

A HIGHLY SENSITIVE FLUORESCENT CHEMOSENSOR FOR SIMULTANEOUS DETERMINATION OF Ag(I), Hg(II), AND Cu(II) IONS

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Abstract

Simultaneous determination of the heavy metal ions at the $\mu\text{g/L}$ level in an aqueous solution has always been attractive to scientists. Here, we present a simple, rapid and highly sensitive fluorescent chemosensor for simultaneous determination of mercury, copper, and silver ions in an aqueous solution in the presence of various competitive metal ions, including Na^+ , K^+ , Ca^{2+} , Ba^{2+} , Mg^{2+} , Pb^{2+} , Cd^{2+} , Co^{2+} , Zn^{2+} , Fe^{2+} , Ni^{2+} , Al^{3+} , and Cr^{3+} ions. The detection limits of the sensor for Hg^{2+} , Cu^{2+} , and Ag^+ ions are 2.8, 0.8, and $1.0\mu\text{g/L}$, respectively. This sensor is potentially suitable for detecting and monitoring these metal ions in drinking water samples according to the regulations of the World Health Organization.

Keywords. Fluorescence, chemosensor, Ag(I), Hg(II), Cu(II), dimethylaminocinnamaldehyde, aminothiourea.

1. INTRODUCTION

Mercury, copper, and silver are toxic heavy metals. Along with the other metals in the gold group including gold, lead and aluminum, they are the least abundant native metals in the earth's crust [1]. In nature, they often appear simultaneously in minerals containing sulfur, tellurium and selenium [2]. These metals also appear together in alloys, high tech trash, and dental amalgams [3].

Mercury is considered a major concern because of its high toxicity. Poisonous inorganic mercury can cause spontaneous abortion, congenital malformation and digestive system disorders [4]. Poisonous organic mercury can cause erethism, acrodynia, gingivitis, stomatitis, neurological disorders, and total damage to the brain and the central nervous system. It is also associated with congenital malformation [5]. Organic mercury is formed mainly as the result of methylation of inorganic mercury by microorganisms in soil and water [6]. Human exposure to organic mercury is via fish consumption, especially predatory fish such as tuna, sword fish, king mackerel, shark, and tilefish [7]. WHO set the upper concentration limit for

inorganic mercury in drinking water at $6\mu\text{g/L}$ [8].

Copper is a heavy metal, but is not as toxic as mercury. Copper is in the essential metals group to many organisms and is considered micronutrients. However, it is also toxic if taken in excess [9]. An excessive intake of copper is thought to be related to Wilson's Disease, Infantile Cirrhosis, and disruption of the metabolism of copper in the human body. Some compound of copper may cause some dermal or eye irritation [10].

Like copper, silver is one of the heavy metals which is highly toxic to microorganisms such as algae, bacteria, viruses, or larval forms of aquatic animals due to its antimicrobial properties. However, high concentrations of silver ions can have adverse effects on the environment and is considered hazardous to human health [11]. Excessive silver intake may discolor the skin and hair. There is no adequate data to derive a health-based guideline value for silver in drinking-water. However, the low levels of silver in drinking water, generally below $5\mu\text{g/L}$, are not relevant to human health. Higher levels of silver, up to $100\mu\text{g/L}$ (special situations exist where silver salts may be used to maintain the bacteriological quality of

drinking water), can be tolerated without risk to health [8].

The development of methods to simultaneously determine the amount of copper, silver and mercury is always attractive to scientists. Some methods such as voltage titration and spectrophotometry, can determine these metal ions at mg/L levels simultaneously [12, 13]. For simultaneous determination of these metals at the $\mu\text{g/L}$ level, several methods, such as anodic stripping voltammetry and solid phase extraction combined with ICP-OES or CV-AAS method, have been used [14,15]. However, these methods often require expensive equipment and complicated techniques. Fluorescence-based analytical methods are gaining popularity because they are sensitive, simple and less expensive [16-18]. Until now, the number of fluorescent sensors for simultaneous determination of metal ions copper, silver and mercury at $\mu\text{g/L}$ levels are still very low. Concerning the ultra small fluorescent method, a recent work has reported a sensor able to determine these metal ions at the $\mu\text{g/L}$ level, but only for simultaneous determination of copper and mercury ions [19].

Chemosensors are molecules of an abiotic origin that interact with the analyte to yield measurable signals with a real-time response. The general operating principle of chemosensors is based on coordination events. Hence, the reaction of a chemosensor with the analyte and the accompanying signal changes are reversible [20].

