EXTRACTION-FLUORIMETRIC DETERMINATION OF TRACE AMOUNTS OF MERCURY BY 1,10-DIAZA-18-CROWN-6 AND ROSE BENGAL

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ABSTRACT : *A simple and sensitive extraction-fluorimetric method for the determination of mercury is reported. The method is based on the quantitative extraction of a ternary crown-mercury-rose bengal complex into chloroform at pH 10 followed by fluorimetric measurements at 580 nm. A linear working range from 0.05 to 2.00 μg/mL is obtained. The relative standard deviation for 1.0μg/mL mercury is 3.5%. The procedure was successfully applied to the determination of mercury in fish and mercurochrome.*

KEY WORDS : *Mercury, Determination, 1,10- Diaza-18-crown-6, Rose bengal, Extraction, Fluorimetry.*

INTRODUCTION

Previous studies on the complex formation between macrocyclic crown ethers and different metal ions revealed that the 18- membered crowns form fairly stable complexes with some alkali, alkaline earth and heavy metal ions such as K^+, Ba^{2+}, Tl^+, Cd^{2+}, Pb^{2+} and Hg^{2+} [1-3]. The resulting 1:1 complexes are extractable into organic solvents using strongly coloured or fluorescent counter-ions [4-6]. Such extraction processes have been successfully employed in the determination of trace amounts of metal ions [7-13].

Because of the widespread use of mercury compounds in agriculture and industry, large amounts of Hg enter the environment as a serious pollutant. Thus, the assay of micro levels of mercury has received large attention [14-16] due to its accumulative effect and high toxicity

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[17,18]. In this paper we report a simple, rapid and sensitive method for the determination of traces of mercury by quantitative extraction of its ternary complex with 1,10- diaza-18- crown-6 and rose bengal into chloroform, followed by fluorimetric measurements at 580 nm. Stractures of the crown ether and the dyestuff are shown in Fig.1.

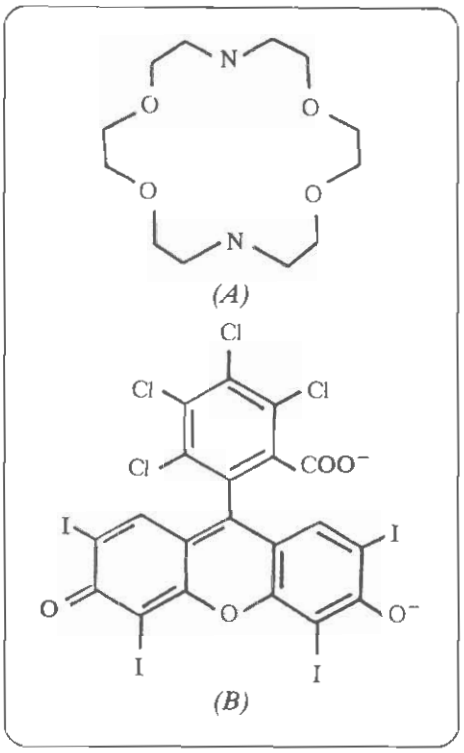

Fig. 1: Structure of DA18C6 (A) and rose bengal (B).

EXPERIMENTAL

Reagents

All chemical were of the highest purity available and used without further purification except for vacuum drying over P_2O_5. Triply distilled, deionized water was used throughout. All volumetric flasks used were already calibrated by weight.

A 1000μg/mL stock solution of mercury(II) was prepared by dissolving 0.2710g of $HgCl_2$ *(Merck)* in a 200mL volumetric flask and diluting to the mark with water. Working solutions were prepared by appropriate dilution of stock solution with water.

Rose bengal (RB) stock solution, 2.0×10^{-4}M, was prepared by dissolving 0.2035g of the dyestuff (*Fluka*, Switzerland) in a 1000mL volumetric flask and diluting to the mark with water.

1,10-Diaza-18-crown-6(DA18C6,Merck) stock solution, 1.0×10^{-3}M, was prepared by dissolving 0.0525g of the crown ether in a 200mL volumetric flask and diluting to the mark with water.

Buffer solution was pH 10 borate buffer (Titrazol).

