DOI: 10.1002/cjoc.201600809

Sensitive and Selective Fluorescent and Colorimetric Sensor for Ag^+ Based on the Supramolecular Self-Assembly in Semi-Water

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Specific recognition of ultratrace levels of ions in semi-water using super-quicker methods is still a challenge for environmental monitoring. Herein we report a fluorescent and colormetric sensor (**ZH**) based on supramolecular self-assembly, whose structure was destroyed by the addition of ultratrace of silver ions. The process promoted either naked eye visible color changes or fluorescence intensity quenched in conjunction with a wide pH range. Systematic studies revealed very high selectivity (0.07 μmol/L) for silver ions, and other common cations, *e.g.*, Hg^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+} had nearly no influence on the sensing behavior. This sensor also served as a multiple use of component in sensing materials by addition of I^- into the mixture of ZH and Ag^+ (about 5 times). What's more, **ZH** containing filter paper emerged distinct color and fluorescence changes upon exposure to silver (Ag^+), which could be used as a portable method to undertake field testing for Ag^+.

Keywords supramolecular self-assembly, silver (Ag^+), dual-channel, aqueous solution

Introduction

In the past few years, quite a lot of research has been dedicated to the area of sensing trace transition metal ions because of their indispensable effects on the biosphere.[1] Silver is one of the most abundant transition metals in mammals and industry. Silver ions play vital roles in various biological processes, such as interaction with DNA, binding to enzymes, destroying bacteria, preventing electron transport, indicating that the accumulation of Ag^+ affects multiple physiological activities in living systems.[2] With the development of science and technology, silver and its compounds were widely used in jewelry, electroplating, imaging industries, and electrical equipment.[3] It is important to our production and life, but even trace amounts of Ag^+ can cause irreversible damage to living body. According to World Health Organization (WHO), the maximum bearable amount of Ag^+ is 0.5 μmol/L in drinking water.[4] Up to now, there are some different methods to detect Ag^+, such as atomic absorption spectrometry, electrothermal atomic absorption spectrometry, inductively coupled plasma mass spectrometry, inductively coupled plasma atomic emission spectrometry, ion chromatography.[5] However, these methods are highly professional and difficult to operate. Therefore, there has been a considerable research effort devoted to developing effective methods for detecting Ag^+ in the environment. Fluorescence spectrometry and naked-eye detection methods are particularly attractive due to their easier operation, obvious comparison, high sensitivity and selectivity.[6]

Most of the fluoroscent sensor molecules are composed of an ion recognition unit modified with a fluorophore. Among the various fluorophores, phenazines have attracted more and more attention due to their excellent photophysical properties.[7] They are found using in many fields, such as pharmaceutical technology, new energy development and industrial dyes.[8] One of the most important aspect of phenazine derivatives is that they can be applied to ions and neutral molecules recognition. What's more, phenazine derivatives served as important and versatile building blocks have been extensively designed to develop the sensors and biosensors over the years.

In this paper, by considering our research interests and selecting the ideal building blocks,[9] we designed and synthesized a phenazine derivative **ZH** (Scheme 1), which could readily selectively recognize Ag^+ in aqueous solution. It was easy to form the intermolecular

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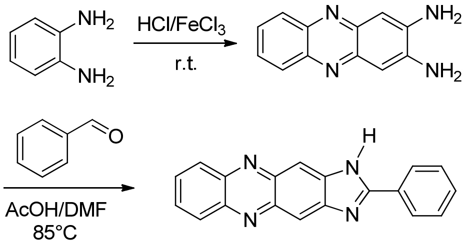
Received November 20, 2016; accepted December 13, 2016; published online XXXX, 2017.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cjoc.201600809 or from the author.

hydrogen bonds and π-π conjugated system among the molecules of **ZH**, as a result it had an excellent optical property to achieve naked-eye colorimetric and fluorimetric recognition. The supramolecular self-assembly, including the intermolecular hydrogen bonds and π-π conjugated system, was rapidly destroyed by addition of Ag^+ leading to significant fluorescent intensity and obvious color changes from yellow to light pink.

