

Colorimetric Nitrite and Nitrate Detection with Gold Nanoparticle Probes and Kinetic End Points

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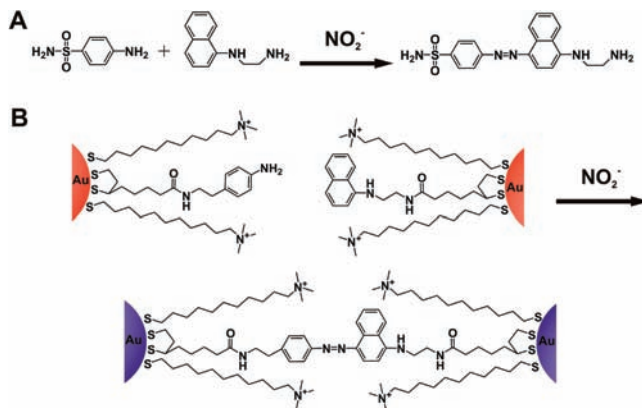
Contamination of rural drinking water supplies with nitrate (NO_3^-) and nitrite (NO_2^-) ions by livestock waste, organic wastes, and chemical fertilizers continues to be a problem throughout the world.¹ One study estimates that greater than 4 million Americans are using self-supplied water with nitrate concentrations exceeding the maximum contaminant level (MCL) defined by the Environmental Protection Agency (EPA).² Epidemiologic studies have associated nitrate and nitrite ion exposure in drinking water to a number of medical issues including spontaneous abortions, intrauterine growth restriction, and birth defects of the central nervous system.^{3–10} While several methods for testing nitrate and nitrite concentrations exist, including those based on organic chromophores¹¹ or fluorophores¹² and ion chromatography,¹³ the use of these methods requires complicated and expensive instrumentation, limiting testing in rural and remote areas where such evaluation is most essential. Therefore, a simple test for dangerous levels of nitrite and nitrate could greatly aid individuals in evaluating the quality of their water source.

Chemically functionalized gold nanoparticles (Au NPs) exhibit distance-dependent optical properties, making them useful in colorimetric detection systems, including those for DNA,^{14–17} proteins,¹⁸ small molecules,¹⁹ metal cations,^{20–22} and cancerous cells.²³ Herein, we have developed novel functionalized Au NPs with nitrite-reactive groups that take advantage of these optical properties and can be used to colorimetrically detect nitrite or nitrate ions in a selective manner using the concept of a kinetic end point (the time required to effect precipitation).

To create a probe that exhibits selectivity for NO_2^- , a bimolecular reaction that is specific for the analyte is needed. The Griess reaction, where sulfanilamide and naphthylethylenediamine are coupled by nitrite (Scheme 1A), is ideal in this regard and has been used to create related detection systems.^{11,24} In this reaction, nitrite ion converts a sulfanilamide into a diazonium salt, which is subsequently coupled with naphthylethylenediamine, producing an azo dye. Detection systems, which rely on the ensuing colorimetric change, have been based upon this reaction, but they require complicated spectrophotometric instrumentation for readout and quantification,¹¹ which is a significant hurdle for on-site use. We hypothesized that polyvalent gold nanoparticles, isotopically functionalized with thiolated precursors for the Griess reaction, could yield a system with a *distinct* color change (observable with the naked eye) and a kinetically controlled end point, which could provide analyte concentration information in the range of the EPA-recommended MCL.

In a typical experiment, two types of Au NP probes were prepared. The first, “aniline Au NPs,” are modified with 5-[1,2]dithi-

Scheme 1. (A) Griess Reaction. (B) Colorimetric Detection of Nitrite with Functionalized Au NPs



olan-3-yl-pentanoic acid [2-(4-amino-phenyl)ethyl]amide (DPAA). We discovered that these Au NPs precipitate in water when they are modified only with the hydrophobic DPAA, so the particles must be cofunctionalized with hydrophilic (11-mercapto-undecyl)-trimethyl-ammonium (MTA) molecules to increase their solubility. A second Au NP probe, “naphthalene Au NPs,” are modified with 5-[1,2]dithiolan-3-yl-pentanoic acid [2-(naphthalene-1-ylamino)ethyl]amide and MTA. The loading of these molecules per nanoparticle has not yet been determined.

