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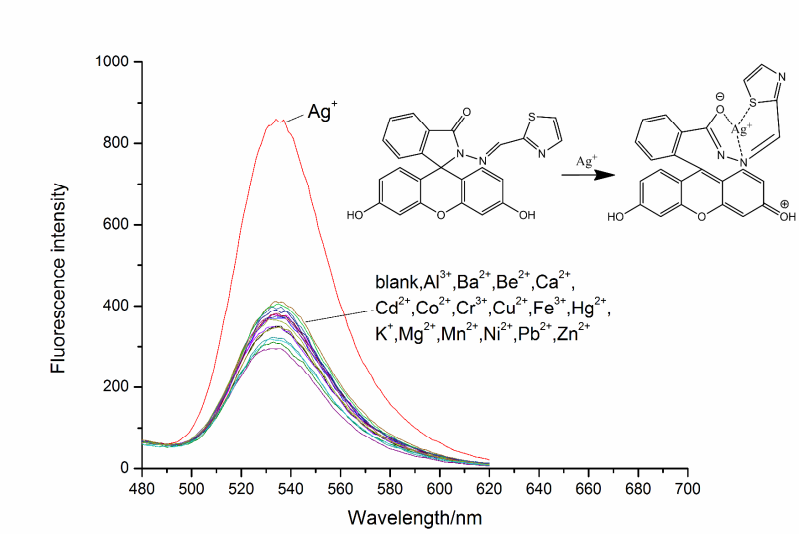
A fluorescein spirolactam derivative (HTMIX) was synthesized and used as a "turn-on" fluorescence probe for the detection of Ag^{+} in water samples. The established method has the advantages of good selectivity, high sensitivity, cost-effective, amenable miniaturization and operating convenience for detection of Ag^{+} in environmental and drinking water.



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A turn-on fluorescein spirolactam derivative as a high selective fluorescence probe for detection of silver ion(I) in water

Dong-Sheng Lin[a], Jia-Ping Lai[a]*, Hui Sun[b]*, Zhou Yang[a], Yue Zuo[a]

Abstract : In this paper, a fluorescein spirolactam derivative, ((E)-3', 6'-dihydroxy-2-((thiazol-2-ylmethylene) amino) spiro [isoindoline-1,9'-xanthen] -3-one) (HTMIX), was synthesized and used as a turn-on fluorescence probe for the detection of silver ion in aqueous solution. The binding mechanism of HTMIX to Ag^+ was evaluated using the Hildebrand-Benesi equation based on a 1:1 binding model with R=0.9993. And the influences of sixteen common metal ions on the fluorescence intensities of HTMIX-Ag^+ solution were investigated in detail. The results showed that HTMIX exhibited high selectivity toward Ag^+ in a 20% ethanol solution. The obtained fluorescence probe was used for quantitative determination of Ag^+ with a good linear range from 0.1 μM to 10 μM (R=0.9969) and a satisfactory detection limit of 0.08 μM. In addition, the present probe has been further used for detection of Ag^+ in tap water, river water and lake water. And the accuracy of the results obtained by the proposed method shows a good agreement with that obtained by flame atomic absorption spectrometry.

Keyword: fluorescence probe; fluorescein derivative; silver ion; interference; environmental water

[a] School of Chemistry & Environment, South China Normal University, Guangzhou, Guangdong, 510006, China. E-mail: laijp@scnu.edu.cn; Fax: +86-20-39310187; Tel.: +86-20-39310257

[b] College of Environmental Science & Engineering, Guangzhou University, Guangzhou, Guangdong, 510006, China. E-mail: sunhuicherry@gmail.com

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1. Introduction

For a long time, silver ion is well known for its ability to kill harmful bacteria.[1-3] And it is always taken to be a precious metal benefit to human. Thus, seldom attentions have been put on the detection of silver in environmental samples. In fact, recent researches show that silver ion can cause severe damages to both environment and human.[4,5] As one of the most toxic heavy metals, surpassed only by mercury, silver ion has been assigned to the highest toxicity class.[6] Silver ions may damage the benign bacteria, and inhibit their growth, thereby affecting their reproduction.[7] Excessive intake of silver ions can also lead to long-term insoluble substance formed in eye and skin cells, which make the eyes secrete mucus and skin dim.[8] Thus, blood silver (argyraemia) and urine silver excretion are useful indices for human silver exposure in clinical. With the rapid development of industry, more and more silver is widely used in electronics, photographic, imaging and pharmaceutical industry. Every year there are above 2500 tons of silver are discharged as industrial wastes into the environment, including 150 tons into the sludge of sewage treatment plants, and 80 tons in surface water.[6] Especially in recent years, with the rapid development of nanoscience, various of silver nanoparticle products, such as nano-Ag catalyzer and antimicrobial reagents, has been widely used in research and clinic. After discharged into environment, a

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part of silver nanoparticles are oxidized to silver ions and dissolved into environmental water. And the other part of them is deposited into mud. Thus they must cause more serious pollution to environment and damage to human health. Therefore, it has great significance to develop a rapid, selective, sensitive and simple detection method for silver ion in environmental and human fluid.