Scheme 1 Synthetic procedures of sensor **ZH**



Experimental

General information and materials

All reagents were gained commercially for synthesis and without further purification during experiment progress. All the cations Fe^{3+}, Hg^{2+}, Ag^+, Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{3+}, and Mg^{2+} were added in the form of perchlorate salts, which were purchased from Alfa-Aesar Chemical, and stored in a vacuum desiccator. Doubly distilled water was used throughout the experiment. ^1H NMR and ^{13}C NMR spectra were recorded on a Mercury-400BB spectrometer at 600 MHz. ^1H chemical shifts are reported downfield from tetramethylsilane (TMS, δ scale with solvent resonances as internal standards). UV-vis spectra were recorded on a Shimadzu UV-2550 spectrometer. The fluorescence spectra were recorded with a Shimadzu RF-5301 spectrofluorimeter. Melting points were measured on an X-4 digital melting-point apparatus (uncorrected). Infrared spectra were performed on a Digilab FTS-3000 FT-IR spectrophotometer. All reagents used were of analytical grade.

Synthesis and characterization of sensor ZH

2,3-Diamino-phenazine (0.42 g, 2.0 mmol), benzaldehyde (0.233 g, 2.2 mmol) and five drops acetic acid (AcOH) were combined in 85 ℃ absolute DMF (20 mL) (Scheme 1). The solution was stirred and refluxed for 8 h, after cooling to room temperature, the brown precipitate was filtrated, washed with hot absolute ethanol three times, recrystallized with DMF to get brown powdery product.

ZH: yield 80%; m.p.>300 ℃; ^1H NMR (DMSO-d_6, 600 MHz) δ: 13.45 (s, 1H), 8.51 (s, 1H), 8.37 (d, $J=6$ Hz, 2H), 8.25 (d, $J=18$ Hz, 3H), 7.89 (d, $J=6$ Hz, 2H), 7.66 (d, $J=6$ Hz, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 159.90, 149.25, 142.25, 142.08, 140.88, 140.86, 140.62, 140.23, 132.22, 130.16, 129.87, 129.86, 129.60, 129.43, 129.23, 128.18, 115.23, 109.87, 106.29; IR (KBr) v: 3115 (NH), 1693 (C=N); ESI-MS *m/z* $[M+H]^+$ calcd for $C_{19}H_{12}N_4$ 296.1135; found 297.114 cm^{-1}.

Results and Discussion

The sensor could only recognize Ag^+ over other metal ions. The colormetric and fluorimetric sensing abilities were primarily investigated by adding DMSO solution of various metal ions to the DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20) solutions of sensor **ZH** separately. The fluorescent intensity was sharply decreased at 548 nm with the addition of Ag^+ (Figure 1). Besides, evident color change (from yellow to light pink) was observed, corresponding to the absorption peaks at 403.5 nm decreased with the addition of Ag^+(Figure 2). The fluorescence ON-OFF behavior and obvious color change, in particular, suggest that the supramolecular self-assembly was disintegrated. No significant UV-vis or fluorescence spectra change was observed when solutions of other cations (Fe^{3+}, Hg^{2+}, Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{3+}, Mg^{2+}) were added to the **ZH** solution. Thus, **ZH** could selectively instantly (dual-channel) detect Ag^+ in DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20).

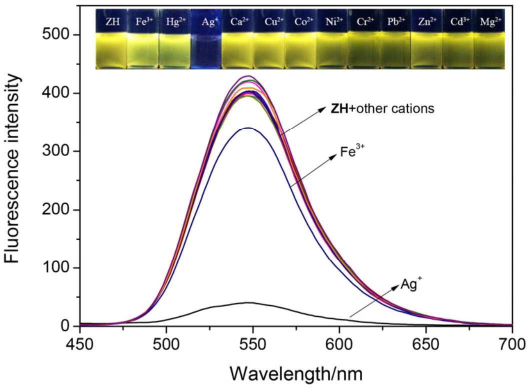


Figure 1 Fluorescence spectra ($\lambda_{ex}=415$ nm) of **ZH** (2×10^{-5} mol/L) with different metal ions: Fe^{3+}, Hg^{2+}, Ag^+, Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{3+}, Mg^{2+} (10 equiv., respectively) in the DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20). Inset: visual fluorescence emission of probe **ZH** upon addition of different metal ions.

