Phenanthroline Derivative Fluorescent Probe for Rapid and Sensitive Detection of Silver(I)

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In the present work, a phenanthroline derivative (2-(2-methoxyphenyl)-4-phenyl-1,10-phenanthroline, MPP), as a fluorescent probe, was synthesized to realize a rapid, simple and sensitive detection of silver(I). The detection conditions of Ag⁺ were optimized. This fluorescent probe has the advantages of a fast reaction time, a wide pH applicable range, and a low detection limit, exhibiting a good linear response between the fluorescence intensity and the concentration in the range of 0.05 – 1.5 μmol/L for Ag⁺. The detection limit is as low as 3.38 × 10⁻⁶ mol/L (S/N = 3). This probe had been used to detect Ag⁺ in real samples, and the recovery efficiency of spiked Ag⁺ had been also tested. The recovery efficiency is satisfactory, ranging from 92.0 to 105.4%. Therefore, this fluorescent probe should provide a new choice for the quantitative detection of silver ions in environmental water samples.

Keywords Silver ions, fluorescence probe, small organic molecule, heavy metal, sensitivity

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Introduction

Silver ion is one of the heavy metal ions with biologically toxicity. In recent years, silvers ion and relative compounds have been widely used in industrial production and daily life, such as electronics, photography, pharmacy and other fields. According to the requirements of the World Health Organization (WHO) document, the safe dose of silver to the human body is 0.05 ppm or less. However, it is estimated from industrial waste and polluted water that the annual release of silver to the environment is about 2500 tons, which far exceeds the legal limit. According to the requirements of the World Health Organization (WHO) document, the safe dose of silver to the human body is 0.05 ppm or less. Therefore, it is estimated from industrial waste and polluted water that the annual release of silver to the environment is about 2500 tons, which far exceeds the legal limit. Moreover, the accumulation of silver ions will damage important organs, such as the liver and kidneys of the human body. Therefore, the development of a simple and effective silver-ion detection method is of great significance to protect the environment and human health.

At present, the conventional detection methods of silver ions include atomic absorption spectroscopy (AAS), inductively coupled plasma–mass spectrometry (ICP-MS), electrochemical methods, and potentiometric methods and so on. Among the usual methods, fluorescence spectrometry has attracted people’s attention due to its fast response time, high sensitivity and easy operation. Recently, there have been many reports concerning silver ions detection methods based on fluorescence spectroscopy, such as a carbon dots-based fluorescence sensor, cyanine-based, graphene-based, rhodamine derivative, tetraphenylethenyl, imidazole derivative, copper nanoparticles, etc. Compared with the reported silver-ion fluorescence probes, the fluorescent probe highlighted in this paper is easy to be synthesized, and exhibits an ultra-fast detection speed and high sensitivity, which can be used for the real-time detection of silver ions in water samples.

Herein, the fluorescent probe A (2-(2-methoxyphenyl)-4-phenyl-1,10-phenanthroline, MPP) was designed and synthesized for the high-sensitivity detection of silver ions. Silver ions can be chelated with MPP, causing the MPP to decrease in the photoluminous intensity. MPP exhibits an ultrafast detection speed and a wide pH applicable range compared to the reported fluorescent probe of silver ions. Under the optimum conditions, the concentration of added Ag⁺ was linear with the final fluorescence intensity. The detection limit was lower than the World Health Organization (WHO) threshold for Ag⁺ in drinking water. Moreover, MPP has been successfully utilized in real water samples to detect Ag⁺ with excellent recoveries. The method of Ag⁺ detection based on this fluorescent probe is of great significance for the efficient detection of silver ions in environmental water samples.

Experimental

Chemicals and instruments

8-Aminoquinoline and phenylacetylene were purchased from Saan Chemical Technology (Shanghai) Co., Ltd. 2-Methoxybenzaldehyde was purchased from Shanghai Bi De Pharmaceutical Technology Co., Ltd. Toluene was obtained from Sinopharm Chemical Reagent Co., Ltd. TA-Au (Triazole
Au(I) catalyst was synthesized with Ref. 29. NaH2PO4 was produced at Beijing Chemical Plant. Na2HPO4 was produced by Tianjin Guanguo Fine Chemical Research Institute. Cationic interfering ion standard solutions (1 mmol/L) used in the experiment (Ag+, Pd2+, Pt2+, Co2+, Cr3+, Zn2+, Ba2+, Mg2+, Hg2+, Al3+, Cu2+, Ni2+, Mn2+, Cu+, Na+, Be2+, Cd2+, Cs+, K+, Fe3+, V3+, Sr2+, Li+, Rb+, Pb2+ and Au3+) were obtained by diluting their nitrates with distilled water.

