Fluorescence Properties of Metal Complexes of 8-Hydroxyquinoline-5-sulfonic Acid and Chromatographic Applications

Krystyna Soroka, Rathnapala S. Vithanage, Denise A. Phillips, Brian Walker, and Purnendu K. Dasgupta*

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409-4260

Seventy-eight metal species are examined for fluorescence properties of their chelates with 8-hydroxyquinoline-5-sulfonic acid (HQS); 42 of these fluoresce, many intensely. The optimum pH, determined by ligand ionization vs. hydroxo complex formation, lies between 5 and 8. Cadmium forms the most fluorescent complex in a purely aqueous solution. Fluorescence is enhanced for many metals in surfactant (hexadecyltrimethylammonium ion, HTA^+) containing media and in a water:dimethylformamide solvent. A number of metal ions quench the fluorescence of other metal–HQS chelates, Fe(III) being by far the most effective, and such quenching is accentuated in media containing HTA^+. The fluorescence properties can be exploited by introducing the ligand through a postcolumn reactor or by incorporating it in the eluent in a chromatographic system. Subpicomole detection limits are attainable for Cd, Mg, and Zn.

Oxine (8-hydroxyquinoline, HQ) and its derivatives occupy a uniquely important place in analytical chemistry, perhaps second only to EDTA and its analogues. Indeed, few analytical reagents have merited multivolume monographs solely devoted to them (*1*) and new applications of oxine and its derivatives in modern analytical chemistry are continually being developed (*2, 3*). Of special interest to us is the intense fluorescence exhibited by many metal complexes of oxine and its derivatives which are themselves nonfluorescent except in concentrated sulfuric and perchloric acids or anhydrous fluoroalcohols (*4, 5*). In a series of papers Rogers and co-workers (*4, 6–8*) and Stevens (*9*) have given an excellent account of the fluorescence or the lack thereof of oxine and its metal complexes and the effects of substituents and solvent media upon such fluorescence.

A comprehensive review of fluorometric methods for the determination of inorganic species containing more than 1400 literature citations has appeared (*10*). For obvious reasons, essentially all previous efforts have been directed to developing specific reagents (and conditions) for the determination of given analytes. However, a generally applicable fluorogenic complexing agent which undergoes a nonfluorescent → fluorescent transition upon complexation with a large number of metal ions (i.e., a nonspecific fluorogenic reagent) will be of particular utility in ultratrace metal ion chromatography, of interest to us. In this regard, oxine and its derivatives are unparalleled in the large number of fluorescent metal complexes formed. Unfortunately, oxine and most of its analogues form complexes that are insoluble in water. Although it is possible to carry out metal ion chromatography involving oxine chelates with hydroorganic (*11, 12*) or micellar (*13*) solvents, it would be desirable to conduct such separations with purely aqueous eluents. From this standpoint, 8-hydroxyquinoline-5-sulfonic acid (HQS) is the ligand of choice—the complexation properties are analogous to those of HQ with essentially the same complexation constants (*14, 15*) but with greatly enhanced aqueous solubility (*16, 17*), sufficient to preclude problems arising from precipitation, at least in neutral and alkaline solutions (*18*).

While there is a substantial amount of literature on the metal complexes of HQS, these largely do not deal with the fluorescence properties. Fluorescence properties of metal–HQS chelates have been exploited in a pioneering work in paper chromatography by Feigl and Heisig (*19*) and later in a series of 10 papers by Bishop, involving general studies on complexation or titrimetric applications; the last of these papers appeared in 1976 (*20*). The fluorescent indicator properties have been used by others as well (*21, 22*). Fluorometric determination procedures have been described for Mg (*23–26*) and Cd (*27*). A kinetic fluorometric procedure was developed for the determination of Al at low pH (*28*) and several oxidizing agents (Ag^+/persulfate, MnO_4^- and Ce(IV) have been determined by their ability to oxidize HQS into an unidentified fluorescent product (*29–31*). The difference in fluorescence lifetimes has been exploited for the individual determination of six group II and group III (group 2 and group 3 in 1985 notation) metals from a mixture (*32*). The enhancement of the metal–HQS fluorescence by a cationic micelle, especially hexadecyltrimethylammonium ion, has been reported by a number of workers (*33–39*). A fiber-optic probe designed to sense several metals based on the fluorescence induced on immobilized HQS has recently been described (*40*).

However, there is a remarkable lack of quantitative data on the relative fluorescence intensities of the various metal chelates in aqueous or mixed aqueous solution. In the few cases where more than one metal has been studied, the reported orders of fluorescence intensity vary (see, for example, ref 6 and 41). For cases where the reports are less quantitative in nature, the observations are even diametrically opposite as to whether a certain metal–HQS chelate fluoresces or not (see, for example, ref 27 and 42). In order to fully assess the potential of HQS as a fluorogenic reagent in metal ion chromatography, we have carried out a systematic investigation of the fluorescence properties of HQS chelates of metal ions across the periodic table up to uranium, in regard to optimal excitation and emission wavelengths, pH, and oxidation state, and have also studied the enhancement of fluorescence intensity in micellar (hexadecyltrimethylammonium) and in water/*N,N*-dimethylformamide (DMF) solvents.

EXPERIMENTAL SECTION

All fluorescence measurements were conducted on a Perkin-Elmer LS-5 spectrofluorometer with both excitation and emission slits set at 10 nm and an integration time of 4 s (except in peak determining scans which employed a response time of 0.5 s). Measurements of pH were made with an Altex PHI 71 pH meter equipped with an Orion Ross combination electrode calibrated by the two point method.