The sensor needed to demonstrate high selectivity for the target analyte over other possible interfering metal ions. The variations of UV-vis absorbance and fluorescence of **ZH** in DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20) binary solutions caused by the cations (Fe^{3+}, Hg^{2+}, Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{3+}, Mg^{2+}, Au^{3+}) were investigated (Figures 3, 4 and Figure S10). Form the bar diagram, we could easily consider that the effects on emission and absorption intensity of **ZH** and Ag^+ solutions upon addition of various cations were almost negligible. Therefore, it was clear that other ions' interference was negligible during the detection of Ag^+. These results further suggested

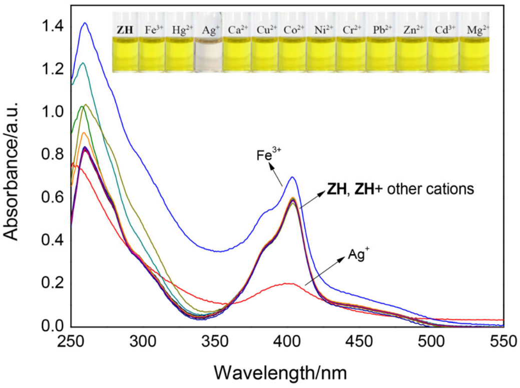


Figure 2 UV-vis spectra responses of **ZH** (2×10^{-5} mol/L) with different metal ions: Fe^{3+}, Hg^{2+}, Ag^+, Ca^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{3+}, Mg^{2+} (10 equiv., respectively) in the DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20). Inset: photograph of **ZH** upon addition of different metal ions.

that **ZH** could be used as a sensor for Ag^+ over a wide range of cations.

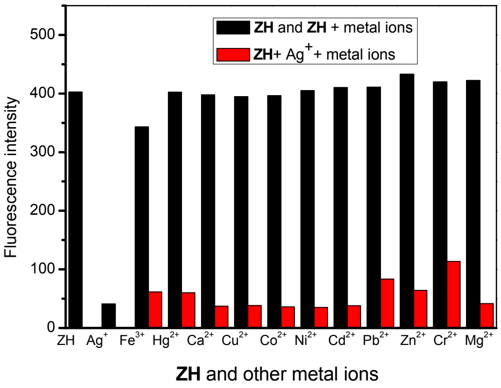


Figure 3 Fluorescence spectra of **ZH** and **ZH**-Ag^+ in the presence of 10 equiv. of various metal ions in DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20) solutions.

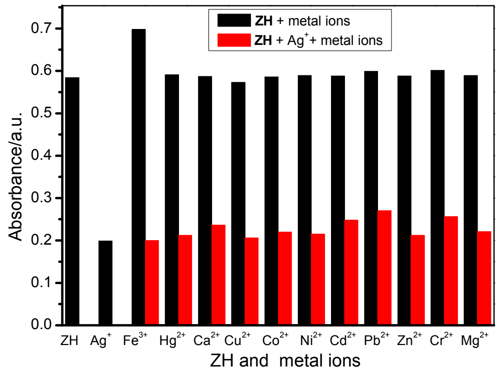


Figure 4 UV-vis spectra of **ZH** and **ZH**-Ag^+ in the presence of 10 equiv. of various metal ions in DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20) solutions.

We also plotted fluorescence and UV-vis absorbance against Ag^+ concentration (Figures 5 and 6). In fluorescence spectra, upon addition of Ag^+ to receptor **ZH**, the emission and the absorption were both gradually decreased at 548 and 403.5 nm respectively. The detection limits of **ZH** for Ag^+ calculated on the basis of $3\sigma/m$ were 2.92×10^{-7} mol/L for fluorescence and 7.73×10^{-8} mol/L for absorption spectra respectively (Figures S6 and S7),[10] which are both far lower than the index of WHO for Ag^+ in drinking water (less than 0.05 μmol/L).

