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# Colorimetric determination of o-phenylenediamine in water samples based on the formation of silver nanoparticles as a colorimetric probe

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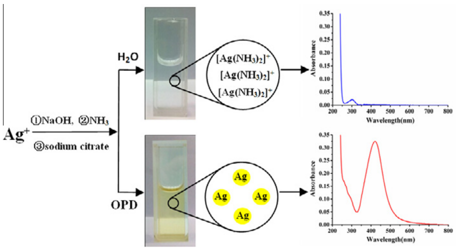
## HIGHLIGHTS

- Detection of o-phenylenediamine based on the formation of silver nanoparticles.
- O-phenylenediamine could be monitored by a UV–vis spectrophotometer or the naked eye.
- This method has been applied to determine o-phenylenediamine in water samples.
- Our strategy opens a new avenue for o-phenylenediamine determination.

## GRAPHICAL ABSTRACT



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## ABSTRACT

A simple, rapid and cost-effective method for visual colorimetric detection of o-phenylenediamine (OPD) based on the formation of silver nanoparticles (AgNPs) has been developed in this paper. Silver ions can be reduced to AgNPs by OPD in a few minutes, causing changes in absorption spectra and color of the reaction system. Therefore, colorimetric detection of OPD could be realized by a UV–vis spectrophotometer or even the naked eye. Results showed that the absorption intensity of AgNPs at 416 nm exhibited a good linear correlation ($R^2 = 0.998$) with OPD concentration in the range from $10^{-6}$ to $8 \times 10^{-5}$ mol $L^{-1}$ and the detection limit ($3\sigma/S$) was calculated to be $1.61 \times 10^{-7}$ mol $L^{-1}$. Furthermore, as low as $4 \times 10^{-6}$ mol $L^{-1}$ OPD can be visualized by the naked eye without the requirement of any complicated or expensive instruments. This proposed method has been successfully applied to determine OPD in water samples, and may provide an innovative platform in the development of sensors for guiding environmental monitoring in the future.

## Introduction

O-phenylenediamine (OPD), which is known as an important chemical intermediate, is widely used to produce pesticides, medicines, dyes, auxiliaries, photosensitive materials, and has broad market prospects. However, OPD has been found to be a mutagenic substance and be hazardous for human body in the case of ingestion, inhalation, eye contact [1]. Heavy exposure to OPD can damage the respiratory system, digestive system, liver and even lead to cancer. OPD is also regarded as one of the serious environmental pollutants owing to its toxicity and carcinogenicity [2]. The American Conference of Governmental Industrial Hygienists (ACGIH) classified OPD dihydrochloride as a confirmed animal carcinogen with unknown relevance to humans and recommended an occupational exposure limit of 0.1 mg $m^{-3}$ to protect workers

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against blood dyscrasia [3]. OPD is soluble in water, which make it easy to contaminate ground and surface water, and it is of great significance to monitor OPD in drinking and environmental water.

Up to now, several analytical methods have been developed for the determination of OPD including spectrophotometry [4,5], high-performance liquid chromatography (HPLC) [6–8] and capillary electrophoresis (CE) [8,9]. These methods have the advantages of high sensitivity and good selectivity. However, these approaches, which usually require specialized equipments or complicated procedures, are somewhat laborious, time-consuming, expensive, and unsuitable for real-time detection. Therefore, it is highly desirable to develop a new sensitive, fast and practical method for the detection of OPD.

Colorimetric sensors have attracted increasing considerations for their convenience of visual observation and simple operations in recent years [10–12]. Metal nanomaterials such as silver nanoparticles (AgNPs) and Au nanoparticles (AuNPs) have been found wide applications as ideal reporters for colorimetric detection owing to their unique optical and electric properties [13–16]. Taking the advantage of their strong localized surface plasmon resonance (LSPR) absorption with extremely high extinction coefficients, systems based on the formation of metal nanoparticles have been employed for the optical-detection of reducing agents. When gold/silver ions are reduced to AuNPs/AgNPs, solutions show a distinctive color attributed to differences in their size and concentration. Besides, surface plasmon resonance bands of noble-metal nanoparticles are typically located in the visible region and the band is strongly dependent on a nanoparticle's size, shape, composition, crystallinity and interparticle spacing [17]. Therefore, colorimetric sensors can be established relying on the color and UV–vis spectrum response of metal nanoparticles suspension. However, the oxidation of silver ions is weaker than gold ions, and only several substances can reduce silver ions to AgNPs. So the method using silver ions has higher selectivity than that of gold ions. Additionally, Ag nanomaterials are more cost-effective in their preparation compared to Au nanomaterials. The AgNPs-based sensor has been applied to determine many substances such as metal ions [18–21], chiral compounds [22,23], drugs [24–27], pesticide [28,29], small molecular [30–32], and proteins [33,34].