All fluorescence tests were performed on a F-2700 fluorescence spectrometer (Hitom, Japan). The nuclear magnetic resonance spectroscopy (NMR) of compounds was identified by 1H NMR (Varian Mercury YH-400 NMR spectrometer), using tetramethylsilane (TMS) as an internal standard. ESI mass spectra were obtained using an Agilent 1190 microTOF Q II (Bruker, Germany) without using the liquid-phase part. MALDI-TOF mass spectra were obtained using a Bruker Microflex LRF20. All pH measurements were tested with a Sartorius PB-10 digital pH meter.

Synthesis of the fluorescent probe MPP
The synthesis of MPP was based on the literature.20-30 8-Aminoquinoline (0.288 g, 2 mmol, 1 eq) and 2-methoxy-benzaldehyde (0.3 g, 2.2 mmol, 1.1 eq) were well mixed in a 100 mL three round-bottom flask with N2. (Heating melt in 60°C, recovery after room temperature for 1 h). Phenylacetylene (0.224 g, 2.2 mmol, 1.1 eq), TA-Au (75 mg, 0.1 mmol, 5 mol%) and 50 mL toluene were added in sequence at room temperature (0.224 g, 2.2 mmol, 1.1 eq), TA-Au (75 mg, 0.1 mmol, 5 mol%) and 50 mL toluene were added in sequence at room temperature for 10 min, then heated at 110°C for 48 h. The product obtained by the reaction was named MPP (2-(2-methoxyphenyl)-4-phenyl-1,10-phenanthroline. Yield, 35%, Scheme 1).

Characterization of MPP: 1H NMR (400 MHz, DMSO-d6): δ 9.16 (dd, J = 4.3, 1.8 Hz, 1H), 8.51 (dd, J = 8.1, 1.8 Hz, 1H), 8.13 (s, 1H), 8.09 (dd, J = 7.6, 1.8 Hz, 1H), 7.98 (d, J = 9.1 Hz, 1H). 7.88 (d, J = 9.1 Hz, 1H), 7.81 (dd, J = 8.1, 4.3 Hz, 1H), 7.68 - 7.56 (m, 5H), 7.53 (ddd, J = 8.7, 7.3, 1.8 Hz, 1H), 7.27 - 7.18 (m, 2H), 3.88 (s, 3H); 13C NMR (100 MHz, DMSO-d6) δ 157.63, 155.44, 150.56, 147.64, 146.47, 146.33, 138.30, 136.56, 132.16, 131.17, 130.11, 129.33, 129.02, 128.66, 127.00, 125.23, 125.20, 123.89, 123.86, 121.24, 112.50, 56.23. HRMS (ESI Positive) calc. for C25H19N2O+: 363.1497, found, 363.1501. 1H NMR, 13C NMR and mass spectrum of MPP were shown in Figs. S1 - S3 (Supporting Information).

Computational methods
All of the calculations were carried out using the GAUSSIAN09 program package.31 In the calculations, LanL2DZ and 6-31G(d) basis sets were adopted to describe metal atoms and other atoms, respectively. The geometries of the MPP have been optimized with the B3LYP density functional theory (DFT) approach, which combines Becke’s three-parameter nonlocal hybrid exchange potential12 with the non-local correlation functional of Lee, Yang, and Parr,13 using the 6-31G(d) and LanL2DZ34,35 basis set. The vibrational frequencies, also calculated at the same level of theory, have been used to characterize the stationary points, zero-point energy (ZPE) corrections calculations. The number of imaginary frequencies for intermediates is 0.

Analytical procedure
In a 5 mL sample tube, 30 μL of a MPP stock solution and a different volume of a Ag+ standard solution was blended and diluted to 3 mL with a DMSO aqueous solution (DMSO and HEPS buffer, v/v = 3/7, HEPES pH = 7.0, 40 mmol/L). The final concentration of MPP was 5 μmol/L, and the final concentration of silver ions ranged from 0 to 10 μmol/L. The fluorescence intensity of each sample solution was measured at room temperature after 20 s. The fluorescence spectral conditions were, λex = 281 nm; λem = 403 nm. A quartz cell of 1 cm path length was applied. The slits were set at 10 and 5 nm.

Results and Discussion
Spectroscopic properties of the fluorescent probe MPP
Figure 1A(a) shows the fluorescence excitation spectrum of MPP (5 μmol/L). It can be seen that the maximum excitation wavelength was 281 nm. Under this excitation wavelength, the fluorescence emission peak of MPP was located at 403 nm in Fig. 1A(b). When 10 μmol/L Ag+ existed in the test solution, the fluorescence intensity of MPP was significantly reduced (Fig. 1A(c)). The quenching efficiency was over 90%. And it also showed obvious fluorescence quenching under ultraviolet-light irradiation.

