

Solid Substrate-Room Temperature Phosphorescence Method for the Determination of Trace Mn(II) Based on Oxidizing Reaction of Hydrogen Peroxide Using α,α' -Bipyridine as Sensitizer

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Abstract A new solid substrate-room temperature phosphorescence (SS-RTP) method for the determination of trace manganese (II) has been established. It bases on the fact that fullerol (R) emits strong and stable room temperature phosphorescence (RTP) on filter paper substrate. H_2O_2 can oxidize R to cause the SS-RTP quenching. But manganese (II) can obstruct H_2O_2 to oxidize R, and enhance the RTP of R. α,α' -Bipyridine (Bipy) can sensitize the RTP. After adding Bipy, the ΔI_p enhances 7 times than that without Bipy. Under the optimum conditions, the linear dynamic range of this method is 0.016–1.12 pg spot^{-1} with a detection limit (L.D.) of 4.6 fg spot^{-1} ($m_{\text{Mn}^{2+}}$ is the absolute mass of Mn^{2+}), and the regression equation of working curve is $\Delta I_p = 25.20 + 63.55 m_{\text{Mn}^{2+}} (\text{pg spot}^{-1})$, $n = 6$, $r = 0.9983$. For 0.016 and 1.12 pg spot^{-1} Mn^{2+} , RSDs are 4.3 and 4.8%, respectively ($n = 7$). This method has been applied to the determination of trace manganese (II) in actual sample with high sensitivity and good selection. And the reaction mechanism of SS-RTP is discussed.

Keywords Manganese (II) · Fullerol · Solid substrate-room temperature phosphorescence · α,α' -Bipyridine · Sensitization

Introduction

The low solubility of fullerene hampers their research and use, especially in biological and pharmacological studies. In recent years, many efforts have been devoted to solving this problem, and some water-soluble fullerenes are synthesized, such as [60]fullerene-cyclodextrin complex, [60]fullerene-calixarenes complex, fullerol, Water-Soluble C_{60} Dendrimer etc [1–3]. In the last decades, functionalized fullerenes have exhibited a wide range of biological and medicinal activities, such as DNA photocleavage, antioxidants, neuroprotective agents, antiapoptotic activity, enzyme inhibition, anti-HIV activity, contrast agents and radiotracers [4–7]. These results are the foundation of use of C_{60} in biology and physic. In recent years, we have succeeded to synthesize smaller fullerene ($\text{C}_{50}\text{Cl}_{10}$) [8], which could emit fluorescence. Therefore, conceiving under the perturbation of weight atom, if fullerenes could emit solid substrate-room temperature phosphorescence (SS-RTP), the results can be used not only to develop the application of fullerenes by its high reactivity and its rigidity, but also to enhance the sensitivity of signal of SS-RTP. Because of hydrophobicity of fullerenes, we take fullerol as an example, and study a new SS-RTP method for the determination of trace manganese (II).

Manganese, which is a necessary trace element for human body, and is very important to human's growth and metabolism. In recent years, many methods have been developed to determine the manganese, such as catalytic kinetic color fading photometry (LD: 4.0×10^{-11} g ml^{-1}) [9], solid-phase extraction on nanometer TiO_2 hyphenated with inductively coupled plasma mass spectrometry (ICP-AES) (LD: 1.5×10^{-11} g ml^{-1}) [10], microwave plasma torch atomic Emission spectrometer (LD: 6.0×10^{-9} g ml^{-1}) [11], luminol chemiluminescence (LD: 2.0×10^{-9} g ml^{-1}) [12], Solid Phase Extraction-High Performance Liquid

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Chromatography (LD: 2.0×10^{-9} g ml⁻¹) [13] and so on. Though these methods have high sensitivity, some need complex operations or dear instruments. So searching for a sensitive, rapid and accurate method with small sampling volume has being the concern for many chemists in country and abroad. SS-RTP has many advantages, such as sensitive, accurate, simple operation, small sampling volume, little interference, long lifetime and big Stokes displacement, etc. It has been widely applied to the determination of trace mercury [14] and human serum protein IgG [15]. Our researches reveal that fullerol emits strong and stable room temperature phosphorescence (RTP) on filter paper substrate. Manganese (II) can obstruct H₂O₂ to oxidize fullerol to cause the SS-RTP quenched. After adding α,α' -Bipyridine (Bipy), the ΔI_p enhances 7 times than that without Bipy. Basing on this, we establishes a new SS-RTP method for the determination of trace manganese (II). This method has been applied to the determination of trace manganese (II) in actual sample with high sensitivity and good selection.