The reported methods for detection of silver include ion selective electrodes,[9,10] atomic absorption spectrometry (AAS),[11,12] inductively coupled plasma mass spectrometry (ICP-MS),[13,14] inductively coupled plasma atomic emission spectrometry (ICP-AES),[15] differential pulse anodic stripping voltammetry,[16] microextraction[17,18] as well as quantum dots.[19,20] Meanwhile, optical probes, especially the fluorescence probes for metal ions have received considerable attentions in recent decades due to the good selectivity, high sensitivity, low detection limit, ease of operation, cost effective and large dynamic concentration range.[21] However, most of the reported fluorescence probes toward Ag^+ are "turn-off" probes,[22-25] and exhibit a quenching response upon binding with silver ions due to the special characters of the outer electronic structure (d10) of silver.[26] Therefore, the development of a "turn-on" probe toward Ag^+ is of great significance to achieve high sensitivity in complex sample detection. Li et al demonstrated the use of carbon nanoparticles obtained from carbon soot by lighting a candle as a cheap,

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effective fluorescent sensing platform for sensitive and selective detection of Ag^+ in a real sample. [27] Wang et al reported a DQAg sensor for selective detection of Ag^+ based on inhibition of the resonance. [28]

In present study, a fluorescein spirolactam derivative, ((E)-3', 6'-dihydroxy-2-((thiazol-2-ylmethylene) amino) spiro (isoindoline-1, 9'-xanthen)-3-one) (HTMIX) was synthesized and used as a "turn-on" probe toward Ag^+ in aqueous media. The working conditions of the Ag^+ probe will be optimized and the factors affecting its analytical performance will be discussed. In light of the results obtained, the applicability of the probe developed for the determination of Ag^+ ion in actual environmental and drinking water is assessed and discussed in detail.

2. Materials and methods

2.1 Chemicals and reagents

All the materials for synthesis were purchased from commercial suppliers and used without further purification. Fluorescein and hydrazine hydrate were purchased from Aladdin Reagent Co., Ltd (Shanghai, China, www.aladdin-reagent.com). 2-Thiazolecarboxaldehyde was purchased from Beijing Ouhe Chemical Technology Co., Ltd (Beijing, China,

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www.ouhechem.com). Aqueous solutions of metal ions were prepared from their nitrate or chloride salts which purchased from Aladdin Reagent Co., Ltd (Shanghai, China, www.aladdin-reagent.com). Buffer solutions were prepared with Tris-HNO_3. Unless otherwise specified, all reagents used were analytical grade, and distilled water was used throughout the experiment.

2.2 Instrumentation

Fluorescence spectra were recorded on a Hitachi FL-2500 fluorescence spectrophotometer (Hitachi, Japan, www.hitachi.com). NMR spectra were taken on a Varian NMR System 400 MHz Spectrometer (Varian, USA, www.varianinc.com) with tetramethylsilane (TMS) as internal standard and dimethyl sulfoxide-d6 (DMSO-d6) as solvent. Mass spectra were obtained on a Finnigan LCQ Deca XP MAX spectrometry (Finnigan, USA, www.thermoscientific.com).

2.3 Procedures

Scheme 1 outlines the syntheses of (E)-3', 6'-dihydroxy-2- ((thiazol-2-ylmethylene) amino) spiro [isoindoline-1, 9'-xanthen]-3-one (HTMIX) by preparing fluorescein hydrazide and reacting it in ethanol with

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thiazole-2-carbaldehyde. The structures were confirmed using ^{1}H NMR and mass spectrometry.

<Scheme 1>

2.3.1 *Synthesis of fluorescein hydrazide* **(1)**

Fluorescein hydrazide was synthesized based on reported literature.[29] Briefly, excess hydrazine hydrate (24 mL; hydrazine content>80 mass%) was added in a 100-mL flask containing a suspension of fluorescein (6 g, 18.1 mmol) in 50 mL methanol. After heated to reflux for 7 h with stirring, the suspended particles were consumed and a clear solution was obtained. The ensuing solution was allowed to cool and poured into 400 mL distilled water, and then the yellow precipitation formed immediately, which was allowed to settle for 2 h. The suspension was filtered, washed with water until the filtrate was colorless, and then washed with 3×10 mL of cold absolute ethanol to get rid of other impurities. The crude product was purified by recrystallization from ethanol to give 3.59 g of compound **1** as an off-white solid (57%). ^{1}H NMR (400 MHz, DMSO-d6), δ(ppm): 4.42 (s, 2H), 6.44 (m, 4H), 6.63 (s, 2H), 7.01 (d, 1H), 7.51(m, 2H), 7.80 (d, 1H), 9.85 (s, 2H). ESI-MS m/z:347.4 $(M + H)^{+}$.