8-Hydroxyquinoline-5-sulfonic acid (Aldrich) was twice recrystallized (as the monohydrate) from large volumes of hot water. The majority of the metal salts used were analytical reagent grade and were typically nitrates, sulfates, or chlorides. In a few cases

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as noted, fluoride complexes were used as well. The rare metal salts used were of the purest grade available; however, details of the extent and nature of any metallic impurities present were not available. While explicit attempts were not made to standardize the solutions of individual metal salts, it is unlikely that the worst case error in the target concentration is greater than 10%. For the majority of metals, this error is likely less than 1%. Hexadecyltrimethylammonium chloride (HTAC) was obtained as a 25% (w/v) concentrate (Fisher, HPLC grade). Dimethylformamide and other solvents used were reagent grade and KOH/HCl used for pH adjustment were of ultrapure quality. Water used in this work is distilled and then deionized; it meets all the specifications of ASTM type I reference reagent water, but no explicit efforts were made to determine the nature and concentrations of residual metal ions in the water.

The purity of reagents is particularly important in this work because some metals form very intensely fluorescent HQS chelates while some others appear to act as powerful quenchers (vide infra). Presence of these metals in the original reagents as impurities even in concentrations as low as 0.1% can completely nullify observations. For this reason, experiments were conducted in relatively dilute solutions, containing 20 μM of the metal ion. Initial experiments were conducted at a fixed pH of 9.05 ± 0.03 (unbuffered), because the published pK values of HQS indicate that the ligand is essentially completely ionized by this pH. To 50 mL of a 1 mM solution of HQS at pH 9.05, 200 μL of a 5 mM solution of the metal was added. The pH was readjusted to 9.05 by the addition of microaliquots of dilute KOH or HCl if it altered by more than 0.03 units. The optimum λ_{ex} and λ_{em} values cited pertain to this pH. The fluorescence intensity as a function of pH (integer pH units between 4 and 10) was determined in an analogous manner at the λ_{ex} and λ_{em} values found to be optimum for pH 9; in a few preliminary studies it was determined that the shifts of the optimal λ_{ex} and λ_{em} values as a function of pH are minimal. The addition of HTAC to an unbuffered HQS solution causes a drop in pH because of deprotonation of HQS to form the HTA^+–QS^- ion pair. In HTAC media, the maximal excitation and emission wavelengths are slightly red-shifted (~5 nm). The emission was measured at the optimum wavelengths after adjusting the pH to the specified optimum value. For water–DMF solvent studies, (10 – x) mL of DMF were added to x mL of the aqueous metal–HQS chelate (100 μM metal ion, 1 mM HQS, except for Cd for which a 10 μM concentration was used) prepared at the optimum pH for the fluorescence of the metal chelate. The fluorescence intensity was measured at the optimum wavelengths for the purely aqueous medium. Wavelengths of maximum emission in water/DMF media are slightly red-shifted (5–10 nm) compared to purely aqueous solutions; the optimal excitation wavelength does not shift noticeably. To calculate the enhancement factor, the dilution by DMF was taken into account.

In all cases, fluorescence intensities were also measured at least at one other metal concentration lower than the concentration specified above, to ensure that the operational range is in the linear domain of fluorescence intensity–concentration relationship. Except for cadmium as indicated, no significant departure from linearity was noted at 20 μM metal concentration in aqueous solutions; more dilute solutions were used for cadmium to avoid the inner filter effect.

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 excitation source. The excitation monochromator was set at 362 nm and the emission filter was high pass type, the 50% cutoff point being 470 nm. Although the optimal (energy corrected) excitation wavelength for most metal–HQS complexes is around 390 nm, 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.

For HQS-bearing eluents, the stationary phase was a surface-sulfonated poly(styrene–divinylbenzene) cation exchanger of unspecified particle size (likely ≥15 μm) and an ion exchange capacity of ~40 μequiv/g, (column type HS, 50 × 4.2 mm Wescan Instruments, Santa Clara, CA). For use of HQS as a postcolumn reagent, a glass-lined column (250 × 4 mm, Scientific Glass Engineering, Austin, TX) packed with 5-μm C_{18}-silica was initially coated with 100 mL of 5 mM sodium octanesulfonate (Eastman Kodak) and then used with an eluent containing 50 mM sodium potassium tartrate and 0.4 mM sodium octanesulfonate, adjusted to pH 3.4 with NaOH, after Cassidy and Elchuk (*43*). The postcolumn reactor was a filament-filled porous membrane type of ~10 μL volume that has been described earlier (*44*). Stainless steel screen-T reactors (*44*) lead to an undesirable degree of iron contamination and fluorescence quenching. The reactor was operated under nitrogen pressure and was adjusted to obtain an eluent:reagent mixing ratio of 5:3. Buffering agents used in the eluents or reagent (Tris, Bicine, MOPS, MOPSO) weere used without further purification (Serva Fine Biochemicals, Westbury, NY).

RESULTS AND DISCUSSION

The data for purely aqueous solutions are presented according to the periodic groups (see Table I).

Group I. The alkali metals are not expected to associate strongly with HQS and this is generally observed. However, solutions containing Rb and Cs decidedly show perceptibly increased fluorescence compared to the K-containing (KOH is used for pH adjustment) blank. The purity specifications on the Rb and Cs salts used are excellent and suggest that the fluorescence is not due to impurities. It is difficult to distinguish with certainty if blank values are lower if the pH is adjusted with LiOH and NaOH compared with KOH because of the greater amount of impurities present in the first two compounds. The blank value, however, is perceptibly lower if tetra-*n*-propylammonium hydroxide is used for pH adjustment.

It is interesting to note that alkali-metal salts of HQ in neat DMF have been reported to exhibit more intense fluorescence than almost any other metal (*45*). Regardless of whether significant complexation occurs with the heavier alkali metals in aqueous media, it appears certain that a significant amount of the "blank" fluorescence is due to association with residual metals in reagents and solvent; the blank is significantly reduced upon the addition of ethylenediaminetetraacetate (EDTA). Group IB metals do not form fluorescent chelates with HQS. Chelates are formed nevertheless, as indicated by UV spectroscopy; a number of these efficiently quench the fluorescence of other metal chelates. If oxidized to the trivalent state (for example with persulfate), Ag oxidizes HQS to an unidentified fluorescent product (*29*), the same as that formed with Ce(IV) or Sb(V).