Experiment

Apparatus and reagents

Phosphorescence measurements were carried out on a Perkin-Elmer LS-55 luminescence spectrophotometer with a front-surface attachment and a solid sample shelf (Perkin-Elmer). The instrument's main parameters are as follows: delay time: 0.1 ms; gate time: 2.0 ms, cycle time: 20 ms, flash count: 1; slit: Ex = Em = 10.0 nm; speed: 1500 nm min⁻¹. pH-3B precision acidometer (Shanghai Medical Laser instrument plant); 85-1 constant temperature magnetic stirrer (ShenZhen TiannanhaiBei company); AE240 electronic analytical balance (Mettler-Toledo instruments Shanghai company); a 0.5 μ L flat head micro-injector (Shanghai Medical Laser Instrument Plant) was used to introduced solution.

Mn(II) working solution: 1.00 mg ml⁻¹ Mn (II) primary standard solution (GSBG 62019-90 2502) was diluted to 1.00, 100.00 ng ml⁻¹ as working solution. 1.0×10^{-5} mol l⁻¹ C₆₀(OH)₂₅ solution, KH(C₈H₄O₄)-NaOH buffer solution (pH = 4.0) (100.00 ml of 0.1 mol l⁻¹ KH(C₈H₄O₄) solution and 0.8 ml of NaOH solution diluted to 200.00 ml), 0.2% α,α' -Bipyridine solution, 5.0 % (w/v) H₂O₂ solution. All reagents are of A.R. grade except for Mn(II), which is of primary standard; the water used was prepared by twice quartz sub-boiling distillation.

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

Experimental method

0.50 ml of R, 2.00 ml of H₂O₂, 1.00 ml of KH(C₈H₄O₄)-NaOH buffer solution, certain amount of Mn(II) were added successively, mixed homogeneously, then diluted to 25 ml with water. The tube was kept at 25°C for 30 min. A blank test was conducted simultaneously. Both test solution and blank solution were placed for determination of phosphorescence.

A ration filter paper (8.0 mm \times 60.0 mm) was immersed in 1 mol l⁻¹ Pb(Ac)₂ solution for 10 s and then dried at $90 \pm 1^\circ\text{C}$ for 2 min; A 0.4 μ L drop of test solution and blank solution was suspended onto the center by a 0.5 μ L flat head micro-injector, respectively, then the wafer was dried at $90 \pm 1^\circ\text{C}$ for 2 min again. The phosphorescence intensity was measured directly at wavelengths $\lambda_{\text{ex}}/\lambda_{\text{em}} = 480/647$ nm. The signal of system (without Mn (II)) was defined as the reagent blank intensity (I_{p1}), and the signal of test solution (containing Mn(II)) was defined as the sample intensity for test solution (I_p). $\Delta I_p = (I_p - I_{p1})$ was calculated.

Result and discussion

Excitation spectra and emission spectra

R can emit strong and stable room-temperature phosphorescence on filter paper at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 480.25/646.90$ ($I_p = 75.03$). H₂O₂ can oxidize R to cause the SS-RTP quenching ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 476.19/645.49$, $I_p = 66.53$). Manganese II can obstruct H₂O₂ to oxidize R, and enhance the SS-RTP of R ($\Delta I_p = 13.92$, Fig. 1). After adding Bipy, the ΔI_p enhances 7 times than that without Bipy. The maximum phosphorescence wavelength is $\lambda_{\text{ex}}/\lambda_{\text{em}} = 480.31/646.69$ ($I_p = 154.24$, $\Delta I_p = 98.03$), so 647 nm is chosen as the working wavelength (Fig. 2).

