

Determination of Trace Mercury by Solid Substrate-Room Temperature Phosphorimetry Quenching Method Based on Catalytic Effect of Hg^{2+} on Formation of the Ion Association Complex $[\text{Sn}(\text{XO})_6]^{4+} \cdot [(\text{Fin})_4]^-$

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Abstract A new method for the determination of trace mercury by solid substrate-room temperature phosphorimetry (SS-RTP) quenching method has been established. In glycine-HCl buffer solution, xylenol orange (XO) can react with Sn^{4+} to form the complex $[\text{Sn}(\text{XO})_6]^{4+}$. $[\text{Sn}(\text{XO})_6]^{4+}$ can interact with Fin^- (fluorescein anion) to form the ion associate $[\text{Sn}(\text{XO})_6]^{4+} \cdot [(\text{Fin})_4]^-$, which can emit strong and stable room temperature phosphorescence (RTP) on polyamide membrane (PAM). Hg^{2+} can catalyze H_2O_2 oxidizing the ion association complex $[\text{Sn}(\text{XO})_6]^{4+} \cdot [(\text{Fin})_4]^-$, which causes the RTP to quench. The ΔIp value is directly proportional to the concentration of Hg^{2+} in the range of 0.016–1.6 fg spot^{-1} (corresponding concentration: 0.040–4.0 pg ml^{-1} , 0.40 $\mu\text{l spot}^{-1}$), and the regression equation of working curve is $\Delta\text{Ip} = 10.03 + 83.15 \text{ mHg}^{2+}$ (fg spot^{-1}), ($r = 0.9987$, $n = 6$) and the detection limit (LD) is 3.6 ag spot^{-1} (corresponding concentration: $9.0 \times 10^{-15} \text{ g ml}^{-1}$, the sample volume: 0.4 μl). This simple, rapid, accurate method is of high selectivity and good repeatability, and it has been successfully applied to the determination of trace mercury in real samples. The reaction mechanism for catalyzing H_2O_2 oxidizing the ion associ-

ation complex $[\text{Sn}(\text{XO})_6]^{4+} \cdot [(\text{Fin})_4]^-$ SS-RTP quenching method to determine trace mercury is also discussed.

Keywords Mercury · Ion association complex $[\text{Sn}(\text{XO})_6]^{4+} \cdot [(\text{Fin})_4]^-$ · Catalytic · Solid substrate-room temperature phosphorescence quenching method

Introduction

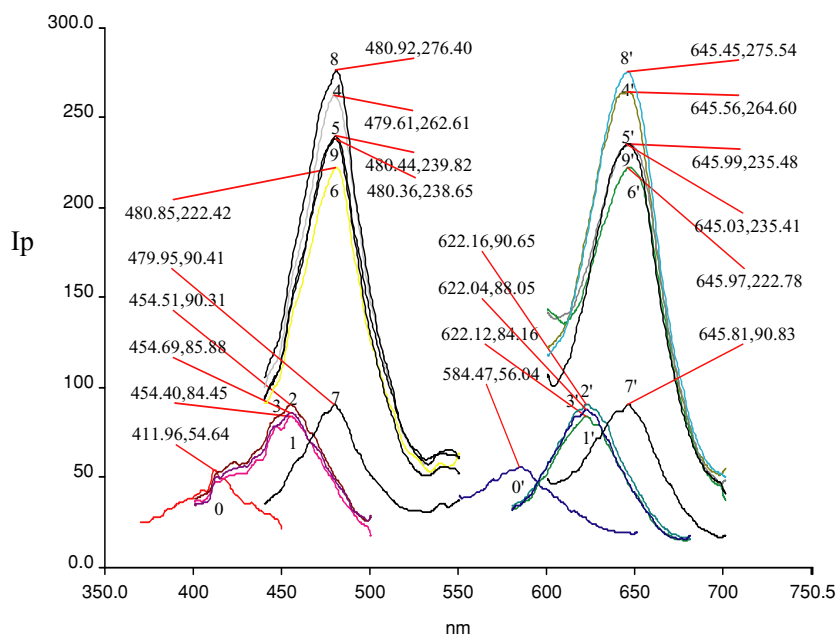
Since mercury and some of its compounds are toxic and they would do great harm to the organs. When they come into human body and accumulate in nerve center, liver and kidney, they will damage and poison related apparatus in human body. Therefore, it is of great significance to determine mercury in fields such as environment, food, medicine, biological science, etc. There have been many methods to determine trace mercury in recent years, such as atomic fluorescence spectrometry (LD: $2.0 \times 10^{-10} \text{ g ml}^{-1}$) [1], inductively coupled plasma atomic emission spectrometry (ICP-AES) (LD: $1.08 \times 10^{-8} \text{ g ml}^{-1}$) [2], flow-injection-vapor generated-non-chromatic dispersion atomic fluorescence spectrometry (FI-VG-AFS) (LD: $5.0 \times 10^{-11} \text{ g ml}^{-1}$) [3], catalytic spectrophotometer (LD: $3.0 \times 10^{-10} \text{ g ml}^{-1}$) [4] and flow-injection catalytic spectrophotometer (LD: $2.0 \times 10^{-8} \text{ g ml}^{-1}$) [5], etc. But the sensitivities of these methods are low, so it is of great significance and academic value for chemists to seek a new method for the determination of trace mercury that has higher sensitivity, repeatability and selectivity, and is accurate, rapid and easy to operate. The method of SS-RTP has been widely applied to the determination of super trace Sn [6], Hg [7], Ag [8], and Ti [9] because of many advantages such as long lifetime, wide Stokes' displacement, light perturbation, good selectivity, high sensitivity and so on. Our research indicated that in