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2.3.2 *Synthesis of (E)-3', 6'-dihydroxy-2-((thiazol-2-ylmethylene) amino) spiro [isoindoline-1, 9'-xanthen]-3-one (HTMIX)* **(2)**

Compound **1** (1.9 g, 5.5 mmol) and 2-Thiazolecarboxaldehyde (0.57 g, 5 mmol) were added to 40 mL absolute ethanol in a 100-mL flask, and the mixture was stirred and refluxed at 83□ for 12 h.[30] Following reaction, the solvent in mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether=1:1) to give 1.94 g of HTMIX (88%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6), δ(ppm): 6.45 (d, 2H), 6.52 (d, 2H), 6.66 (s, 2H), 7.13 (d, 1H), 7.63 (dd, 2H), 7.73 (s, 1H), 7.82 (d, 1H), 7.95 (d, 1H), 8.65 (s, 1H), 10.00 (s, 2H). ^{13}C NMR (400 MHz, DMSO-d6), δ(ppm): 65.14, 102.53, 109.17, 112.54, 122,10, 123.44, 123.79, 127.76, 127.87, 129.23, 134.59, 140.22, 143.97, 150.68, 151.93, 158.81, 163.95, 164.52. ESI-MS m/z：442.4 $(M+H)^{+}$.

2.3.3 *Fluorescence measurements*

All fluorescence spectra studies were performed on a Hitachi FL-2500 fluorescence spectrophotometer. Different amount of metal ions were added to HTMIX (10 μM) in a 20% ethanol solution at pH 9.0 (0.05 M Tris-HNO_3). The resulting solution was shaken well and detected

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immediately. The emission spectra were recorded from 480 nm to 620 nm by excitation at 319 nm. Excitation and emission slits were set at 5.0 nm and the voltage of photomultiplier was set at 700 V.

3. Results and Discussion

3.1 Fluorescence characterization of HTMIX

Fluorescence probes are powerful tools for monitoring environmental and biological sample in *vitro* and/or in *vivo*, because of their simplicity, high selectivity and sensitivity. However, seldom fluorescence probes for Ag^+ have been reported,[23,24,31] because the toxicity and damage of silver to human has been ignored for a long time. In fact, as mentioned in introduction, recent researches have proved that Ag^+ ions are one of the most toxic heavy metals only after mercury, and have been assigned to the highest toxicity class recently. To develop a novel "turn-on" fluorescence probe to Ag^+, the selectivity of HTMIX to Ag^+ was investigated in this work. Ten μM of Ag^+, Al^{3+}, Ba^{2+}, Be^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} solution were respectively added to 10 μM HTMIX in a 20% ethanol solution at pH 9.0. The blank sample was prepared in the same way without the addition of metal ions. The fluorescence emission intensity was recorded

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and shown in Fig. 1. It can be seen that the fluorescence intensity of HTMIX can be obviously enhanced after the addition of Ag^+ while there is no significant fluorescence response due to the addition of other metal ions. It indicates that HTMIX exhibits obvious fluorescent enhance to Ag^+ and can be used as a "turn-on" fluorescence probe for Ag^+. Therefore, in the subsequent work, the interaction mechanism of HTMIX to Ag^+ and the analytical performance of the HTMIX probe will be discussed in detail.

<Fig.1>

3.2 Response mechanism investigation

The response of HTMIX toward Ag^+ is assumed to be based on the opening-closing function of the spirolactam ring.[30,32,33] The proposed binding mechanism is shown in Scheme 2. In general, with the addition of Ag^+, the spirolactam ring in HTMIX is opened and the ternary complex is formed between Ag^+ ion and O, S, N atoms, which leads to the effect of fluorescence enhancement. Since Ag^+ has similar ionic radius (Ag^+: 115 pm) as S atom (S: 110 pm), it is speculated that Ag^+ ion is more easily to form coordinate bond with S atom on thiazole ring. Thus, HTMIX exhibits high selectivity toward Ag^+ ion in comparison to other metal ions.

