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Fluorimetric Metal Ion Sensing Using *N*-Methyl-9-Anthrylhydroxamic Acid

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We have synthesized *N*-methyl-9-anthrylhydroxamic acid, which is a fluorescent analogue of *N*-methylbenzohydroxamic acid. Complexation with various di- and trivalent metal ions occurs (log *K* from 4 to 5) in water with resulting fluorescence quenching. Because the Fe(III) and Al(III) complexes substituted rather slowly, the addition of EDTA provides a temporal method for obtaining some selectivity in the chemosensor.

KEY WORDS: Hydroxamic acid; metal ion; quenching; complexometry; anthracene.

INTRODUCTION

As a tool for the detection of species in solution, fluorescence methods proffer several useful characteristics: sensitivity, low background noise, and application to fiber optics remote sensing techniques [1-4]. Because most applications of chemical sensors will take place in aqueous solution, an understanding of the interplay between binding and fluorescence phenomena in water is requisite to the design of selective fluoroionophores. Specifically, we have examined two intensity-based signaling phenomena—chelation-enhanced fluorescence (CHEF) and chelation-enhanced quenching (CHEQ)—and the mechanisms by which they may be instituted [5]. Anthrylazamacrocycles such as **1a** bind Zn(II) and Cd(II) in water with net CHEF, while the same compound binds Cu(II) and Hg(II) with net CHEQ [6]. However, as a sensor for many other ions, anthrylazamacrocycle **1a** proves ineffectual; for example, the fluorescence of **1a** is unchanged upon the addition of Al(III). Benzohydroxamic acid has been used as a complexing colorimetric reagent for Fe(III) and other metal ions [7], but with a selectivity different from that demonstrated by polyamines. We now report that anthrylhydroxamic acid **4** likewise displays complexation reactivity, but with signal transduction in the form of chelation-enhanced quenching of fluorescence.

EXPERIMENTAL

General. Melting points were taken on an Electrothermal melting-point apparatus and are uncorrected. Microanalyses were carried out at Atlantic Microlab Inc., Norcross, Georgia. Mass spectra were obtained by use of a Kratos-30 mass spectrometer. FT-NMR spectra were obtained at 11.75 tesla (500 MHz) or 7.0 tesla (300 MHz). UV spectra were obtained on a Hewlett-Packard 8451A diode array spectrophotometer; all wavelength data reported are ±1 nm. Fluorescence measurements were made on a Perkin-Elmer LS-5 luminescence spectrometer with excitation at 345 nm; both emission and excitation slit widths varied from 3 to 20 nm, depending on the measurement. Anthracene-9-carboxylic acid (**2**) was purchased from the Aldrich Chemical Company, Milwaukee, WI. All metal ion perchlorates were obtained from GFS Chemical, Columbus, OH.

9-Anthroyl Chloride (3). 9-Anthrylcarboxylic acid

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(10.1 g, 45 mmol) was dissolved in thionyl chloride (100 ml) under argon. The solution was heated to reflux for 7 h, and excess thionyl chloride was evaporated under reduced pressure. After coevaporation with benzene, the greenish-yellow solid was dried under reduced pressure (10.8 g, 99%); ^{1}H NMR ($CDCl_3$) d 7.45–7.70 (m, 4, Ar-H), 8.0–8.2 (d, 4, Ar-H), 8.55 (s, 1, Ar-H): EI mass spectrum, *m/e* 240.0348 [M^+, Calcd. 240.0342.]

