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COMMUNICATION

Highly sensitive label-free colorimetric sensing of nitrite based on etching of gold nanorods†

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A simple colorimetric method with high sensitivity and selectivity was developed for sensing of nitrite as low as 4.0 μM by naked eyes, which is based on etching of gold nanorods accompanied by shape changes in aspect ratios (length/width) and a visible color change from bluish green to red and then to colorless with the increase of nitrite.

As type A inorganic contaminants in drinking water, nitrite and nitrate have proven to be of great threat to human health and may result in diseases like methemoglobinemia, esophageal cancer, *etc.*^{1–3} Many methods, such as ion chromatography,⁴ capillary electrophoresis,⁵ chemiluminescence⁶ and fluorescence spectrum,⁷ have been developed for the detection of NO_2^- and NO_3^- . However, all these methods have poor applicability to field tests. As the largest developing country, China is encountering a great drinking water crisis resulting from the worsening quality of source water. The situation is even worse in its rural regions where people mainly rely on simply prepared tap water or even untreated surface water, shallow groundwater, cellar water, *etc.* The concentrations of NO_2^- and NO_3^- in most of such water may have reached dangerous level^{8–10} and have barely been monitored due to the lack of funds, equipments and qualified persons. It is urgent to develop a simple test method that can be quickly handled by local inhabitants.

Colorimetric assays have proven to be effective for the inspection of NO_2^- in food and wastewater.^{11–13} It is unfortunate that photo-absorption coefficients of employed organic dyes are rather low, which withhold further applications of most colorimetric assays in the quality assurance of drinking water. Benefiting from high photo-absorption coefficients, nano-materials have been widely applied in colorimetric assays for the detection of various targets,^{14–23} as well as NO_2^- in drinking water.^{24,25} The first attempt was made by Mirkin *et al.*²⁵ based on the induced aggregation of two kinds of

functionalized gold nanoparticles (GNPs). Their method yielded a detection limit of 22 μM NO_2^- by naked eyes. Thereafter, using 4-aminothiophenol modified gold nanorods (GNRs) as a color producing-reagent, Yu and Xiao²⁴ developed a non-crosslinking colorimetric NO_2^- sensor based on deamination reaction induced by NO_2^- which resulted in the GNRs aggregation. This method yielded a detection limit of about 10 μM for NO_2^- within 10 minutes. The above attempts suggested nano-materials as a promising regulator in the sensing of NO_2^- in drinking water by colorimetric assays. The methods will be more practical if related processes are simplified and detection ranges are extended.

Herein we developed a much simpler label-free colorimetric method for sensing of NO_2^- based on etching of GNRs. Its applicability, as tested, is suitable for the detection of NO_2^- as low as 4.0 μM by naked eyes.

As shown in Scheme 1, the solution of GNRs (length/diameter ratio about 1.3 : 1) appeared bluish green owing to the intense longitudinal surface plasmon resonance (SPR) absorption of GNRs around 630 nm. The addition of 10 μM NO_2^- to the colloidal solution caused a color change from bluish green to red within 20 minutes. More NO_2^- (40 μM) caused the solution to be almost colorless. The progressive color change corresponds to the partial to complete dissolution of GNRs.

To confirm the dissolution of GNRs caused by NO_2^- , the absorption spectra of GNRs after incubation in NO_2^- solutions (pH $\approx$ 0) at different intervals were examined. As shown in Fig. 1, the absorption spectrum of GNRs (curve a) exhibited strong SPR absorption at bands of 530 and 630 nm corresponding to the

Scheme 1 Schematic illustration for the colorimetric sensing of NO_2^- based on etching of GNRs.

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Fig. 1 Absorption spectra of GNRs after incubation with $10\ \mu\text{M}\ \text{NO}_2^-$ for (a) 0, (b) 3, and (c) 10 min, respectively.

transverse and longitudinal SPR absorption, respectively. The absorption band at 630 nm gradually bleached and shifted to short wavelength (curves b and c) with the further dissolution of GNRs. Our results, together with those of Yang²⁶ and Stucky,²⁷ suggest that the blue-shift should be attributed to the decrease of aspect ratios (length/width) of GNRs and a preferential shortening along the axial direction. The difference between TEM images before and after incubation of GNRs with NO_2^- (Fig. 2) showed that GNRs were transformed from rods into spherical particles, indicating that GNRs were progressively etched by NO_2^- .

