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Rapid-Response and Highly Sensitive Noncross-Linking Colorimetric Nitrite Sensor Using 4-Aminothiophenol Modified Gold Nanorods

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A novel colorimetric nitrite ion sensor was developed utilizing 4-aminothiophenol (4-ATP) modified gold nanorods (GNR). In the presence of nitrite ions, the deamination reaction was induced by heating the 4-ATP modified GNR in ethanol solution, resulting in the reduction of the GNR surface charges, which led to aggregation of GNRs and a colorimetric response that was quantitatively correlated to the concentration of nitrite ions. This simple assay was rapid (≤10 min) and highly sensitive (<1 ppm of nitrite), and it can be used for rapid monitoring of drinking water quality.

Nitrate and nitrite, as part of nitrogen cycle, widely exist in ground and surface water systems, due to contaminations of water sources by fertilizer, waste of livestock, and other organic wastes.1–4 Even a trace amount of nitrate and nitrite ions is unsafe to human health, and both are type A inorganic chemicals in water quality parameters which require close monitoring by health authorities due to their potential toxicity.5 Infants drinking nitrate or nitrite contaminated water may suffer shortness of breath and blue baby syndrome and, if untreated, may die.1,3,5–7 The maximum containment levels (MCLs) of nitrate and nitrite, as recommended by the U.S. Environmental Protection Agency (EPA), are 10 ppm and 1 ppm (measured as nitrogen), respectively.1

In the past 2 decades, various methods have been developed to monitor the nitrate and nitrite levels in fresh water. Some of the typical methods are summarized in Table 1.4 Nitrate and nitrite ions could be directly analyzed by UV spectroscopy and electrochemical methods,8,9 but these methods are susceptible to interference from other compounds and particles in water. Ion chromatography10,11 or other pretreatment methods (i.e., a sulfonated DuPont Nafion fiber, precolumns, and an additional valve)12,13 were used to separate nitrate and nitrite from other inorganic anions, and led to highly sensitive detection of low levels of nitrate and nitrite ions, but the high instrumentation cost excluded them from being viable in-field detection choices. Capillary electrophoresis is another direct detection method for nitrite and/or nitrate14 that is highly sensitive; recently a portable capillary electrophoresis system was developed by Hauser and co-workers15 that could potentially support in-field monitoring of nitrite/nitrate, but it was still quite sophisticated and costly.

Nitrite can be indirectly detected in colorimetric assays by utilizing the highly selective diazotation reaction between nitrite and sulphanilamide,16,17 and the subsequent coupling of the product with N-(1-naphthyl)-ethylenediammonium dichloride to form an azo dye that can be detected with high sensitivity using spectroscopic methods, such as photometry and fluorescence spectroscopy. Since nitrate can be easily reduced to nitrite using enzyme or metallic catalysts, colorimetric assays for nitrite eventually can be used for nitrate detection as well.16,18

Colorimetric assay is highly desirable when in-field monitoring of nitrite and/or nitrate is needed, as in many environmental, agricultural, and food control applications, due to its simplicity and portability. However, most colorimetric assays based on chemical dyes are not sensitive enough for detecting nitrite and/or nitrate at low levels. Recently, Mirkin and co-workers developed a cross-linking colorimetric method based on gold nanoparticles to detect a low level of nitrite ions.18 However, in their approach, two types of gold nanoparticles functionalized differently were needed to react with nitrite ions to trigger the colorimetric response, and each type of nanoparticles needed specific chemical