Group II. All of the metals belonging to group IIA (radium was not studied) produce fluorescent chelates with HQS with the optimal pH being around 8, except for Be which optimally fluoresces at pH 7. At high pH and in the presence of excess HQS, polymeric complexes are reportedly formed with Be (*20*). The fluorescence intensities decrease from Mg to Ba, with Ba being only marginally fluorescent. While the trend is expected due to both the internal "heavy atom effect" (*45*) as well as the decreasing complexation constant (*14*, *15*), the drop in fluorescence is rather steep along the series. The fluorescence of the heavier metal chelates is more susceptible to quenching by adventitious quenchers present as impurities (vide infra) and the observed low fluorescence may actually be partially due to quenching.

Among group IIB metals, Hg forms nonfluorescent chelates in either of its oxidation states. In contrast, both Cd and Zn form strongly fluorescent chelates. The Cd chelate is a factor of 5 to 10 more fluorescent than virtually all other fluorescent HQS chelates. The pH dependence of the fluorescence of group II metal–HQS chelates is shown in Figure 1.

Group III. Among group IIIA metals, trivalent Al, Ga, and In are strongly fluorescent, all optimally at pH 6, with in-

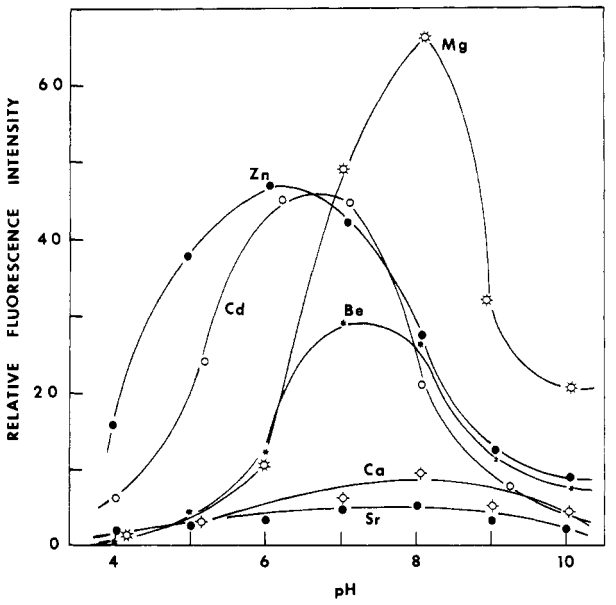

Figure 1. pH dependence of the fluorescnece intensities of group II metal–HQS chelates: Cd, 2 μM; all other metals in this and following figures, 20 μM; HQS, 1 mM.

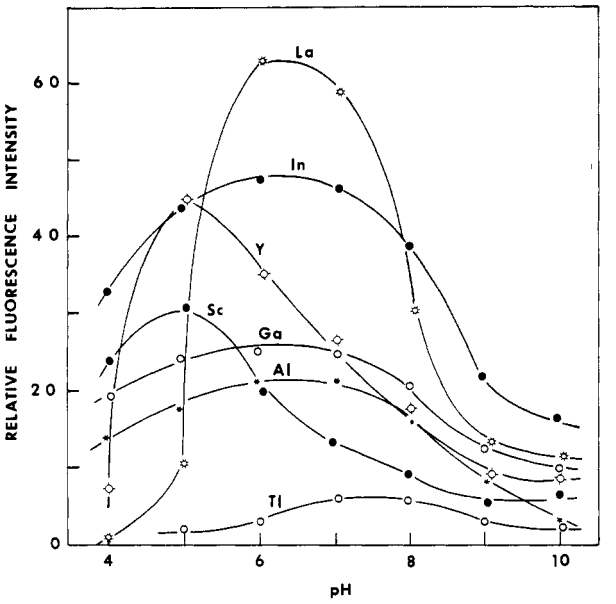

Figure 2. pH dependence of the fluorescence intensities of group III metal–HQS chelates.

creasing fluorescence intensity along the series. Boron as the borate anion is nonfluorescent. Trivalent Tl is nonfluorescent and is a powerful quencher, but univalent Tl is moderately fluorescent. The fluorescence of the Tl(I) chelate, which fluoresces optimally at pH 7, indicates that caution should be excercised with the adage that metals with more than one commonly occurring oxidation state do not form fluorescent chelates (see ref 4, other notable examples being Ti(IV) and Sn(IV)).

Among group IIIB metals, Sc, Y, and La all form strongly fluorescent chelates at optimal pH values of 5 to 6 and the fluorescence increases along the series. The pH dependence of the fluorescence of group III metal–HQS complexes is shown in Figure 2.

Lanthanides and Actinides. The only two strongly fluorescent HQS chelates among the lanthanides are those of Pr and Lu. Eu and Dy chelates are marginally fluorescent at best; the data for the two oxidation states of Ce indicate that the complexes are nonfluorescent; however, Ce(IV) ox-

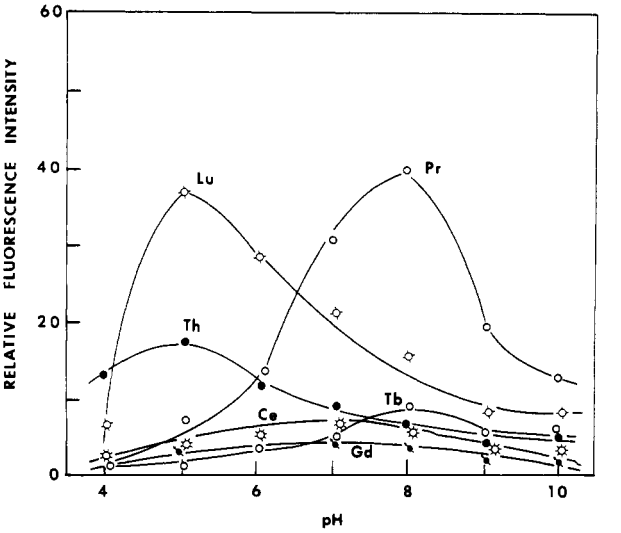

Figure 3. pH dependence of the fluorescence intensities of some lanthanide and actinide metal–HQS reaction products.

