injection analysis in configuration II; reaction coil 1, 510 cm of 0.5 mm I.D. tubing; reaction coil 2, 50 cm of 0.25 mm I.D. tubing; carrier, 0.5 ml/min distilled water; Mg–CDTA, 0.4 ml/min of 1 m*M* unbuffered (pH ~ 6); HQS, 0.5 ml/min of 1 m*M* HQS in 0.3 *M* bicine (pH 8.0).

[a] Sensitivity is defined as the slope of the plot of peak area *versus* the picomoles of metal ion injected. The values have been multiplied by $1 \cdot 10^4$.

[b] nd = Not detected.

eqns. 9 and 10. Additional stopped-flow measurements of $M(EDTA)^{2-}$ complexes indicate that the dissociation rate of the $M(EDTA)^{2-}$ complex of most metals is sufficiently slow that similar behavior will be observed. A notable exception is Cu^{2+} for which we observed a dissociation half-life of 20 s for the EDTA complex. Therefore if EDTA were used rather than CDTA, it is important that reaction coil 2 be maintained short or else reduced sensitivities will be observed for kinetically labile metals such as Cu^{2+}.

In Table 4 the characteristics of calibration curves run using sequential addition of $Mg(CDTA)^{2-}$ and HQS^{2-} are reported for a number of metals. The sensitivity observed for the alkaline earth metals decreases with increasing atomic mass, as would be predicted by eqn. 10 based on the decreasing stability of the $M(CDTA)^{2-}$ complex down the group. Likewise a strong response is observed for Mn^{2+}, Cu^{2+}, Ni^{2+} and Co^{2+}, as predicted by eqn. 10. Only Hg^{2+}, which was predicted to respond strongly to the reagent, did not display the expected behavior. Further studies are being conducted to determine the cause of this anomaly. Nevertheless, the results shown in Table 4 indicate that the sensitivity of the Mg–CDTA–HQS reagent will depend on the relative stabilities of the CDTA complexes when the reagent components are added sequentially.

During the studies of simultaneous addition of $Mg(CDTA)^{2-}$ and HQS^{2-}, the kinetics of the displacement reaction, and thus the sensitivity, improved upon decreasing the pH of the reagent from 10 to 8. Unfortunately with simultaneous reagent addition, it was not possible to lower the pH further without decreasing the intensity of the Mg–HQS fluorescence [7,15]. With sequential addition, it is possible to adjust the pH of the Mg–CDTA reagent to optimize the displacement reaction kinetics and to adjust the pH of the HQS reagent to optimize the fluorescence response. Fig. 1 shows the detector response (relative to that for Mg^{2+}) at various $Mg(CDTA)^{2-}$ reagent pH values for a number of metals. The detection sensitivity of all of the metals passes through an optimum. Acid catalyzed decomplexation of the $Mg(CDTA)^{2-}$ complex results in enhancement of the sensitivity transition metals and calcium as the pH decreases from 10 to 6. However, further decreases in the pH result in reduced sensitivity, since not only is the $Mg(CDTA)^{2-}$ complex is weakened by the high H^+ concentration, but so is the stability of the metal CDTA complex. That is, while the Mg^{2+} is rapidly released by the CDTA, the other metals cannot form a stable complex with the CDTA. Thus for most metals the optimum pH is approximately 6.