In this paper, a colorimetric sensor for OPD based on its reducing property has been proposed. In the presence of OPD, silver ions can be reduced to AgNPs in a few minutes, accompanied by changes in color and absorption spectra of the reaction system. These changes are proportional to the concentration of OPD. Therefore, OPD can be directly detected by monitoring the color change, scanning UV–vis spectroscopy, or even the naked eye. The proposed method offers many advantages of simplicity, economy, high sensitivity, selectivity and wide linear range. More importantly, our present sensor has been successfully applied to determine OPD in spiked water samples and satisfactory results were obtained. To the best of our knowledge, our strategy is the first example of detecting OPD with visual colorimetric method, and opens a new avenue for OPD determination.

## Material and methods

### Reagents

OPD (⩾98.5%), sodium dodecyl sulfate (SDS) and polyvinyl pyrrolidine (PVP) were all purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Silver nitrate ($AgNO_3 \geqslant 99.8\%$) and NaOH were received from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Sodium citrate was obtained from Shanghai Rongrun Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals used were of analytical reagent grade and used as received without further purification. Milli-Q-purified distilled water was used throughout the experiments.

### Apparatus

TEM analysis was performed on a FEI Tecnai G2 F20 transmission electron microscope (TEM). UV–vis absorption spectra were measured on Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cells. All pH measurements were handled with a pHS-25 digital pH-meter (Shanghai Wei Ye Instrument Factory, China).

### Colorimetric detection of OPD

Prior to use, all glassware was soaked in aqua regia (1:3 $HNO_3$/HCl) and rinsed thoroughly with Milli-Q water and dried in air. 0.5 mL of 15 mmol $L^{-1}$ $AgNO_3$, 0.6 mL of 2 mmol $L^{-1}$ NaOH, 0.25 mL of 0.1 mol $L^{-1}$ $NH_3$, 0.15 mL of 1% sodium citrate solution and 1 mL of different concentrations of OPD were sequentially added into a 5.0 mL calibrated test tube. Then, the mixture was diluted to 2.7 mL with Milli-Q water and mixed thoroughly to incubate for a certain time. Finally, the reaction solution was transferred into a 1 cm spectrometric cell to record the absorbance. The absorbances were measured at 416 nm ($\lambda_{max}$ of AgNPs LSPR peak) against a blank solution. It should be noted that the order of addition of reagents is very critical.

## Results and discussion

### Mechanism of colorimetric detection

Fig. 1 describes the principle of the colorimetric determination of OPD. Obvious color change of the solution can be seen after adding OPD into other reagents. Besides, the absorption of AgNPs was not observed in the presence of OPD alone or other reagents without OPD in the region of 330–700 nm. After the reaction between other reagents and OPD, an absorption peak appeared at about 416 nm, indicating the formation of AgNPs. Based on these phenomenons, a colorimetric method for the determination of OPD could be realized by the naked eye or UV–vis spectroscopy during the formation of AgNPs. Even the presence of trace OPD could result in visual color and absorption spectra changes. The transmission electron microscopy image (Fig. 2) of the reaction system also confirmed the formation of AgNPs. Mechanisms of the formation of AgNPs in the presence of OPD that is based on the similar reaction of resorcinol with silver ions were suggested according to the following equations [35]:

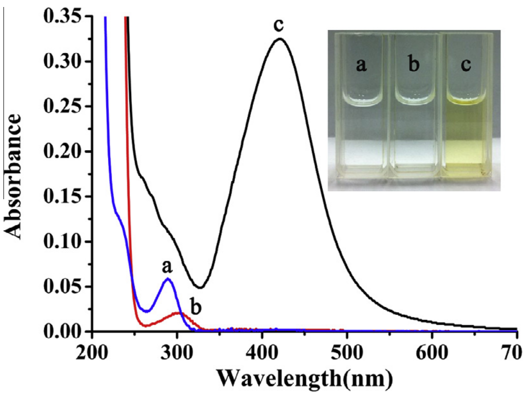

**Fig. 1.** The absorption spectra and photographs of OPD alone (a), 0.5 mL $AgNO_3$ + 0.6 mL NaOH + 0.25 mL $NH_3$ + 0.15 mL sodium citrate solution + 1.2 mL $H_2O$ (b), 0.5 mL $AgNO_3$ + 0.6 mL NaOH + 0.25 mL $NH_3$ + 0.15 mL sodium citrate solution + 1 mL OPD + 0.2 mL $H_2O$ (c). The concentration of OPD is $2 \times 10^{-5}$ mol $L^{-1}$.

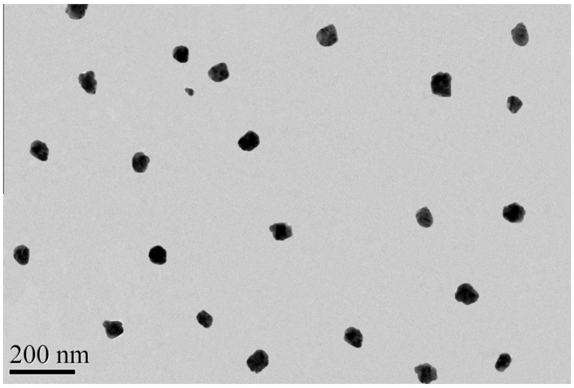

**Fig. 2.** TEM micrograph of the formed AgNPs with $6 \times 10^{-5}$ mol L$^{-1}$ OPD.

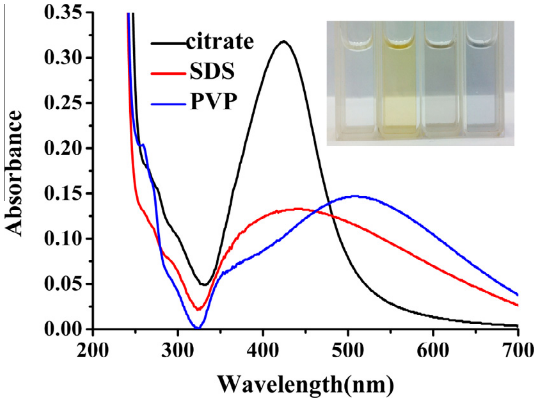

**Fig. 3.** The color and UV–vis absorption spectra of the reaction system stabilized by sodium citrate, SDS or PVP. From left to right on the photograph, they are water (control), citrate-stabilized AgNPs, SDS-stabilized AgNPs and PVP-stabilized AgNPs.

$$2\mathrm{Ag}^+ + 2\mathrm{OH}^- \rightarrow \mathrm{Ag_2O_{(S)}} + \mathrm{H_2O}$$

$$\mathrm{Ag_2O_{(S)}} + 4\mathrm{NH_3} + \mathrm{H_2O} \rightarrow 2\left[\mathrm{Ag(NH_3)_2}\right]^+_{(aq)} + 2\mathrm{OH}^-$$

$$\left[\mathrm{Ag(NH_3)_2}\right]^+_{(aq)} + \mathrm{OPD_{(Red)}} + e^- \rightarrow \mathrm{Ag_{(S)}} + 2\mathrm{NH_3} + \mathrm{OPD_{(Ox)}}$$

where $\mathrm{OPD_{(Red)}}$ is the reduced form of OPD and $\mathrm{OPD_{(Ox)}}$ is its oxidized form.

### *Optimization of experimental conditions*

In order to establish the optimum conditions for the colorimetric detection of OPD, several parameters including stabilizer, the amount of NaOH, the amount of ammonia, reaction time were systematically investigated and optimized.

