Highly Sensitive and Selective Detection of Nitrite Ions Using $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ Magnetic Nanoparticles by Surface-Enhanced Raman Spectroscopy

Juhong Chen^{1,2}, Shintaro Pang¹, Lili He¹, and Sam R. Nugen^{1,2*}

¹Department of Food Science, University of Massachusetts, 102 Holdsworth Way, Amherst, Massachusetts 01003, United States

²Department of Food Science, Cornell University, Stocking Hall, Ithaca, New York 14853, United States

*Corresponding Author

*E-mail: snugen@cornell.edu

ABSTRACT

A novel and pragmatic method was developed to detect the concentration of nitrite ions using $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ magnetic nanoparticles (MNPs) by surface-enhanced Raman scattering (SERS). The as-prepared bifunctional nanocomposites can be used to simultaneously purify target molecules using external magnetic field and produce Raman fingerprint spectrum with trace level of target molecules. In acidic media, 4-aminothiophenol (4-ATP) molecules conjugated on

Fe₃O₄@SiO₂/Au MNPs were triggered by nitrite ions to form azo bonds, resulting in three new marker peaks on the SERS spectrum. Under optimized conditions, the detection limit based on the three marker peaks were 15.63, 13.69, and 17.77 μ M, which was much lower than the maximum NO₂⁻ concentration of 1.0 mg L⁻¹ (71.4 μ M) allowed in drinking water as defined by U.S. Environmental Protection Agency (EPA). The specificity of this proposed method to detect nitrite ions was demonstrated using common ions as competitors. In addition, the SERS-based technique was successfully employed to detect nitrite ions in pond water, urine solution, and pickle brine. Considering its good sensitivity and selectivity, the detection method is well suited for the detection of nitrite ions in real samples without pretreatment steps.

KEYWORDS: magnetic nanoparticle, gold nanoparticle, nitrite ion, diazotization reaction, surface-enhanced Raman scattering

1. Introduction

Nitrite, as a nitrogen-oxygen chemical unit combining with various inorganic and organic compounds, is a part of nitrogen cycle in the environmental and biological conditions (Kim et al. 2012). Due to the contaminations of water sources by fertilizer, livestock waste, and other organic waste, it is not surprising to find a trace amount of nitrite ions in surface and ground water systems (Liu et al. 2015). Unfortunately, even a trace amount of nitrite ions can be deleterious to human health. On the other hand, nitrite is important in biochemistry as a source of vasodilator nitric oxide, and in meat curing as preservatives to prevent bacterial growth (Gladwin et al. 2004; Honikel 2008). However, after reacting with degraded amino acid under certain conditions, nitrites can become carcinogenic. Therefore, as a safety measure, the concentration of nitrite ions is treated as an important water quality parameter. The accepted maximum

contaminant levels (MCL) for nitrite ions in drinking water are regulated to be 3.0 mg L^{-1} ($214.2 \text{ }\mu\text{M}$) by the World Health Organization (WHO) and 1.0 mg L^{-1} ($71.4 \text{ }\mu\text{M}$) by the U.S. Environmental Protection Agency (EPA). In addition, the presence of nitrite ions in the urine solution can be used as an indicator of urinary tract infections. Hence, accurate and early detection of nitrite ions in both environmental, biological, and food samples is of great significance.

The most common method to detect nitrite ions is based on the Griess-Ilosvary reaction. In the past decades, various analytical methods have been reported for the detection of nitrite ions, including colorimetry, fluorometry, electrochemistry, chromatography, and electrophoresis (Ahmed et al. 1996; Büldt and Karst 1999; Daniel et al. 2009; Everett et al. 1995; Guan et al. 1996; Stanley et al. 1994; Xiao and Yu 2010). Although most of these methods have high sensitivity, they are susceptible to interferences from the physical and chemical properties of detection samples, such as color, turbidity, and presence of other compounds. For example, Xiao *et al.* reported a colorimetric method to detect nitrite ions using gold nanorod, which was limited to detection samples with a background color of purple (Xiao and Yu 2010). Guan *et al.* used high-performance capillary electrophoresis to detect nitrite ions in river water after filtration to remove solid particles (Guan et al. 1996). In order to overcome these problems, pretreatment steps were applied to separate nitrite ions from the interferences (Jackson and Jones 1991). Recently, magnetic particles have gained a wide attention to be used to separate targets in a diagnostic field (Chen et al. 2015a; Wang et al. 2016a). Moreover, due to the large surface area of nanomaterials, magnetic nanoparticles (MNPs) have shown a potential to be used to purify and concentrate nitrite ions.