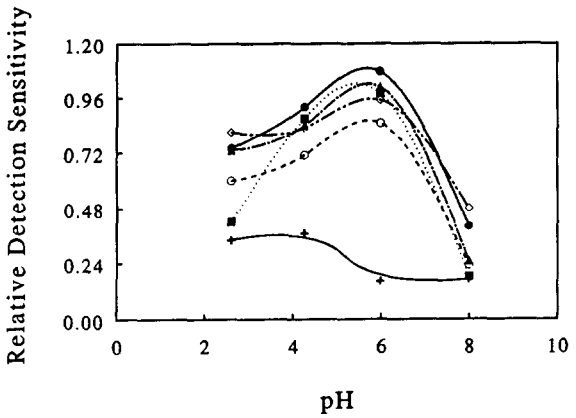

Fig. 1. Effect of the pH of the Mg–CDTA solution on the detection sensitivity for Cu^{2+}(◇), Mn^{2+} (●), Co^{2+} (▲), Ni^{2+} (■), Ca^{2+} (○) and Sr^{2+} (+). Experimental conditions: mode, flow injection analysis in configuration II; reaction coil 1, 510 cm of 0.5 mm I.D. tubing; reaction coil 2, 48 cm of 0.25 mm I.D. tubing; carrier flow, 0.5 ml min^{-1} of 0.01 *M* tartaric acid at various pH; Mg–CDTA reagent flow, 0.16 ml min^{-1} of 0.001 mol l^{-1} $Mg(CDTA)^{2-}$; HQS reagent, 0.22 ml min^{-1} of 0.001 mol l^{-1} HQS in 0.6 mol l^{-1} bicine buffer (pH 8.0); sensitivity, relative to that for Mg^{2+}.

Strontium is an exception to the above behavior. It displays optimal sensitivity between pH 2 and 4. Strontium forms a weaker complex with CDTA than Mg^{2+}, and so thermodynamically only a small portion of the Sr^{2+} would displace Mg^{2+}, as indicated by the low sensitivity observed in Table 3. Under acidic conditions, all of the Mg^{2+} and Sr^{2+} dissociates from the CDTA. When the pH is then increased by addition of the buffered HQS solution, CDTA will complex with the nearest metal ion. Thus the proportion of $Sr(CDTA)^{2-}$ formed depends primarily on the relative concentration of Sr^{2+} to

Mg^{2+}, although complexation kinetics can play a significant role as indicated by the low sensitivity for Ni^{2+} at pH 2.6. At higher pH values, the $Mg(CDTA)^{2-}$ is not fully dissociated, and so the sensitivity is dictated by the relative stabilities of the Mg^{2+} and Sr^{2+} complexes.

Fig. 2 shows the isocratic separation of transition metals and Fig. 3 shows a gradient separation of the lanthanide metals. Detection in both cases is by fluorescence after sequential addition of $Mg(CDTA)^{2-}$ and then HQS^{2-}. The sensitivity of the reagent is similar for most of the metals shown, and corresponds to a detection limit of approximately 5 ng injected (based on three times the baseline noise). These detection limits are comparable to those achievable with 4-(2-pyridylazo)resorcinol (PAR) using absorbance detection [19] and Mg–EDTA–HQS using fluorescence detection [8]. Decreasing the reagent concentration would further improve the detection limit by decreasing the intensity of the background. An enhanced sensitivity is expected for Zn^{2+} and Lu^{3+} as the HQS^{2-} complexes of these metals are strongly fluorescent, and so will contribute to the fluorescence from

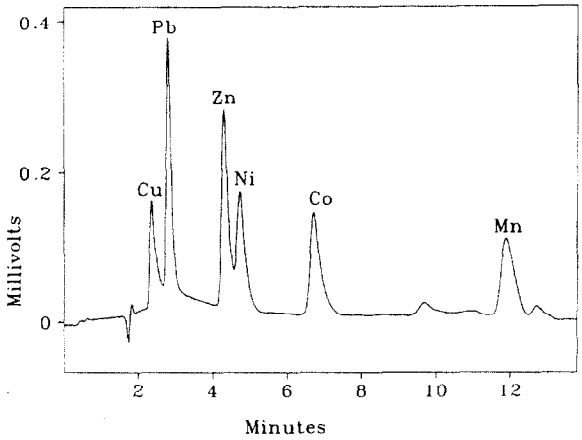

Fig. 2. Isocratic separation of transition metals. Column 5-μm Delta Pak C_{18}, 150 × 3.9 mm I.D.; eluent 0.05 mol l^{-1} tartarate (pH 3.4), 0.0024 mol l^{-1} $C_8SO_3^-$; flow-rate, 1.0 ml min^{-1}; injection, 50 μl of 3×10^{-5} mol l^{-1} each of Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+}; post-column reaction by sequential addition of 0.43 ml min^{-1} of 0.001 mol l^{-1} $Mg(CDTA)^{2-}$ followed by 0.43 ml min^{-1} of 0.001 mol l^{-1} HQS in 0.6 mol l^{-1} bicine (pH 12.17) (final solution pH 8.0); fluorescence excitation 360 nm, emission, 500 nm.