Optimum measurement condition for SS-RTP

Concentration and volume

For the system of 1.12 pg spot⁻¹ Mn²⁺, the reagents amount or concentration was changed, respectively (Figs. 3–9). The results showed that the optimal volume of reagents were as following: 0.50 ml of 1.0×10^{-5} mol l⁻¹ C₆₀(OH)₂₅, 2.00 ml of 5.0% H₂O₂, 1.00 ml of KH(C₈H₄O₄)-NaOH buffer solution (pH = 4.0), 0.50 ml of 0.2% Bipy. At this time, the pH value of reaction solution was 5.00, the ΔI_p reached the maximum and remained stable.

Fig. 1 Solid substrate room temperature phosphorescence (SS-RTP) spectra for the System. 1,1' paper; 2,2' 4,4' + 70 ng Mn²⁺; 3,3' 0.50 ml R; 4,4' 5,5' + 2.00 ml H₂O₂; 5,5' 3,3' + 1.00 ml Buffer

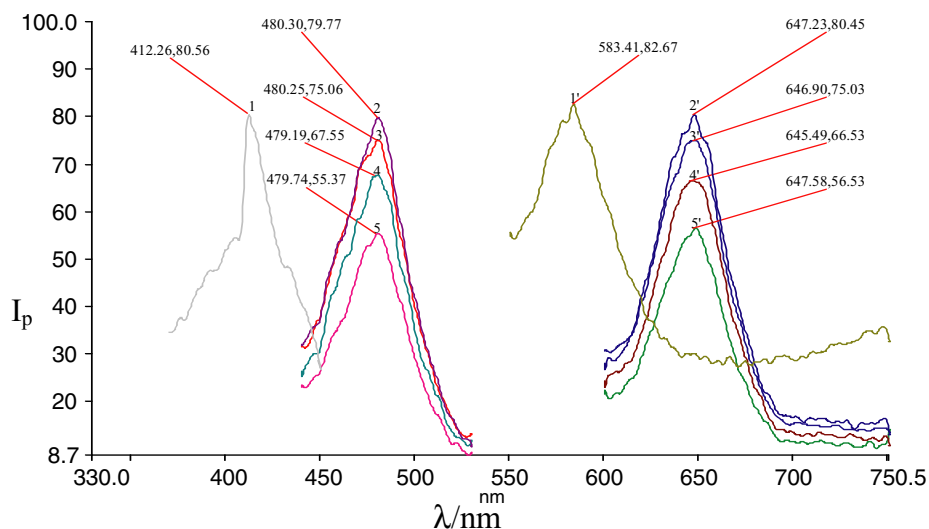


Fig. 2 Solid substrate room temperature phosphorescence (SS-RTP) spectra for the System. 1,1' 2,2' + 70 ng Mn²⁺; 2,2' 0.50 ml R + 1.00 ml Buffer + 2.00 ml H₂O₂ + 0.50 ml α,α-bipyridyl; 3,3' paper

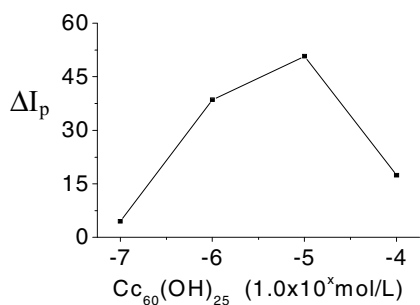
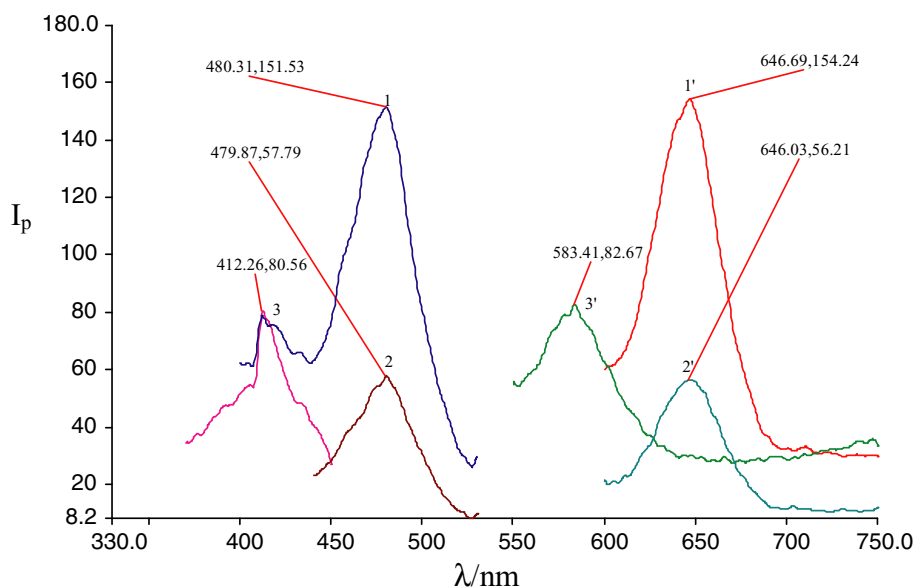