At pH = 0, the practical redox potential of HNO_2/NO is 1.0 V, while the standard redox potentials of $\text{AuCl}_2^-/\text{Au}$, $\text{AuCl}_4^-/\text{Au}$, $\text{AuBr}_2^-/\text{Au}$ and $\text{AuBr}_4^-/\text{Au}$ are 1.15, 1.00, 0.85, and 0.93 V relative to a standard hydrogen electrode (NHE), respectively. The thermodynamic differences theoretically suggest that gold can be oxidized to AuBr_4^- or AuBr_2^- by NO_2^- under similar conditions, although no direct evidence was reported. However, it was reported that gold could be leached in the presence of certain sulfur-containing chemicals and oxidants.^{28–30} The leaching was based on the formation of a Au^+ -sulfur-containing chemical complex which reduced the activity of Au^+ and therefore reduced the redox potential of Au^+/Au . Other research showed that cetyl trimethylammonium bromide (CTAB) could also reduce the activity of AuBr_2^- by the formation of a AuBr_2^- -CTA⁺ complex and consequently reduce the redox potentials of $\text{AuBr}_2^-/\text{Au}$ or $\text{AuCl}_2^-/\text{Au}$ to less than 0.4 V relative to

Fig. 2 TEM images of GNRs (A) before and (B) after incubation with $20\ \mu\text{M}\ \text{NO}_2^-$.

NHE.³¹ Theoretically, the presence of CTAB could promote the redox reaction between gold and NO_2^- . To verify the fact, the following experiments were conducted. Gold nanoparticles (GNPs) were first stabilized by 0.4% Tween-20 to stabilize them in 1.0 M HCl. Under such conditions, negligible difference of the absorption spectra of GNPs was observed with the coexistence of 1.0 mM NO_2^- and NaBr (Fig. 3, curves a–d). It indicates that Br^- did not promote the redox reaction between gold and NO_2^- . In contrast, the addition of CTAB would result in dramatic decrease of the absorbance of GNPs (Fig. 3, curves e–g), suggesting that the etching of GNRs by NO_2^- was accelerated by CTAB. Since AuCl_4^- can oxidize Au to produce a AuBr_2^- -CTA⁺ and/or AuCl_2^- -CTA⁺ complex in the presence of CTAB,³¹ we presume that the GNRs were also oxidized to AuBr_2^- -CTA⁺ and/or AuCl_2^- -CTA⁺ instead of a $\text{Au}(\text{III})$ complex.

The absorption band at 630 nm gradually bleached and shifted to short wavelength, which indicated that the chemical etching of GNRs by NO_2^- is not isotropic in all directions. The preferential shortening along the axial direction can be attributed to less surface passivation and/or higher reaction activities at the tips of gold nanorods.³²

To evaluate whether this response is selective toward NO_2^- , the influence of other common ions was investigated. As shown in the inset of Fig. 4, among the tested ions, NO_2^- and Fe^{3+} caused obvious color changes. The decrease of absorption areas from 480 to 840 nm ($\text{Area}_{\text{blank}} - \text{Area}_{\text{sample}}$) of GNRs after incubation with such ions ($100\ \mu\text{M}$ except for Cu^{2+} and Fe^{3+} , $10\ \mu\text{M}$) served as another reference. NO_3^- , PO_4^{3-} , Ac^- , Br^- , SCN^- , ClO_4^- , S^{2-} , Pb^{2+} , Hg^{2+} , Ca^{2+} , Al^{3+} , Mn^{2+} , Ag^+ , Cr^{3+} , Cu^{2+} and Cd^{2+} gave almost no response. A slight decrease was observed for SO_4^{2-} and Zn^{2+} . Mg^{2+} likely inhibited the oxidation of GNRs by dissolved oxygen and caused a slight increase (GNRs would be partially dissolved at a certain extent of oxygen, see Fig. S1 in the ESI†). At identical concentrations, NO_2^- and Fe^{3+} ($10\ \mu\text{M}$) presented remarkable responses. Fe^{3+} can also oxidize GNRs and lead to an absorption spectrum change of GNRs, which would interfere with the sensing of NO_2^- .³² The interference of Fe^{3+} could be eliminated by the addition of 0.01 M 1,2-cyclohexylenedinitrilotetraacetic acid (DCTA) to form