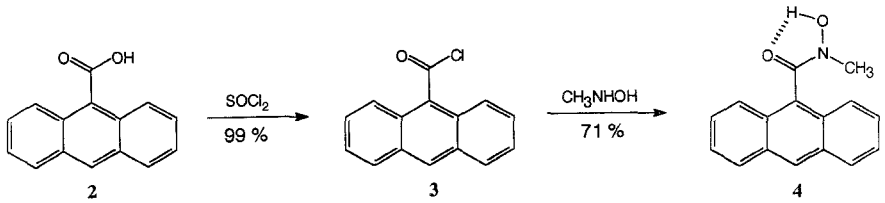
N-*Methyl 9-anthrylhydroxamic Acid (4).* *N*-Methylhydroxylamine hydrochloride (3.93 g, 42 mmol) and sodium bicarbonate (3.71 g, 44 mmol) in dry acetonitrile (500 ml) were stirred for 2 h in an ince bath under argon. 9-Anthroyl chloride (2.5 g, 10 mmol) was added into the ice-cold solution and stirred for 6 h. After filtration of the white precipitate, the filtrate was evaporated. The brown oil was dissolved in chloroform (70 ml) and extracted with 0.4 *N* Na_2CO_3 solution (5 × 200 ml). The basic aqueous solution was neutralized with 5% HCl solution and extracted with chloroform (5 × 125 ml). The chloroform layer was dried over sodium sulfate and evaporated to give a yellow solid (1.86 g, 71%), which was recrystalized from chloroform: mp 187–188°C, ^{1}H NMR ($CDCl_3$) d 3.1 (s, 3, CH_3), 7.45–7.55 (m, 4, Ar-H), 7.9–8.1 (m, 4, Ar-H), 8.55 (s, 1, Ar-H), 9.6-9.8 (s, 1, OH): EI mass spectrum, *m/e* 251.0950 [M^+, Calcd. 251.0946]. *Anal.* Calcd. for $C_{16}H_{13}NO_2 \cdot 0.2H_2O$: C, 75.40; H, 5.30; N, 5.50. Found: C, 75.38; H, 5.14; N, 5.49.

RESULTS AND DISCUSSION

Anthrylhydroxamic acid **4** was prepared using the route shown in Scheme I. The reaction of thionyl chloride with 9-anthrylcarboxylic acid affords the corresponding acid chloride (**3**) in a 99% yield as a yellow-green solid. Condensation with *N*-methylhydroxylamine in acetonitrile solution gave **4**, which could be recrystallized from chloroform to yield transparent crystals (mp 187–188°C) that turned opaque on standing for several days. Characterization of this sample was fully consistent with the structure assignment; microanlaysis of this hygroscopic material indicated 0.2 mol of water. While two rotamers might have been observed using NMR, only one was seen in $CDCl_3$; we anticipate that the most stable conformation of **4** involves intramolecular hydrogen bonding of the acidic proton. We also expect an orthogonal arrangement of the hydroxamic acid mean plane with respect to that of the anthracene, which is likewise predicted by molecular mechanics methods. Some metal ion complexes of benzohydroxamate are hydrolytically labile [8]; those of **4** appear not to be over the time frame of 24 h, a fact we attribute in part to steric protection by the anthracene perihydrogens that flank both faces of the carbonyl group in the orthogonal conformation.

Anthrylhydroxamic acid **4** displays an anthracene-like fluorescence emission, with λ_{max} at 414 nm in mildly acidic media. At 25 μ*M* in pH 7 bis-trispropane (BTP) buffer (0.01 *M*), the emission is approximately 24 times lower in intensity than that observed for anthrylazamacrocycle **1a**; the hydroxamic acid moiety is oxidized more easily than is an ammonium ion (which exists at pH 7), resulting in greater initial fluorescence quenching. Deprotonation of **4** leads to increased quenching (Figs. 1 and 2), and **4** is 13 times more fluorescent than is its conjugate base (**5**). The known pK_a of *N*-methylbenzohydroxamic acid is 8.5 [9], which correlates qualitatively with the calculated fluorescence titration of **4** of 7.94.

Solutions of anthrylhydroxamic acid (**4**; 25 μ*M*) in pH 7 BTP buffer (1 m*M*) were prepared, and to each was added 10 equiv of various metal ions as their chloride [Hg(II), Sn(IV)], nitrate [Ag(I)], or perchlorate [all others] salts. The resulting fluorescence changes, shown in Fig. 3, demonstrate metal ion coordination with concomitant CHEQ effects for A1(III), Cd(II), Co(II), Cu(II), Fe(III), In(III), Pb(II), UO_2(II), and Zn(II) ions. Complex formations are complete within 10 min for all these ions except for Al(III) and Fe(III); Fig. 4 shows the rate of complexation with 5 equiv of several CHEQ metal ions under otherwise identical conditions. It is apparent that after 10 min, only the Al(III) reaction is not near



2 3 4

Scheme I

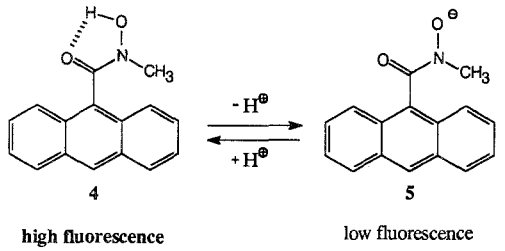


Fig. 1. Fluorescence of **4** as a function of ionization state.