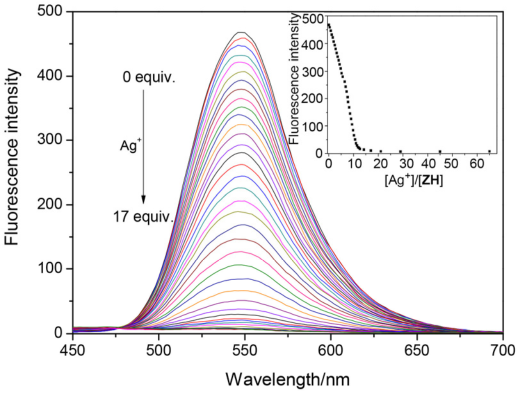


Figure 5 Fluorescence spectra of **ZH** in the presence of different concentrations of Ag^+ in DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20) solutions. Inset: a plot of fluorescence intensity depending on the concentration of Ag^+ in the range from 0 to 17 equivalents, the detection wavelength was 415 nm.

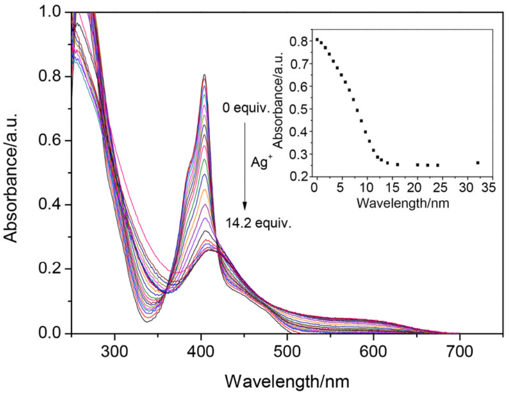


Figure 6 UV-vis spectra of **ZH** in the presence of different concentrations of Ag^+ in DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20) solutions. Inset: a plot of absorbance depending on the concentration of Ag^+ in the range from 0 to 14.2 equivalents.

For better understanding the Ag^+ binding properties of **ZH**, ^1H NMR titration, and ESI-MS methods were used to illustrate the characteristic structural changes occurring upon interaction with Ag^+. As shown in Figure 7, the ^1H NMR chemical shifts of **ZH** show two strong peaks at δ 13.45 and 8.6, which could be assigned to the N-H (H_b) proton and Ar-H (H_a) proton respectively. After addition of 0.1 equivalent of Ag^+, the N-H peak at δ 13.45 disappeared. At the same time, the Ar-H (H_a) peak at δ 8.6 gradually decreased. From what we have discussed above, we can safely draw a conclusion that Ag^+ coordinates with sensor **ZH** through pyrazine group, which shields H_a proton because its peak decreased.

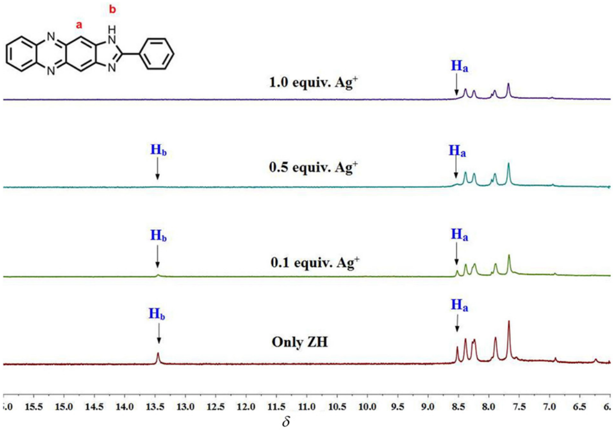


Figure 7 Partial ^1H NMR spectra of free **ZH** and **ZH** in the presence of varying amounts of Ag^+.