Here, we present a simple, rapid and highly sensitive fluorescent chemosensor for simultaneous determination of mercury, copper, and silver ions in an aqueous solution. This sensor is potentially suitable for detecting and monitoring these metal ions in drinking water samples according to the regulations of WHO.

2. INSTRUMENTS AND MATERIALS

2.1. Instruments

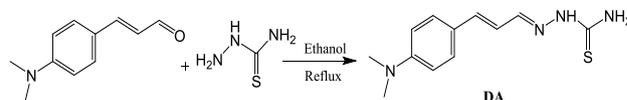
The ^{13}C and ^1H NMR spectra were obtained on a Bruker-400 instrument operating at 100 MHz and 400 MHz, respectively; mass spectra were acquired and analyzed on a Finnigan 4021C using Bruker Daltonics Flex Analysis software; elemental analysis was carried out on a Flash EA 1112 instrument; absorption and fluorescence spectra were recorded on a Shimadzu UV-1800 UV-vis spectrophotometer and a Shimadzu RF-5301 PC Series fluorescence spectrometer.

2.2. Reagents

4-N,N-Dimethylaminocinnamaldehyde (DACA), aminothiurea, and all cations (Zn^{2+} , Cu^{2+} , Cd^{2+} , Pb^{2+} , Ag^+ , Fe^{2+} , Cr^{3+} , Co^{2+} , Ni^{2+} , Ca^{2+} , Ba^{2+} , Al^{3+} , Mg^{2+} , Hg^{2+} , K^+ and Na^+) were obtained from Aldrich. Perchlorate counter ion was used for all metals in the system. Tetrasodium ethylenediaminetetraacetate (Na_4EDTA), $\text{Na}_2\text{S}_2\text{O}_3$, and all solvents were purchased from Merck and used as received. Spectroscopic studies were performed using HPLC reagent grade solvents without fluorescent impurities.

2.3. Synthesis of dimethylaminocinnamaldehyde-aminothiurea

DACA and aminothiurea were mixed in absolute ethanol. The reaction mixture was heated at reflux for 6 hours under a nitrogen atmosphere and then stirred for 2 more hours at room temperature. The resulting precipitate was filtered and washed with ethanol several times, then recrystallized from absolute ethanol to give the final product DA (scheme 1) in 83.0 % yield. The structure of dimethylaminocinnamaldehyde-aminothiurea was obtained from investigation into the ^1H NMR, ^{13}C NMR, and MS spectra.



Scheme 1: The synthetic route to chemosensor DA

3. RESULTS AND DISCUSSION

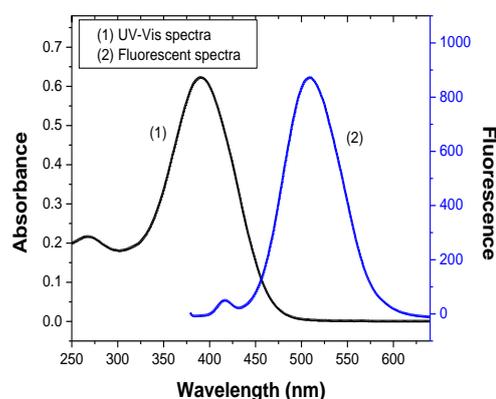


Fig. 1: UV-Vis (DA 15 μM) and fluorescent (DA 3 μM , $\lambda_{\text{ex}} = 390 \text{ nm}$) spectra of **DA** in $\text{C}_2\text{H}_5\text{OH}/\text{H}_2\text{O}$ solution (1/9, v/v) at pH \sim 7

The free DA exhibited a characteristic absorption band peaked at 390 nm and gave rise to an extensively green emission at 510 nm in aqueous solution with a quantum yield of 0.25 (Fig. 1).

Satisfying expectations in the design of fluorescent sensors for HTM ions, the fluorescence intensity of DA was quenched when Ag^+ , Cu^{2+} , or Hg^{2+} ions was added to the solution. In contrast, no distinct change in the fluorescence spectra was observed when 5 equiv of various competing metal ions including Na^+ , K^+ , Ca^{2+} , Ba^{2+} , Mg^{2+} , Pb^{2+} , Cd^{2+} , Co^{2+} , Zn^{2+} , Fe^{2+} , Ni^{2+} , Al^{3+} , and Cr^{3+} ions, were added to an aqueous solution of DA (Fig. 2).