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<Scheme 2>

To further confirm that the binding between HTMIX and Ag^+ is based on a 1:1 binding model. The binding equilibrium constant has been estimated with the Hildebrand-Benesi equation.[34-36]

$$H + G \overset{K_{obs}}{\rightleftharpoons} HG$$

Here H stands for HTMIX and G stands for Ag^+. I and I_0 are the fluorescent emission intensity at the presence and absence of Ag^+ in a HTMIX aqueous solution (20% ethanol, pH 9.0). As shown in Fig. 2, the plot of $I_0/(I-I_0)$ versus $[Ag^+]^{-1}$ exhibits a very good linearity with R=0.9993, which strongly supports the 1:1 (HTMIX:Ag^+) binding model. In addition, the equilibrium binding constant (K_{obs}) has been obtained from the slope of the plot. The high binding constant ($K_{obs}=2.04\times10^5$ $L\cdot mol^{-1}$) indicates that HTMIX exhibits a strong affinity toward Ag^+ under the optimal experimental conditions.

<Fig.2>

3.3 Effect of pH on the fluorescence intensity of HTMIX probe

It is well known that the responses of most optical probes, especially fluorescence probes, are affected by H^+ concentration of testing

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solution.[31] At the optimum pH, HTMIX should give the most sensitive response toward Ag^+. On the other hand, the selection of pH value of solution should ensure that Ag^+ is not precipitated. Thus, to investigate the effect of pH on the fluorescence response of HTMIX to Ag^+, a series of HTMIX solution with different pH adjusted with Tris-HNO_3 were prepared. The variation of fluorescence intensity difference ($I-I_0$) of the probe (10.0μM) in the presence (I) and absence (I_0) of Ag^+ (10.0μM) as a function of pH has been studied and outlined in Fig. 3. It can be seen that HTMIX shows no significant response to Ag^+ below pH 7.3; however, when the pH is above 7.5, the signal ($I-I_0$) enhances evidently and reaches its maximum at pH 9.0; when pH is above 9.0, the signal ($I-I_0$) decreases gradually. Based on the response mechanism as discussed in section 3.2, we speculate that the basic medium is favorable to the combination of HTMIX toward Ag^+. An increased pH would help to open the spirolactam ring and enhance the fluorescence emission intensity of HTMIX-Ag^+ chromophores. However, too high pH value would lead to the precipitation of Ag^+. Thus, the fluorescence intensity of HTMIX-Ag^+ solution decreased markedly when the pH value of the solution is above 9 (Fig. 3). Therefore, the Tris-HNO_3 solution at pH 9.0 has been chosen as the optimum buffer solution for all detections in the following studies.

<Fig.3>

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3.4 Quantitative determination of silver ion

In order to investigate the possibility of quantitative detection of Ag^+ with HTMIX fluorescent probe, the fluorescence emission spectra of HTMIX in a 20% ethanol solution at pH 9.0 has been recorded respectively with gradual addition of different amounts of Ag^+. As shown in Fig. 4, it can be seen that the fluorescent emission intensity of HTMIX increased with the increase of the amount of Ag^+ (Fig. 4a). It reached its maximum value when the concentration of Ag^+ was as the same as HTMIX. Then the addition of excess silver ion could not cause significant effect on the fluorescence intensity of HTMIX (Fig. 4b). The fluorescent response has been calculated to cover a good linear range (R=0.9969) from 0.1 μM to 10 μM (Fig. 4b, insert) with a detection limit of 0.08 μM (3σ/slope).

<Fig.4>

For wastewater, the maximum allowable emission of Ag^+ is 0.5 mg/L(4.63 μM) stipulated by the "Integrated Wastewater Discharge Standard" of China **[GB 8978-1996]**. And as to the drinking water, the limit of Ag^+ in drinking water is 0.05 mg/L (0.463 μM) stipulated by the "Standards for Drinking Water Quality" of China **[GB5749-2006]**. Therefore, the developed probe in present work is sensitive enough for the detection of Ag^+ in not only environmental water, but also in drinking

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water samples.

3.5 Interference investigation

To further confirm the selectivity of the HTMIX probe for Ag^{+} detection and investigate the possibility for detection of Ag^{+} in real water samples with HTMIX probe, the interferences of sixteen metal ions including Al^{3+}, Ba^{2+}, Be^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} on the fluorescence intensities of HTMIX probe were investigated in detail. Firstly, 10 μM of Ag^{+} solution was added to 10 μM of HTMIX in a 20% ethanol solution at pH 9.0. And then, 1 equiv, 5 equiv and 10 equiv of interference ions were added into the above solutions, respectively. The effects of interference ions on the fluorescence intensity of HTMIX-Ag^{+} solution are shown Fig. 5. It can be clearly seen that the addition of most other metal ions have no significant influence on the fluorescence intensity of the HTMIX-Ag^{+} probe except for Cu^{2+}, Co^{2+} and Ni^{2+} (Fig.5a). It must be mentioned that Cu^{2+} and Co^{2+} can only cause a slight decrease of fluorescence intensity of HTMIX-Ag^{+} probe even when the concentration of Cu^{2+} and Co^{2+} increase to 10 equiv of Ag^{+}. However, an obvious decrease of fluorescence intensity affected by Ni^{2+} was observed especially while the concentration of Ni^{2+} increases to 10 equiv of Ag^{+}. This is possibly ascribed to the quenching effect of

