Fluorometric Determination of Zinc

SIR: Although the fluorescent characteristics of many zinc compounds have been investigated, only a few fluorometric methods for the determination of zinc have been reported. The fluorescence of a stabilized dispersion of zinc 8-hydroxyquinolate has been used for the determination of zinc in the concentration range of 0.4 to 12 p.p.m. (*3*). The fluorescing ether extract of a zinc–Rhodamine *s*-thiocyanate complex permits the determination of zinc at a 0.2-p.p.m. lower concentration limit (*1*). In a 70% ethanol–water mixture, the fluorescent zinc dibenzothiazolylmethane complex has been employed for the determination of zinc in the concentration range of 0.5 to 50 p.p.m. (*5*). Studies on three fluorescing zinc 2-methyl-8-hydroxyquinolate complexes have not resulted in an analytical application (*2*).

The present report describes the fluorometric determination of zinc using picolinealdehyde 2-quinolylhydrazone as a complexing agent. Characteristics of the polydentate ligand and of the zinc chelate are reported. Variables associated with metal ion complexation, with extraction of the zinc complex, and with fluorometric measurement of the zinc complex are discussed. Results on the determination of zinc in selected samples are presented. Data are included on the effects of many common ions on the fluorometric measurement.

EXPERIMENTAL

Apparatus. All fluorescence measurements were made with an Aminco-Bowman spectrophotofluorometer using 10-mm. square quartz cells. The fluorometer was equipped with a Hanovia Xenon lamp source and a 1P21 photomultiplier detector. The slits were arranged as follows: excitation beam, $^1/_8$, $^1/_{16}$, and $^1/_8$ inch; fluorescence beam, $^1/_8$, $^1/_{16}$, $^1/_8$, and $^1/_{16}$ inch. Measurements of pH were made with a Corning Model 12 pH meter.

Reagents. The chelating agent, picolinealdehyde 2-quinolylhydrazone, PAQH, was synthesized and purified in this laboratory. The starting material, 2-quinolylhydrazine, was prepared by the method described by Perkin and Robinson (*4*).

Picolinealdehyde 2-quinolylhydrazone was prepared by the addition of 3.18 grams (0.02 mole) of 2-quinolylhydrazine and 2.14 grams (0.02 mole) of 2-pyridinecarboxaldehyde to 75 ml. of absolute ethyl alcohol in a 100-ml. beaker. The solution was heated on a hot plate for 15 minutes and then allowed to cool to room temperature. The yellow product, which first started to precipitate early in the heating period, was filtered and redissolved in benzene. The desired product was obtained from benzene as a microcrystalline solid, yield 3.15 grams (64%), m.p. 204 to 204.5° C.

Anal. calcd. for $C_{15}H_{12}N_4$: C, 72.55; H, 4.88; N, 22.57. Found: C, 72.63; H, 4.67; N, 22.43.

A $8.00 \times 10^{-3}M$ solution of picolinealdehyde 2-quinolylhydrazone was prepared by dissolving 0.4968 grams of reagent in a minimum volume of 0.1*M* HCl with gentle heating. The cooled solution was diluted to 250 ml. with deionized water.

A standard zinc solution, $5.049 \times 10^{-3}M$ was prepared by dissolving 0.3301 gram of pure granular zinc in a minimum volume of dilute hydrochloric acid and diluting the resultant solution to 1 liter with deionized water.

A pH 8.0 buffer was prepared by the addition of concentrated hydrochloric acid to a solution 0.5*M* in tris(hydroxymethyl)aminomethane.

Deionized water was prepared by passing distilled water through a Barnstead Demineralizer equipped with a 0802 type cartridge.

Chloroform and all other reagents were of reagent grade quality.

Procedure. Dissolve the sample containing zinc by appropriate means. Adjust the pH of the solution to approximately 7.

To a 5-ml. aliquot containing from 1.3 to 16 μg. of zinc in a 125-ml. separatory funnel, add at least a 70-fold excess of PAQH, 10 ml. of pH 8.0 buffer, and dilute to approximately 75 ml. with distilled water.

Extract the aqueous solution with three 5-ml. portions of chloroform. Dilute the combined extracts to a volume of 50 ml. with additional chloroform. Measure the fluorescence of the chloroform solution at 535 mμ using an excitation wavelength of 485 mμ.