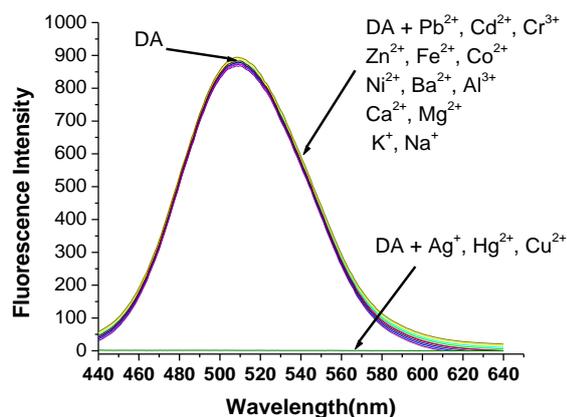


Fig. 2: Changes in fluorescence intensity of DA (3 μM) in EtOH/ H_2O solution (1:9, v/v) at pH 7 upon the addition of different metal ions including Ag^+ , Hg^{2+} , Cu^{2+} , Pb^{2+} , Cd^{2+} , Cr^{3+} , Zn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Ba^{2+} , Al^{3+} , Ca^{2+} , Mg^{2+} , K^+ and Na^+ ions (10 μM each) with an excitation wavelength of 390 nm

The possibility of using DA to quantify one of the three ions (Ag^+ , Hg^{2+} , Cu^{2+}) in absence of the other two in an aqueous solution was also investigated. Fig. 3 shows that there are good linear relationships between the change in fluorescence intensity of DA against different Ag^+ , Hg^{2+} or Cu^{2+} concentration. The correlation coefficients of the linear regression are 0.999. The following equations were developed from calibration curves: $I_{510} = (874.0 \pm 2.2) - (3.4 \pm 0.0) \times [\text{Ag}^+]$, $I_{510} = (879.9 \pm 3.2) - (2.6 \pm 0.0) \times [\text{Hg}^{2+}]$, and $I_{510} = (882.9 \pm 2.6) - (10.5 \pm 0.1) \times [\text{Cu}^{2+}]$. The detection limits and quantitation limits were 1.0 $\mu\text{g/L}$ and 3.5 $\mu\text{g/L}$, 2.8 $\mu\text{g/L}$ and 9.2 $\mu\text{g/L}$, 0.8 $\mu\text{g/L}$ and 2.7 $\mu\text{g/L}$, respectively.

The pH range of the chemosensor was also investigated (Fig. 4). The results indicate that the ideal pH range for Ag^+ , Hg^{2+} , and Cu^{2+} detection is between 5 and 9. The broad pH range makes DA useful for a variety of applications, including the detection of heavy metal ions in wastewater,

industrial trade analysis, and physiological processes.

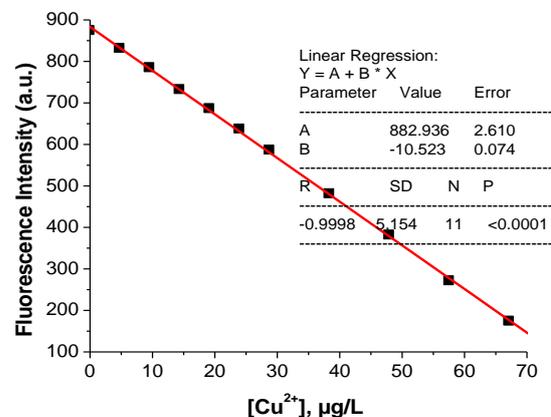
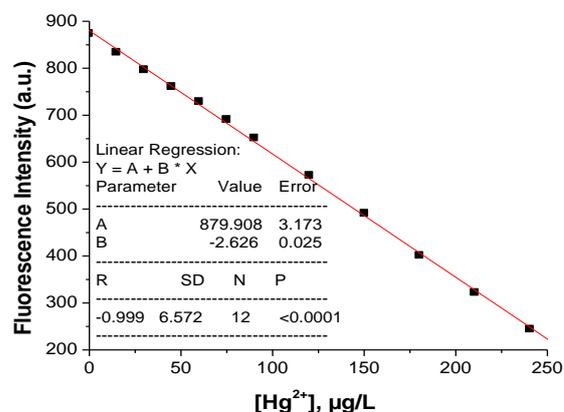
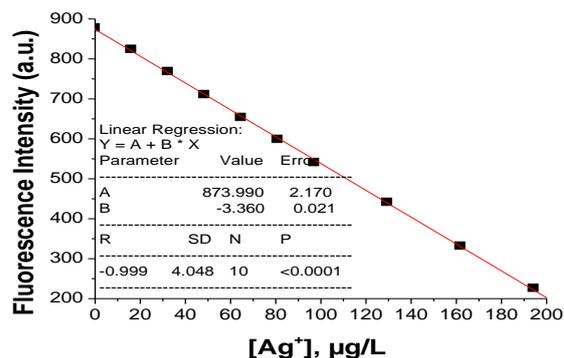