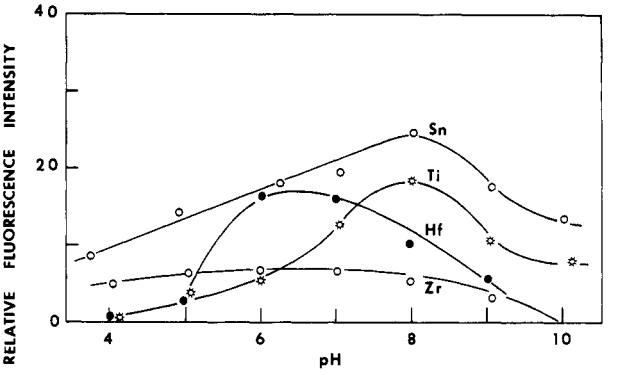

Figure 4. pH dependence of the fluorescence intensities of some group IV metal–HQS chelates.

idizes HQS to some unidentified fluorescent product. Among the actinides, only the naturally occurring elements, Th and U, were studied. Th(IV) forms a moderately fluorescent chelate, the UO_2^{2+} chelate is nonfluorescent. The pH dependence of the fluorescence intensities of the metal–HQS reaction product for several lanthanides and actinides is shown in Figure 3. For the case of Ce(IV), the reaction was allowed to proceed for 30 min at pH 8; the pH was then adjusted.

Group IV. Among group IVA metals, Sn(IV) forms a strongly fluorescent chelate whereas the Sn(II) chelate is nonfluorescent. The Pb(II) chelate is marginally fluorescent and also appears to be the least soluble among the HQS chelates in that precipitation occurs at concentrations of 1 mM and above. The Pb(IV) chelate of HQ has been reported to be nonfluorescent in DMF (*45*). In group IVB, quadrivalent Ti, Zr, and Hf chelates are all moderately fluorescent; Ti(III) is nonfluorescent. the pH dependence of the fluorescence intensities of the metals in group IV is shown in Figure 4.

Group V. Among group VA elements, As(III), As(V), or Bi(V) all as the oxo anions, and Sb(III) or Bi(III) as the oxo cations, do not form fluorescent HQS chelates. The fluorescent product formed with Sb(V) appears to be an oxidation product, because both the development of fluorescence is slow and the fluorescence behavior is identical with that of the product formed with Ce(IV). Among group VB metals, V(V) as the metavanadate and V(IV) as the VO^{2+} cation did not form fluorescent HQS chelates. Although intense fluorescence was observed from pentavalent Nb and Ta chelates, the purity specifications on the compounds used were rather poor and the data should be regarded as tentative.

Table I. Fluorescence Properties of Metal-HQS Chelates

metal (oxidation state)	$\lambda_{ex,max}$,[a,b] nm	$\lambda_{em,max}$,[a,b] nm	pH_{max}[c]	rel fluorescence intens[d]
Group IA				
Li(I)[e]				nfl[f]
Na(I)[e]				nfl
K(I)[e]				nfl
Rb(I)[e]	393	506	8	3.9
Cs(I)[e]	393	506	8	4.1
Group IB				
Cu(I)[g]				nfl
Cu(II)[h]				nfl
Ag(I)[e]				nfl
Au(III)[i]				nfl[j]
Group IIA				
Be(II)[h]	392	507	7	29.2
Mg(II)[e]	393	506	8	66.4
Ca(II)[e]	394	512	8	9.6
Sr(II)[e]	395	506	8	5.4
Ba(II)[e]	394	512	8	2.9
Group IIB				
Zn(II)[i]	393	506	6	46.9
Cd(II)[h]	387	522	7	450[k]
Hg(I)[e]				nfl
Hg(II)[e]				nfl
Group IIIA				
Al(III)[h]	395	500	6	21.4
Ga(III)[e]	397	512	6	24.9
In(III)[e]	398	517	6	47.5
Tl(I)[e]	393	511	7	6.0
Tl(III)[e]				nfl[j]
Group IIIB				
Sc(III)[e]	394	511	5	30.7
Y(III)[e]	394	508	5	45.1
La(III)[e]	393	511	6	63.0
Lanthanides				
Ce(IV)[h]	396	507	7	6.7[l]
Ce(III)[h]				nfl[m]
Pr(III)[e]	395	503	8	40.0
Nd(III)[e]	396	509	8	3.7
Sm(III)[e]	394	510	7	3.2
Eu(III)[e]	392	506	8	2.3
Gd(III)[e]	396	509	8	8.8
Tb(III)[e]	394	509	6	4.9
Dy(III)[e]	394	508	8	2.3
Ho(III)[e]	397	510	7	3.2
Er(III)[e]	394	508	8	4.2
Tm(III)[e]	395	505	8	4.2
Yb(III)[e]	394	509	8	4.4
Lu(III)[e]	394	508	5	37.1
Actinides				
Th(IV)[i]	395	506	5	17.3
U(VI)[n]				nfl
Group IVA				
Sn(II)[i]				nfl
Sn(IV)[o]	397	516	8	24.3
Pb(III)[e]	396	514	8	4.3
Group IVB				
Ti(III)[i]				nfl[j]
Ti(IV)[p]	394	505	8	18.3
Zr(IV)[q]	396	508	6	6.5
Hf(IV)[r]	393	507	6	16.7
Group VA				
As(III)[s]				nfl
As(V)[t]				nfl
Sb(III)[u]				nfl
Sb(V)[v]	396	507	7	6.3
Bi(III)[e]				nfl
Bi(V)[w]				nfl
Group VB				
V(IV)[x]				nfl
V(V)[y]				nfl
Nb(V)[z]	394	506	7	60.3
Ta(V)[z]	395	502	7	76.6
Group VIB				
Cr(III)[e]				nfl[j]
Cr(VI)[aa]				nfl
Mo(II)[i]				nfl[j]
Mo(VI)[ab]				nfl
W(VI)[ac]	394	522	7	17.3
Group VIIB				
Mn(II)[e]				nfl
Mn(VII)[ad]				nfl
Re(III)[i]				nfl
Group VIIIB				
Fe(II)[h]				nfl
Fe(III)[e]				nfl[ae]
Co(II)[e]				nfl[j]
Ni(II)[e]				nfl
Ru(III)[i]				nfl
Rh(III)[i]				nfl
Pd(III)[i]				nfl
Os(III)[i]	393	504	7	8.9
Ir(IV)[i]	394	508	8	28.5
Pt(II)[af]	395	518	8	5.7
Pt(IV)[i]				nfl