### *Effect of stabilizer*

Metal nanomaterials are unstable and have a tendency to aggregate. Therefore, the use of stabilizer is very significant to prevent the aggregation and maintain the stability of AgNPs in aqueous solution [36]. In this study, we selected SDS, PVP and sodium citrate as AgNPs stabilizer. SDS and PVP are common surfactants that have been used as stabilizers to prevent nanoparticle agglomeration because they provide steric stabilization. Citrate-capped AgNPs with electronegative charged surface can also be dispersed from each other in the water symmetrically by the electrostatic repulsion [37]. As shown in Fig. 3, when sodium citrate was used as stabilizer, the color of the solution changed to yellow and peaked at about 416 nm. However, when SDS or PVP was used, the color changed to brown or blue, and the absorption peak was at about 443 nm or 507 nm with poor symmetry. Moreover, in the presence of SDS or PVP, the maximum absorption intensity of AgNPs decreased in comparison with when sodium citrate was used at same OPD concentration. Therefore, sodium citrate was chosen and used as stabilizer. Different amounts of sodium citrate

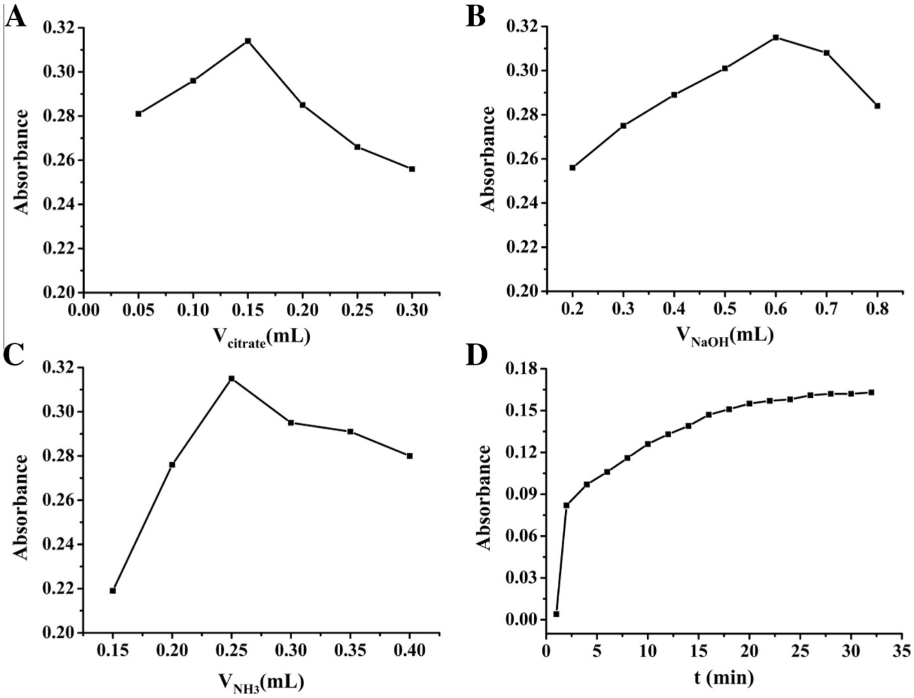

**Fig. 4.** (A) Effect of volume of sodium citrate solution (1%, w/v) on the LSPR absorbance. Experimental condition: OPD ($2 \times 10^{-5}$ mol L$^{-1}$); 0.5 mL of 15 mmol L$^{-1}$ $\mathrm{AgNO_3}$; 0.6 mL of 2 mmol L$^{-1}$ NaOH; 0.25 mL of 0.1 mol L$^{-1}$ $\mathrm{NH_3}$; reaction time: 20 min. (B) Effect of volume of NaOH solution (2 mmol L$^{-1}$) on the LSPR absorbance. Experimental condition: OPD ($2 \times 10^{-5}$ mol L$^{-1}$); 0.5 mL of 15 mmol L$^{-1}$ $\mathrm{AgNO_3}$; 0.25 mL of 0.1 mol L$^{-1}$ $\mathrm{NH_3}$; 0.15 mL of 1% sodium citrate solution; reaction time: 20 min. (C) Effect of volume of ammonia solution (0.1 mol L$^{-1}$) on the LSPR absorbance. Experimental condition: OPD ($2 \times 10^{-5}$ mol L$^{-1}$); 0.5 mL of 15 mmol L$^{-1}$ $\mathrm{AgNO_3}$; 0.6 mL of 2 mmol L$^{-1}$ NaOH; 0.15 mL of 1% sodium citrate solution; reaction time: 20 min. (D) Effect of reaction time from the addition of OPD on the LSPR absorbance. Experimental condition: OPD ($10^{-5}$ mol L$^{-1}$); 0.5 mL of 15 mmol L$^{-1}$ $\mathrm{AgNO_3}$; 0.6 mL of 2 mmol L$^{-1}$ NaOH; 0.25 mL of 0.1 mol L$^{-1}$ $\mathrm{NH_3}$; 0.15 mL of 1% sodium citrate solution.