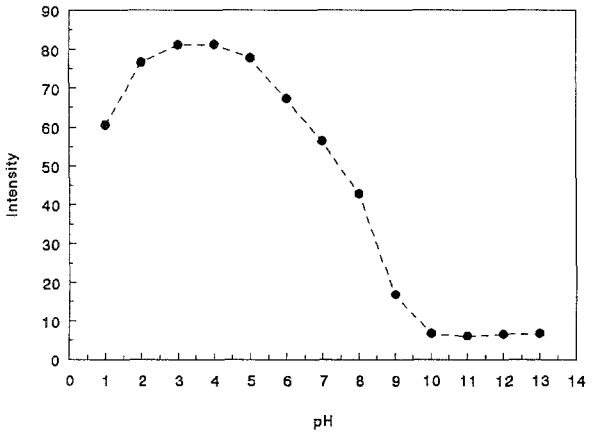


Fig. 2. pH–fluorescence profile of anthrylhydroxamic acid, **4** (27 μ*M*).

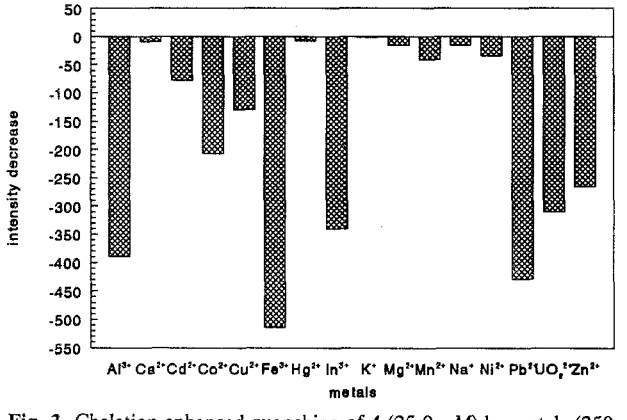


Fig. 3. Chelation-enhanced quenching of **4** (25.0 μ*M*) by metals (250 μ*M*). Bis-tris propane buffer (1 m*M*) at pH 7 was used in all solutions. The metal ion-free emission intensity was 550; intensity decreases are shown. All emission intensities were measured 2 h after metal ion addition.

completion. Titrations using these metal ions were likewise conducted under the same conditions; as shown in Fig. 5, we observed that Fe(III) affords the largest CHEQ

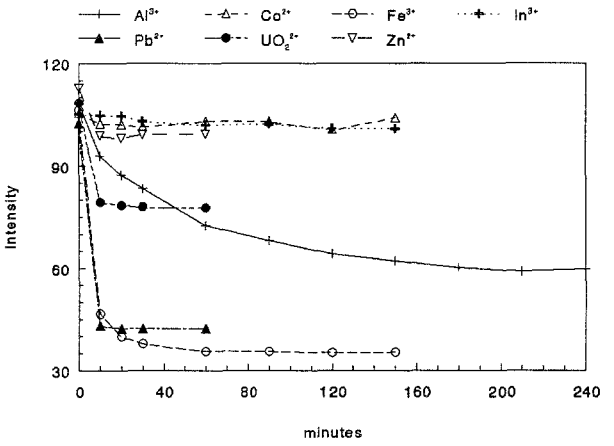


Fig. 4. Time dependence of fluorescence quenching of **4** (5.0 μ*M*) by metals (25 μ*M*). Bis-tris propane buffer (1 m*M*) at pH 7 was used in all solutions.