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Fig. 1 Room temperature phosphorescence (RTP) spectra for the system



glycine-HCl buffer solution, XO can react with Sn^{4+} to form complex $[\text{Sn}(\text{XO})_6]^{4+}$ [10]. Meanwhile, Hg^{2+} has a catalytic effect on the reaction of H_2O_2 oxidizing ion association complex $[\text{Sn}(\text{XO})_6]^{4+} \cdot [(\text{Fin})_4]^-$ which can emit strong and stable room temperature phosphorescence (RTP) on polyamide membrane (PAM) to cause RTP quenching. Based on the facts above, a new method for the determination of trace mercury by solid substrate-room temperature phosphorimetry (SS-RTP) quenching method has been established. The reducing value of phosphorescence intensity (ΔI_p) is directly proportional to the concentration of Hg^{2+} in the range of 0.016–1.6 fg spot^{-1} (0.40 $\mu\text{l spot}^{-1}$, corresponding concentration: 0.040–4.0 pg ml^{-1}), and the regression equation of working curve is $\Delta I_p = 10.03 + 83.15 \text{ m Hg}^{2+}$ (fg spot^{-1}), $r = 0.9987$, $n = 6$. And the detection limit is 3.6 ag spot^{-1} (0.4 μl , corresponding concentration: $9.0 \times 10^{-15} \text{ g ml}^{-1}$), which is 5.6×10^3 times lower than that of FI-VG-AFS [3]. This simple, rapid, accurate, selective and reproducible method has been applied to the determination of trace mercury in real samples with satisfactory results. And the determination of trace mercury by SS-RTP quenching method is rarely reported before.

Experiment

Apparatus and reagents

LS-55 luminescence spectrophotometer (Perkin Elmer Corporation. Main parameters are: delay time: 0.1 ms, gate time: 2.0 ms, cycle time: 20 ms, flash count: 1, Ex. Slit: 10 nm, Em. Slit: 10 nm, scan speed: 1500 nm min^{-1}); solid sample shelf (Perkin-Elmer) 85-1 constant temperature mag-

netic stirrer (Shenzhen Tiannanhai Company); 0.5 μl flat head micrometer syringe (Shanghai Medical Laser Instrument Plant).

Hg^{2+} working solution: Hg^{2+} primary standard solution (GSBG 62039-90 4701) is diluted to 100 pg ml^{-1} as working solution. $1.0 \times 10^{-4} \text{ mol L}^{-1}$ XO solution, glycine-HCl buffer solution (pH = 1.1), Sn^{4+} (2.0 g L^{-1}), 1% (w/v) cetyltrimethyl ammonium bromide (CTMAB), $1.0 \times 10^{-4} \text{ mol l}^{-1}$ fluorescein, 0.3% (w/v) H_2O_2 solution.