There should exist a strong coordination between silver ion and two N atoms on MPP. To study the coordination situation of MPP and silver ions, the complex ratio was tested. Keeping the total concentration of MPP and silver ions constant, and then the concentration ratio of MPP and silver ions was changed, and the corresponding fluorescence intensity was recorded. The data in Fig. 2A indicates that the complex ratio of MPP to Ag+ was 2:1. Meantime, mass spectrometry was used as an auxiliary means to verify the coordination between MPP and silver ions.
The molecular ion peak at \( m/z \) 833.2 was observed in Fig. S4, which corresponds to \([2\text{MPP}+\text{Ag}^+]\) peak. This result supports the structure of the complex and confirms the stoichiometry of the metal chelate as being the ML\(_2\) type. In Fig. 1B, the strong coordination between silver ions and two MPP molecules should lead to photo-induced electron transfer (PET) from fluorescent groups to silver ions, and the fluorescence of MPP was significantly quenched.36 Otherwise, the computational methods were used to simulate the stable structure of this coordination compound. The result is shown in Fig. 2B. It shows that the Out-cis structure is calculated to be the most stable and the stable coordination structure of MPP and silver ions based on Out-cis structure is simulated.

**Effect of the water content and pH of test solutions**

The nature of solvents and the pH of the test system will affect the fluorescence intensity.11 In Fig. 3A, the fluorescence intensity of the probe MPP in five pure solvents (DMSO, DMF, MeOH, EtOH, and THF) was tested separately. The results show that the fluorescence intensity of the probe MPP in the DMSO solvent was the strongest. This is due to the MPP’s “negative solvato-kinetic effect”, which often appears in the spectra of compounds where \( \pi-\pi^* \) and \( n-\pi^* \) transitions exist simultaneously. Many compounds with \( n \) electrons (such as \(-\text{N}=, =\text{O}\) groups) have multiple excited state energy levels formed when excited, and there are often \( \pi-\pi^* \) and \( n-\pi^* \) transition energy levels close to each other to cause proximity. This overlap and interaction between the energy levels will cause energy loss and reduce the emission quantum yield. Since the solvent has different effects on different energy levels, for example, an increase in the polarity of the solvent can strongly cause a decrease in the \( \pi-\pi^* \) energy level; but the increase in the polarity of the solvent has almost no effect on the change of the \( n-\pi^* \) energy level. Therefore, when the polarity of the solvent gradually increases, the \( \pi-\pi^* \) and \( n-\pi^* \) energy levels can be continuously separated, so that the original proximity effect is greatly reduced, and the energy loss effect caused by the proximity effect is also reduced. As a result, the fluorescence intensity increases as the solvent polarity increases.37 Among the five solvents, DMSO has the strongest polarity, and the fluorescence intensity of MPP is the strongest.

Next, the water content in an organic solvent, the pH of the buffer solution and the kind of buffer solution were investigated. MPP was dispersed in the DMSO solvent and the effect of the water content was tested. When silver ions existed, the fluorescence quenching efficiency of MPP (5 \( \mu\text{mol/L} \)) was detected under different water contents, respectively. According to Fig. 3B, the fluorescence quenching efficiencies gradually increased, and then decreased with the water content increasing.
The fluorescence quenching efficiency was the highest when the water content for 70% of the total solvent. Therefore, the optimized water content was 70% of the total solvent.

Since the change in the pH has an important influence on the accuracy of the experimental results, the fluorescence quenching efficiencies of MPP under different pH values were tested in mixed acid solutions (phosphoric acid, acetic acid, boric acids were all 0.04 mol/L). It can be seen from Fig. 3C that the fluorescence quenching efficiencies were relatively low for pH 3.0, because the protonation of MPP weakened its complexation with silver ions, resulting in lower fluorescence quenching efficiencies. From pH 4.0 to 12.0, the quenching efficiencies of silver ions to the MPP were greater than 90%. This result indicates that MPP can be used to detect Ag⁺ in a wide pH range, which is very useful for analyzing complex real samples. Otherwise, the fluorescence quenching efficiencies of MPP were also tested in different kinds of buffer solutions, and the result is shown in Fig. 3D. MPP has a better response in the HEPES buffer solution than other buffer solutions. Therefore, DMSO/HEPES = 3/7 (v/v, pH = 7.0) was selected as the test condition in following experiments.