Fig. 3: Changes in fluorescence intensity ($\lambda_{em} = 510$ nm) of DA (3 μM) in EtOH/ H_2O solution (1:9, v/v) at pH ~ 7 vs the concentration of Ag^+ ions (16-195 $\mu\text{g/L}$), Hg^{2+} ions (15-240 $\mu\text{g/L}$), and Cu^{2+} ions (5-67 $\mu\text{g/L}$)

The possibility of using DA to quantify one of the three ions (Ag^+ , Hg^{2+} , and Cu^{2+}) in presence of other two ions in an aqueous solution was also investigated. $[\text{Hg}(\text{S}_2\text{O}_3)_4]^{6-}$ and $[\text{CuEDTA}]^{2-}$ are very stable complexes with formation constants of $10^{33.24}$ and $10^{18.70}$, respectively [21]. $\text{Na}_2\text{S}_2\text{O}_3$ and Na_4EDTA were chosen to form complexes with Hg^{2+} and Cu^{2+}

ions. Fig. 5 shows that the change in fluorescence intensity of DA by Hg^{2+} and Cu^{2+} can be prevented if 10 equiv of $Na_2S_2O_3$ and 10 equiv of EDTA are added to the solution (against respective metal ions).

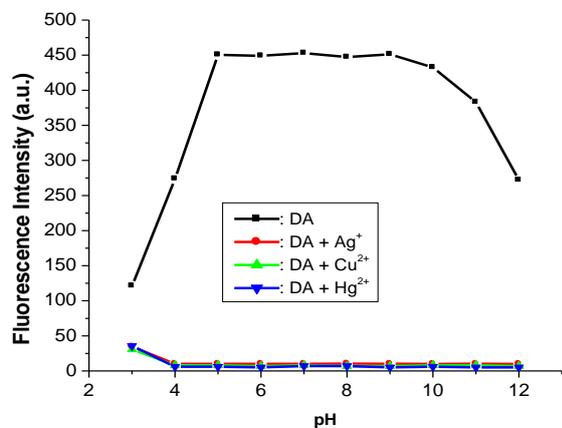


Fig. 4: Fluorescence intensity ($\lambda_{em} = 510\text{ nm}$) of DA ($3\ \mu\text{M}$) in the absence and presence of 5 equiv of Ag^+ , Hg^{2+} , and Cu^{2+} ions in EtOH/ H_2O solution (1:9, v/v) at different pH levels

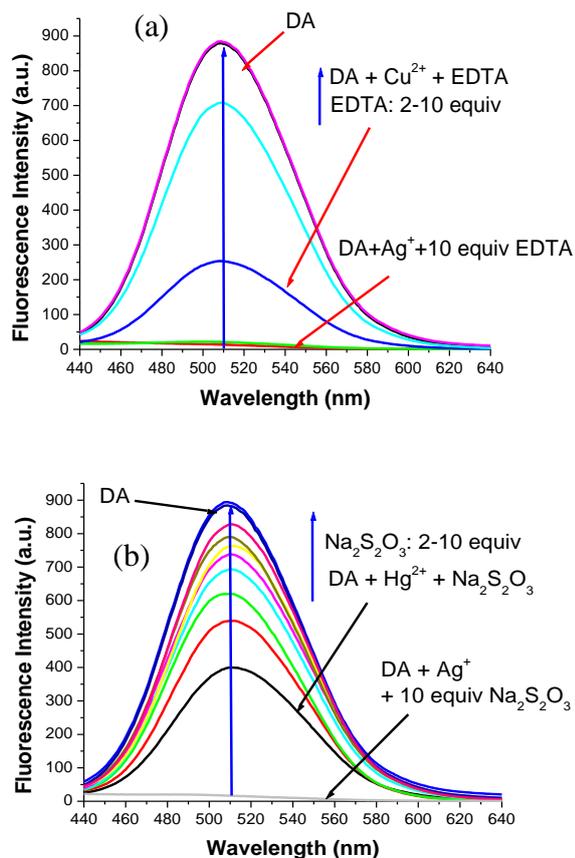


Fig. 5: Changes in fluorescence intensity of DA ($3\ \mu\text{M}$) in EtOH/ H_2O solution (1:9, v/v) at pH 7 upon addition of (a) Cu^{2+} and EDTA, (b) Hg^{2+} and $Na_2S_2O_3$ with an excitation wavelength of 390 nm