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paramagnetic metal ions on HTMIX.[37,38] To reduce or eliminate the interferences of Cu^{2+}, Co^{2+} and Ni^{2+} on the detection of Ag^{+} in real sample, 100 μM of EDTA (1 equiv of interfering ions) was added to solution as masking reagent to prevent interferences from Cu^{2+}, Co^{2+} and Ni^{2+}. As can be seen from Fig.5b that the negative effect of Cu^{2+}, Co^{2+} and Ni^{2+} on the fluorescence intensity of HTMIX-Ag^{+} can be easily eliminated by the addition of EDTA in detection solution (Fig.5b blue column). Thus, the HTMIX probe described herein can be used for the detection of Ag^{+} in real water sample with high selectivity.

<**Fig.5**>

3.6 Applicability study

To test the applicability of the proposed fluorescence probe in practical samples, the Ag^{+} in tap water, Zhujiang River water and Centre Lake water in Guangzhou have been detected with this method. The water samples were filtered with 0.22 μm membrane filter for 3 times before use and then detected directly under the optimal conditions. Since no Ag^{+} was detected in those water samples, all the water samples were spiked with Ag^{+} standard solution to the concentrations of 0.8 and 2.0 μmol L^{-1}, respectively. The recoveries obtained using the proposed fluorescence probe were found to vary from 87.02% to 107.26%. Details on the

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recovery test on real samples are tabulated in Table 1. The accuracy of the proposed method was also checked by use of flame atomic absorption spectrometry (FAAS) with results shown in Table 1. It can be seen that the HTMIX fluorescence probe exhibits satisfactory results and shows a good agreement with the results obtained by FAAS. In summary, the fluorescence probe developed has shown to provide a sensitive and accurate monitoring method for onsite determination of Ag^+ in real environmental and drinking water samples with satisfactory recoveries at practical concentration range.

<**Table 1**>

4. Conclusions

To meet with the need for a fast, accurate and sensitive onsite monitoring method in the determination of Ag^+ in environmental water, a fluorescein spirolactam derivative (HTMIX) has been synthesized and used as a "turn-on" fluorescence probe for the detection of Ag^+ in water sample. The obtained probe is found to give a fast, stable, sensitive and selective signal for Ag^+. It has been successfully applied for direct determination of Ag^+ in actual water samples. The accuracy of the present method shows a good agreement with the results obtained by flame atomic absorption spectrometry. In summary, with the advantages of good selectivity, high sensitivity, cost-effective, amenable miniaturization and operating

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convenience, the established method provide a practical way for the determination of Ag^+ in complex environmental samples, and the development of a miniaturized on-line determination system is on the way in our research group.

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Legends of Schemes, Figures and Table

Scheme 1 Synthetic route of fluorescein hydride(**1)** and HTMIX(**2)**

Scheme 2 Proposed binding mechanism of HTMIX toward Ag^{+}

Fig. 1 Fluorescence emission spectra of HTMIX (10 μM) toward metals. (in pH9.0 C_2H_5OH/water(1/4,V/V) solution adjusted with Tris-HNO_3 buffer)

Fig. 2 Hildebrand-Benesi plot of HTMIX toward $[Ag^{+}]^{-1}$

Fig. 3 Effects of pH value on fluorescence intensity of HTMIX-Ag^{+}.

Fig. 4 Fluorescence response of HTMIX toward Ag^{+} (a: Fluorescence emission spectra of HTMIX with gradual addition of different amounts of Ag^{+}. b: Calibration curve of Ag^{+} detected with HTMIX probe)

Fig. 5 Effects of interference ions on the fluorescence intensity of HTMIX-Ag^{+} solution (a: without EDTA masking reagent; b: with EDTA masking reagent for Cu^{2+}, Co^{2+} and Ni^{2+})

Table 1 Comparison of the results for Ag^{+} detection in water samples (n=3) with HTMIX probe and FAAS