Fig. 3 Effect of the concentration of C₆₀(OH)₂₅ on ΔI_p for the system

Solid substrate

For the system containing 1.12 pg spot⁻¹ Mn²⁺, according to the method described above, the SS-RTP spectra of the same

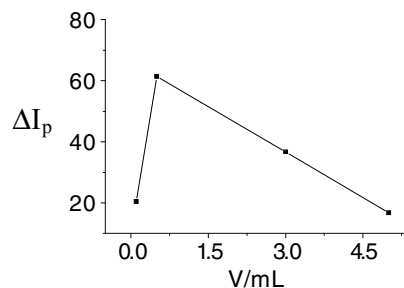


Fig. 4 Effect of the dosage of C₆₀(OH)₂₅ on ΔI_p for the system

reaction solution was measured at working wavelength on three kinds of substrates, which were filter paper, polyamide membrane (PAM), and acetic cellulose membrane (ACM), respectively (Fig. 10). The result showed that ΔI_p of system

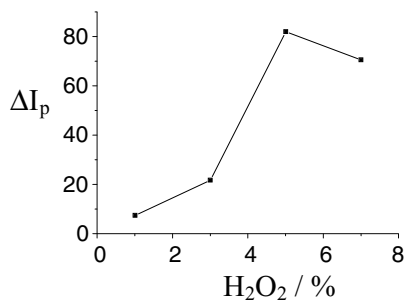


Fig. 5 Effect of the concentration of H₂O₂ on ΔI_p for the system

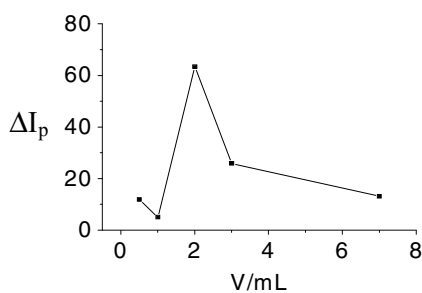


Fig. 6 Effect of the dosage of H₂O₂ on ΔI_p for the system

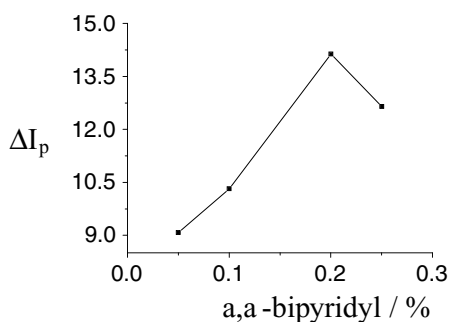


Fig. 7 Effect of the concentration of α,α-bipyridyl on ΔI_p for the system

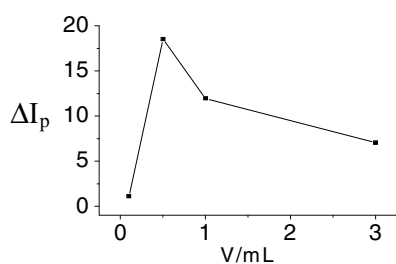


Fig. 8 Effect of the dosage of α,α-bipyridyl on ΔI_p for the system

on the filter paper reached the highest, so it was chosen as the solid substrate in the following experiment.