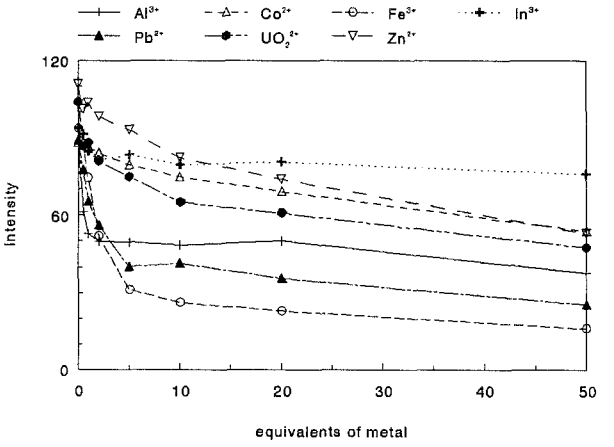


Fig. 5. Fluorescence titrations of **4** (5 μ*M*) by metals. Bis-tris propane buffer (1 m*M*) at pH 7 was used in all solutions. All emission intensities were measured 3 h after metal ion addition.

at 10 equiv of metal ion, while Al(III) forms the most stable complex. Association constants under these conditions could be calcuated for (ion, log *K*): Co(II), 4.1; Fe(III), 5.0; Pb(II), 4.7; UO_2(III), 4.5; and Zn(II), 4.2. The complexation equilibrium is a function of buffer type and concentration. Fe(III) titration succeeds in bis-trispropane buffer but fails in the less strongly complexing buffer HEPES (Fig. 6); the addition of 20 μ*M* cyclen (**1b**) to the HEPES solution presumably complexes transition metal ion impurities and permits visualization of the Fe(III). More concentrated buffer yields more gradual saturation by FE(III), as shown in Fig. 7; because the concentration of **4** is higher than that used in Fig. 6, HEPES buffer without cyclen is

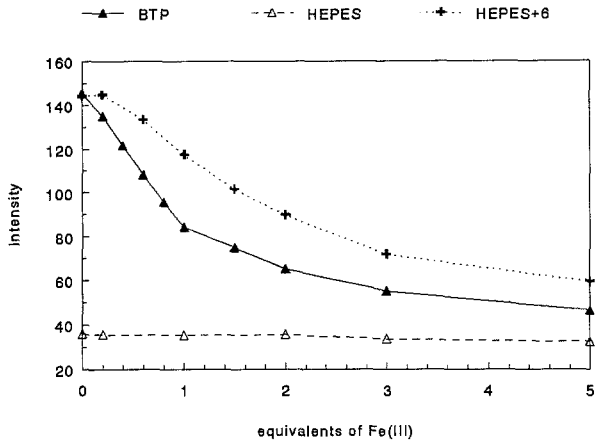


Fig. 6. Fe(III) titration of **4** (5.0 μM) as a function of buffer and cobuffer. Titrations were carried out under three conditions: bis-tris propane buffer (1 mM) at pH 7, HEPES buffer (1 mM) at pH 7, and HEPES buffer (1 mM) at pH 7 with added **6** (20 μM). All emission intensities were measured without incubation after sequential metal ion additions to a single solution.

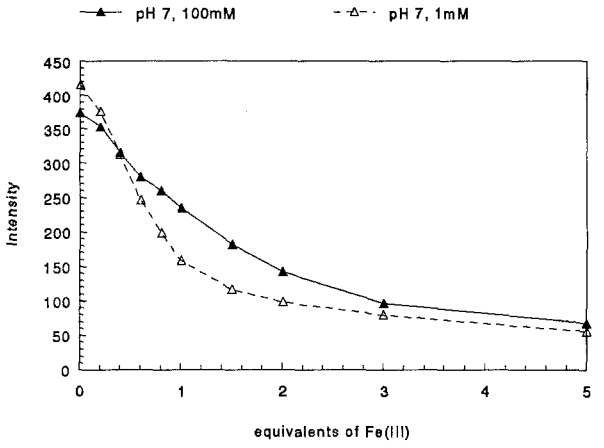


Fig. 7. Fe(III) titration of **4** as a function of buffer concentration. Titrations were carried out under two conditions: HEPES buffer (100 mM at pH 7 and HEPES buffer (1 mM) at pH 7. All emission intensities were measured without incubation after sequential metal ion additions to a single solution.