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longitudinal surface plasmon resonance peaks (SPRlong), were nanorods with different aspect ratios, as indicated by different ATP,19 peptides,20 and DNA.21 Electrostatic aggregation can occur due to electrostatic manipulation of the surface charges has also functionalization of the nanoparticles. Cross-linking is not always molecular cross-links adds to the complexity of the chemical response may develop. Also the need to chemically form the rate-determining step that limits how rapidly a colorimetric nanoparticles. The formation of the molecular cross-links can be the formation of molecular cross-links that connect individual nanoparticles. The formation of the molecular cross-links can be the rate-determining step that limits how rapidly a colorimetric response may develop. Also the need to chemically form the molecular cross-links adds to the complexity of the chemical functionalization of the nanoparticles. Cross-linking is not always required for a colorimetric assay. Aggregation of nanoparticles due to electrostatic manipulation of the surface charges has also been utilized to detect a variety of molecular targets, such as ATP,19 peptides,20 and DNA.21 Electrostatic aggregation can occur more rapidly than cross-linking,20 leading to faster assays. With the utilization of electrostatic aggregation of gold nanorods (GNR), a more rapid colorimetric nitrite sensing system was developed in this report. Taking advantage of the higher plasmonic sensitivity of the large aspect ratio GNR than the spherical gold nanoparticles, a truly colorimetric detection of nitrite at as low as 0.25 ppm (significantly below the EPA recommended MCL) was achieved with simple visual inspection in less than 10 min. Since the plasmonic properties of GNRs can be tuned by controlling their aspect ratios, the sensitivity of the GNR—nitrite sensor can be further improved.

**EXPERIMENTAL SECTION**

Fabrication and Functionalization of Gold Nanorods. Gold nanorods with different aspect ratios, as indicated by different longitudinal surface plasmon resonance peaks (SPRlong), were synthesized via the seed-mediated growth method.22,23 Details of the procedure were reported elsewhere.27 Hexadecyltrimethylammoniumbromide (C16TAB, 99%), and benzyldimethylammonium chloride hydrate (BDAC, 99%), l-ascorbic acid, gold(III) chloride hydrate (>99%), and silver nitrate (>99%) were all purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Nanopure deionized and distilled water (18.2 MΩ) was used for all experiments.

The GNPs were further functionalized by 4-aminophenol (4-ATP). Briefly, 4 mL of 3 nM gold nanorods were reacted with 0.5 mL of 10 mM 4-ATP dissolved in acidic water (pH = 2) under vigorously stirring at 60 °C for 3 h. The solution was then centrifuged and washed twice with 3 mM CTAB acidic aqueous solution (pH = 4). Finally, 4-ATP modified gold nanorods were resuspended in 2.5 mL of acidic water (pH = 4).

**NitrO Sensing Using 4-ATP Functionalized GNR.** In a typical experiment for detecting nitrite, 0.8 mL of sample was placed in a 1.5 mL eppendorf tube. A volume of 8 µL of 1 M phosphoric acid was added to keep the sample under acidic conditions. Then 4-ATP modified GNR was added to make the final extinction of SPRlong equal to 0.9. After a quick vortex mixing, 0.2 mL of ethanol was added to the mixture. The solution was incubated at 95 °C in a water bath incubator for a certain amount of time (heating time is quantitatively recorded and correlated to the nitrite concentration in the sample) and then put on ice briefly to cool down the solution.

The whole detection strategy is illustrated in Scheme 1. Under acidic conditions, amines attached to the benzene ring are ionized yielding ammonium cations. GNR colloids are stable under such conditions due to the electrostatic repulsion among positively charged ammonium cations. In the presence of nitrite ions, the primary aromatic amines react with nitrate ions to form diazonium salt in the presence of mineral acid. In aqueous solution, diazonium salts are unstable and tend to lose the diazonium groups. In the presence of ethanol, amine groups in aromatic amines are subsequently dissociated from benzene rings under heating in the deamination reaction, replaced by hydrogen in the primary products, leading to the reduction of surface charges of the GNRs. The disruption of the surface charge balance leads to aggregation of gold nanorods, yielding a dramatic color change, which can be observed via the naked eye.