[a] At pH 9 but does not shift markedly with pH. [b] Because of the relatively large slit widths (10 nm) employed and the natural width of both the absorption and emission bands, these values should be regarded accurate to only within ±2 nm. The values are corrected for lamp output and phototube response. [c] The pH at which highest fluorescence is observed, to the nearest integer unit. [d] At the optimum pH, at the cited values of $\lambda_{ex,max}$ and $\lambda_{em,max}$. The blank value reaches a maximum at pH 8 and is 1.8 at that pH (λ_{ex} 393, λ_{em} 506). [e] Nitrate salt. [f] Nonfluorescent, the fluorescence is equal to or below blank value. [g] Solid CuCl dissolved in deoxygenated 1 mM sulfoxine at pH 9 to reach a concentration of 20 μM Cu(I). [h] Sulfate salt. [i] Chloride or chloro complex. [j] This metal ion exerts a significant quenching action on the fluorescence of other metal–sulfoxine chelates. [k] A value of fluorescence intensity of 45 in relative units was obtained at 2 μM Cd^{2+}. The experiment was carried out at this concentration to avoid the inner filter effect. The value has been multiplied by 10 to correspond to the concentration of other metal ions. At 20 μM Cd^{2+}, the relative fluorescence intensity is somewhat less than 450. [l] A transient red-violet species is formed immediately on Ce(IV) addition and slowly disappears. The decreasing visible absorption is accompanied by increasing fluorescence. The value quoted is taken after 30 min, at which point the fluorescence intensity is stable. [m] Ce(III) is itself intensely fluorescent (λ_{ex} 253, λ_{em} 358); however, the sulfoxine chelate is not, either at wavelengths characteristic of uncomplexed Ce(III) or at wavelengths typical of other sulfoxine chelates. [n] As uranyl nitrate. [o] As sodium stannate. [p] As titanyl sulfate. [q] As zirconyl nitrate. [r] As hafnyl nitrate. [s] As sodium arsenite. [t] As sodium arsenate. [u] As antimonyl sulfate. [v] Liquid $SbCl_5$ directly added to 1 mM sulfoxine to reach a concentration of 20 μM Sb(V). [w] As sodium bismuthate. [x] As vanadyl sulfate. [y] As sodium metavanadate. [z] As fluoride, HF digest of the metal pentoxide. [aa] As sodium chromate. [ab] As sodium molybdate. [ac] As sodium tungstate. [ad] As potassium permanganate. [ae] The only stable sulfoxine complex that displays an intense visible absorption, visually the color is dark green. [af] As the *cis*-diamine dichloride.

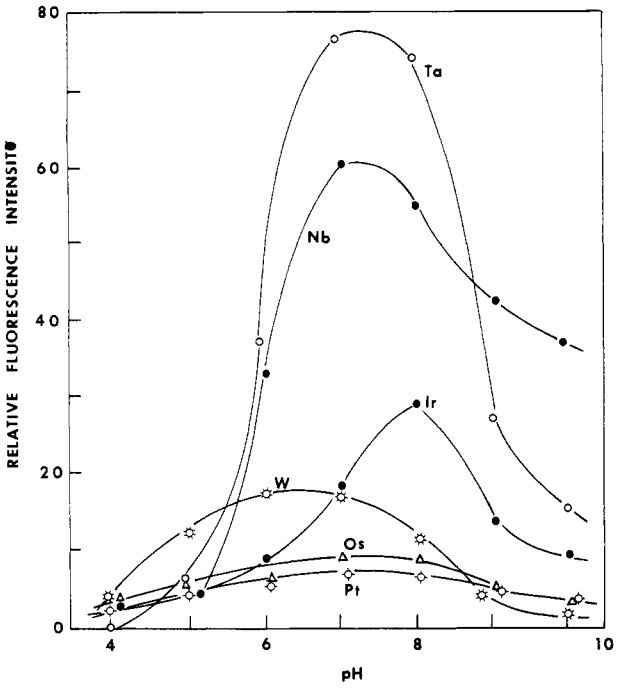

Figure 5. pH dependence of the fluorescence intensities of some group V–VIII metal–HQS chelates.

Group VI. Among the metals in group VIB, only W(VI) forms a fluorescent chelate, whether added as a salt of the tungstyl cation or as a tungstate. Neither hexavalent Mo nor Cr as the anions react with HQS, both cationic Mo(II) and Cr(III) form nonfluorescent chelates and act as effective quenchers.

Groups VII and VIII. The majority of the metals belonging to these groups do not form markedly fluorescent chelates. Indeed, a number of these nonfluorescent chelates are highly efficient quenchers, Fe(III) being the most notable (vide infra). Mn as MnO_4^- has been reported to oxidize HQS to a fluorescent product (*30*); however in the present experiments (pH ≥4), oxidation was not observed. Divalent Pt, trivalent Os, and quadrivalent Ir form fluorescent HQS chelates, the last being markedly so. However, Ir(IV) is a strong oxidizing agent; it is possible that the fluorescence is due to the HQS oxidation product rather than the metal chelate. The pH dependence of the fluorescence of metal–HQS complexes is shown in Figure 5.

