Determination of trace amounts of Mercury (II) by Total Fluorescence Quenching using 1,10-phenanthroline and eosin

Application Note

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Introduction

Mercury in various forms constitutes a serious environmental pollutant;[1,2] hence there is the need for its detection and determination, particularly at trace levels. The most widely used procedure for the determination of mercury is cold-vapour atomic absorption spectrometry (cold vapor AAS). Although AAS may suffer from some interferences, detection and determination of mercury at extremely low levels (0.02 µg/L) has been achieved by the use of this method.[3] The traditional approach to the determination of Hg(II) is to use spectrophotometric methods. Most of these methods involve extraction of a mercury-chelating agent complex into an organic phase followed by measurement of its absorbance.[4-7] These procedures are cumbersome and are limited by the solubility of the ligand and the complex in aqueous medium. Although analytical methods based on ternary complex formation can offer superior sensitivity and selectivity, the limitations aforementioned may make them unattractive. These limitations have been overcome by the introduction of micellar systems,[8,9] which avoid the extraction step.

A molecular-absorption spectrophotometric procedure for the trace determination of Hg(II) ions in micellar medium has been described.[8] This method involves the formation of a ternary complex, 1,10-phenanthroline-mercury(II)-eosin with empirical composition $[Hg(o\text{-}phen.)_2]R$ where R represents a divalent eosin anion. Frei, et. al[10] noted that the sensitivity of Ag(I) measurement based on ternary complexes of eosin, silver(I) and o-phenanthroline can be enhanced by the use of the fluorescence-quenching phenomenon in aqueous solution. Since selective quenching effect was observed in their system, a similar effect would be expected for the $[Hg(o\ phen.)_2]R$ ternary complex in a micellar medium.

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In order to improve the molecular-absorption spectrophotometry of [Hg(o-phen)2]R in micellar media, the fluorescence-quenching phenomenon observed with PVA solubilized 1,10-phenanthroline-Hg(II)-eosin system was investigated. The detection limit in this work (0.01 mg/L) is not comparable to that of cold vapour AAS; however, it is lower than that reported for the molecular absorption spectrophotometry.[8] We report here a simple, rapid and sensitive spectrofluorimetric method for the determination of Hg(II) ions in a semi-micellar medium.

Experimental

Equipment

- Cary 5E UV-Vis-NIR spectrophotometer fitted with a Cary Total Fluorescence Accessory.
- pHScan 2 pH meter.

Reagents

- Hg(II) stock solutions, 50 mg/L and 5 mg/L.
- 1,10-phenanthroline, 2.5 x 10^{-3} M.
- 2,4,5,7-tetrabromofluorescein, 7.3 x 10^{-4} M (eosin).
- Ethylenediamine tetraacetic, disodium salt, 0.05 M.
- Polyvinyl alcohol (PVA) solution (freshly prepared), 1.0%.
- Acetate buffer (pH 4.5)
- Deionized (Milli Q) water was used.

Procedure

A series of solutions containing aliquots of Hg(II) ions (0.1-10 µg) was prepared containing 1mL of 0.05 M EDTA solution, 5 mL of acetate buffer (pH 4.5), 5mL of 2.5 x 10^{-3} M 1,10-phenanthroline solution and 1ml of freshly prepared 1% PVA solution. The solutions were mixed thoroughly and then 1 mL of 7.3 x 10^{-4} M eosin solution was added. The contents were again mixed well and diluted to the mark in 25 mL volumetric flasks with Milli Q water. After mixing the solutions, aliquots of each solution were pipetted into the mirrored 10 mm cuvette.

The total fluorescence was measured using the excitation wavelength, lex = 509 nm. No standing time was necessary.

The order of addition of the reagents, both for analysis and for interference studies is metal ion(s) followed by the solutions of EDTA, buffer, 1,10-phenanthroline, PVA and finally eosin.

Results and discussion

Factors affecting fluorescence

The ratio of sample to blank intensity is essentially constant over the pH range 4.0-4.7, with maximal quenching over the range 4.3-4.7.

The fluorescence intensity is reagent concentration dependent. At high eosin concentration, the phenomenon of self-absorption is observed which also affects sensitivity, hence as a compromise, 1 mL of eosin solution was selected. On the other hand, as large a concentration as possible of eosin and an excess of 1,10-phenanthroline with respect to Hg(II) would give better results. (Figures 1 and 2 indicate the difference in the self absorption).