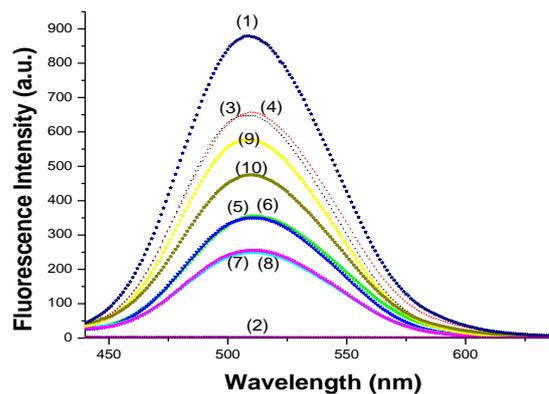


Fig. 6: The possibility of identifying individual ions in the mixture: (1) DA; (2) DA + Ag^+ + Hg^{2+} + Cu^{2+} ; (3) DA + Ag^+ + Hg^{2+} + $Na_2S_2O_3$ + Cu^{2+} + EDTA; (4) DA + Ag^+ ; (5) DA + Ag^+ + Hg^{2+} + Cu^{2+} + EDTA; (6) DA + Ag^+ + Hg^{2+} ; (7) DA + Ag^+ + Hg^{2+} + $Na_2S_2O_3$ + Cu^{2+} ; (8) DA + Ag^+ + Cu^{2+} ; (9) DA + Hg^{2+} ; (10) DA + Cu^{2+} (the concentrations of DA, metal ions, Na, and ED: $3\ \mu\text{M}$, $0.6\ \mu\text{M}$, $100\ \mu\text{M}$, and $100\ \mu\text{M}$, respectively)

Fig. 6 shows that it is possible to quantify the Ag^+ ions in the metal ions mixture (Ag^+ , Hg^{2+} , and Cu^{2+}) based on the variation of fluorescence intensity between the free DA solution (solution 1) and the DA solution containing the metal ions with $Na_2S_2O_3$ ($100\ \mu\text{M}$) and EDTA ($100\ \mu\text{M}$) (solution 3). Following that the Hg^{2+} ions concentration can be quantified based on the variation of fluorescence intensity between the solution 3 and the solution with the removal of $Na_2S_2O_3$ ($100\ \mu\text{M}$) (solution 5). Finally, the Cu^{2+} ion concentration can be quantified based on the variation of fluorescence intensity between the solution 3 and the solution with the removal of EDTA ($100\ \mu\text{M}$) (solution 7).

There are good linear relationships between the variation of fluorescence intensity of DA and Ag^+ , Hg^{2+} or Cu^{2+} concentration. The correlation coefficients of the linear regression are 0.999. The following equations were developed from calibration curves: $\Delta I_{510} = (3.1 \pm 2.1) + (3.4 \pm 0.0) \times [Ag^+]$, $\Delta I_{510} = (-6.6 \pm 3.2) + (2.6 \pm 0.0) \times [Hg^{2+}]$, and $\Delta I_{510} = (-9.7 \pm 2.6) + (10.5 \pm 0.1) \times [Cu^{2+}]$.

Fig. 7 shows the fluorescence titration spectra of DA upon the addition of Ag^+ , Hg^{2+} , and Cu^{2+} . The fluorescence intensity of DA was gradually quenched by increasing the concentration of the metal ions. Emission was almost completely quenched upon addition of 1 equiv of Ag^+ , 0.5 equiv of Hg^{2+} and 0.5 equiv of Cu^{2+} . When 5 equiv of Na_2S , 10 equiv of $Na_2S_2O_3$, and 10 equiv of EDTA were added to the previous solutions, respectively, the fluorescence intensity increased and was restored to

its original value. These results indicate that Ag^+ , Hg^{2+} , and Cu^{2+} reversibly binds to DA to form complexes with stoichiometry of 1:1, 1:2, and 1:2, respectively. These results indicate that DA acts as an ON-OFF fluorescent chemosensor and DA can be used to quantify each individual ion in a mixture of Ag^+ , Hg^{2+} , and Cu^{2+} in an aqueous solution. Remarkably, DA can detect Ag^+ , Hg^{2+} , and Cu^{2+} ions with high sensitivity (detection limits = 1.0, 2.8, and 0.8 $\mu\text{g/L}$, respectively) over a wide pH range (from 5 to 9) and with only a small percentage of organic solvent in the solution (1:9 EtOH:H₂O). These features make DA a promising candidate for practical determination of Ag^+ , Hg^{2+} , and Cu^{2+} ions.