Ion perturbation

For the system of 1.12 pg spot⁻¹ Mn²⁺, the effect of ions such as Mg²⁺, Li⁺, Ag⁺, Pb²⁺ and I⁻ on ΔI_p were examined

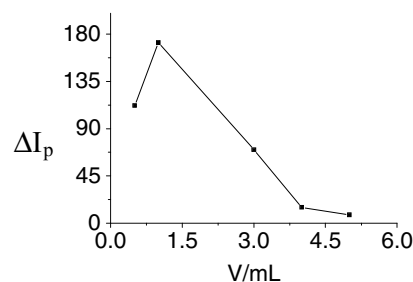


Fig. 9 Effect of the dosage of KH(C₈H₄O₄)-NaOH on ΔI_p for the system

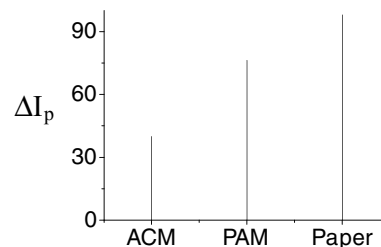


Fig. 10 Effect of solid substrate on ΔI_p for the system

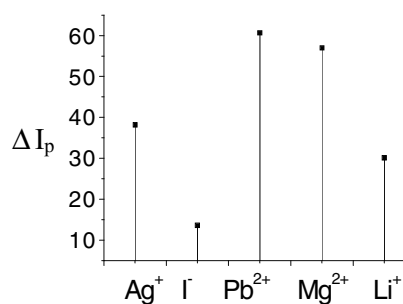


Fig. 11 Effect of ion perturbation on ΔI_p for the system

respectively. The results showed that ΔI_p of Pb²⁺ was the highest, so Pb²⁺ was chosen as ion perturber (Fig. 11).

Sensitizer

For the system containing 1.12 pg spot⁻¹ Mn²⁺, the effect of different surface reactive agents on ΔI_p were examined respectively (Fig. 12). The results showed that among Polyvinyl Alcohol (PVA), Bipy, polyethylene glycol (PEG), polyacrylamide (PAM), Bipy had the maximum quenching effect. So Bipy was chosen as activating agent. After adding Bipy, the ΔI_p enhanced 7 times than that without Bipy.

Reaction acidity

For the system containing 1.12 pg spot⁻¹ Mn²⁺, the effect of pH value on ΔI_p was examined. When the reaction pH value was 5.0, the ΔI_p remained stable and reached the maximum (Fig. 13).

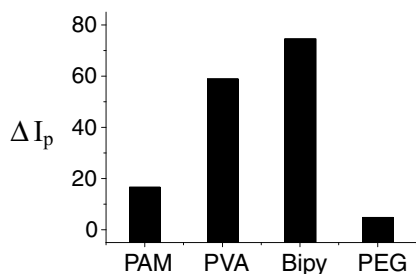


Fig. 12 Effect of sensitizer on ΔI_p for the system

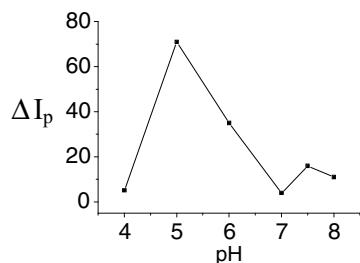


Fig. 13 Effect of reaction acidity on ΔI_p for the system

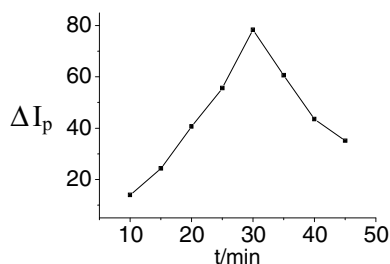


Fig. 14 Effect of reaction time on ΔI_p for the system

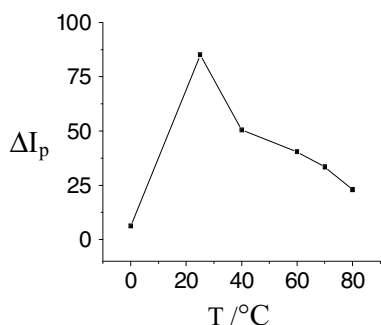


Fig. 15 Effect of reaction temperature on ΔI_p for the system

Reaction temperature and time

For the system containing $1.12 \text{ pg spot}^{-1} \text{ Mn}^{2+}$, the effect of reaction temperature and time on ΔI_p were examined. When the reaction was carried out at room temperature (25°C) for 30 min, its ΔI_p reached the maximum (Figs. 14 and 15).