Filter paper was purchased from XinHua Paper Corporation (Hangzhou, China). Polyamide membrane, acetic acid cellulose membrane and nitric acid cellulose membrane were purchased from LuQiaoSiJia biochemical plastic plant (Hangzhou, China).

Experiment method

Phosphorescent chemical reactions

Certain amount of Hg^{2+} (1.0–100 pg), 3.00 ml of glycine-HCl solution, 2.00 ml of XO, 5.00 ml of Sn^{4+} , 1.00 ml of

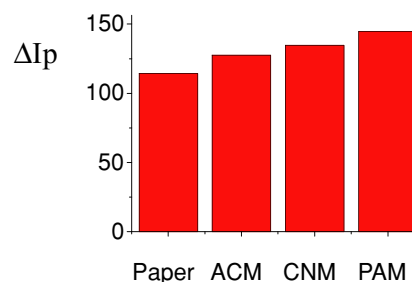


Fig. 2 Effect of solid substrate on ΔI_p for system

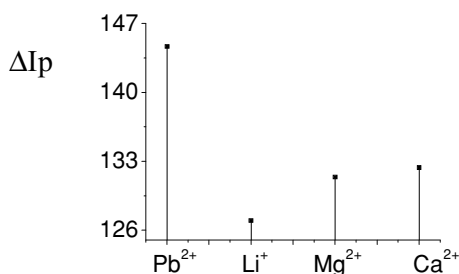


Fig. 3 Effect of ion perturbation on ΔIp for system

CTMAB, 2.00 ml of Fin⁻¹ (fluorescein anion), 1.00 ml of H₂O₂ were added into a 25 ml test tube, mixed homogeneously, then diluted to 25 ml with water. The tube was kept at 60°C for 10 min, and then cooled by flowing water for 5 min until 25°C to stop the reaction.

Measurement of phosphorescence

The PAM was immersed in 1.0 mol l⁻¹ Pb(Ac)₂ solution for 10 s, and dried at 90°C for 2 min. A 0.40 μl drop of test solution and blank solution was suspended onto the center. The phosphorescence intensity was measured directly at wavelengths λ_{ex}/λ_{em} = 480/646 nm. The signal of [Sn(XO)₆]⁴⁺·[(Fin)₄]⁻-H₂O₂ system (without Hg²⁺) was defined as the reagent blank intensity (Ip₁), and the signal of Hg²⁺-[Sn(XO)₆]⁴⁺·[(Fin)₄]⁻-H₂O₂ system was defined as the sample intensity for test solution (Ip). ΔIp (= Ip-Ip₁) was calculated.

Result and discussion

Excitation spectra and emission spectra

The RTP spectra of the system are showed in Fig. 1. The results indicate that in glycine-HCl buffer solution XO can react with Sn⁴⁺ and form complex [Sn(XO)₆]²⁺, which can emit strong and stable RTP on the PAM at λ_{ex}/λ_{em} = 454.51/622.16 nm (Ip = 90.65). [Sn(XO)₆]⁴⁺ and Fin⁻ can form the ion association

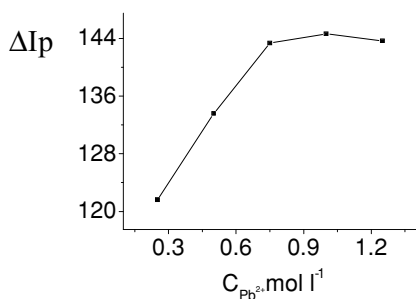


Fig. 4 Effect of C_{Pb²⁺} on ΔIp for the system

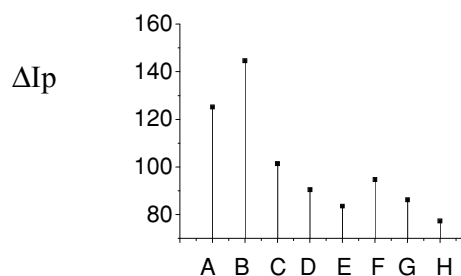


Fig. 5 The effects of different surfactants on ΔIp for the system

complex [Sn(XO)₆]⁴⁺·[(Fin)₄]⁻ (λ_{ex}/λ_{em} = 479.61/645.56, Ip = 264.60). The red shift of λ_{em} is 23.4 nm. H₂O₂ oxidizes the ion association complex [Sn(XO)₆]⁴⁺·[(Fin)₄]⁻, which causes the RTP to quench, and the Ip value declined continuously at λ_{ex}/λ_{em} = 480.44/645.99 nm (Ip = 235.48). Meanwhile, Hg²⁺ can accelerate the quenching of RTP at λ_{ex}/λ_{em} = 479.95/645.81 nm (Ip = 90.83), so 480/646 nm was chosen as the working wavelength. The ΔIp of the system with CTMAB was 3.6 times higher than that without CTMAB, which shows that CTMAB has a spike effect on catalytic action.