Surface-enhanced Raman scattering (SERS), recognized as a highly powerful analytical technique, has been widely employed to detect a number of chemical and biological agents

(Chen et al. 2015b; Guerrini and Graham 2012; Pang et al. 2014; Wang et al. 2016b; Yang et al. 2010; Yang et al. 2015; Zavaleta et al. 2009). As known, the formation of hotspots at the interstitial junctions or nanogaps between plasmonic metallic nanostructures contributes the giant SERS signal enhancement (Ward et al. 2007). Although, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) with rough surface have been used to detection aqueous nitrite ions by SERS strategy, these methods cannot avoid the above-mentioned interferences from samples (Huang et al. 2010; Kim et al. 2012; Liu et al. 2015). Fortunately, hybrid or core-shell nanostructures can combine different properties in one to form multifunctional nanomaterials. In particular, the integration of MNPs and plasmonic nanoparticles in composites can result in simultaneous magnetic separation and SERS-based analysis (Cai et al. 2014; Zhang et al. 2014).

In this work, we synthesized and prepared $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs for highly sensitive and selective SERS-based detection of nitrite ions. To accomplish this, these as-prepared bifunctional nanocomposites, having a Fe_3O_4 core and a layer of deposited AuNPs, were functionalized with 4-aminothiophenol (4-ATP). In acidic media, nitrite ions triggered primary aromatic amine from 4-ATP molecules on $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs to form a diazonium salt, which further reacted with another aromatic amine to form azo bonds. The intensities of three newly-observed SERS peaks, which were assigned to the formed azo bonds, were directly related to the concentration of nitrite ions. To the best of our knowledge, it is the first study to detect nitrite ions using $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs.

2. Experimental section

2.1 Chemicals and Materials

Polyethylene glycol 600 was purchased Alfa Aesar (Ward Hii, MA). Ammonium hydroxide and (3-aminopropyl)triethoxysilane (APTES) were purchased from Acros Organics (Morris

Plains, NJ). Gold chloride trihydrate, tetraethyl orthosilicate (TEOS), hydrogen chloride, and cetyltrimethyl ammonium bromide (CTAB) were purchased from Sigma Aldrich (Saint Louis, MO). The pond water was collected from campus pond at the University of Massachusetts, Amherst. Synthetic urine solution was purchased from RICCA chemical (Arlington, TX). Pickle brine was purchased form local market. All other chemicals were purchased form Fisher Scientific (Fair Lawn, NJ). Aqueous solutions were prepared using Mill-Q water with 18.2 MΩ·cm at 25 ° C from Thermo Scientific (Asheville, NC). All chemicals were reagent grade and used as received.

2.2 Instrumentation

Transmission electron microscopy (TEM) images of all nanoparticles were obtained using a JEOL JEM-2000FX TEM (Peabody, MA). The energy-dispersive X-ray (EDX) elemental mapping images of individual Fe₃O₄@SiO₂/Au MNP were obtained using JEOL JEM-2200FS (Peabody, MA). The UV-vis spectra were recorded using a UV-vis spectrophotometer from Biotek synergy 2 (Winooski, VT).

2.3 Synthesis of Fe₃O₄@SiO₂/Au MNPs

AuNPs with 13 nm in diameter were synthesized according to previous literature report (Hu et al. 2014). Briefly, HAuCl₄ solution (100 mL, 1 mM) was heated to boil under vigorous stirring. A solution of trisodium citrate (10 ml, 38.8 mM) was rapidly added and boiling continued for additional 30 minutes. The wine red solution was cooled down to room temperature and stored at 4 °C until use.