Enhancement of Fluorescence by HTAC. Significant increases in fluorescence intensities were observed in the presence of HTAC for all the metals so studied, Mg, Ca, Sr, Ba, Al, Cd, and Zn. This is similar to the observations of Meshkova et al. (*35*) for the lanthanides. The enhancement factors (at the optimum pH) range from ~1.2 for Sr to nearly an order of magnitude for Al; also the optimum pH is reduced by 1–2 units in the micellar system. Further, in general, the pH range for maximal fluorescence is extended. Maximum enhancement is reached in all cases in the 0.25–1.0 mM HTAC concentration range. For any given metal, this optimum HTAC concentration does not change upon a 2-fold change in the metal concentration. A summary of these results is given in Table II; details of the fluorescence intensity dependence upon pH and HTAC concentration will be reported elsewhere.

Fluorescence Intensities of Metal–HQS Chelates in Water/DMF Media. Because significant enhancement was reported for DMF (neat) as solvent for the Mg–HQS chelate compared to water (*8*), it was of interest to us to investigate the effect of mixed water/DMF solvents on the HQS chelates of some representative metals, Al, Cd, Zn, and Ca. The results are shown in Table III. Although the degree of enhancement, if any, varies a great deal from metal to metal, even among the limited subset tested, the enhancement at high DMF content is remarkable, most notably for Al. It should be emphasized that throughout the entire range of solvent composition, the changes in the value of the blank are negligible, and in going from purely aqueous to 90 vol % DMF media, the optimal excitation wavelength does not shift and only a

Table II. Enhancement of Fluorescence by a Cationic Micelle[a]

metal	optimum HTAC concn, mM	optimum pH range	enhancement factor over pure aqueous solution (at optimum pH)
Mg	0.5–1	6.8–7.2	1.6
Sr	0.25	6.7–7.5	1.2
Ba	0.5	6.6–6.9	2.6
Al	0.5	5.4–7	10.2
Zn	0.5–1	4–7.5	1.8
Cd	0.25	6.2–6.8	1.6

[a] All of the systems studied contain 1 mM HQS, the metal concentrations used were 500 μM (Ba, Sr), 100 μM (Mg, Al, Zn), and 10 μM (Cd).

Table III. Enhancement of Fluorescence in Water/DMF Media

vol % DMF	rel fluorescence intens enhancement factor[a] Al	Cd	Zn	Ca
10	2.65	1.71	1.33	1.24
20	5.80	1.91	1.31	0.60
30	9.14	1.74	1.39	0.59
40	14.9	1.65	1.62	0.59
50	22.3	1.81	2.02	0.82
60	31.4	2.22	2.77	1.13
70	41.6	2.79	3.86	1.55
80	48.1	3.09	5.20	3.10
90	82.5	2.96	7.05	7.98

[a] For each metal, the relative fluorescence intensity is normalized to unity for a purely aqueous solution. The absolute value can be estimated from the data in Table I.

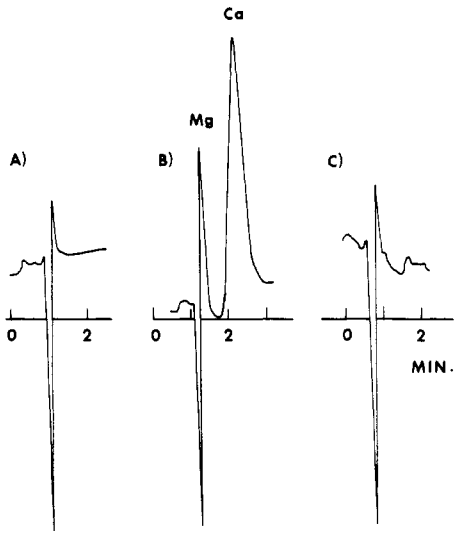

Figure 6. Ultratrace detection of magnesium: eluent, 1 mM HQS, 10 mM Tris, pH 8.0, 150 mM KNO_3; flow rate, 1 mL/min; column, Wescan HS, 50 × 4.2 mm; PMT voltage, 660 V, 0.05 μA full scale. Samples used were as follows: (a) water, (b) aqueous solution of 500 fmol of Mg^{2+} and 500 pmol of Ca^{2+}, (c) water. Note the slight increase in the water response in (c) due to cross contamination in the sample valve from injection (b).

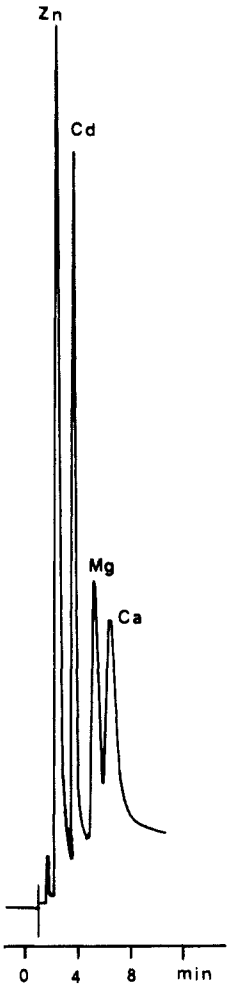

Figure 7. Gradient separation of Zn, Cd, Mg, and Ca: column, Wescan HS (50 × 4.2 mm); flow rate, 1 mL/min; eluent A, 250 μM HQS, 2.5 mM MOPS, pH 7.0; eluent B, A + 500 mM KNO_3; program, linear gradient from 0 to 65% B in 0–2.35 min; sample injected electropneumatically at 1 min into the program, 65–100% B in 2.35–4.80 min, hold at 100% B; samples, 135 pmol of Zn, 33 pmol of Cd, 135 pmol of Mg, 20 nmol of Ca; PMT voltage, 375 V, 0.02 μA full scale.

slight red shift (5–10 nm) is observed for the emission maximum.