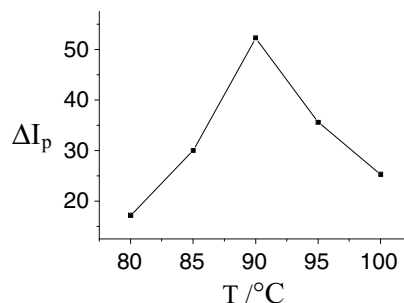


Fig. 16 Effect of desiccate temperature on ΔI_p for the system

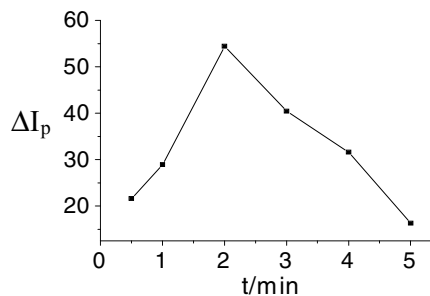


Fig. 17 Effect of desiccate time on ΔI_p for the system

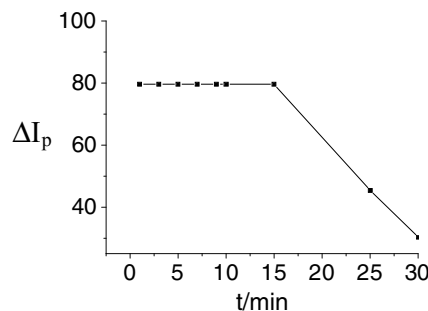


Fig. 18 Effect of standing time on ΔI_p for the system

The desiccation temperature and time

Under the optimal experiment conditions described above, when the filter paper was dried at $90 \pm 1^\circ\text{C}$ for 2 min, ΔI_p reached the maximum and remained stable (Figs. 16 and 17).

The stability of phosphorescence emission spectra

Under the optimal conditions above, the ΔI_p of the system remained stable in the following 15 min after reacting at 25°C for 30 min (Fig. 18).

Linear range, working curve, limit determination

Under the optimal experiment conditions described above, determined the ΔI_p of system by experimental method. The results showed that ΔI_p was directly proportional

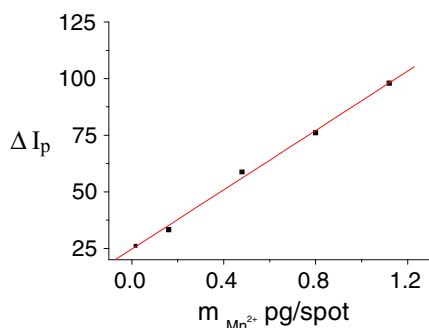


Fig. 19 Working curve

to the concentration of Mn^{2+} in the range of $0.016\text{--}1.12\text{ pg spot}^{-1}$ (or $0.040\text{--}2.8\text{ ng ml}^{-1}$, $0.4\text{ }\mu\text{l spot}^{-1}$). The regression equation of working curve could be expressed as $\Delta I_p = 25.20 + 63.55 m_{\text{Mn}^{2+}} (\text{pg spot}^{-1})$, (Fig. 19) ($m_{\text{Mn}^{2+}}$ is the absolute mass of Mn^{2+}), $n = 6$, $r = 0.9983$. The blank solution was measured repeatedly for 11 times, and the detection limit calculated by 3 Sb/k was 4.6 fg spot^{-1} (or $1.2 \times 10^{-11}\text{ g ml}^{-1}$). For 0.016 and $1.12\text{ pg spot}^{-1}\text{ Mn}^{2+}$, RSDswere 4.3 and 4.8% , respectively ($n = 7$). That is to say, the repeatability of the method was satisfying.