Optimum measurement condition for SS-RTP

The concentration and amount of reagents

For the system of 4.00 pg ml⁻¹ Hg²⁺, the amount and concentration of reagents were changed, respectively. The results showed that the optimal volume of reagents were as follows: 1.00 ml of XO, 3.00 ml of glycine-HCl, 5.00 ml of Sn⁴⁺, 1.00 ml of CTMAB, 2.00 ml of Fin⁻ and 1.00 ml of H₂O₂. At this time, the pH value of reaction solution was 1.10 and the ΔIp reached the maximum and remained stable.

The selection of substrate

For the system of 4.00 pg ml⁻¹ Hg²⁺, according to the method described above, effect of four different substrates on the ΔIp of the system was examined. The four kinds of substrates were quantitative filter paper, nitric acid cellulose

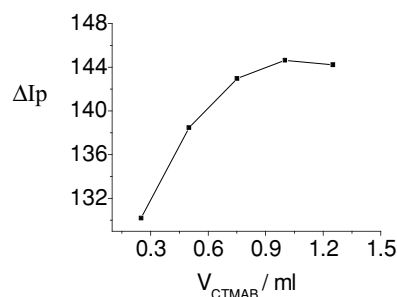


Fig. 6 Effect of V_{CTMAb} (ml) on ΔIp for the system

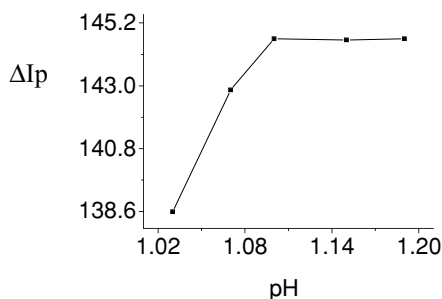


Fig. 7 Effect of pH on ΔI_p for the system

membrane (CNM), acetic cellulose membrane (ACM), and polyamide membrane (PAM), respectively. The results showed that the ΔI_p on PAM was the largest (Fig. 2), so PAM was selected as the substrate in the following experiment.

Heavy atom perturbation

For the system of $4.00 \text{ pg ml}^{-1} \text{ Hg}^{2+}$, the effect of ions such as Pb^{2+} , Li^+ , Mg^{2+} , and Ca^{2+} on ΔI_p of the system were examined, respectively. The results showed that the ΔI_p of Pb^{2+} was the highest, so Pb^{2+} was chosen as ion perturber (Fig. 3). Meanwhile, the effect of the concentration of Pb^{2+} on ΔI_p was examined (Fig. 4). When 1.0 mol l^{-1} of Pb^{2+} was used, the ΔI_p reached the maximum.

Selecting activating agent

For the system of $4.00 \text{ pg ml}^{-1} \text{ Hg}^{2+}$, the effect of different surface reactive agents on ΔI_p were examined. The results showed that among A (polyvinyl alcohol, PVA), B (cetyltrimethyl ammonium bromide, CTMAB), C (Triton X-100), D (Tween-80), E (Polyoxyethylene), F (sodium polypropylene acid, PAA-Na), G (sodium dodecylbenzene sulfate, DBS) and H (cetyl pyridinium chloride, CPC), CTMAB had the maximum quenching effect (Fig. 5). So CTMAB was chosen as activating agent. Meanwhile, the effect of the amount of CTMAB on ΔI_p was examined. When