Quenching of the Fluorescence of Metal–HQS Chelates. Some quantitative data on the quenching action of a few metal ions, which were observed to act as quenchers upon the fluorescence of the Mg–HQS chelate, are presented in Table IV. These experiments weere conducted in the presence of excess HQS with the quencher concentration significantly lower than the fluorescing metal chelate concentration. This is in contrast to the study of Meshkova et al. (*35*) on the quenching of the fluorescence of the La–HQS chelate by quencher ions present in an order of magnitude greater concentration. The very powerful quenching action exhibited by Fe(III) on the fluorescence of HQS chelates has not been reported previously and is particularly important in view of its wide occurrence in real samples and may indeed limit the usefulness of the recently reported innovative fiber-optic sensors which employ immobilized HQS (*40*).

Quenching by iron clearly has important consequences on the use of HQS in trace metal ion chromatography. Modern high-performance liquid chromatographic hardware makes extensive use of stainless steel as fluid contact parts. Iron is inevitably leached off in trace amounts as complexing ligands are commonly incorporated in aqueous eluents (which contain significant amounts of dissolved oxygen even after ordinary degassing) for metal ion chromatography. While details of the quenching effects of iron will be reported elsewhere, we

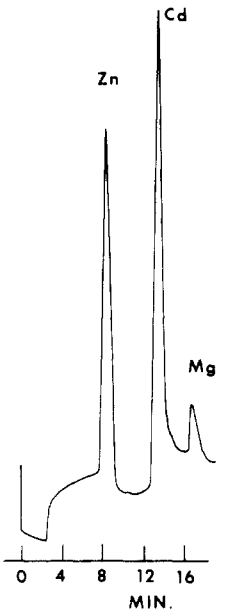

Figure 8. Detection of Zn, Cd, and Mg by postcolumn reaction with HQS: column, SGE-C18 5 μm (250 × 4 mm), coated with octanesulfonate; eluent, 50 mM sodium potassium tartrate, 0.4 mM octanesulfonate, pH 3.4; flow rate, 0.5 mL/min; postcolumn reagent, 1 mM HQS, 300 mM bicine, pH 9.3, 0.3 mL/min; reactor effluent, pH ~8.0; sample, 25 pmol of Zn, 6 pmol of Cd, 500 pmol of Mg; PMT voltage, 590 V, 0.02 μA full scale.

wish to note a few salient points here. Among the HQS chelates of Ca, Mg, Cd, and Al in purely aqueous medium, the quenching effect decreases in the order Ca > Mg > Cd > Al; this may very well simply be mirroring the corresponding increase in the respective complexation constants (ref 14 and 15; the complexation constant for the Al–HQS system has not been reported; however, we estimate it to be the highest among the four metals above on the basis of the reported complexation constants with HQ).

The presence of HTAC greatly accentuates the quenching effect; the anionic Fe–HQS complex is likely concentrated at the micellar interface and thus exhibits greater quenching ability. The quenching effect is sufficiently large so as to wipe out all gains of the surfactant enhancement of the original fluorescence at all but very low quencher concentrations. Indeed, it is possible that the rapid drop of fluorescence intensity at high HTAC concentrations for some metals may partly be due to the presence of adventitious quenchers that are present in the HTAC reagent itself. These factors limit the usefulness of HTAC enhancement of the fluorescence intensity of HQS chelates. In contrast, the DMF/water medium does not appear to be nearly as suceptible to quenching; the enhancement factor is still sufficiently large for most quencher concentrations so as to outweigh the greater susceptibility to quenching.

Chromatographic Applications. HQS can be used successfully as an integral component of the eluent similar to the use of dithiocarbamates (*46*) of diazo dyes (*47*) in metal ion chromatography. However, careful optimization of column-eluent combinations are often necessary to achieve a desired separation. A chromatogram of 10^{-8} M Mg^{2+} and 10^{-5} M Ca^{2+} on a short column (50 mm) is shown in Figure 6, with preceding and succeeding injections of water. The water dip is not noticeable at lower detector sensitivity and may be completely separated from the Mg peak on a 100-mm column; however, the attainable limit of detection will deteriorate. This example constitutes the first demonstration, to our knowledge, of subpicomole detection of Mg. If ultratrace determinations are not essential, HQS-bearing eluents easily permit gradient elution. An example separation of Zn, Cd, Mg, and Ca is

Table IV. Effect of Selected Quenching Metal Ions upon the Fluorescence of the Mg-HQS Complex[a,b]

quenching ion	% original fluorescence remaining	quenching ion	% original fluorescence remaining
Cr(III)	94.1	Au(III)	59.4
Ag(I)	91.0	Tl(III)	17.7
Ti(III)	62.4	Fe(III)	1.0

[a] At a quencher concentration of 50 μM. [b] 100 μM Mg, 1 mM HQS, pH 8, λ_{ex} 390 nm, λ_{em} 510 nm.

shown in Figure 7.

By and large, however, introducing HQS postcolumn is a more versatile approach, for much the same reasons that the vast majority of metal ion chromatography is currently carried out by introducing chromogenic reagents in a postcolumn mode. The chromatogram in Figure 8 showing the separation of Zn, Cd, and Mg was obtained with a porous membrane postcolumn rector (PCR). Detection limits for both Zn and Cd in this mode are routinely well below 10^{-8} M and approaches 10^{-9} M under optimum conditions. Another example of a postcolumn application involving HQS is the detection of metals such as iron which act as quenchers by introducing a fluorescent HQS metal complex in a micellar solvent through the PCR and monitoring the decrease in fluoresence. An example is shown in Figure 9. Detection limits in this mode are not nearly as good as in direct fluorescence detection, as may be expected.