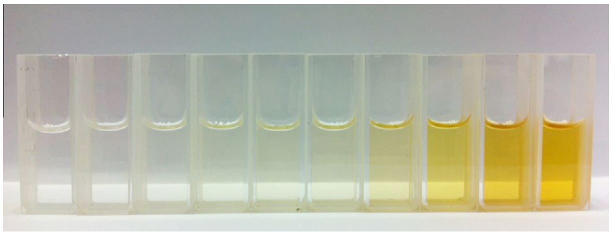

**Fig. 5.** Color changes of the reaction system in the presence of various concentrations of OPD as reducing agent. OPD concentrations from left to right in mol L$^{-1}$: $10^{-6}$, $2 \times 10^{-6}$, $4 \times 10^{-6}$, $6 \times 10^{-6}$, $8 \times 10^{-6}$, $10^{-5}$, $2 \times 10^{-5}$, $4 \times 10^{-5}$, $6 \times 10^{-5}$, $8 \times 10^{-5}$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Effect of interfering ions on the determination of OPD. Experimental condition: OPD ($2 \times 10^{-5}$ mol L$^{-1}$); 0.5 mL of 15 mmol L$^{-1}$ $AgNO_3$; 0.6 mL of 2 mmol L$^{-1}$ NaOH; 0.25 mL of 0.1 mol L$^{-1}$ $NH_3$; 0.15 mL of 1% sodium citrate solution; reaction time: 20 min.

| Interfering species | Tolerated ratio [$C_{species}$]/[$C_{OPD}$] |
|---|---|
| $Na^+$, $K^+$, $SO_4^{2-}$, $NO_3^-$, $CO_3^{2-}$ | 200 |
| $Ca^{2+}$ | 150 |
| $Mg^{2+}$ | 100 |
| $Cu^{2+}$ | 20 |
| $Cl^-$ | 5 |
| $Fe^{3+}$ | 2.5 |
| m-Phenylenediamine, p-Phenylenediamine, o-Nitroaniline | 1 |

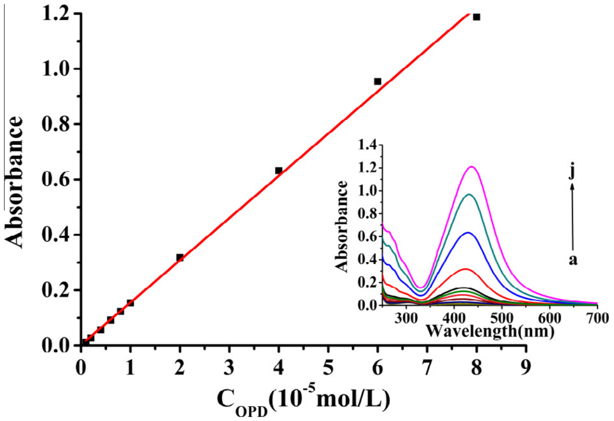

**Fig. 6.** The linear relationship between the absorption intensity and concentration of OPD. Experimental condition: OPD ($10^{-6}$-$8 \times 10^{-5}$ mol L$^{-1}$); 0.5 mL of 15 mmol L$^{-1}$ $AgNO_3$; 0.6 mL of 2 mmol L$^{-1}$ NaOH; 0.25 mL of 0.1 mol L$^{-1}$ $NH_3$; 0.15 mL of 1% sodium citrate solution; reaction time: 20 min. The insert is UV–vis absorption spectra of the reaction system with OPD concentration ranging from a to *j* in mol L$^{-1}$: $10^{-6}$, $2 \times 10^{-6}$, $4 \times 10^{-6}$, $6 \times 10^{-6}$, $8 \times 10^{-6}$, $10^{-5}$, $2 \times 10^{-5}$, $4 \times 10^{-5}$, $6 \times 10^{-5}$, $8 \times 10^{-5}$.

solution (1%) were added to the reaction mixture. Results were shown in Fig. 4A and they demonstrated that the absorbance was the highest with 0.15 mL sodium citrate solution. When the addition amount was more than or less than 0.15 mL, the absorbance declined. Therefore, 0.15 mL of 1% sodium citrate solution was chosen in the subsequent experiments.