Determine the amount of zinc in the sample from a calibration curve prepared under identical conditions.

RESULTS AND DISCUSSION

Characteristics of the Reagent. The reagent, picolinealdehyde 2-quinolylhydrazone, is slightly soluble in organic solvents, insoluble in water, but soluble in dilute acidic solution. A stable dihydrochloride salt can be obtained as a bright yellow solid from glacial acetic acid upon treatment with gaseous hydrogen chloride. The reagent is readily extractable out of aqueous solution into chloroform over the pH range of 3.65 to 11.60. At pH 8.0, a distribution coefficient of 96 was calculated.

The reagent in chloroform exhibits weak fluorescence (Figure 1). The fluorescence is minimal relative to the strongly fluorescent zinc complex. In the analytical procedure, the fluorescence of excess reagent remains constant.

The reagent acts as a nonselective chelating agent toward many metal ions. Colored reaction products are formed with divalent cobalt, copper, iron, nickel, palladium, and zinc ions. Fluorescence is exhibited by the zinc complex. All of the reaction products are insoluble in chloride solutions and are readily extractable into common nonaqueous solvents.

Characteristics of the Zinc Complex. Zinc ion forms a water-insoluble complex with the reagent in the presence of chloride ion. The complex, with an experimentally determined empirical formula of $Zn(PAQH)Cl_2$, is soluble in benzene, carbon tetrachloride, chloroform, dimethylsulfoxide, isoamyl alcohol, and nitrobenzene. Conductance data obtained on the solid dissolved in dimethylsulfoxide indicate that the complex is essentially nonionic and undissociated in this solvent.

The zinc complex is readily extractable out of basic solution into chloroform. The resultant chloroform extract exhibits a high fluorescence intensity. Maxima in the excitation and fluorescence spectra occur at 485 mμ and 535 mμ, respectively (Figure 1). Fluorescence spectra of the solid complex dissolved in chloroform and of the complex extracted out of aqueous solution into chloroform were identical. It is assumed that the extracted fluorescent species is $Zn(PAQH)Cl_2$.

The distribution coefficient for the extraction of the zinc complex out of aqueous solution at pH 8.0 into chloroform was about 11. Three successive extractions with 5-ml. portions of chloroform are sufficient for the recovery of 99% of the complex from an aqueous volume of 75 ml.

Effects of pH and Excess Reagent. A pH study (Figure 2) showed that the zinc complex exhibits the highest fluorescence intensity at a pH of 8.9. A relatively sharp decrease in fluorescence intensity was observed at both higher and lower pH values.

At pH 8.9, maximum and reproducible fluorescence intensity was obtained over a 200- to 500-fold excess of reagent over zinc ion. At pH 8.0, maximum intensity was obtained with a 67:1 ratio of reagent to metal ion. The lower pH is to be preferred since less reagent is required.

Effect of Zinc Concentration. The zinc–picolinealdehyde–2-quinolylhydrazone complex displayed a linear relationship between concentration and fluorescence intensity over the concentration range of 0.404 to $4.847 \times 10^{-6}M$ (0.026 to 0.31 p.p.m.) zinc ion. Negative deviation from linearity was

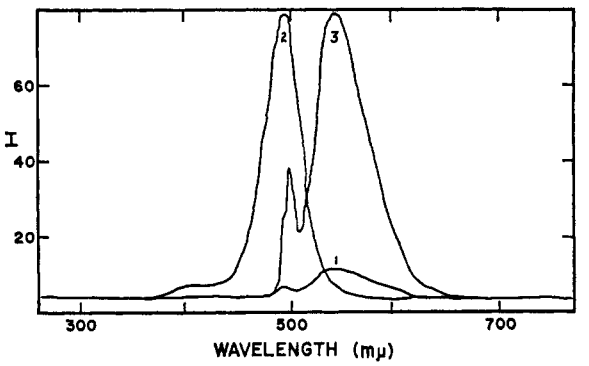

Figure 1. Fluorescence spectra of picolinealdehyde 2-quinolylhydrazone, each solution 7.775×10^{-4}M, pH 8.0