Apparatus

The fluorescence spectra were recorded on a RF 5000 spectrofluorometer and the fluorescence intensity measurements were made with a *Perkin-Elmer* LS-2B filter fluorimeter. Measurements of pH were made with a Corning 125 pH meter using a combined *Metrohm* electrode (6.0202.000).

Procedure

An aliquot of the sample solution containing 0.5-20μg of Hg(II) ion was placed in a 10mL volumetric flask. Two mL of 1.0×10^{-3}M DA18C6, 1.0mL of 2.0×10^{-4}M rose bengal and 0.4mL buffer pH 10 were added and the solution was diluted to the mark with water. The solution was then transferred into a 60mL separatory funnel, 10mL chloroform was added and the mixture was shaken vigorously for 5 min. The phases were allowed to separate and the fluorescence intensity of the organic phase was measured at 580nm against a reagent blank.

RESULTS AND DISCUSSION

Distribution coefficient of DA18C6 and the overall extraction constant

It is well known that DA18C6 forms a fairly stable 1:1 complex with Hg^{2+} ion in aqueous solution with log K_s=17.85 [19]. Application of a strongly fluorescent anionic dye such as rose bengal could lead to a sensitive and useful method for the trace metal ion determination.

The distribution coefficient of DA18C6 was determined in the crown concentration range $10^{-3}-10^{-4}$M. The aqueous solution of crown

ether (10mL) and 10mL of chloroform were shaken in a separatory funnel for 5 min. After the separation of phases, the crown concentration in the aqueous phase was determined by a spectrophotometric titration method [20]. The value of the distribution coefficient, D_L , was calculated as 2.45.

The overall extraction equilibrium can be written as:

$$Hg^{2+} + (DA18C6)_O + RB^{2-} \overset{K_{ex}}{\rightleftharpoons} (HgDA18C6\ RB)_O \qquad (1)$$

where the subscript O and the lack of subscript designate the organic and the aqueous phases, respectively. The following equilibria must be considered in the extraction process:

$$DA18C6 \overset{D_L}{\rightleftharpoons} (DA18C6)_O$$

$$Hg^{2+} + DA18C6 \overset{K_s}{\rightleftharpoons} HgDA18C6^{2+}$$

$$HgDA18C6^{2+} + RB^{2-} \overset{D_c}{\rightleftharpoons} (HgDA18C6RB)_O$$

$$(HgDA18C6RB)_O \overset{K_D}{\rightleftharpoons} (HgDA18C6^{2+})_O + (RB^{2-})_O$$

The overall extraction constant, K_{ex} , is expressed by $K_{ex}=K_sD_c / D_L$. Under the experimental conditions used, it can be assumed that $[HgDA18C6^{2+}] >> [Hg^{2+}]$ and $[HgDA18C6^{2+}] \approx C^o_{Hg} - [HgDA18C6RB]_O$, where C^o_{Hg} is the total concentration of Hg^{2+} ion. In order to calculate $[HgDA18C6RB]_O$, K_D has to be known. If it is assumed that there is no dissociation of the ternary complex in the organic phase (i.e. $K_D=0$), one can write $[(HgDA18C6RB)_O]=\Delta I/A=I_i$, where ΔI is the fluorescence intensity of the organic phase measured against a reagent blank and A is the fluorescence constant of the ternary complex.

An expression for the apparent D_c , D'_c , can be derived as:

$$D'_c = I_i / (C^o_{Hg} - I_i)(C^o_{RB} - I_i) \qquad (2)$$

when the D'_c value is independent of crown / RB ratio, the assumption of $K_D=0$ is true and $D_c=D'_c$. Otherwise, a trial-and-error method must be employed to calculate D_c. In this work, an expression similar to that derived by *Frensdorff* [21] used to evaluate the values of D_c and K_D:

$$D_c = [2I_i + K_D - (K^2_D + 4K_DI_i)^{1/2}] / [2(C^o_{Hg}-I_i)(C^o_{RB}-I_i)] \qquad (3)$$

The resulting values of D_c and K_D are $\log D_c = 5.09$ and $\log K_D = -17.4$. Thus, the overal extraction constant was calculated as $\log K_{ex}=22.55$.