The nature of the complex was investigated. Earlier studies had shown that under experimental conditions in the presence of excess phenathroline, bis (phenanthroline) complex formation was favored (i.e. $HgCl_2(phen)_2$).[8,11]

Applying the continuous variation (Job's plot) and the mole ratio plot method, we confirmed that the mole ratio of mercury to phenanthroline is 1:2; while a 1:2 ratio of the counter ion (eosin) to Hg(II) was determined. In the presence of Hg(II), the ratio of henanthroline to the eosinate counter-ion is thus 4:1.

Fluorescence spectra

Figure 1 shows the excitation spectra of varying concentrations of mercury(II)-1,10-phenanthroline-eosin complex at pH 4.5. The excitation peak maximum is at 509 nm. The excitation spectrum of eosin only or in combination with 1, 10-phenanthroline is similar.

The addition of small microgram amounts of Hg(II) ions to eosin solution produces no change in the spectrum; but when Hg(II) ions (5 mg/L) and phenanthroline are added to the eosin solution, a sharp decrease in fluorescence intensity of the dye at 509 nm occurs (Figure 3A, (a) and (b)). This shows that fluorescence quenching of eosin by Hg(II) ions takes place only in the presence of 1,10-phenanthroline. Figures 3A and 3B compare the absorption and excitation spectra under these experimental conditions.

The reaction between Hg(II), phenanthroline and eosin is instantaneous, resulting in the formation of a precipitate on standing, which is solubilized by the addition of 1 mL of 1% PVA solution. Except for the effect of PVA which was optimized to be 1 mL in 25 mL, 5 mL of phenanthroline and 1 mL of eosin, the other experimental variables (EDTA and buffer) were used as recommended previously.[8]

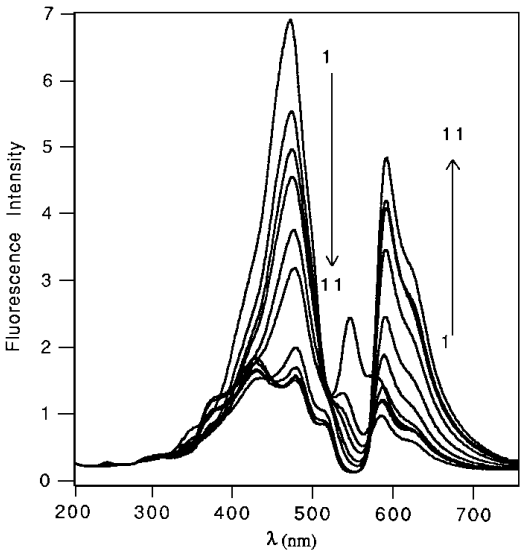

Figure 1. Excitation spectra of 1,10-phenanthroline-eosin at varying concentrations of mercury(II) (high eosin concentration, 5 mL) showing self-absorption {curve 1 = 0 mg/L Hg(II); curves 2 to 11 = 0.3 to 13 mg/L}

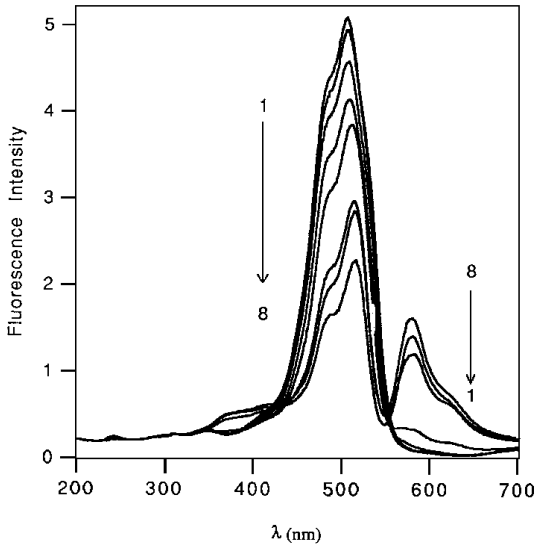

Figure 2. Excitation spectra of 1,10-phenanthroline-eosin at varying concentrations of mercury(II) (low eosin concentration, 1 mL) {curve 1 = 0 mg/L Hg(II); curves 2 to 8 = 0.3 to 13 mg/L}

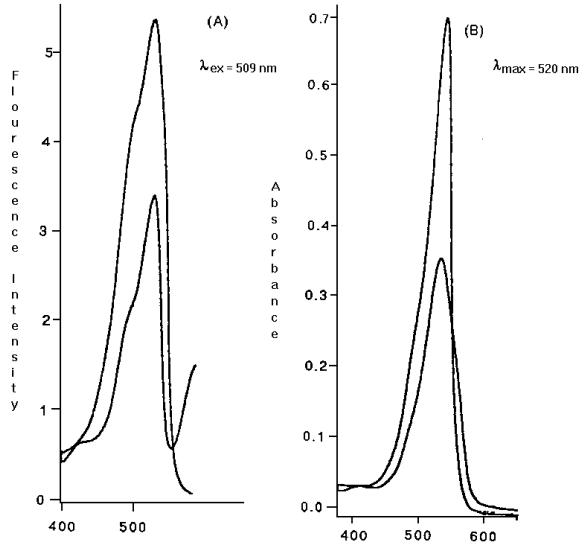

Figure 3. Excitation (A) and Absorption spectra (B) of 1,10-phenanthroline-eosin in the presence (a) and absence (b) of Hg(II) ions