The aniline and naphthalene Au NPs are red when dispersed in aqueous solution due to an intense surface plasmon resonance at 520 nm. In the presence of nitrite ion under acidic conditions, however, the amine groups on the aniline Au NPs are converted to a diazonium salt, which then couples with the naphthalene Au NPs to form covalently interconnected nanoparticle probes (Scheme 1B). This reaction causes the formation of crosslinked particle networks which precipitate rapidly, causing the solution to change from red to colorless (Figure 1A). The diazo bond formation was confirmed with MALDI MS and surface enhanced Raman spectroscopy (Supporting Information). The EPA MCL for nitrite ion in drinking water is 1 ppm (21.7 μM). This system is well-designed to identify nitrite concentrations above 21.7 μM . For example, for concentrations of nitrite ion above $\sim 30 \mu\text{M}$, nearly all of the aniline and naphthalene Au NPs react and precipitate, causing the solution to appear clear. At concentrations between ~ 22 and $30 \mu\text{M}$, the color varies as a function of nitrite ion concentration. Nitrite ion concentrations below $\sim 22 \mu\text{M}$ do not cause a color change. The onset of the color change can be controlled kinetically. In this example, an incubation time of 25 min at a temperature of 95 °C will cause the onset of the color change to occur at the EPA limit (Figure 1B). This end point can be moved between ~ 20 to $35 \mu\text{M}$ simply by changing the incubation time (Supporting Information), allowing one to adjust the assay for different threshold concentra-

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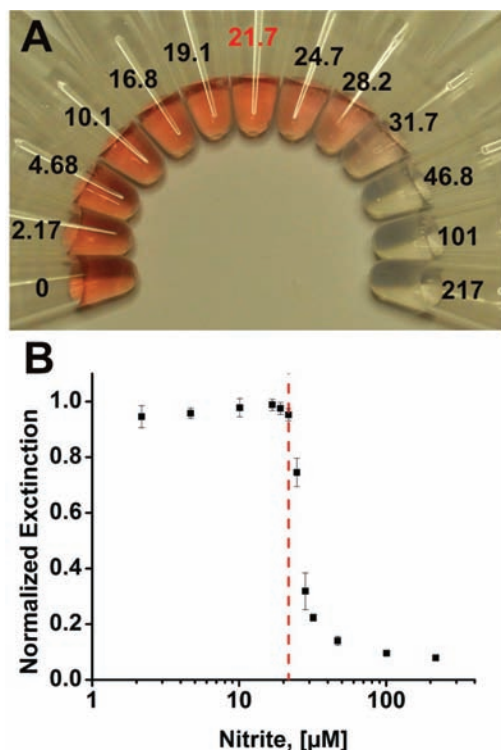


Figure 1. (A) Photograph of particle solutions after incubation with various concentrations of nitrite. The nitrite concentrations, in μM , are listed next to the respective solutions. The MCL of nitrite in drinking water ($21.7 \mu\text{M}$) is highlighted in red. (B) Particle solution extinction at 524 nm after incubation as a function of nitrite concentration. The red dashed line indicates the nitrite MCL.

tions. The range of end points accessible is not particularly broad but is highly relevant given the EPA recommendations for this analyte. Importantly, when tested in complex environmental samples that had been spiked with nitrite ions (filtered water from Lake Michigan), the assay responded the same as in distilled water (Supporting Information).

Importantly, we extended the capability of this Au NP based method to the detection of nitrate ion by adding a simple enzymatic reduction step. To facilitate this reduction, samples of the nitrate ion in water were mixed with nitrate reductase and its cofactors and then incubated at room temperature for 15 min, producing nitrite ions.²⁵ Aliquots of these solutions were removed and mixed with the particles. After 16 min of incubation at 95°C , the onset of particle precipitation occurs approximately at the EPA limit (10 ppm or $161 \mu\text{M}$) for nitrate ion in drinking water (the reduction step dilutes the original sample). Higher concentrations result in particle aggregation and colorless solutions (Supporting Information).

The selectivity of the assay was evaluated by challenging it with other environmentally relevant anions. All of the interfering anions tested, including NO_3^- , F^- , SO_4^{2-} , Br^- , ClO_4^- , CH_3COO^- , $\text{S}_2\text{O}_3^{2-}$, $\text{C}_2\text{O}_4^{2-}$, N_3^- , and HCO_3^- , do not elicit a response relative to the blank (Supporting Information). Even at relatively high concentrations (3 mM, 2 orders of magnitude greater than the nitrite concentration), the system still shows no color change, demonstrating its high selectivity for nitrite ions.

In conclusion, we have synthesized novel Au NP probes for colorimetrically detecting nitrite and, when coupled to an enzymatic reduction step, nitrate. The design of these materials takes advantage of the intense optical properties of a gold colloid to create a convenient assay readout. In contrast to other systems, the readout of this test requires no instrumentation. Whereas other Au NP based anion detection systems lack specificity,^{26,27} the chemistry on these novel nanoparticles is highly selective, as the crosslinking reaction between the particles occurs only in the presence of the nitrite ion. Taken together, these advantages make this assay simple, robust, inexpensive, and therefore promising for on-site water testing.

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Supporting Information Available: Synthetic methods for functionalized Au NPs and related compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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