CONCLUSIONS

HQS appears to be an unusually promising reagent for postcolumn addition or other applications in metal ion chromatography—an ideal fluorometric complement to (pyridylazo)resorcinol (PAR), which is in wide use in metal ion chromatography today. Indeed, PAR works best with those metals which form nonfluorescent or very weakly fluorescent chelates with HQS. HQS cannot compete with PAR as an absorptiometric reagent, since the molar absorptivities of the chelates (λ_{max} 360–400 nm) are uniformly below 10^4, at least a factor of 4 lower than the corresponding PAR chelates. Although the effects of further substitution, specifically at the 2 and 7 positions in HQS, on metal chelation selectivity and stability, have been studied (*18*, *48*), there is no evidence to believe that any advantage is to be gained over HQS in regard to fluorescence properties. Further, except for the 7-iodo derivative (Ferron), they are not commercially available and must be synthesized. For the 7-iodo derivative, our preliminary studies confirm what may be expected on the basis of the internal heavy atom effect: the fluorescence intensities obtained with this compound are uniformly lower than those obtained with HQS itself, typically by a factor of 8 to 10.

Meaningful further improvements along this line should involve the development of a ligand that forms complexes with much greater molar absorptivities, without sacrificing the fluorescence properties, such that tandem or even same cell (*49*) absorptiometric detection can be sensitively performed for metals that do not form fluorescent chelates. Most of the more intensely absorbing azo derivatives (azoxines) do not form fluorescent complexes, however. The sole exception are derivatives in which 1-amino-2-naphthol or its sulfonated derivatives are diazotized and coupled to the 7 position in HQS or HQ, as originally reported by Badrinas (*50*). These form metal complexes that fluoresce an intense pink and may be worthy of further studies.

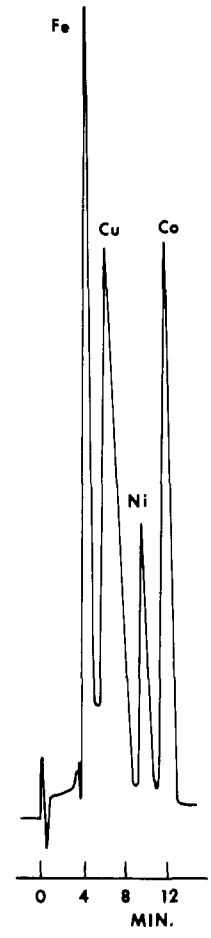

Figure 9. Detection of Fe, Cu, Ni, and Co by fluoresence quenching, postcolumn reaction with the Al–HQS complex: column and eluent conditions, as given in Figure 8; postcolumn reagent, 300 μM Al, 900 μM HQS, 500 μM HTAC, 300 mM MOPSO, pH 7.9, 0.3 mL/min; reactor effluent pH ~7.0; sample, 500 pmol each of Fe and Cu, 2 nmol of Co, 5 nmol of Ni.

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Artifacts Arising from the Improper Preparation and Use of Nonaqueous Ion Exchange Resins

Michael G. Strachan[1] and R. B. Johns*

Department of Organic Chemistry, University of Melbourne, Parkville, Victoria 3052, Australia

This paper reports on the consequences of inadequate conditioning of nonaqueous macroreticular ion-exchange resins in the separation of coal-derived liquids and/or petroleum substitutes. In such cases any residual resin decomposition products will be incorporated into the fractions eluted from the resins. These contaminants have certain spectral and analytical characteristics similar to the complex fossil fuel derived fractions from the resin separation procedure which make their presence less obvious. Indeed, some characteristic features, such as $f(a)$ values, pyrogram data, very high H/C and O/C ratios, and high H_{Ali}/H_{Ar} ratios from the Brown–Ladner equation are different enough to bias the quantitative data and lead to incorrect structural compositional conclusions. The data suggest that it is an essential step, in such a fractionation procedure, to adequately precondition the resins.

The increasing use of nonaqueous ion exchange resins for the separation of complex mixtures such as coal-derived liquids (*1–5*) and petroleum (*6–10*) raises the issue of the extent to which contaminants released from the resins may bias the analytical data eventually obtained. Current resins in use have much improved resistance to mechanical attrition and do not show the fragmentation in either polar or nonpolar organic solvents characteristic of conventional exchange resins. Accordingly they are open to a wider application in analytical separations. The more widely used resins are the Rohm and Haas Amberlyst series, A-21 and A-27 (weak and strong anion exchangers, respectively) and A–15 (strong cation exchanger). Previously A-29 was used (*6–8*) but in the literature it has been replaced by Amberlite IRA-904, also a macroreticular resin with exchange characteristics and properties similar to A-27.

Much time is required to activate and condition these resins into their particular functionalized form and to remove resin decomposition products. The literature reports developments in understanding the methods of conditioning and packing and the use of these resins, but characteristically the reasons for the changes recommended are not elaborated upon (*7, 8, 10*). In a previous paper the use of resins A-21, A-27, and A-15 was described (*4*) for the separation of a coal-derived liquid on the basis of functionality. Since A-27 had not previously been reportedly used for such a purpose, the introduction of the combination as described (*4*) raised the need to know the potential bias which may be introduced into the range of physicochemical analyses of the product fractions from contaminants derived from improperly prepared resin columns. This paper reports our assessments.

EXPERIMENTAL SECTION

Resins and Chemicals. The Amberlyst anion (A-21 and A-27) and cation (A-15) exchange resins were obtained from Rohm and Haas. Methanol (May and Baker, Ltd., Sydney, Australia) and methylene dichloride (Ajax Chemicals, Sydney, Australia) were both analytical reagent grade and redistilled prior to use.

Extraction and Preparation of Resin Samples. The only modifications to the procedures published (*4*) for the activation and conditioning of the resins was that fresh conditioning solvent (i.e., methanol or methylene dichloride) was used every 50 h rather than after the 200-h washing cycle used for each solvent. Coloration was observed until the third batch of methanol for all three resins, the fourth batch was colorless in each case. Methylene dichloride extracted little material from all resins, coloration only being observed in the first 50-h washing. However, washing was continued for the 200-h period. The resins were washed for a total of 400 h each. The methanol and methylene dichloride washings of each resin were combined, and the solvent was removed by rotary evaporation under high vacuum. They were then concentrated in vials. The residual solvent was removed by a nitrogen

[1] Present address: Petroleum Geochemistry Group, School of Applied Chemistry, W.A.I.T., Kent St., Bently 6102, W.A., Australia.

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