### *Effect of NaOH*

The addition amount of NaOH has great effects on the formation of AgNPs. The absorbance of the reaction system with a variety volumes of 2 mmol L$^{-1}$ NaOH solution was given in Fig. 4B. Lower absorption intensity was obtained with less NaOH. However, the absorbance dropped down steeply when the volume of NaOH solution was more than 0.6 mL, which might be due to the formation of $Ag_2O$ precipitate [35]. Thus, 0.6 mL was selected as the optimum amount for 2 mmol L$^{-1}$ NaOH solution.

### *Effect of ammonia*

The amount of ammonia can also affect the formation of AgNPs. As we all know, the addition of NaOH can cause silver ions to precipitate. But in the presence of ammonia, silver ions can exist in the complex form and this prevents precipitation. Fig. 4C showed the changes of the absorbance at 416 nm with different volumes of 0.1 mol L$^{-1}$ ammonia solution. It revealed that by adding more than 0.25 mL of ammonia solution, the absorbance of the reaction mixture decreased. Because excess ammonia made it slightly difficult to reduce the silver complex and OPD could not reduce silver ions. Thus, 0.25 mL of 0.1 mol L$^{-1}$ ammonia solution was chosen as the optimum value.

### *Effect of reaction time*

The reaction time is a key point that affects the formation of AgNPs. The absorption spectra of the reaction mixture were recorded at different times. As shown in Fig. 4D, the absorption intensity quickly increased within the initial 20 min, whereas it became weak and slow when the incubation time was more than 20 min. These results indicated that the formation of AgNPs almost completed within 20 min. For detective convenience, all the absorbance measurements were performed within 20–21 min from the addition of OPD solution.

### *Colorimetric assay of OPD*

Under the optimum conditions, the colorimetric assay was processed for the quantitative determination of OPD, based on

**Table 1**
Comparison of the proposed method for the determination of OPD with some other previously reported methods.

| Analytical method | Reagents/detection or technique | Linear range (μmol L$^{-1}$) | Sample matrix | LOD (μmol L$^{-1}$) | References |
|---|---|---|---|---|---|
| HPLC[a] | Chemiluminescence detection | 0.92–92.59 | Oxidative hair dyes | 0.27 | [7] |
| HPLC | – | 4.63–185.19 | Commercial permanent hair dyes | 2.59 | [8] |
| MEKC[b] | – | 4.63–555.56 | Commercial permanent hair dyes | 2.96 | [8] |
| CZE[c] | Amperometric detection | 1.00–100.00 | Hair dyes | 0.22 | [9] |
| SERS[d] | α-CD-AgNPs | 0.10–1.00 | Tap water, lake water, reservoir water and river water | 0.03 | [38] |
| UV | The formed AgNPs | 1.00–80.00 | Boiling and cooling tap water, lake water | 0.16 | This work |

[a] High performance liquid chromatography.
[b] Micellar electrokinetic chromatography.
[c] Capillary zone electrophoresis.
[d] Surface-enhance raman spectroscopy.

**Table 3**
Results of the determination of OPD in spiked water samples. Experimental condition: 0.5 mL of 15 mmol $L^{-1}$ $AgNO_3$; 0.6 mL of 2 mmol $L^{-1}$ NaOH; 0.25 mL of 0.1 mol $L^{-1}$ $NH_3$; 0.15 mL of 1% sodium citrate solution; reaction time: 20 min.

| Samples | Added amount (μmol $L^{-1}$) | Found amount (μmol $L^{-1}$) | Std Dev (μmol $L^{-1}$) | RSD (%, n = 3) | Recovery (%, n = 3) |
|---|---|---|---|---|---|
| boiling tap water | 0 | 6.09 | - | - | - |
| | 10 | 16.34 | 0.159 | 1.55 | 102.5 |
| | 50 | 55.69 | 1.518 | 3.06 | 99.2 |
| cooling tap water | 0 | 3.5 | - | - | - |
| | 10 | 13.41 | 0.130 | 1.31 | 99.1 |
| | 50 | 51.55 | 1.168 | 2.43 | 96.1 |
| lake water | 0 | 1.84 | - | - | - |
| | 10 | 12.1 | 0.108 | 1.05 | 102.6 |
| | 50 | 51.74 | 1.387 | 2.78 | 99.8 |