(1) Fluorescence of picolinealdehyde 2-quinolylhydrazone, excitation wavelength—485 mμ
(2) Excitation spectrum of zinc complex, 4.039×10^{-6}M, fluorescence wavelength—535 mμ
(3) Fluorescence spectrum of zinc complex, 4.039×10^{-6}M, excitation wavelength—485 mμ
The spectra are uncorrected

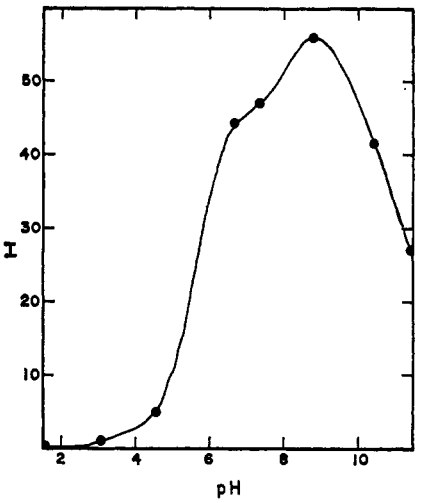

Figure 2. Effect of pH on formation and extraction of zinc–picolinealdehyde 2-quinolylhydrazone complex

1×10^{-4}M PAQH and 6×10^{-6}M zinc
Wavelength settings, 485 mμ and 535 mμ

observed at higher zinc concentrations. Concentration quenching may be the limiting factor in the applicable range of zinc concentration.

Effect of Diverse Ions. The effects of diverse ions on the formation, extraction, and measurement of the zinc complex are presented in Table I. Relative errors were calculated as indicated.

A number of metal ions interfere in the formation and measurement of the zinc complex. Cadmium, cobalt, copper, iron, mercury, and nickel ions form colored reaction products with the reagent. Reagent is consumed and although the complexes do not fluorescence, they are extracted into chloroform and absorb the excitation radiation. The addition of EDTA to eliminate metal ion interferences was detrimental to the formation of the zinc complex.

Both cyanide and thiocyanate ions enhance the fluorescence intensity of the zinc complex. However, extremely high concentrations of cyanide ion, sufficient to mask the interference of the above metal ions served to destroy the zinc complex also. Other common anions have little effect upon the system.

Analysis of Synthetic Zinc Samples. Results on synthetic samples, employing the recommended procedure, are presented in Table II. These data demonstrate that in the absence of interfering ions, the method is applicable to the determination of zinc in microgram quantities.

Table I. Effect of Diverse Ions on Fluorescence Intensity of the Zinc Complex

Zinc concentration = 0.105 p.p.m.

Ion	Concentration, p.p.m.	Source	Relative error, %[a]
Fe^{+2}	98	$Fe(ClO_4)_2$	Emulsion
Co^{+2}	104	$Co(NO_3)_2$	>−40
Ni^{+2}	100	$NiCl_2$	>−40
Cu^{+2}	100	$Cu(NO_3)_2$	>−40
Au^{+3}	184	$HAuCl_4$	Ppt. in $CHCl_3$
Pt^{+4}	48	H_2PtCl_6	−1.9
Mn^{+2}	102	$MnSO_4$	0
Ca^{+2}	99	$CaCl_2$	−1.9
Mg^{+2}	101	$MgCl_2$	−1.9
Al^{+3}	87	$AlCl_3$	−3.8
Cd^{+2}	100	$CdSO_4$	>−30
Hg^{+2}	98	$HgCl_2$	>−30
Cr^{+3}	108	$CrCl_3$	0
NH_4^+	99	NH_4Cl	+1.9
$S_2O_3^{-2}$	101	$Na_2S_2O_3$	−1.9
F^-	98	KF	−0.9
PO_4^{-3}	102	K_2HPO_4	−1.8
CN^-	99	KCN	+18
SCN^-	96	KSCN	+2.8

[a] Rel. error $= \dfrac{\text{I observed} - \text{I standard}}{\text{I standard}}$

Table II. Results on the Analysis of Selected Zinc Samples

Sample	Zinc present, μg.	Zinc found, μg.[a]
1	1.3	1.3
2	2.6	2.6
3	5.2	5.4
4	10.4	10.4
5	15.6	15.4

[a] Average of 3 determinations.

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WORK supported by the National Science Foundation. First Midwest Meeting, The American Chemical Society, Kansas City, Mo., Nov. 5, 1965.

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