The effect of reaction time

Under the above conditions, the reaction time of silver ions and MPP was determined. The probe MPP (5 μmol/L) was added into different concentrations of silver ion solutions, respectively. The continuous variation trend of the intensity of the fluorescent emission peak with the time increasing was recorded. As can be seen from Fig. 4, the fluorescence emission intensities of the test solutions decreased rapidly, and the intensities reached a steady state after 20 s. This indicates that the reaction time of MPP with Ag⁺ is very short and it is very useful in real sample analysis. Therefore, 20 s was selected as the reaction time.
Selectivity and anti-interference ability of MPP

In order to investigate the selectivity of MPP for silver ions, the fluorescent emission intensities of MPP with some common metal ions were detected. As shown in Fig. 5, Ca²⁺, Mg²⁺, Na⁺, K⁺ and other 16 metal ions (10 μmol/L) have almost no influence on the fluorescence intensity of MPP. But, unfortunately, five metal ions (Co²⁺, Zn²⁺, Ni²⁺, Cu²⁺, and Cd²⁺) have a certain degree of the quenching effect on MPP.

Since EDTA is a widely used masking agent, we wanted to mask these interfering metal ions using EDTA as the masking agent. At first, the effect of adding different concentrations of EDTA on the fluorescence intensity of MPP and MPP + Ag⁺ was tested. When the concentration of EDTA in the test solution ranges from 0.05 to 0.5 mmol/L, there was unobviously an effect on the fluorescence intensity of MPP and MPP + Ag⁺ (see Fig. S5). The red columns in Fig. 5 shows that five kinds of interfering ions (10 μmol/L) could be masked by 0.05 mmol/L EDTA without changing the fluorescence intensity of the probe. Therefore, 0.05 mmol/L EDTA as masking agent was added to the test solution to improve the selectivity of MPP.

In Fig. 6, the anti-interference ability of MPP was also examined. The black columns show that the addition of common interfering metal ions (10-times the concentration of Ag⁺) has no significant effect on the fluorescence quenching efficiency of MPP with silver ions except these interfering metal ions (Co²⁺, Zn²⁺, Ni²⁺, Cu²⁺, and Cd²⁺). The red columns show that Co²⁺, Zn²⁺, Ni²⁺, Cu²⁺ and Cd²⁺ (all 10 μmol/L, 10-times the concentration of Ag⁺) were almost masked by 0.05 mmol/L EDTA. The above results show that MPP has a satisfactory selectivity for the detection of silver ions when masking these interfering ions with EDTA. Some literature reported the concentration of these five interfering metal ions in environmental water samples. The concentration of Co²⁺ in industrial wastewater and mining wastewater, the concentration of copper ions, zinc ions, cadmium ions and nickel ions in tap water, river water and drinking water did not exceed 10 μmol/L. In Fig. S6, 0.05 mmol/L EDTA as a masking agent was used to eliminate the interference of these five interfering metal ions mixtures, and the result is satisfactory. Therefore, 0.05 mmol/L EDTA would be used to mask interfering metal ions in real samples.

Fluorescence titration experiments

The fluorescence spectral titration experiment of silver ions was performed under the addition of 0.05 mmol/L EDTA. As shown in Fig. 7, the fluorescence intensity of MPP decreased gradually when the concentration of Ag⁺ was changed from 0.05 to 1.5 μmol/L in test solutions. The concentrations of silver ions and the fluorescence emission intensity of MPP have satisfactory linear correlation, and the linear correlation coefficient ($R^2$) is 0.995. The limit of quantification of Ag⁺ is 0.05 μmol/L. The detection limit (LOD) can be calculated by the following formula (1):

$$\text{LOD} = \frac{3\alpha}{K}.$$  \hspace{1cm} (1)

Here, $\alpha$ is the standard deviation of the blank probe MPP solution for 11 times; $K$ is the slope of the calibration curve. The detection limit of silver ions for MPP can be found to be $3.38 \times 10^{-8}$ mol/L.

Table 1 gives a comparison of the detection range, the response time and the pH applicable range of silver ions detected by fluorescence spectroscopy in other literature and this work. The results indicate that MPP has a shorter detection range compared to other methods, but it has a shorter response time and a wider pH applicable range.
response time to silver ions, a wide pH applicable range and the detection limit is lower than some reported literature.