Fig. 3 The absorption spectra of GNPs after incubation with 0.1 mM NO_2^- (a), 0.1 mM NO_2^- + 4 mM Br^- (b), 0.1 mM NO_2^- + 5 mM Br^- (c), 0.1 mM NO_2^- + 6 mM Br^- (d), 0.1 mM NO_2^- + 4 mM CTAB (e), 0.1 mM NO_2^- + 5 mM CTAB (f), 0.1 mM NO_2^- + 6 mM CTAB (g), respectively.

Fig. 4 Decrease of absorption areas and color changes of tested solutions for common ions at 100 μM (except Cu^{2+} , Fe^{3+} and NO_2^- , 10 μM).

a complex of DCTA-Fe^{3+} and reduce the reaction activity of Fe^{3+} (the conditional stability constant K_{FeDCTA}' is $10^{6.24}$). The present system provides excellent selectivity for NO_2^- over other ions in the presence of 0.01 M DCTA. It should be attributed to the relatively high redox potential of $\text{AuBr}_4^- - \text{CTA}^+/\text{Au}$ which excludes the oxidation of GNRs by other common ions.

Fig. 5 shows the analytical performance of our proposed method. The absorption area of GNRs decreased gradually with the increase of NO_2^- . Under optimal conditions (pH $\approx$ 0, incubation temperature 55 $^\circ\text{C}$, incubation time 20 min, see Optimization of experimental conditions section in the ESI,[†] Fig. S1–S3), a good linear relationship

Fig. 5 Absorption spectra of GNRs after incubation with different concentrations of NO_2^- for 20 min. Insets show the decrease of absorption area response to different concentrations of NO_2^- and the colour change with the increase of NO_2^- concentration from left to right, respectively.

Table 1 The recovery of spiked NO_2^- in local drinking water. The standard deviation of each sample was obtained by three measurements

Sample	Added (μM)	Detected (μM)	RSD (%)	Recovery (%)
1	2.50	2.62	0.9	104.6
2	4.00	3.77	2.8	94.2
3	7.00	5.41	1.0	77.3
4	10.0	7.56	1.0	75.6

between the decrease of absorption area from 400 to 800 nm ($\text{Area}_{\text{blank}} - \text{Area}_{\text{sample}}$) and NO_2^- concentrations was obtained within the range of 1.0 to 15.0 μM ($R = 0.997$). The detection limit was calculated to be 0.5 μM ($23 \mu\text{g L}^{-1}$, $S/N = 3$), which is far below the maximum containment level as recommended by U.S. EPA (1 mg L^{-1} measured as nitrogen, $\sim 71 \mu\text{M}$ for NO_2^-).²⁴ The digital photo in the inset of Fig. 3 indicates that a containment level of 4.0 μM ($184 \mu\text{g L}^{-1}$) can be easily read out by naked eyes.

The practical performance of the proposed method was further tested by samples of local drinking water. No NO_2^- was detected in such samples. The method was alternatively tested by standard addition and the recovery of spiked NO_2^- ranges from 75.6 to 104.6% (Table 1). It indicates that such a method is applicable to the quantification of NO_2^- in aqueous solutions.

In summary, this study reported a new convenient colorimetric method for the detection of NO_2^- based on the etching of GNRs. The method is characterized by its high selectivity and sensitivity, with a considerably low detection limit of $56 \mu\text{g L}^{-1}$ (measured as nitrogen). The method is more sensitive and simpler compared with those proposed by Mirkin *et al.*²⁵ and Yu and Xiao.²⁴ The highlight of this method is the obvious visible color change of GNR solutions correlated to different concentrations of NO_2^- . Hence, it is particularly applicable to field tests and can be expected to be further developed into economical products (e.g. NO_2^- test-papers). Since NO_3^- can be easily reduced to NO_2^- by enzyme or metallic catalysts, the proposed colorimetric assays for nitrite can be used for nitrate detection, theoretically.

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