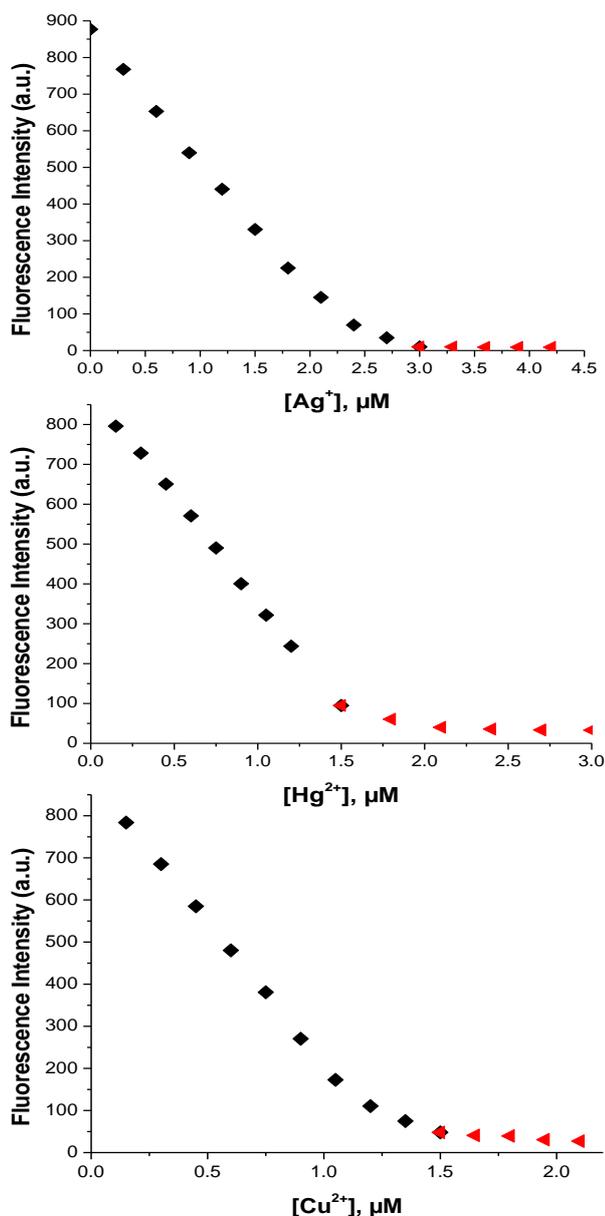


Fig. 8: The changes of the fluorescence intensity of DA (3 μM) with increasing the concentration of the metal ions: (a) 0-4.5 μM of Ag^+ ions; (b) 0-3.0 μM of Hg^{2+} ions; (c) 0-2.5 μM of Cu^{2+} ions

4. CONCLUSIONS

A new fluorescent chemosensor for simultaneous quantification of silver, copper, and mercury ions in an aqueous solution has been reported. The concentrations of individual ions were quantified from the variation of fluorescence intensity of DA solution upon the addition of these metal ions. The amount of silver ions was observed by using both $\text{Na}_2\text{S}_2\text{O}_3$ and EDTA to prevent the reaction of Hg^{2+} and Cu^{2+} with DA. The amount of mercury or copper ions was then observed by removing $\text{Na}_2\text{S}_2\text{O}_3$ or EDTA, respectively. The limits of detection for Hg^{2+} , Cu^{2+} , and Ag^+ ions are 2.8, 0.8, and 1.0 $\mu\text{g/L}$, respectively. DA was found to be able to detect metal ions in a wide pH range (from 5 to 9) and with the presence of a small amount of organic solvent (1:9, EtOH:H₂O). This simple, rapid and highly sensitive fluorescent chemosensor is potentially suitable for detecting and monitoring these metal ions in drinking water samples according to the regulations of WHO.

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