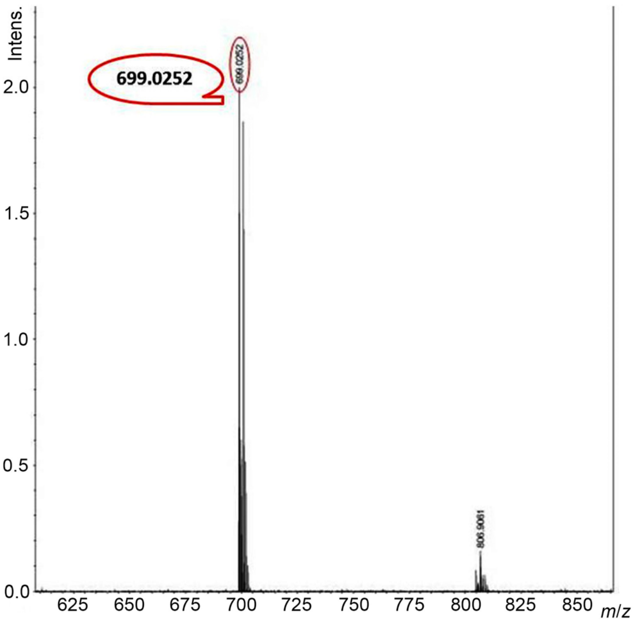


Figure 8 ESI-MS spectrum of **ZH**-Ag^+.

A key to explore mechanism was the assembling structure of **ZH** and **ZH**-Ag^+. The SEM images of **ZH** showed regular spindle shape (Figure 9a). As shown in Figure 9b, upon addition of Ag^+, the regular spindle shape of **ZH** changed to an irregular cluster structure. From their X-ray diffraction (XRD) (Figure 10), we obtained a d-spacing of 3.51 Å by $2\theta = 25.33°$, suggesting that π-π stacking of intermolecular may exist. Unexpectedly, the π-π stacking was destroyed after adding Ag^+. Based on all of above tests, we rationally inferred the same groups repelled from each other due to the electrostatic repulsion interactions. The formation of spindle shapes must relate to the perfect cooperation between the electrostatic repulsion interactions and the π-π stacking interactions. Importantly, hydrogen bonding played a crucial role in this perfect cooperation.[11] With the addition of Ag^+, the perfect cooperation was destroyed which caused the optical properties to change (Figure 11).

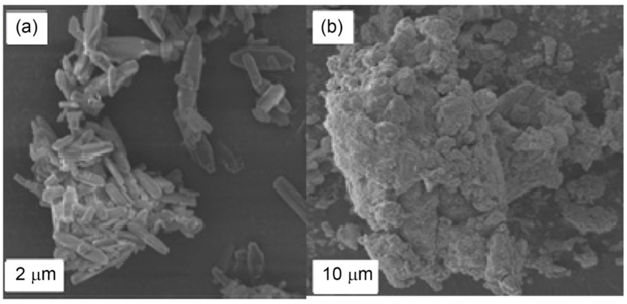


Figure 9 The SEM images of (a) **ZH** and (b) **ZH**-Ag^+.

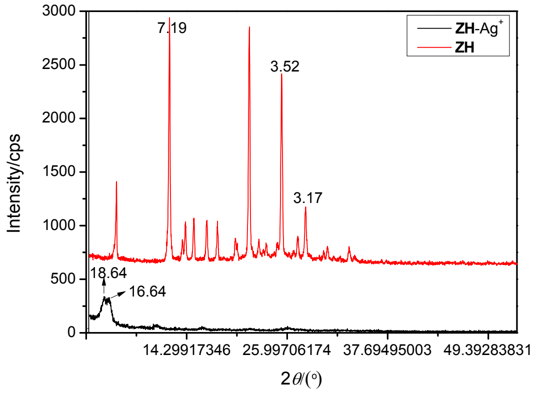


Figure 10 XRD diagrams of **ZH** and **ZH**-Ag^+.

