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Poly(acrylic acid)-templated silver nanoclusters as a platform for dual fluorometric turn-on and colorimetric detection of mercury (II) ions

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ABSTRACT

An easy prepared fluorescence turn-on and colorimetric dual channel probe was developed for rapid assay of Hg^{2+} ions with high sensitivity and selectivity by using poly(acrylic acid)-templated silver nanoclusters (PAA-AgNCs). The PAA-AgNCs exhibited weak fluorescence, while upon the addition of Hg^{2+} ions, AgNCs gives a dramatic increase in fluorescence as a result of the changes of the AgNCs states. The detection limit was estimated to be 2 nM, which is much lower than the Hg^{2+} detection requirement for drinking water of U.S. Environmental Protection Agency, and the turn-on sensing mode offers additional advantage to efficiently reduce background noise. Also, a colorimetric assay of Hg^{2+} ions can be realized due to the observed absorbance changes of the AgNCs. More importantly, the method was successfully applied to the determination of Hg^{2+} ions in real water samples, which suggests our proposed method has a great potential of application in environmental monitoring.

1. Introduction

Mercury is one of the most potent toxicants in the world. The exposure to mercury even at very low concentration would result in digestive, kidney and especially neurological diseases [1,2]. Therefore, the probe of Hg^{2+} ions has been a focus of many research endeavors. Traditional techniques for the detection of Hg^{2+} ions share the drawbacks of requiring expensive and sophisticated instrumentation and complicated sample preparation processes [3]. Although many of these limitations are now being addressed by the development of new assays using inorganic materials [4–8], small organic molecule [9,10], DNA [11], DNAzyme [12], protein [13], etc. as sensing elements, there is still an urgent demand for the design of Hg^{2+} ions sensors with high sensitivity and selectivity.

Recently, there has been an explosion of interest in the use of nanomaterials for the development of novel molecular analytical tools [14]. Especially, owing to their unique optical properties, excellent stability, good biocompatibility and water solubility, noble metal nanoclusters have attracted extensive research interests and have been developed to provide a wide range of novel applications [15–18]. For example, they have been successfully employed as novel sensing materials for detection of protein [19], DNA [20], metal ions [21–28], and various small molecules [29,30]. Very recently, these nanoclusters have also been used for Hg^{2+} detection [22–28]. For example, BSA-templated Au nanoclusters (AuNCs) have been used in assaying Hg^{2+} ions based on the high-affinity metallophilic Hg^{2+}–Au^{+} interactions [26]. Also, the dihydrolipoic acid-stabilized Ag nanoclusters (AgNCs) have been used for Hg^{2+} detection through the aggregation of the Ag nanoclusters [27]. However, all of these methods are limited by their fluorescence turn-off nature, which might significantly compromise the specificity since other quenchers or environmental stimulus could also lead to fluorescence quenching and give "false positive" results. Herein, for the first time, we describe the use of the poly(acrylic acid)-templated silver nanoclusters (PAA-AgNCs) as a novel platform for highly sensitive and selective fluorescence turn-on and colorimetric sensing of Hg^{2+} ions.

2. Materials and methods

2.1. Chemicals and materials

Silver nitrate ($AgNO_3$) was purchased from Alfa Aesar. Poly(acrylic acid, sodium salt) (PAA, average Mw = 15,000) was bought from Sigma–Aldrich. All other reagents were of analytical reagent grade, and used as received. Ultrapure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiment.

2.2. Instrumentation

The UV–Vis absorption spectra and fluorescence spectra were recorded using a JASCO V-550 UV/Visible and a JASCO FP6500

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spectrophotometer (JASCO International Co., LTD., Tokyo, Japan). TEM images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV.

2.3. Preparation of Ag nanoclusters

For the synthesis of fluorescent Ag nanoclusters, 20 μL PAA (35%) was introduced into a freshly prepared 280 μL of 5 mM $AgNO_3$ solution under stirring. After 5 min, the mixture was subjected to the UV irradiation using a Cole Parmer 97500-50 Transilluminator (365 nm, 7000 μW/cm^2) for 30 s. All the experiments were performed at room temperature (298 K).