The lifetime of phosphorescence

For the system containing $1.12\text{ pg spot}^{-1}\text{ Mn}^{2+}$, its lifetime was determined by phosphorescence attenuation method (Delay time $0.1\text{--}2.0\text{ ms}$, Gate time 2.0 ms). The phosphorescence lifetime was obtained by RTP attenuation curve (Fig. 20). According to the method in literature [16], the regression equation of the attenuation curve could be expressed as $\ln I_p = 4.8380 - 0.01254 \times t$, $r = -0.9858$. According to the definition of the SS-RTP lifetime, $\tau = 79.74\text{ ms}$ was obtained.

Interference test

For the system containing $1.12\text{ pg spot}^{-1}\text{ Mn}^{2+}$, the allowed concentrations (multiple) of coexistent ions or coexistent

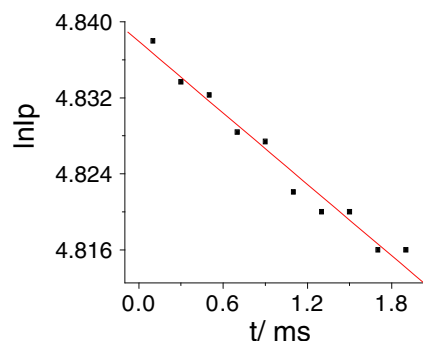


Fig. 20 Attenuation curve of SS-RTP lifetime

materials ($E_r = \pm 5\%$) are listed in Table 1. The multiple of coexistent ions are higher, which indicated high selectivity of this method.

Analysis of samples

1.00 ml of mineral water (A), river water (B) from Jiulong river and tap water (C) were sucked to dilute to 100.0 ml , respectively, and mixed homogeneously. 1.00 ml of diluted solution was measured each time for the content of Mn^{2+} . A standard addition recovery rate experiment was also conducted. The results were listed in Table 2.

Mechanism of reaction

R can emit strong and stable solid substrate room temperature phosphorescence (SS-RTP) signal on filter paper substrate. H_2O_2 can oxidize R to form R' , which causes the room temperature phosphorescence quenching. The reaction can be expressed as follows:



When Mn^{2+} is present, it reduces H_2O_2 to form H_2O .

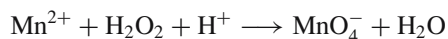


Table 1 Effect of foreign substances on the determination of the system containing $1.12\text{ pg spot}^{-1}\text{ Mn}^{2+}$

Coexistent ions	Allowed concentrations (Multiple)	Relative error (%)	Coexistent ions	Allowed concentrations (Multiple)	Relative error (%)
Al^{3+}	5×10^5	1.4	SCN^-	1×10^5	2.4
Ca^{2+}	5×10^5	4.4	Fe^{2+}	1×10^5	-2.4
Cd^{2+}	5×10^4	4.5	Br^-	2×10^4	-0.8
Cu^{2+}	2×10^4	0.5	PO_4^{3-}	2×10^4	2.6
K^+	2×10^3	3.4	Cl^-	2×10^4	2.7
Bi^{3+}	2×10^4	3.5	Cr^{3+}	2×10^4	-4.1
Ni^{2+}	2×10^2	-1.2	Ba^{2+}	2×10^4	-3.1
Hg^{2+}	2×10^4	-2.6	Zn^{2+}	2×10^3	3.4
SO_4^{2-}	2×10^4	3.2			

Table 2 The analytical results of manganese ($n = 6$)

Sample	Present method ($\mu\text{g/L}$)	Added ($\mu\text{g/L}$)	Found ($\mu\text{g/L}$)	Recovery (%)
A	1.15	0.11	1.27	100.9
B	0.137	0.14	0.24	73.6
C	1.08	0.11	1.16	74.6

Manganese(II) can obstruct H_2O_2 to oxidize R, and enhance I_p of the system ($\Delta I_p = 13.92$, Fig. 1). So trace manganese(II) can be measured by SS-RTP method.

Conclusion

α, α' -Bipyridine can sensitize manganese(II) to obstruct H_2O_2 to oxidize fullerol, and cause to increase I_p of the system. Based on this fact, a new method for the determination of trace manganese (II) by solid substrate-room temperature phosphorescence quenching method has been established. This method has higher sensitivity. It is not only applied to the determination of trace manganese (II) in living samples, but also widen the application of fullerene by its higher

reactivity. For example, fullerene as perturber can enhance sensitivity of SS-RTP method by use of its rigidity.

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