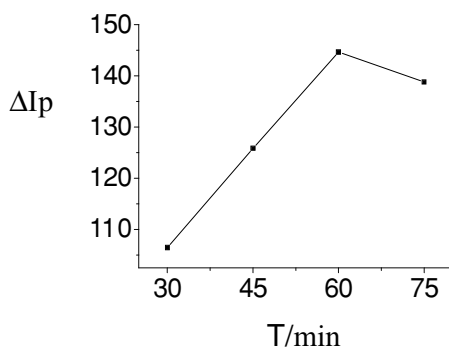


Fig. 8 Effect of reaction temperature on ΔI_p for system

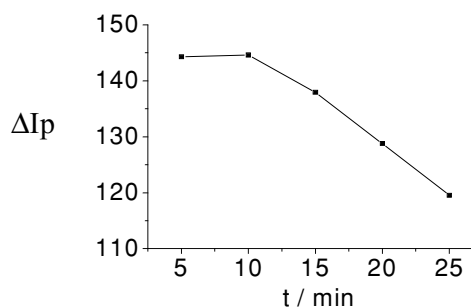


Fig. 9 Effect of reaction time on ΔI_p for system

1.00 ml of CTMAB was used, the ΔI_p reached the maximum (Fig. 6).

Acidity for reaction

For the system of $4.00 \text{ pg ml}^{-1} \text{ Hg}^{2+}$, the effect of pH value on ΔI_p was examined (Fig. 7). Results showed that when pH was 1.10, the ΔI_p reached the maximum and remained stable. So 3.00 ml of glycine-HCl buffer solution ($\text{pH} = 1.1$) was used to control acidity.

Temperature and time for catalytic reaction

Under the optimum concentration, for the system containing $4.00 \text{ pg ml}^{-1} \text{ Hg}^{2+}$, the effects of reaction temperature and time on ΔI_p were examined, respectively (Figs. 7 and 8). The results showed that when the reaction temperature was $60 \pm 0.2^\circ\text{C}$ and the time was 10 min, the ΔI_p reached the maximum and remained stable.

Time and temperature for drying before SS-RTP determination

For the system of $4.00 \text{ pg ml}^{-1} \text{ Hg}^{2+}$, when the drying temperature was 75, 80, 85, 90 and 95°C , the ΔI_p were 119.74, 128.59, 137.48, 144.65 and 144.59, respectively; when the drying time was 0.5, 1.0, 1.5, 2.0 and 2.5 min, the ΔI_p were 87.51, 90.84, 109.67, 144.66 and 144.60, respectively. Result

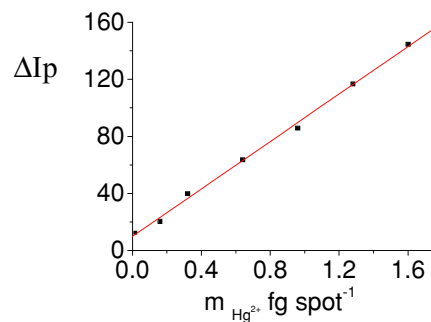


Fig. 10 Working curve

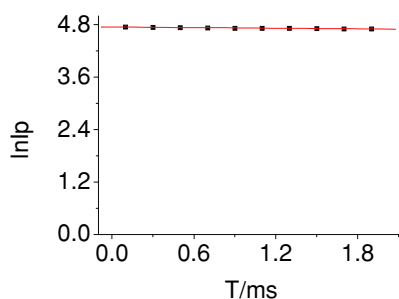


Fig. 11 Attenuation curve of SS-RTP lifetime

showed that when the temperature and time was $90 \pm 1^\circ\text{C}$ and 2 min, the ΔIp reached the maximum and remained stable.

Stability of system

Under the optimum conditions above, when the determination time was 5, 10, 15, 20 and 2.5, the ΔIp of the system were 144.65, 144.67, 144.65, 144.65 and 144.63, respectively. The ΔIp remained stable in the following 20 min, which indicated that this method had good reproducibility (Fig. 9).