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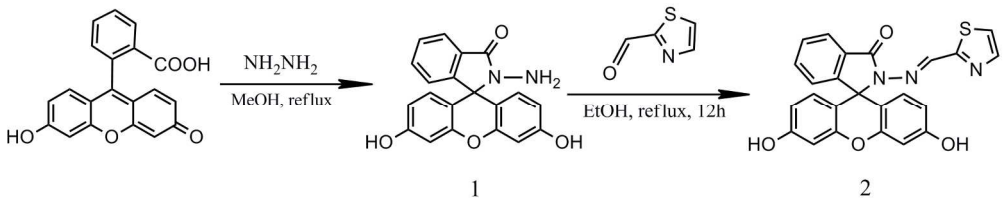

Scheme 1 Synthetic route of fluorescein hydride(1) and HTMIX(2)
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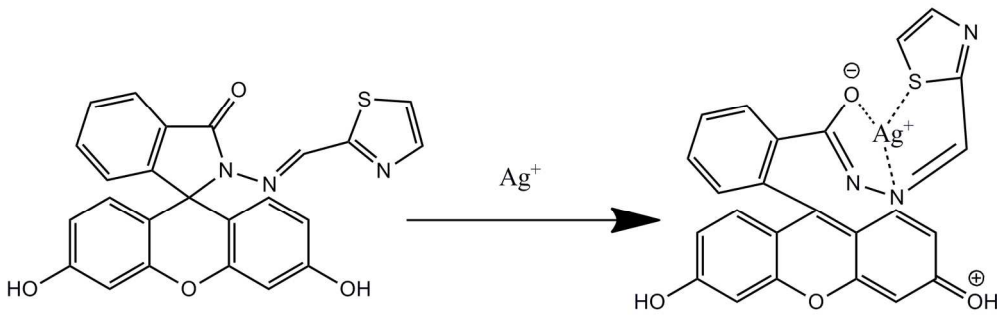

Scheme 2 Proposed binding mechanism of HTMIX toward Ag+
165x51mm (300 x 300 DPI)

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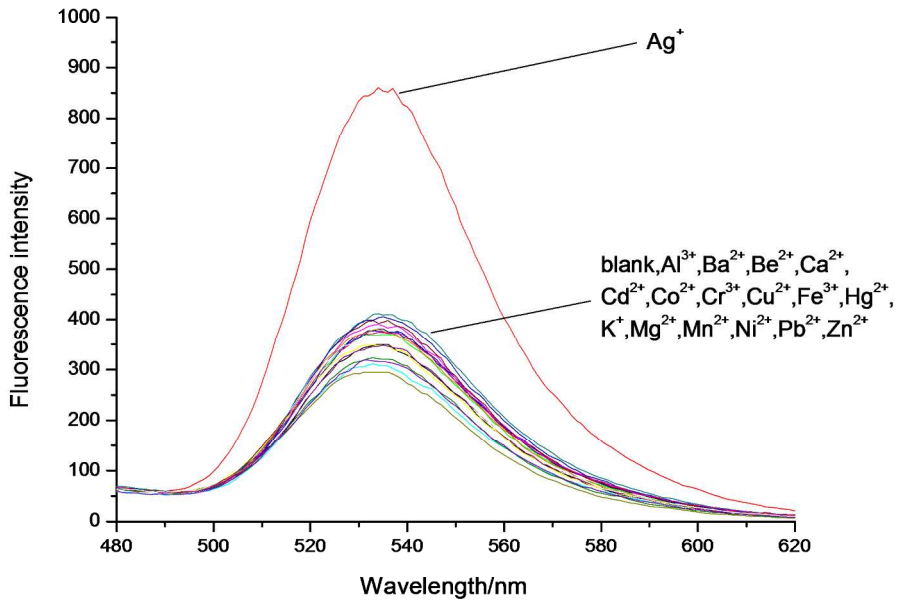

Fig.1 Fluorescence emission spectra of HTMIX (10 μM) toward metals. (in pH9.0 C2H5OH/water(1/4,V/V) solution adjusted with Tris-HNO3 buffer)
201x143mm (300 x 300 DPI)

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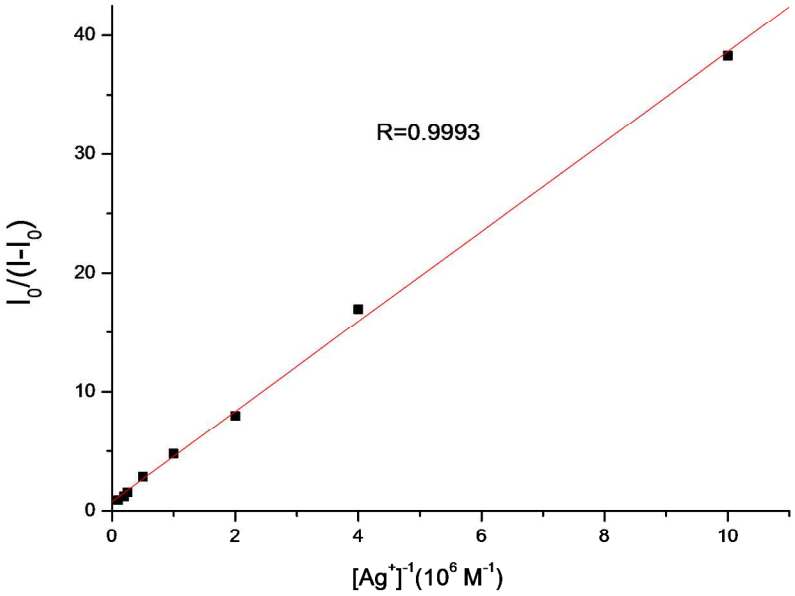