The preparation of Fe₃O₄@SiO₂ MNPs has been reported in a previously published literature (Cai et al. 2014). Firstly, FeCl₃·6H₂O (0.540 g) was dissolved in a mixture solution containing ethylene glycol (16 mL) and polyethylene glycol 600 (16 mL). NaAc·3H₂O (0.656 g) was added

under stirring to form a transparent solution. The mixture was then transferred into a Teflon-line stainless-steel autoclave and heated at 180°C for 8 hours. After cooling down to room temperature, the Fe₃O₄ MNPs were washed three times with Mill-Q water and dried under vacuum. Fe₃O₄ MNPs (10 mg) were dispersed in ethanol solution (100 mL). After adding NH₄OH (1.5 mL) and TEOS (100 µL), the mixture was mechanically stirred for 18 hours at room temperature.

Prior to forming AuNPs deposition, Fe₃O₄@SiO₂ MNPs were functionalized with amine group by incubating with APTES (200 µL) under mechanical stirring for 8 hours at room temperature. After washing three times with Mill-Q water, the amine functionalized Fe₃O₄@SiO₂ were added into sonicated AuNPs solution and stirred under mild shake overnight. The formed Fe₃O₄@SiO₂/Au MNPs were washed three times and dispersed in Mill-Q water at a concentration of 0.5 mg mL⁻¹ for further use.

2.4 Preparation of 4-ATP Capped Fe₃O₄@SiO₂/Au MNPs

The obtained Fe₃O₄@SiO₂/Au MNPs were functionalized with 4-ATP using a modification of a previously reported method (Liu et al. 2015). Briefly, Fe₃O₄@SiO₂/Au MNP solution (25 µL, 0.5 mg mL⁻¹) was added into 0.5 mL centrifuge tube. 4-ATP solution (50 µL, 0.1 mM) and HCl solution (50 µL, 0.1 M, containing 1 mM CTAB) were added. After incubating under stirring for 5 hours at room temperature, 4-ATP capped Fe₃O₄@SiO₂/Au MNPs were washed three times using magnet and dispersed in HCl solution (90 µL, 0.1 M, containing 1 mM CTAB) until use.

2.5 Detection of Nitrite Ions (NO₂⁻) Using 4-ATP Capped Fe₃O₄@SiO₂/Au MNPs

The nitrite ion stock solution was diluted into varying concentrations using Mill-Q water. Each concentration (50, 100, 200, 400, 600, 1000, and 5000 µM, 10 µL) was added into mentioned-above centrifuge tube containing 4-ATP capped Fe₃O₄@SiO₂/Au MNPs (90 µL).

Mill-Q water without nitrite ions was used as control. After incubating under stirring for 5 minutes in room temperature, $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs were washed three times using magnet and dispersed with HCl solution (100 μL , 0.1 M, containing 1 mM CTAB). The solution (20 μL) was dropped on gold-coated glass slide, and a magnet was placed under the glass slide to aggregate these $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. The spectrum of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs was recorded using DXR Raman spectro-microscope.

The specificity to detect nitrite ions was investigated using competing ions (Cl^- , CH_3COO^- , PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- , CO_3^{2-} , HCO_3^- , $\text{S}_2\text{O}_3^{2-}$, SO_4^{2-} , NO_3^-). In order to detect nitrite ions in environmental, biological, or food samples, the nitrite ions were spiked into pond water, urine solution, or pickle brine to form varying concentrations, respectively.

2.6 Raman Instrumentation

Prior to drying, the spectra of each sample were recorded using a Thermo Scientific DXR Raman spectro-microscope (Madison, WI) with a laser source of 780 nm. A minimum of 10 spots were randomly measured for each sample using the following conditions: a 10 $\times$ confocal microscope objective, 5 mW laser power, 3.1 μm spot diameter, and 50 μm slit width for 2 seconds integration time. The Raman spectra within the wavelength range of 800-1700 cm^{-1} were analyzed using Thermo Scientific TQ Analyst software version 8.0, and each Raman intensity was averaged from at least three independent samples.