2.4. Detection of Hg^{2+} ions

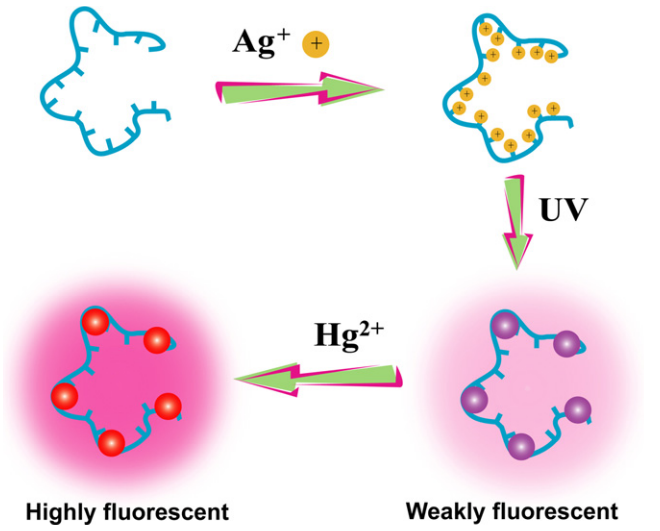
In a typical test, 20 μL AgNCs was mixed with 180 μL of 20 mM NH_4Ac buffer at pH 7.0. 2 μL of different concentrations of Hg^{2+} ions was added, and equilibrated for 10 min at room temperature before the spectral measurements. UV–Vis absorption spectra were recorded using a JASCO V-550 UV/Vis spectrophotometer. Fluorescence measurements were carried out on an FP-6500 spectrofluorometer. The emission spectra were recorded in the wavelength of 500–750 nm upon excitation at 490 nm. Each spectrum was the average of three scans. A 1-cm path length rectangular quartz cell was used for these measurements.

Recovery of spiked Hg^{2+} ions in different aqueous samples was also studied. The tap water and mineral water samples were used without any pretreatment, while the river water was filtered using a syringe filter (pore diameter 0.22 μm) to remove oil and other organic impurities prior to analysis. The results showed that no Hg^{2+} existed in real water samples. Thus, using standard addition method, water samples spiked with various concentrations of Hg^{2+} ions were added to an equal volume of the PAA-AgNCs. The mixture was incubated at room temperature for 10 min and then fluorescence spectra were recorded under an excitation wavelength of 490 nm.

3. Results and discussion

3.1. Preparation and characterization of the PAA-AgNCs

The working principle of our work is schematically represented in Scheme 1. We use poly(acrylic acid) (PAA) as a template to generate fluorescent Ag nanoclusters. Firstly, PAA carries carboxylic acid groups capable of coordinating with Ag^+ ions [31], which can serve as Ag source for further reduction reactions. After 30 s of UV irradiation, the color of the solution gradually changed from colorless to purple, which associated with $Ag_n{}^+$ nanoclusters, where n varied from 2 to 8, according to previous studies [32,33]. The as-prepared PAA-AgNCs exhibited weak fluorescence, while the fluorescence intensity of the AgNCs was significantly enhanced upon the addition of Hg^{2+} ions. Meanwhile, the AgNCs showed color changes from purple to pale red in the presence of Hg^{2+} ions.



Scheme 1. Schematic illustration of the poly(acrylic acid)-templated silver nanoclusters (PAA-AgNCs) formation and the fluorescence and colorimetric response of the PAA-AgNCs to Hg^{2+} ions.

A series of tests were carried out to confirm the feasibility of strategy discussed above. Firstly, the PAA-AgNCs were synthesized according to previous reports [34–36]. The as-prepared AgNCs were studied by transmission electron microscopy (TEM) imaging (Fig. S1), which suggests that there is no formation of larger Ag nanoparticles or aggregation. Fig. S2A displays the absorption spectra of AgNCs, and the bands featured both at 350 nm and at 510 nm should be assigned to the absorption of the photoreduced silver nanoclusters [32,33]. The fluorescence spectrum of the AgNCs over a range of excitation wavelengths was then carried out. As shown in Fig. S2B, the emission band of AgNCs was found to be red shifted with increasing excitation wavelengths between 350 and 550 nm, and it exhibited maximum emission at 575 nm when excited at 490 nm. These results indicated the presence of a distribution of AgNCs with different numbers of Ag atoms [15]. Fig. S3A compares the fluorescence spectra of AgNCs before and after addition of Hg^{2+} ions. Interestingly, it was found that the fluorescence intensity of AgNCs could be enhanced by addition of Hg^{2+} ions. Moreover, it takes less than 1 min to reach a constant value (Fig. S3B), the reaction time was shorter than most of other Hg^{2+} ions assays [4,37]. These features denoted that PAA-AgNCs could serve as a novel and facile platform for rapid and turn-on detection of Hg^{2+} ions.