Fig.2 Hildebrand-Benesi plot of HTMIX toward [Ag+]-1
200x141mm (300 x 300 DPI)

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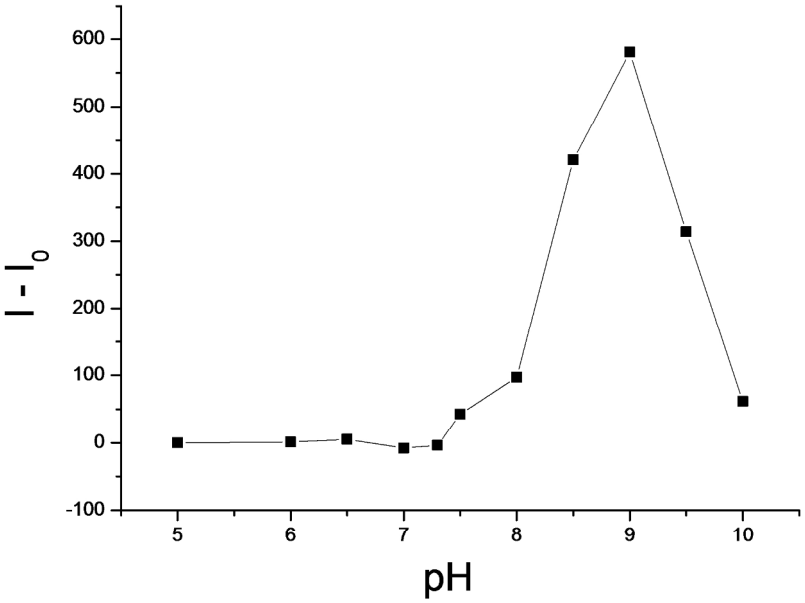

Fig.3 Effects of pH value on fluorescence intensity of HTMIX-Ag+
205x145mm (300 x 300 DPI)

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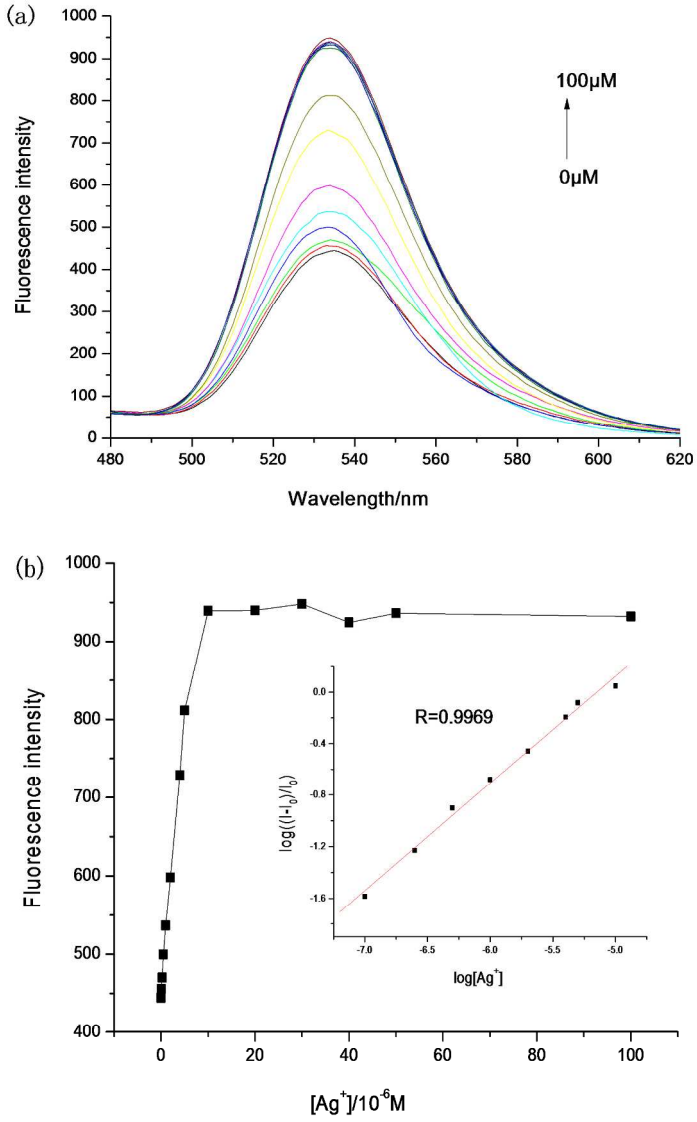