3. Results and discussion

3.1 Principle of Nitrite Ions Detection by Azo Bonds

The mechanism for the detection of nitrite ions by surface-enhanced Raman scattering strategy is shown in Figure 1. Based on the strong interaction between thiol group and gold surface, 4-ATP molecules absorbed on the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs can be readily triggered by nitrite ions

in acidic media. The small gaps formed between AuNPs provided plenty of SERS-active hotspots to enhance Raman signal. The two peaks at the wavelength of 1593 and 1080 cm^{-1} assigned to the a_1 modes of the 4-ATP molecules are observed (Huang et al. 2010; Kim et al. 2012). In the presence of nitrite ions in acidic media, the 4-ATP molecules on the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs are triggered to form azo bonds. Meanwhile, three new peaks at 1436, 1391, and 1140 cm^{-1} appeared, which had been reported to be assigned to the b_2 modes of the azo bonds (Huang et al. 2010; Ward et al. 2008; Zhou et al. 2006). In order to reduce the experimental errors, the intensities at the three new peaks were normalized using the intensity at the peak of 1080 cm^{-1} to calculate the concentration of nitrite ions. In this study, Fe_3O_4 magnetic cores were used to purify and concentrate nitrite ions in complex samples, as well as to enhance SERS signal by aggregating using magnet (Cai et al. 2014).

Figure 1. Schematic illustration of the detection of nitrite ions using $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs capped with 4-ATP (The spectra of 4-ATP on $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs before (red line) and after (blue line) addition of nitrite ions (NO_2^-) in acidic media).

3.2 Characterization of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs

The $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs consisted of a Fe_3O_4 magnetic core, a shell of SiO_2 , and a layer of AuNP deposition. The SiO_2 shell was used to link the magnetic core and AuNP deposition, resulting in bifunctional $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. The size and morphology of nanoparticles at each stage were characterized using transmission electron microscopy (TEM) images. The average size of AuNPs, synthesized using the classical citrate reduction method, was 13 nm in diameter (Figure 2a). The diameter of the synthesized Fe_3O_4 magnetic core was approximately 100 nm (Figure 2b). These particles were then coated with a SiO_2 shell. As shown in Figure 2c, a smoother surface morphology was observed, indicating the successful coating SiO_2 shell on the surface of Fe_3O_4 MNPs. After being functionalized with positively charged amine-rich groups using APTES, the negatively charged citrate-reduced AuNPs were added to form a high-density layer of AuNPs on the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs by the strong affinity between amine group and AuNPs (Figure 2d) (Xia et al. 2006).

Figure 2. TEM images of (a) AuNPs, (b) Fe_3O_4 MNPs, (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs, and (d) $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. (e) UV-vis absorption spectra of (i) AuNPs, (ii) Fe_3O_4 MNPs, (iii) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs, and (iv) $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs (insert: the corresponding photograph). (f) EDX elemental mapping images of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs based on Fe, Si, O, and Au element.

The UV-vis absorption spectra of AuNPs, Fe_3O_4 MNPs, $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs, and $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs were measured (Figure 2e). As shown, AuNPs displayed an absorption peak at the wavelength of 520 nm. A comparison of the spectra for Fe_3O_4 MNPs before and after SiO_2 coating revealed only a slightly stronger absorption near 400 nm after the SiO_2 coating. Following deposition of the AuNP layer on the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs, a broad peak could be observed at the wavelength of 530 nm. The slight red shift of the feature peak from AuNPs was mainly due to the strong interparticle interaction, indicating the presence of AuNPs on the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs (Fang et al. 2008). Meanwhile, the corresponding solution colors of these nanoparticles are shown (Figure 2e, insert).

To further confirm their nanostructure and atomic composition, the obtained individual $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNP was analyzed using energy-dispersive X-ray (EDX)-TEM images. The elemental distributions are shown using different color areas in the elemental mapping images, where red, green, blue and yellow areas corresponded to elemental Fe, Si, O, and Au atoms, respectively (Figure 2f). Compared to the red area, a larger green area was observed, indicating the coating of SiO_2 on the surface of the Fe_3O_4 MNPs. In addition, an obvious yellow circle on the outside of this individual MNP suggested the Au-enriched area. These results also indicated that a high-density layer of AuNPs was deposited on the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs.