Interference studies

The effect of foreign ions on the fluorescence intensity of Hg(II)-phenanthroline-eosin complex was investigated. The addition of up to 500-fold EDTA excess over Hg(II) did not affect the reaction, so EDTA was used as a mass-masking agent. In the presence of EDTA, the interference of cations which would normally interact with 1,10-phenanthroline were examined, e.g. Cu(II), Fe(II), Fe(III), Al(III) Pb(II). The tolerance limits for these ions were100-fold molar excess at a Hg(II) concentration of 5 mg/L. On the other hand, only 0.5 mg/L of Ag(I) can be tolerated; hence its interference must be removed by extraction for this method to be applicable to Hg(II) determination.

Calibration curve, limit of detection and precision

The calibration curve was prepared by plotting the concentration (mg/L) of Hg(II) against the reciprocal of fluorescence intensity at 509 nm (figure 4a), while figure 4b indicates the plot against the relative fluorescence intensity. A linear relationship was obtained over the range 4.98 x 10^{-7} to 2.49 x 10^{-5} M of Hg(II) corresponding to 0.10 to 5 mg/L.

The reproducibility of the method was obtained by analyzing 10 replicates containing 5 mg/L of Hg(II). The relative standard deviation was 1.0% with a detection limit of 0.01 mg/L.

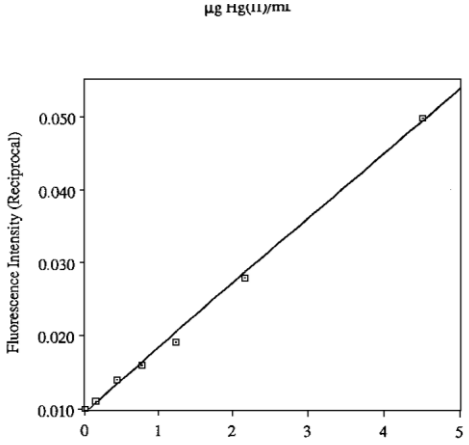

Figure 4a. Calibration: Fluorescence - Hg(II) concentration dependence - fluorescence intensity (0-5 mg/L)

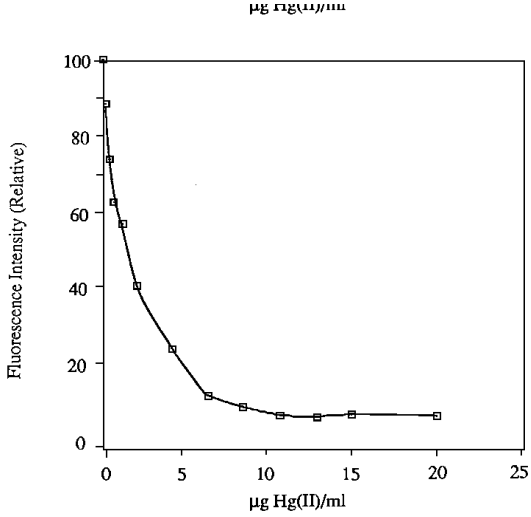

Figure 4b. Calibration: Fluorescence - Hg(II) concentration dependence - reciprocal of fluorescence intensity (0-20 mg/L)

Conclusion

Using the Cary 5E Fluorescence Accessory, an analytical procedure (not limited by the sensitivity of the instrument) for the indirect fluorimetric trace determination of Hg(II) ions in micellar medium has been developed, with a detection limit of 0.01 mg/L which is >10 times more sensitive than the molecular-absorption spectrophotometry previously reported. The proposed method is simple, reasonably sensitive and rapid. Most interferences could be removed by masking, while the most troublesome ones [Ag(I), Cu(II)] would have to be eliminated by extraction techniques. The method is potentially useful for the analysis of Hg(II) in water samples and factory effluent.

Although cold vapour AAS is the most important technique for mercury analysis, it requires specialized instruments. The increasing importance of mercury and its toxicity in environmental health requires the development of simple and rapid analytical methods for its determination. The other advantage of this method over the absorbance method is that a large excess of phenanthroline is not necessary for fluorescence quenching to take place.

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