**RESULTS AND DISCUSSION**

SERS Characterization of Surface Chemistry of the Gold Nanorods. Surface enhanced Raman spectroscopy (SERS) was used to confirm diazo bond formation and deamination reaction.
As shown in Figure 1, SERS spectra of GNR in 20% ethanol were recorded after modified with 4-ATP, reacted with nitrite ions, and heated to 95 °C. The strongest band at 1074 cm\(^{-1}\) is identified as the stretching vibration of C–S, and the C–C stretching vibration of benzene rings is observed at 1577 cm\(^{-1}\). Importantly, N\(=\)N stretching vibration of diazonium salt is shown in 1394 cm\(^{-1}\), which only appears in nitrite treated GNR-4ATP. The 1142 and 1439 cm\(^{-1}\) bands are assigned as \(b_2\) modes of benzene ring vibrations. The intensities of these two bands are directly correlated to the charge transfer from the gold nanorods to the diazo ions attached to their surfaces, which is influenced by the conjugation between the benzene ring and diazo bond. The absence of these two bands in 4-ATP modified GNR and GNR-4ATP after deamination reaction confirmed the formation of diazo bonds between 4-ATPs and nitrite ions, as illustrated in step 2 of Scheme 1.

Aggregation Induced Colorimetric Nitrite Sensing Using the 4-ATP GNR Sensors. In Figure 2A, differences between the UV–vis spectra of GNR sensors treated with different samples clearly showed the aggregation of the GNR sensors when nitrite ions were present in the samples. Surface functionalization of the GNRs with 4-ATP under acidic conditions did not introduce

Figure 1. Raman spectra of 4-ATP modified GNR (black) and GNR-4ATP reacted with nitrite (red) and heated at 95 °C (blue) in 20% ethanol.

Figure 2. (A) Absorption spectra of CTAB covered GNR (black), GNR-4ATP in 20% ethanol solution (red), and aggregated GNR after heating in 20% ethanol (blue). (B) Photograph of GNR-4ATP reacted with various concentrations of nitrite after incubation in 20% ethanol at 95 °C.

Figure 3. (A) Extinction of GNR-4ATP at wavelength of its SPR\(_{\text{long}}\) band, 785 nm, reacted with 30 \(\mu\)M nitrite, as a function of incubation time at 95 °C. (B) Extinction of GNR-4ATP at wavelength of its SPR\(_{\text{long}}\) band, 680 nm, after 5 min incubation at 95 °C as a function of nitrite concentration.

significant change to the surface charges of the GNRs, the slightly blue-shifted longitudinal plasmonic band indicated stable GNR colloids in aqueous solution. Without the presence of nitrite ions, the GNR-4ATP sensors remained stable for weeks. However, after reaction with nitrite ions and the subsequent deamination reaction, neutralized surface molecules (benzene) significantly reduced the overall surface charges on the GNR surfaces and led to aggregation of the GNRs, as evidenced by the significant red shift and broadening of the longitudinal band (SPR$_{\text{long}}$) (The peak at 1150 nm is identified as the ethanol absorption peak). A colorimetric response was recorded (red to purple), as shown in Figure 2B.

The amount of diazonium ions undergoing deamination reaction was dependent on the heating time of the mixture. As the heating time increased, more diazonium ions were decomposed with the elimination of the $-\text{N}=$ groups, which led to the reduction of the positive surface charges of the GNRs. When the surface charges were lowered down to a critical level, aggregation of the GNRs was induced. The extinction intensity of the SPR$_{\text{long}}$ band of the GNR colloids was continuously monitored throughout the heating process. As shown in Figure 3A, extinction of the SPR$_{\text{long}}$ band started to decrease significantly after 2 min of heating, indicating the onset of the aggregation of GNRs. The onset and progression of the aggregation was also dependent on the nitrite concentration in the sample. More nitrite led to more diazonium ions being formed, which subsequently resulted in a higher rate of deamination reaction and the earlier onset of the aggregation. As shown in Figure 3B, at a fixed heating time, a critical coagulation concentration (CCC) of nitrite could be identified that represents the minimum concentration of nitrite that induces the aggregation of the GNRs.