3.2. Hg^{2+} detection using the PAA-AgNCs as fluorescent probes

The specific feature that Hg^{2+} ions could induce fluorescence enhancement of PAA-AgNCs was then employed for the highly sensitive detection of Hg^{2+} ions. As shown in Fig. 1A, the fluorescent intensity of PAA-AgNCs emitted at 575 nm increased gradually with increasing concentrations of Hg^{2+} ions. Fig. 1B presents the fluorescence data as a function of enhancement factor $(F - F_0)/F_0$ versus the concentration of Hg^{2+} ions, where F and F_0 are fluorescence intensity of AgNCs excited at 490 nm in the absence and presence of Hg^{2+} ions, respectively. The value of $(F - F_0)/F_0$ increased gradually and reached a plateau when the concentration of Hg^{2+} raised from 0 to 50 μM. A linear region is observed between 0 and 20 μM, where Hg^{2+} can be quantitatively measured. The detection limit in our work was estimated to be 2 nM, which is much lower than the Hg^{2+} detection requirement for drinking water of U.S. Environmental Protection Agency with the maximum contamination level at 10 nM. Also, it is better than most of the values obtained by nanoclusters-based assays [25,28]. More importantly, the turn-on sensing mode offered additional advantage to efficiently reduce the likelihood of a false positive signal.

For an excellent chemosensor, high selectivity is a matter of necessity. To investigate whether our system is specific for Hg^{2+} ions, the fluorescence responses of PAA-AgNCs to the other metal ions were monitored and compared with that of Hg^{2+} ions. The results shown in Fig. 2 present the relative photoluminescence intensity of the PAA-AgNCs in the presence of 10 μM Hg^{2+} and other metal ions with a concentration ten times higher than that of Hg^{2+}. We found that only Hg^{2+} ions could result in a significant fluorescent enhancement of the PAA-AgNCs, whereas, no obvious fluorescence changes were observed in the presence of other metal ions. Moreover, the response of our sensing system to the presence

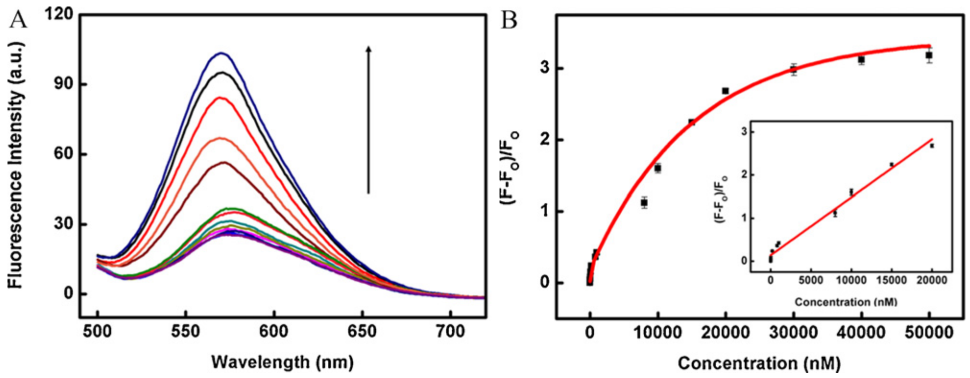


Fig. 1. (A) Representative fluorescence spectra of PAA-AgNCs in the presence of increasing Hg^{2+} ions concentrations (from 2 nM to 50 μM). (B) Plots of the fluorescent intensity of AgNCs versus the concentration of Hg^{2+} ions, with excitation and emission wavelengths of 490 and 575 nm. The error bars represent the standard deviation of three measurements. Inset is a linear region.

of various environmentally relevant anions was investigated (Fig. S4). Our results showed that all the anions presented only slight and negligible effects on the fluorescence of the PAA-AgNCs. These observations indicated that our sensing system exhibited excellent selectivity for Hg^{2+} ions in aqueous solution.