Analytical parameters

Under the optimal experiment condition described above, the ΔIp of system was measured according to the experimental method. The results showed that the reducing value of phosphorescence intensity (ΔIp) is directly proportional to the concentration of Hg^{2+} in the range of $0.016\text{--}1.6 \text{ fg spot}^{-1}$ (or $0.040\text{--}4.0 \text{ pg ml}^{-1}$, $0.40 \mu\text{l spot}^{-1}$). The regression equation of working curve can be expressed as $\Delta\text{Ip} = 10.03 + 83.15 \text{ m Hg}^{2+}$ (fg spot^{-1}), $n = 6$, $r = 0.9987$. For samples containing $0.016 \text{ fg spot}^{-1}$ and $1.6 \text{ fg spot}^{-1} \text{ Hg}^{2+}$, the relative standard deviations (RSDs) are 2.6% and 3.5% ($n = 6$), respectively. It showed that this method had good precision. The reagent blank was measured repeatedly for 11 times and the LD calculated by $3\text{Sb}/k$ was 3.6 ag spot^{-1} (sample volume:

$0.40 \mu\text{l}$, corresponding concentration: $9.0 \times 10^{-15} \text{ g ml}^{-1}$) (Fig. 10).

The component of the ion association complex

The component of the ion association complex was determined by equi-molar continuous variation method and molar ratio method. The result showed that the mole ratio in the ion association complex for $\text{Sn}^{4+}:\text{XO}$ was 1:6 and $[\text{Sn}(\text{XO})_6]^{4+}:\text{Fin}^-$ was 1: 4, thus the component can be expressed as $[\text{Sn}(\text{XO})_6]^{4+} \cdot [(\text{Fin})_4]^-$.

Lifetime of phosphorescence

For the system containing $1.6 \text{ fg spot}^{-1} \text{ Hg}^{2+}$, the lifetime was determined by phosphorescence attenuation method (Delay time: $0.1 \sim 2.0 \text{ ms}$, Gate time: 2.0 ms). The phosphorescence lifetime was obtained by RTP attenuation curve (Fig. 11). According to the method described in literature [11], the regression equation of the attenuation curve can be expressed as $\ln \text{Ip} = 4.745 - 0.02275 t \text{ (ms)}$, $r = -0.9954$. According to the regression equation, the lifetime is $\tau_p = 43.96 \text{ ms}$.

Interference test

For the sample containing $4.00 \text{ pg ml}^{-1} \text{ Hg}^{2+}$, the allowed concentration (multiple) of coexistent ions ($\text{Er} \pm 5\%$) are as follows: Na^+ , K^+ , F^- , Cl^- , Br^- and SO_3^{2-} (1.5×10^4); S^{2-} , Ni^{2+} , PO_4^{3-} , $\text{C}_2\text{O}_4^{2-}$ and SO_4^{2-} (1.0×10^4); NO^{2-} and NO_3^- (5.0×10^3); ClO_4^- , BrO_3^- , As(III) , and As(V) (2.4×10^3); Cd^{2+} , Mn^{2+} , Cr(IV) and Cr(III) (1.0×10^3), Al^{3+} , SCN^- , Fe^{3+} , Fe^{2+} and Cu^{2+} (800), Bi^{3+} and Ba^{2+} (500); Co^{2+} and Zn^{2+} (350).

Sample analysis

Hair: human hair sample was immersed in acetone for 30 min, washed by water and then dried. $0.8 \text{ g} (\pm 0.1 \text{ mg})$ of sample was weighed accurately. Then the sample was

Table 1 Parameters of the spectra

System	$\lambda_{\text{ex}}^{\text{max}}$ (nm)	$\lambda_{\text{em}}^{\text{max}}$ (nm)	Ip	ΔIp
0.0'.PAM	411.96	584.47	56.04	
1.1' 3.00 ml glycin-HCl + 2.00 ml XO	454.40	622.12	84.16	
2.2'.1.1' + 5.00 ml Sn^{4+}	454.51	622.16	90.65	
3.3' 2.2' + 1.00 ml CTMAB	454.69	622.04	88.05	
4.4' 3.3' + 2.00 ml Fin^-	479.61	645.56	264.60	
5.5' 4.4' + 1.00 ml H_2O_2	480.44	645.99	235.48	
6.6' 5.5' + 1.0 pg Hg^{2+}	480.85	645.97	222.76	
7.7' 5.5' + 100 pg Hg^{2+}	479.95	645.81	90.83	144.65
8.8' 2.2' + 2.00 ml Fin^- + 1.00 ml H_2O_2	480.92	645.45	275.54	
9.9' 8.8' + 100 pg Hg^{2+}	480.36	645.03	235.41	40.13