the formation of AgNPs. Color changes of the reaction system in the presence of various concentrations of OPD in the range from $10^{-6}$ to $8 \times 10^{-5}$ mol $L^{-1}$ was shown in Fig. 5. We can see the color of the reaction mixture became deeper and deeper with the increase of OPD concentration. And as low as $4 \times 10^{-6}$ mol $L^{-1}$ OPD can be visualized by the naked eye without the requirement of any complicated or expensive instruments. Furthermore, to quantitatively detect OPD with the developed method, UV–vis absorption spectra were also recorded. As shown in Fig. 6, the absorbance linearly increased with the increase of OPD in the range from $10^{-6}$ to $8 \times 10^{-5}$ mol $L^{-1}$. The standard regression equation was $A_{416} = 0.152c + 0.003$, $R^2 = 0.998$. The detection limit ($3\sigma/S$) was calculated to be $1.61 \times 10^{-7}$ mol $L^{-1}$. The relative standard deviation (RSD) for the determination of $5 \times 10^{-6}$ mol $L^{-1}$, $10^{-5}$ mol $L^{-1}$ and $5 \times 10^{-5}$ mol $L^{-1}$ of OPD were 0.73%, 0.93% and 1.55% ($n = 6$), respectively. A comparison of the proposed method for the determination of OPD with some other previously reported methods is presented in Table 1. We can see that the suggested method exhibited higher sensitivity with a lower detection limit and wider linear range. Even more important, our analytical method has distinctive advantages such as simplicity, rapidness and low cost.

### *Interferences studies*

The influence of some possibly coexisting foreign substances including inorganic ions ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $SO_4^{2-}$, $NO_3^-$, $Cl^-$ and $CO_3^{2-}$) and organic compounds (m-phenylenediamine, p-phenylenediamine and o-nitroaniline) were investigated for the analysis of real samples. A fixed amount of OPD ($2 \times 10^{-5}$ mol $L^{-1}$) was taken with different amounts of foreign species and the recommended procedure was followed. A relative error of 5% was considered tolerable. Results showed that no significant interference was observed with ions including $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, $NO_3^-$ and $CO_3^{2-}$, even at a 100–200 fold higher concentration than that of OPD (Table 2). Some species ($Fe^{3+}$, $Cu^{2+}$ and $Cl^-$) did not interfere with the OPD determination until in lower concentrations. But m-phenylenediamine, p-phenylenediamine and o-nitroaniline interfered with the OPD detection even in an equal concentration with OPD. Above results indicated that species usually found in water samples do not have severe effects on the determination of OPD, and the proposed method can be applied to detect trace OPD in water samples.

### *Determination of OPD in water samples*

To further explore the practical application of the proposed method, it was applied to detect OPD in water samples such as boiling tap water, cooling tap water and lake water (was taken from Xuanwu Lake, China). The former needed no pretreatment. The other two were filtered and diluted 2 times with Milli-Q water before detection. Then certain amounts of OPD standard solution were spiked into the water samples and analyzed according to the proposed method. The experimental results were listed in Table 3. It displayed that the recoveries for OPD in three different water samples ranged from 95% to 105%. The RSD of three parallel experiments were all below 5%. The high analytical precision and good recovery indicated that this colorimetric method was reliable and could be applied to detect OPD in water samples rapidly and conveniently.

## Conclusion

This study introduced a novel method to selective colorimetric detection of o-phenylenediamine based on the formation of AgNPs. Silver ions can be reduced to AgNPs by o-phenylenediamine in a few minutes, accompanied by changes in color and absorption spectra of the reaction system. Thus the concentration of o-phenylenediamine can be monitored by the naked eye or UV–vis spectrophotometer. The proposed method possesses several advantages compared with some other reported detection methods. First, this method is simple in design, and as low as $4 \times 10^{-6}$ mol $L^{-1}$ o-phenylenediamine can be visualized by the naked eye without the requirement of any complicated instruments. Second, this approach is fast in manipulation, and the detection can be completed within 20 min. Third, this strategy is cost-effective, and no special reagents or expensive instruments are needed. Forth, this sensor with high sensitivity and selectivity has been successfully applied for the detection of o-phenylenediamine in water samples. Finally, the assay is carried out during the formation of AgNPs, which provides not only a new detection method of o-phenylenediamine but also a novel synthesis method of AgNPs. We hope that this method may be exploited as an effective means for guiding environmental monitoring in the future.

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