The CCC represents the concentration of nitrite that would trigger the colorimetric response from the GNR sensors. As shown in Figure 4A, the CCC is dependent on the heating time. By adjustment of the heating time, the GNR sensors can be used to detect nitrite at different concentrations, with a sensitivity of $\sim 10 \mu\text{M}$ reached at 10 min of heating. A response curve was created for GNR sensors made from GNRs with an aspect ratio of 1.5 ($\lambda_\text{long} = 680 \text{ nm}$), as shown in Figure 4B. The CCC was correlated with the heating time of the sample--sensor mixture. With the time recorded when the aggregation occurs, which can be easily determined through simple visual inspection, the concentration of the nitrite in the sample can be quickly determined. It should be noticed that the CCC appeared to be exponentially correlated to the heating time, hence to increase the sensitivity of the detection beyond 10 $\mu$M, the heating time will need to be significantly increased.

**Figure 4.** (A) GNR, with SPR$_{\text{long}}$ at 680 nm, particle solution extinction at 680 nm after heating at different nitrite concentrations and different heating times. (B) CCC of GNR-4ATP with SPR$_{\text{long}}$ at 680 nm as a function of heating time.

**Figure 5.** CCC of GNR-4ATP with different aspect ratios as a function of the SPR$_{\text{long}}$ band.

**Figure 6.** Selectivity of GNR nitrite sensor toward nitrite and other anions. (The concentration of SO$_4^{2-}$ and S$_2$O$_3^{2-}$ were 0.1 mM, the concentration of NO$_2^-$ was 30 $\mu$M, and the concentrations of the other anions were 1 mM.)
To further improve the sensitivity of the GNR sensors, we took advantage of the tunable plasmonic properties of the GNRs by controlling their aspect ratios. It is well-known that larger aspect ratio GNRs are more sensitive toward changes in their dielectric environment. As the aspect ratio increases, a lower concentration of nitrite is needed to trigger the aggregation of the GNRs. At fixed heating time (5 min), the CCC (representing the sensitivity of the assay) decreases as the aspect ratios of the GNRs increase (as demonstrated by the red-shift of the longitudinal plasmonic band), as shown in Figure 5. The lowest CCC observed in this study was 5.2 µM (~0.25 ppm) using GNR sensors with λ\text{log} = 806 nm (data not shown), which is significantly lower than the recommended EPA standard (1 ppm or 21.7 µM). It is reasonable to believe the sensitivity can be further improved if GNRs with larger aspect ratios are used.

Any detection schemes based on surface charge balance would inevitably be interfered by other anions in the sample if they were present at high enough concentrations. To evaluate the selectivity of the GNR sensing scheme, its responses toward other anions, including HCO₃⁻, CH₃COO⁻, F⁻, Cl⁻, Br⁻, NO₃⁻, ClO₃⁻, H₂PO₄⁻, C₂O₄²⁻, SO₄²⁻, and S₂O₃²⁻, were investigated. No colorimetric responses were observed for anions other than SO₄²⁻ and S₂O₃²⁻ up to the concentration of 1 mM. For SO₄²⁻ and S₂O₃²⁻, 0.1 mM appeared to be the limit without triggering a colorimetric response. Therefore, for anionic concentration below 100 µM, the GNR sensors are specific toward nitrite. Combining it with an enzymatic or metallic-catalytic reduction of nitrate, the GNR sensing scheme can be applied to highly sensitive nitrate detection as well (see Figure 6).

CONCLUSIONS
In conclusion, a novel GNR nitrite sensor, taking advantage of the tunable optical property of GNR, was developed. This noncross-linking aggregation assay was rapid and highly sensitive with a detection range of 5.2–100 µM (0.25–5 ppm). With the colorimetric response, no sophisticated instrumentation is required. It can serve as an easily applicable, user-friendly, portable assay to monitor low-level nitrite and/or nitrate contaminations in drinking water or relatively high-purity water.

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