Table 2 The analytical results of mercury in hair and tea

Sample	Average found ($\mu\text{g}\cdot\text{g}^{-1}$, $n=6$)	Added ($\mu\text{g}\cdot\text{g}^{-1}$)	Obtained ($\mu\text{g}\cdot\text{g}^{-1}$)	Recovery (%)	RSD (%, $n=7$)	Dithizone ($\mu\text{g}\cdot\text{g}^{-1}$, $n=6$)
Hair	0.33	0.30	0.31	103.3	3.1	0.32
Tea	0.019	0.020	0.020	100.0	2.0	0.020

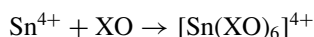
digested at low temperature by 32.0 ml of mixture solution of HClO_4 and HNO_3 (1:8 v/v). The digested solution was dried under elevated temperature, and then several drops of H_2SO_4 (1:1, v/v) were added to the residue. After leached with water, the residue was transferred to a 100 ml measuring flask, diluted to the mark with water. 1.00 ml of test solution was sucked, adjusted pH value to 1.10, and diluted to 100 ml. And 1.00 ml of diluted solution was measured each time.

Tea: the tea sample was treated in the same way as hair, and 1.00 ml of diluted solution was measured each time (Table 1).

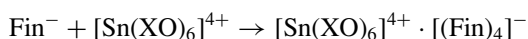
The content of Hg^{2+} in solution was determined according to the above procedure, and a standard addition recovery rate experiment and a comparative test with dithizone were also conducted. The analytical results for Hg^{2+} are listed in Table 2.

Mechanism of reaction

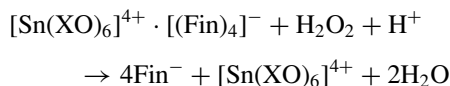
In glycine-HCl buffer solution, xylenol orange(XO) can react with Sn^{4+} to form complex $[\text{Sn}(\text{XO})_6]^{4+}$, which can emit strong and stable room temperature phosphorescence at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 454.51/622.16$ nm ($\text{Ip} = 90.65$) on the polyamide membrane:



Fin^- can react with $[\text{Sn}(\text{XO})_6]^{4+}$ to form the ion association complex $[\text{Sn}(\text{XO})_6]^{4+}\cdot[(\text{Fin})_4]^-$, which can cause a red-shift of λ_{em} for 23.40 nm ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 479.61/645.56$ nm, $\text{Ip} = 264.60$):

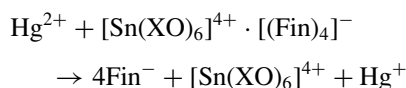


H_2O_2 can oxidize $[\text{Sn}(\text{XO})_6]^{4+}\cdot[(\text{Fin})_4]^-$ to form $[\text{Fin}^-]$ and $[\text{Sn}(\text{XO})_6]^{4+}$, which causes the room temperature phosphorescence quenching of ion complex ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 480.44/645.99$ nm, $\text{Ip} = 235.48$):



With the existence of Hg^{2+} also oxidizes $[\text{Sn}(\text{XO})_6]^{4+}\cdot[(\text{Fin})_4]^-$ to form Fin^- and $[\text{Sn}(\text{XO})_6]^{4+}$, which

deoxidizes Hg^{2+} to Hg^+ and causes the room temperature phosphorescence quenching of ion complex. ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 479.95/645.81$ nm, $\text{Ip} = 89.20$):



Meanwhile, Hg^+ was oxidized to Hg^{2+} by H_2O_2 :



During the reaction, Hg^{2+} can catalyze H_2O_2 oxidizing ion association complex $[\text{Sn}(\text{XO})_6]^{4+}\cdot[(\text{Fin})_4]^-$. Based on these facts, a new solid substrate-room temperature phosphorimetry for the determination of trace mercury has been established.

Conclusion

Based on the facts that Hg^{2+} can catalyze H_2O_2 oxidizing the ion association complex $[\text{Sn}(\text{XO})_6]^{4+}\cdot[(\text{Fin})_4]^-$ which can accelerate the solid substrate room temperature phosphorescence quenching above, a new method for the determination of trace mercury by solid substrate-room temperature phosphorimetry quenching method has been established. This sensitive method has been applied to the determination of trace mercury in living samples with satisfactory result.

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