Fig.4 Fluorescence response of HTMIX toward Ag+ (a: Fluorescence emission spectra of HTMIX with gradual addition of different amounts of Ag+. b: Calibration curve of Ag+ detected with HTMIX probe)
187x267mm (300 x 300 DPI)

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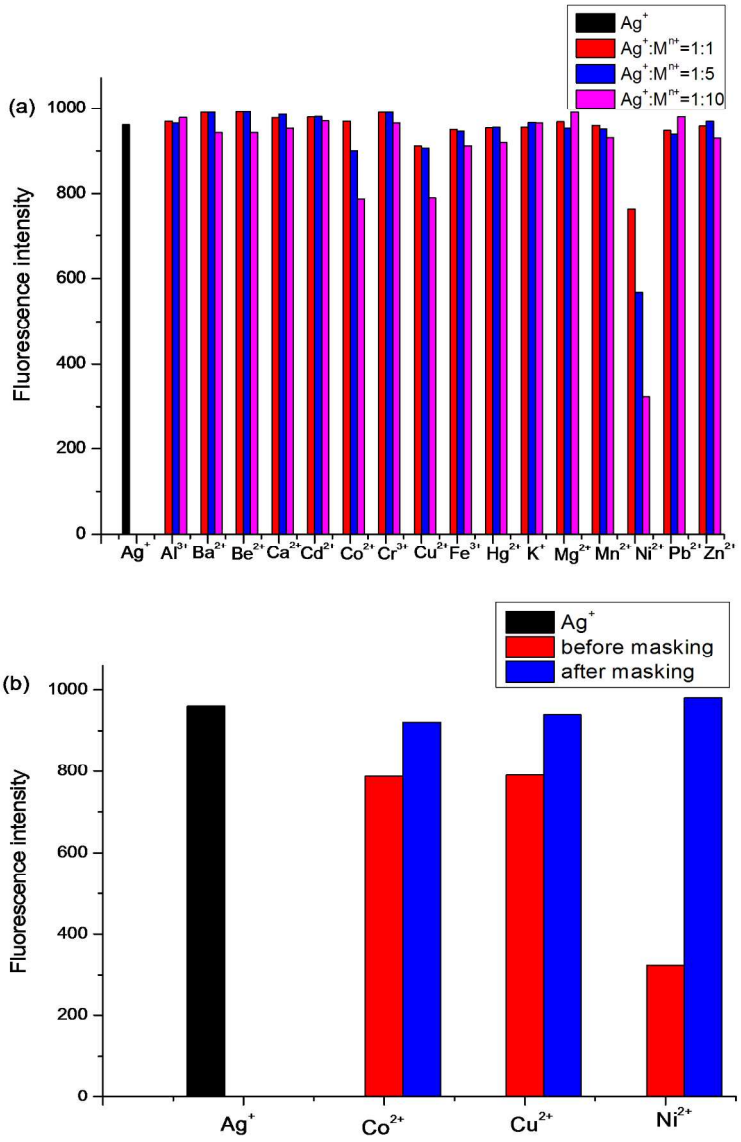

Fig.5 Effects of interference ions on the fluorescence intensity of HTMIX-Ag+ solution (a: without EDTA masking reagent; b: with EDTA masking reagent for Cu2+, Co2+ and Ni2+)
182x234mm (300 x 300 DPI)

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Table 1 Comparison of the results for Ag^{+} detection in water samples (n=3) with the proposed fluorescence sensor and FAAS

Samples	Added（μM）	found（μM）		recovery	
		AAS	FS	AAS	FS
tap water	0.00	ND	ND	—	—
	0.80	0.847	0.823	105.89%	102.85%
	2.00	1.735	1.864	86.75%	93.20%
river water	0.00	ND	ND	—	—
	0.80	0.810	0.858	101.27%	107.26%
	2.00	1.772	1.740	88.60%	87.02%
lake water	0.00	ND	ND	—	—
	0.80	0.884	0.823	110.52%	102.85%
	2.00	1.883	2.040	94.15%	102.02%

Note: FS, Detected by the proposed method；ND, Not detected.

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