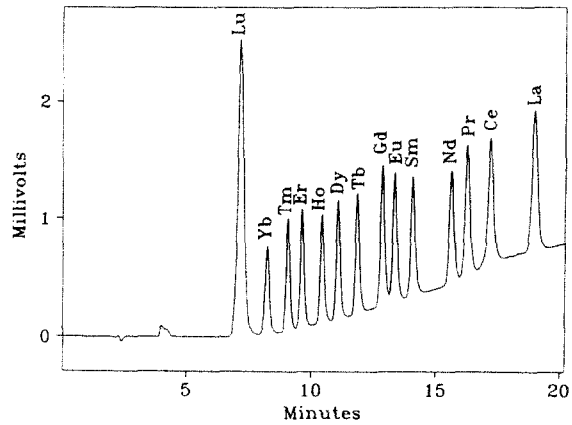

Fig. 3. Gradient separation of the lanthanide metals. Column 5-μm Delta Pak C_{18}, 150 × 3.9 mm I.D.; eluent 0.025 mol l^{-1} α-hydroxyisobutyric acid (HIBA) (pH 5.3) for 4 min then linearly increasing to 0.15 mol l^{-1} HIBA (pH 5.3) over 15 min, $[C_8SO_3^-]$ constant at 0.003 mol l^{-1}; flow-rate, 1.0 ml min^{-1}; injection, 50 μl of 7.5 mg l^{-1} each of Lu^{3+}, Yb^{3+}, Tm^{3+}, Er^{3+}, Ho^{3+}, Dy^{3+}, Tb^{3+}, Gd^{3+}, Eu^{3+}, Sm^{3+}, Nd^{3+}, Pr^{3+}, Ce^{3+} and La^{3+}; post-column reaction by sequential addition of 0.14 ml min^{-1} of 0.001 mol l^{-1} $Mg(CDTA)^{2-}$ in 0.25 mol l^{-1} MOPSO (pH 6.3) followed by 0.14 ml min^{-1} of 0.001 mol l^{-1} HQS in 0.6 mol l^{-1} 2-amino-2-methyl-1-propanol (AMP) (pH 12.17) (final solution pH 8.0); fluorescence excitation 360 nm, emission, 500 nm.

$Mg(HQS)_2^{2-}$. Such behavior has previously been observed in a similar detection system [8]. However, the Lu^{3+} sensitivity exceeds that predicted from the individual fluorescence contributions. The cause of this anomalous behavior is under further investigation.

4. Conclusions

A non-specific fluorescent reagent suitable for post-column reaction detection of metal ions is obtained from 8-hydroxyquinoline-5-sulfonic acid (HQS) by using the displacement mechanism. However, the system behavior is complex due to the presence of multiple interdependent equilibria, such as observed previously for the Zn–EDTA–PAR displacement reagent [9]. For the Mg–CDTA–HQS reagent, the governing equilibria are the complex formation of the Mg^{2+} and displacing metal ion with both $CDTA^{4-}$ and HQS^{2-}. Thus for simultaneous addition of

$Mg(CDTA)^{2-}$ and HQS^{2-}, the sensitivity is determined solely by the equilibrium between the various chelates.

In addition to the equilibrium effects, the displacement kinetics can also play an important role in the sensitivities observed for Mg–CDTA–HQS. Upon sequential addition of $Mg(CDTA)^{2-}$ and HQS^{2-} the slow decomplexation kinetics of the metal–CDTA complex precludes involvement of the HQS^{2-} complexes in the equilibrium governing detection, and thus the sensitivity is dependent solely upon the relative stabilities of the CDTA complexes with Mg^{2+} and displacing metal ion. Since Mg^{2+} forms a weaker CDTA complex than most other metal ions, sequential addition of reagent yields the desired non-specific fluorescent reagent.

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