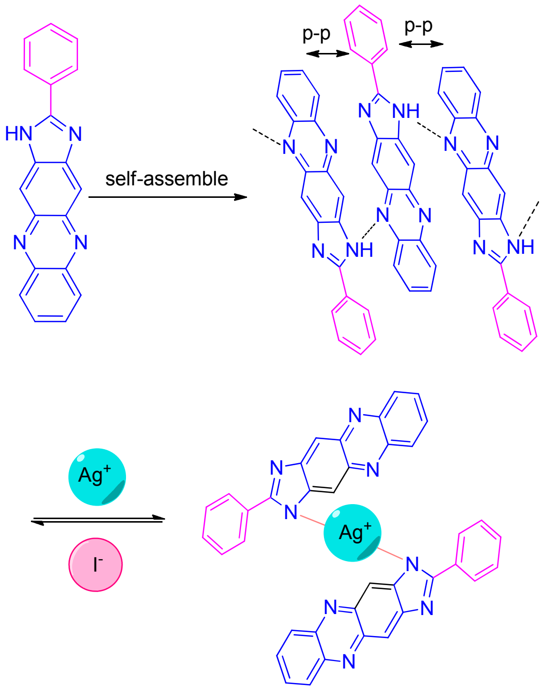


Figure 11 Proposed sensing mechanism of the sensor **ZH** for the detection of silver ion.

The reversibility of the sensor function was tested by addition of I^- to the silver-sensor complex. Upon the addition of I^-, the optical fluorescence intensity gradually increased. The addition of I^- to the **ZH**-Ag^+ complex shows that the process could be repeated several times (Figure S9). Therefore, sensor **ZH** could be considered as a good ON-OFF fluorescent switch.

The practical application of sensor is particularly important for its development and research, so we studied pH scope for **ZH** application and made comparison test strips. As shown in Figure 12, **ZH** can be used in a wide range of 7 to 13. Test strips were prepared by immersing filter paper into a DMSO solution of **ZH** (2×10^{-5} mol/L) and then drying them in air. The test strips containing **ZH** were utilized to sense Ag^+. As shown in Figure 13 and Figure S5, when Ag^+ was added on the test kits, the obvious fluorescence quenching and color changing were observed. Therefore, the test strips could conveniently detect Ag^+ in solution.

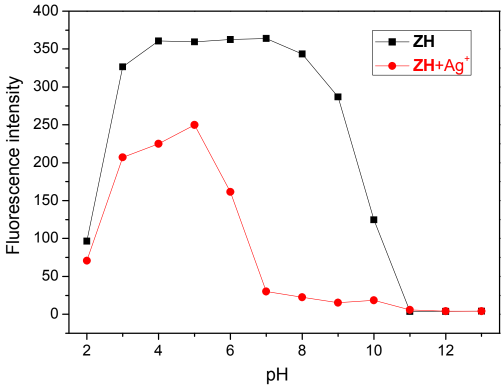


Figure 12 The pH response of **ZH** in the DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES) solution.

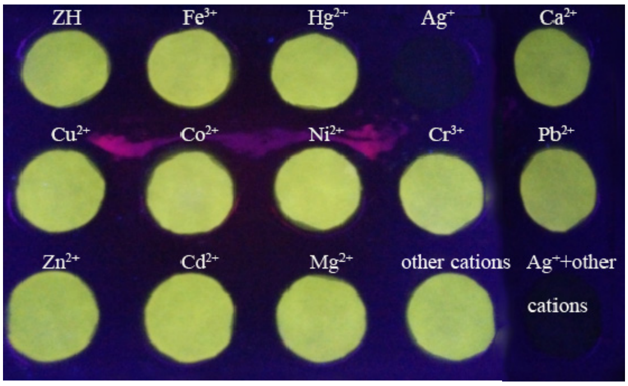


Figure 13 Photographs of **ZH** and the mixture of **ZH** and different cations on the test papers in the DMSO/H_2O (*V*/*V*, 1∶1; buffered with HEPES, pH=7.20) solution with irradiation at 365 nm.

Conclusions

In conclusion, we have presented a low-cost and efficient phenazine-based sensor with high sensitivity for Ag^+. The distinct color changes under day light and rapid fluorescence extinction can be detected by naked eyes. The detection of Ag^+ by using **ZH** is found to be totally free of interference from any other ions, and the mechanism of the supramolecular self-assembly was investigated by XRD and SEM. Moreover, this sensor can be used multiple times during the detection process. The experiment proved that this sensor can be used above 5 times. Taking advantage of the mechanism of the supramolecular self-assembly and the successful application to paper testing, we believe that the sensor **ZH** has the potential applications in environment and physiological systems for Ag^+ detection.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (Nos. 21161018, 21262032, 21574104), the Program for Chang Jiang Scholars and Innovative Research Team in University of Ministry of Education of China (No. IRT1177).

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