usable. Oxidizing metal ions Ag(I) and Sn(IV) result in comparatively small fluorescence increases; chemical oxidation of the hydroxamic group would be expected to yield a less quenching and, therefore, more highly fluorescent form of **4**. Reactions of **4** (5 μM) with 5 equiv of these metal ions at pH 8.5 displays net CHEF, completed within 10 min (Fig. 8). Our observation that only Ag(I) and Sn(IV) yield fluorescence changes not reversed

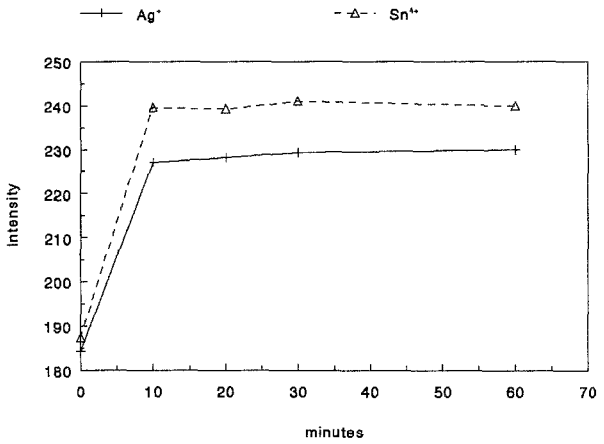


Fig. 8. Titrations of **4** (5.0 μM) by oxidizing metals (25 μM). Bis-tris propane buffer (1 mM) at pH 8.5 was used in all solutions.

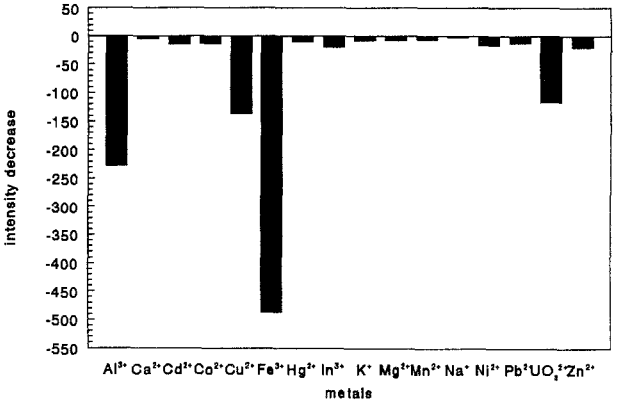


Fig. 9. Effect of added EDTA on the CHEQ of **4** by metals. Bis-tris propane buffer (1 mM) at pH 7 was used in all solutions. The data shown are those from Fig. 3 to which EDTA (67 mM) was added; spectra were recorded 1 h after EDTA addition.

upon EDTA introduction argues for reaction other than simple coordination.

Complete binding selectivity is seldom observed in complexing indicators, and a secondary selectivity mode must be employed to observe one or two of the multiple ions that bind. Thus, when 70 equiv of EDTA was added to the solutions described in Fig. 3, the fluorescence emissions displayed after an hour (Fig. 9) returned to near-baseline levels for all metal ions except Al(III), Fe(III), Cu(II), and UO_2(II). The emission intensities of these solutions do continue to recover as decomplexation occurs, but with half-lives for Al(III) and Fe(III) under the conditions indicated of about 3 h. This approach thus permits

some ions to be visualized in the presence of other ions that complex to anthrylhydroxamic acid **4** with CHEQ.

CONCLUSION

N-Methyl-*O*-anthrylhydroxamic acid (**4**), which is a fluorescent analogue of *N*-methylbenzohydroxamic acid, similarly binds metal ions from aqueous solution but with signaling in the form of chelation-enhanced quenching. Hydroxamate form **5** quenches fluorescence more strongly than does conjugate acid form **4**, likely due to its greater ability for electron transfer in this photoinduced process. Because the nonquenching metal ions Zn(II) and Cd(II) demonstrate CHEQ, we conclude that a principal quenching mechanism is the generation of hydroxamate **5** (in the form of its metal ion complexes) at pH 7. While **4**, like benzohydroxamic acid, complexes many metal ions from water, additional selectivity is obtained by employing their differential rates of substitution by added EDTA.

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