Effect of variables on the extraction

The fluorescence emission spectra of $Pb^{2+}-DA18C6-RB^{2-}$ ternary complex and the corresponding reagent blank in the organic phase are shown in Fig.2. As it is seen, the complex shows a maximum at 580nm, where the reagent blank has a negligible fluorescence at this wavelength.

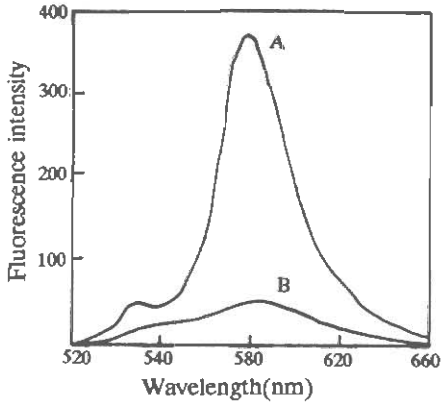

Fig. 2: Fluorescence spectra of the ternary complex (A) and the reagent blank (B) at λ_{ex}=55nm.

The effect of pH on the extraction was studied over the pH range 6-11 and the results are given in Fig.3. It is seen that maximum extraction occurs in the pH range of 7-10. Outside this range, the extent of extraction decreases drastically. At pH values>10, the relatively strong competitive effect of hydroxyl ion with rose bengal for mercury would be the main reason for the decreased extraction. On the other hand, at pH values<7, as the pH decreases

the fraction of RB^{2-} decreases, and so does the percentage of extraction. A pH of 10 was chosen as the optimum working pH, because of the minimum fluorescence intensity of the reagent blank at this pH. It was found that addition of 0.4mL of the borate buffer solution is adequate to reach the best extraction efficiency.

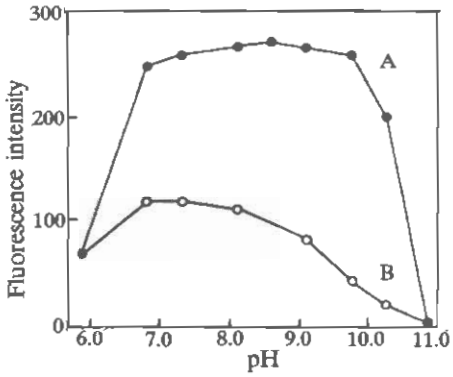

Fig. 3: Effect of pH on the extraction of mercury; sample (A), blank (B). Conditions: Hg^{2+}, 1.0μg/mL; rose bengal, 0.5mL of 2.0×10^{-4}M; DA18C6, 0.5mL of 1.0×10^{-3}M.

The ionic strength of aqueous phase, adjusted by NaCl and $NaNO_3$, was found to influence the extraction of mercury with DA18C6 and rose bengal into chloroform. There is an inverse relationship between the extent of extraction and the ionic strength of the aqueous phase. Similar results have already been reported for the extraction of the ternary complexes of K^+ ion with 18- crowns and dye molecules into organic solvents [13,22].

The influence of rose bengal and DA18C6 concentrations on the extraction of Hg^{2+} ion was studied and the results are shown in Figs. 4 and 5, respectively. As it is seen, the fluorescence intensity of the ternary complex in the organic phase, relative to that of the corresponding reagent blankes, increases with an increase in both rose bengal and DA18C6 concentrations in the aqueous phase. Maximum extraction occurs when the reagent to mercury molar ratio is about 2, for rose bengal, and about 20, in the case of DA18C6. A further excess of both reagents has no considerable effect on the ΔI measured.

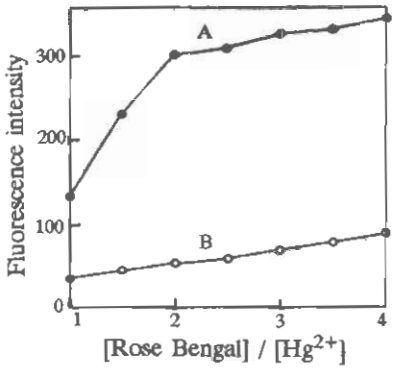

Fig. 4: Effect of rose bengal concentration on the extraction of mercury; sample (A), blank (B). Conditions: Hg^{2+}, 1.0μg/mL; DA18C6, 0.5mL of 1.0×10^{-3}M; buffer, 0.4mL pH 10.