3.3. Hg^{2+} detection using the PAA-AgNCs as colorimetric probes

Another advantage of our system is that a colorimetric assay of Hg^{2+} ions is also possible by taking advantage of the observed absorbance changes of the PAA-AgNCs upon addition of Hg^{2+} ions, which might be due to the changes of the AgNCs state [15,32–36]. As shown in Fig. S5, the absorption peak of PAA-AgNCs at 350 nm decreased, meanwhile, the absorption band at 510 nm increased in the presence of Hg^{2+} ions. Moreover, we observed that the absorption spectrum changes were also sensitive to Hg^{2+} concentrations. The increasing absorbance of the 510 nm peaks correlated well with the increasing concentrations of Hg^{2+} ions (Fig. 3A and B), and the detection limit in our work was measured to be 5 nM. Meanwhile, the absorbance changes of AgNCs resulted in a solution color change from purple to pale red in the presence of Hg^{2+} ions (Fig. S5). This phenomenon indicated that PAA-AgNCs could be used as colorimetric indicators to detect Hg^{2+} ions. Furthermore, the remarkable selectivity toward Hg^{2+} ions over other metal ions could also be observed by the naked eye. As shown in Fig. 3C, Hg^{2+} can cause the change of the solution color from purple to pale red, while the solution retained its original purple color in the presence of other tested metal ions, indicating the high selectivity of this visual method for Hg^{2+} detection. This clearly indicated that PAA-AgNCs can effectively differentiate Hg^{2+} ions and other metal ions with naked eye, thus serving as very simple and promising colorimetric probes.

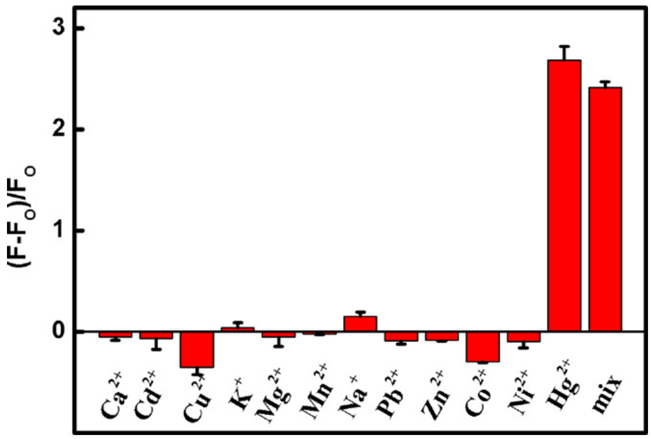


Fig. 2. The effect of different metal ions on the fluorescence emission of the PAA-AgNCs emitted at 575 nm. Concentration of Hg^{2+} ions 10 μM; concentration of other metal ions 100 μM. The sensor response to Hg^{2+} ions (10 μM) in the presence of a mixture of all other metal ions (100 μM) is also presented.

3.4. Detection of Hg^{2+} ions in real water samples

The excellent specificity combined with high sensitivity and fast response of PAA-AgNCs to Hg^{2+} ions suggested that our method might be directly applied for detecting Hg^{2+} ions in real samples. Therefore, we further examined the practicality of the assay for fluorescence detection of Hg^{2+} ions in three different real water samples. Different concentrations of Hg^{2+} ions were, respectively, spiked in filtered water samples and analyzed with the standard addition method. Table 1 showed the results obtained in additional experiments concerning recovery studies on samples spiked with known amounts of Hg^{2+}. One can see that the recovery study of spiked Hg^{2+} determined by our methods exhibited satisfactory results. We also used ICPMS to validate the reliability of the sensor. A water sample collected from South Lake (Changchun, China) was filtered through a 0.22 μm membrane and then analyzed separately using both ICPMS and the developed sensing technique. Neither our sensing materials nor the ICP-MS-based system detected the presence of Hg^{2+} ions in the water sample. So we added 10 μM Hg^{2+} ions in the water sample and then analyzed it again (Table S1). The *F*-test value for the correlation between the two methods was 1.44 (the *F*-test value is 6.39 at a 95% confidence level), suggesting that our present approach are in good agreement with those obtained using ICP-MS. All these results reveal the practicality of using our PAA-AgNCs for the determination of Hg^{2+} ions in real water samples.

Table 1
Determination of Hg^{2+} in different water samples.

Sample	Hg^{2+} spiked (10^{-5} M)	Hg^{2+} recovered (10^{-5} M)	Recovery (%)	RSD ($n = 3$, %)
River water				
1	1	0.97	97	2.52
2	5	5.13	102.6	3.51
3	10	10.9	109	2.26
Tap water				
1	1	1.01	101	1.83
2	5	4.89	97.9	4.15
3	10	10.1	101	1.54
Mineral water				
1	1	1.02	102	1.0
2	5	4.92	98.4	2.65
3	10	9.9	99	2.18