**Applications of MPP in real samples**

Inspired by the ability of MPP to detect Ag⁺ by fluorescence spectroscopy, MPP was applied to detect Ag⁺ in environmental water samples. The content of silver ions in the four real water samples were detected, and 0.05 mmol/L EDTA was added to each sample to mask Co²⁺, Zn²⁺, Ni²⁺, Cu²⁺ and Cd²⁺. The result was shown in Table 2. It shows that the silver ions content is too low to be detected in each water sample. Subsequently, we carried out a spike and recovery test in order to verify the accuracy of this fluorescent probe. The different volume of silver ions standard solutions was added into the sample solution, respectively, and the fluorescence intensity of MPP and the linear equation, the measured concentrations of silver ions in sample solution were calculated and compared to the actual spiked amount. The results are also shown in Table 2. With the addition of the Ag⁺ concentration, the detection amount was basically the same as the addition amount. The recoveries in four water samples were between 92.0 and 105.4%. These results indicate that the detection of Ag⁺ in real water samples is satisfactory and reliable by this fluorescent probe.

### Table 1 Comparison of literature on detection of silver ions by fluorescence spectroscopy

| Probe                                | Linear range | LOD/mol L⁻¹ | Time | pH range | Ref. |
|--------------------------------------|--------------|-------------|------|----------|------|
| Dual carbon dots                     | 0.1–0.5 mmol/L | ——          | 10 min | ——       | 15   |
| Heptamethine cyanine dye-based probe | 0–7 μmol/L   | 3 × 10⁻⁴   | 1 min | 6–7      | 20   |
| Rhodamine derivative                 | 0–5 μmol/L   | 1.3 × 10⁻⁷ | 10 min | ——       | 22   |
| Bisphenazimiazole-based              | 0–1 mmol/L   | 4.2 × 10⁻⁷ | 10 s  | 6–13     | 24   |
| Graphene quantum dots                | 0.1–130 μmol/L | 3.0 × 10⁻⁴ | 10 min | 5–10     | 46   |
| Dithioureapended naphthalimide       | 0–24 μmol/L  | 1.2 × 10⁻⁸ | 15 min | 7–12     | 47   |
| Phenanthroline derivative            | 0.05–1.5 μmol/L | 3.4 × 10⁻⁴ | 20 s  | 4–12     | This work |

a. Not mentioned. b. The optimized pH range of the probe in each literature can be applied to detect silver ions.

### Table 2 Results of the detection of Ag⁺ in real water samples (0.05 mmol/L EDTA was added to each sample)

| Sample          | Ag⁺ added/μmol L⁻¹ | Ag⁺ found/μmol L⁻¹ | Recovery, % | RSD, % |
|-----------------|--------------------|-------------------|-------------|-------|
| Tap water       | 0.0                | 0.0               | ——          | ——    |
|                 | 0.3               | 0.304            | 101.4       | 0.72  |
|                 | 1.0               | 1.008            | 100.8       | 0.38  |
|                 | 1.2               | 1.201            | 100.1       | 0.71  |
| Lake water 1    | 0.0                | 0.0               | ——          | ——    |
|                 | 0.3               | 0.314            | 104.7       | 1.68  |
|                 | 1.0               | 0.920            | 92.0        | 0.62  |
|                 | 1.2               | 1.153            | 96.1        | 0.40  |
| Lake water 2    | 0.0                | 0.0               | ——          | ——    |
|                 | 0.3               | 0.296            | 98.7        | 2.46  |
|                 | 1.0               | 1.021            | 102.1       | 0.95  |
|                 | 1.2               | 1.189            | 99.0        | 2.67  |
| Lake water 3    | 0.0                | 0.0               | ——          | ——    |
|                 | 0.3               | 0.316            | 105.4       | 2.28  |
|                 | 1.0               | 0.944            | 96.4        | 3.64  |
|                 | 1.2               | 1.262            | 103.7       | 2.40  |

a. Recovery is amount of Ag⁺ found except amount of Ag⁺ added. b. RSD is the ratio of standard deviation except the arithmetic average of three times measurement.

### Conclusions

In summary, a phenanthroline derivative probe (MPP) was synthesized for the rapid and sensitive detection of silver ions in water solution. The reaction time of MPP with silver ion is very short, about 20 s. This fluorescent probe can detect silver ions over a wide pH range, and it is from 4.0 to 12.0. In this pH range, the fluorescence quenching efficiency of MPP with silver ion is about 95% and very stable. The linear range of silver ion is from 0.05 to 1.5 μmol/L, and with a detection limit of 3.38 × 10⁻⁸ mol/L. A spike and recovery test for Ag⁺ in four water samples was performed and the recovery rate is satisfactory, ranging from 92.0 to 105.4%. The results show that this fluorescent probe is expected to be applied to the highly sensitive, selective and fast detection of silver ions in environmental water samples.

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### Supporting Information

The Supporting Information is the synthesis and characterization data of intermediate and the final product and supplementary spectral data (PDF). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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