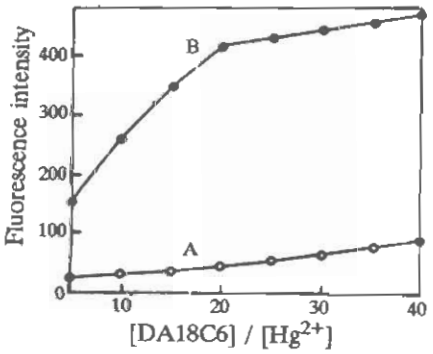

Fig. 5: Effect of DA18C6 concentration on the extraction of mercury; sample (A), blank (B). Conditions: Hg^{2+}, 1.0μg/mL; rose bengal, 0.5mL of 2.0×10^{-4}M; buffer, 0.4mL pH 10.

The extraction of Hg^{2+} ion with DA18C6 and rose bengal under the conditions recommended in the procedure is rapid. A shaking time of 3-5 min. was found sufficient for the extraction. Longer shaking times did not show any considerable effect on the fluorescence intensity measured.

Calibration

Under the optimum conditions described above, a linear calibration graph of ΔI vs$[Hg^{2+}]$ was obtained in the concentration range 0.05-2.00μg/mL with a correlation coefficient of 0.9984 (11 experimental points) and a regression equation of $\Delta I=37+450\,[Hg^{2+}]$. The relative standard deviation of twelve replicate measurements is 3.5% for a 1μg/mL mercury solution.

Effect of diverse ions

In order to determine the sensitivity and utility of the proposed method, the recommended procedure for the extraction of mercury was followed in the presence of different amounts of several cations and anions. The results are given in Table 1. As it is seen, all anions and most of the cations used have no considerable effect on the determination of Hg^{2+} ion. However, Pb^{2+} ion was found to interfere seriously.

Application

In order to assess the applicability of the proposed method to the real samples, it was applied to the determination of mercury in fish and a pharmaceutical preparation (mercurochrome).

The lypophilized fish samples were digested with the least amount of concentrated sulfuric acid followed by addition of hydrogen peroxide for clearity of solution. Excess of hydrogen peroxide was then removed with potassium permangenate. The pH of solution was adjusted to 10 and, after appropriate dilution, the recommended procedure was followed. The experiment was repeated three times and the results were averaged. The concentration of

Table 1 : Effect of foreign ions (FI) on the determination of 1μg/mL of mercury.

Ion	FI:Hg (molar ratio)	Apparent recovery,%	Ion	FI:Hg (molar ratio)	Apparent recovery,%
Na^+	2000	100	Cr^{3+}	50	92
K^+	2000	100			
Cs^+	2000	101		20	98
Ag^+	100	92	Hf^{4+}	10	87
	10	98		3	96
Hg^{2+}	50	112	SO_3^{2-}	50	81
				20	98
	10	103	SO_4^{2-}	50	88
				20	99
Ni^{2+}	1000	98	CO_3^{2-}	50	90
Pd^{2+}	50	113		20	96
	3	103	PO_4^{3-}	50	90
Cu^{2+}	50	80		8	96
	10	92			
	4	96	ClO_4^-	50	89
				10	98
CO^{2+}	100	80			
	10	96	BrO_3^-	50	91
Zn^{2+}	10	104		25	96
			IO_4^-	10	93
Pb^{2+}	1	198		5	96
			SCN^-	100	75
Ce^{3+}	20	104		15	96
Al^{3+}	10	85	F^-	3500	95
	3	96	Tartrate	2000	97

mercury in the fish sample was 2.2±0.2μg/g which is in satisfactory agreement with that obtained by cold vapor AAS (2.4±0.1μg/g).

Mercury concentration in mercurochrome (merbromine) preparation was determined by the proposed method. After the appropriate dilution of the sample with water, the pH of solution was adjusted to 10 and the recommended procedure was followed. The concentration of mercury in the stock sample solution, obtained from three different determinations, was 132±6μg/mL which agrees well with that obtained by cold vapor AAS (130±5μg / mL).

CONCLUSIONS

The method proposed is simple, rapid and sensitive. It compares very favorably with most published methods for the determination of mercury by use of ion- association compounds [14,23,24], and it can certainly be placed among the most sensitive.

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