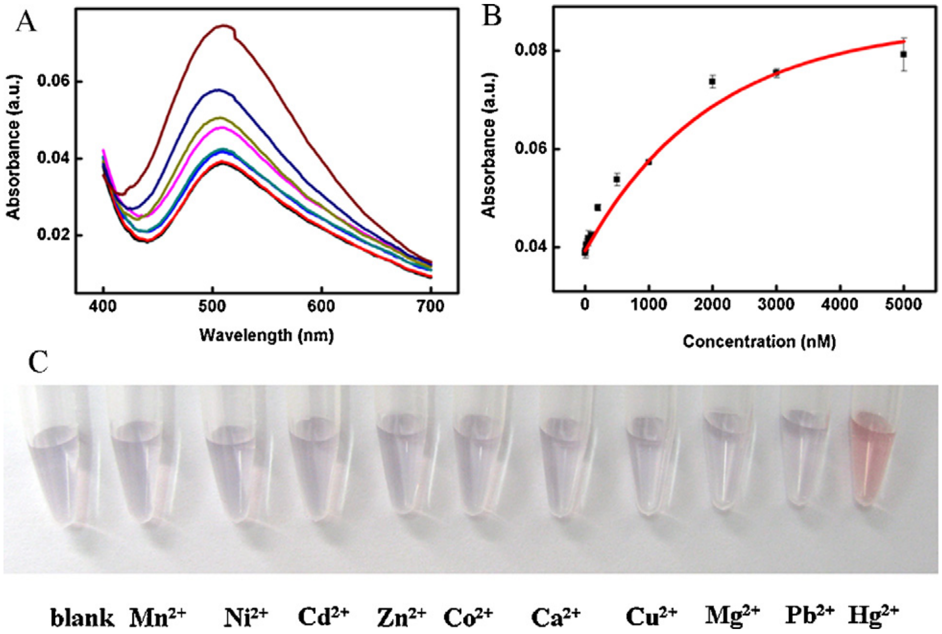


Fig. 3. (A) UV–Vis absorption spectra of PAA-AgNCs in the presence of increasing Hg^{2+} concentrations (0–2 μM). (B) Plots of the absorbance of AgNCs at 510 nm as a function of Hg^{2+} concentrations (0–5 μM). The error bars represent the standard deviation of three measurements. (C) Visual color changes of solutions of AgNCs after addition of Hg^{2+} and other metal ions (50 μM). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.5. Mechanism of Hg^{2+} induced fluorescence enhancement of the PAA-AgNCs

Based on the above findings, we proposed a possible mechanism for the observed fluorescence enhancement and absorbance changes. It is well known that the reduced nanoclusters and oxidized nanoclusters have remarkably different fluorescence properties and can transform into each other in the presence of reducing or oxidizing agents [38]. Also, previous reports have showed that the redox reaction between Ag^0 and Hg^{2+} may become possible, especially when the electrochemical potentials of Ag^0 are rather electronegative in silver nanoparticles or nanoclusters [39,40]. So the possible explanation to the turn-on pattern might be due to the changes of the AgNCs state. Evidence for this explanation was indicated by UV measurement. The absorption peaks located at 350 nm and 510 nm are due to the absorption of photoreduced AgNCs [32,33]. Firstly, upon addition of Hg^{2+} ions, the absorption peak at 350 nm decreased, and the band at 510 nm increased (Fig. S5), which might imply the state changes [15,32–36]. The previous reports and the results of the experiments all supported our inference that the absorbance changes and the fluorescent turn on mechanism might be due to the changes of the AgNCs states. We noted that, due to the high complexity of the PAA-AgNCs system and the lack of adequate understanding of chemical and spectral properties of the AgNCs, it is still a challenge to fully understand the detailed mechanism driving the turn-on response pattern.

4. Conclusions

In summary, we have demonstrated a rapid, highly sensitive, and selective fluorescence turn-on and colorimetric sensor for Hg^{2+} ions detection by using PAA-templated silver nanoclusters. This assay has several important features. The assay is based on the fluorescence turn-on mode, which reduces the likelihood of a false positive signal and enhances the detection sensitivity. It is simple in design and offers a convenient "mix-and-detect" protocol for homogeneous and rapid detection with high sensitivity and selectivity. Furthermore, the assay is easy to implement for visual detection due to the absorbance changes. More importantly, successfully analyses of real water samples proved the practicality of this method. Therefore, we expect that this strategy may offer a new approach for developing low cost, sensitive and rapid sensor for detecting of Hg^{2+} ions, and will be highly useful in a wide range of applications within fluorescence imaging, bioanalysis, and nanotechnology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.10.043.

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