3.3 Finite-Difference Time-Domain (FDTD) Simulation of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs

It is well known that plasmonic nanoparticles can enhance an electromagnetic field and generate localized surface plasmon resonances, providing SERS signals (Chen et al. 2015b; Gandra and Singamaneni 2013; Liang et al. 2009). As previously reported, the SERS intensity of

individual raspberry-like AuNP was mainly confined to built-in hotspots (Qian et al. 2015). Due to the size variations of small gaps between AuNPs, the intensities varied at the different locations of raspberry-like AuNP. However, a new hotspot was obtained between the gaps of two raspberry-like AuNPs (dimer-gap), providing a higher Raman intensity than that of build-in hotspots (Qian et al. 2015). Their results indicated that Raman enhancement contributed by the dimer-gap was at least an order of magnitude higher than that of build-in gaps. Recently, the significance of hotspots formed between the dimer-gap has been widely investigated to enhance SERS performance.

Figure 3. (a) Modeled $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNP dimer ($\text{Diameter}_{\text{Core}} = 100$ nm, $\text{Thickness}_{\text{Shell}} = 10$ nm, and $\text{Diameter}_{\text{AuNP}} = 13$ nm). Electric field intensity map using FDTD simulation at the excitation laser wavelength of 780 nm for $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNP dimer at (b) XY view, (c) XZ view, and (d) YZ view.

In order to investigate the SERS performance of our prepared $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs, the electric field distribution was simulated using FDTD Solutions (Lumerical Solution, Inc.). The schematic illustration of modeled $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNP dimer is shown in Figure 3a. Two

$\text{Fe}_3\text{O}_4@\text{SiO}_2$ MNPs with core diameter of 100 nm and shell thickness of 10 nm, both of which were coated by a layer of AuNPs with diameter of 13 nm, were simulated at the excitation laser wavelength of 780 nm. The electric field intensity map of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNP dimer at XY, XZ, and YZ view are shown in Figure 3b-d. As shown, a strong SERS intensity was obtained from the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNP dimer-gap, mainly contributing to the total Raman enhancement.

3.4 Control Experiments and Optimization of Experimental Conditions for Nitrite Ion (NO_2^-) Detection

In this work, the detectable SERS signal mainly derived from the NO_2^- -triggered azo bonds formed on 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. To demonstrate this point, the spectra of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs from different treatment types are shown in Figure 4. Before being functionalized with 4-ATP, the two spectra of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs and $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs + NO_2^- in acidic media (containing 1mM CTAB) didn't show any obvious differences (Figure 4a,b). The $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs were then incubated with 4-ATP, and excess 4-ATP molecules were washed away with 0.1 M HCl solution containing 1mM CTAB. Two strong peaks at the wavelength of 1593 and 1080 cm^{-1} were observed, indicating the strong binding of 4-ATP to the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs (Figure 4c). Upon addition of nitrite ions, three typical peaks at the wavelength of 1436, 1391, and 1140 cm^{-1} immediately appeared (Figure 4d), which were similar with the previously reported spectra of azo bonds (Correa-Duarte et al. 2015; Kang et al. 2013; Sun and Xu 2012). It is well known that the azo bonds are formed in acidic media. In order to clarify this theory, the reaction was performed in Mill-Q water containing 1 mM CTAB. As expected, no typical peaks caused by the azo bonds were observed (Figure 4e). Thus, these results demonstrated that 4-ATP Capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs were able to be used to detect nitrite ions in 0.1 M HCl solution containing 1mM CTAB.

Figure 4. SERS spectra of control treatment types: (a) $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ + 0.1 M HCl, (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ + nitrite ions + 0.1 M HCl, (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ + 4-ATP + 0.1 M HCl, (d) $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ + 4-ATP + nitrite ions + 0.1 M HCl, and (e) $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ + 4-ATP + nitrite ions + Mill-Q water. (All solutions contained 1 mM CTAB).

To further demonstrate our principle and to optimize experiment conditions, several parameters and conditions were investigated. Firstly, the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs were incubated with 4-ATP at a range of concentrations (10^{-6} to 10^{-1} M, Mill-Q water without 4-ATP molecules was selected as a control). After removing the excess 4-ATP molecules, the SERS spectra were recorded and shown in Figure S1a. The typical peak intensities at the wavelength of 1593 and 1080 cm^{-1} were plotted against the concentration of 4-ATP molecules (Figure S1b). The concentration of 10^{-4} M gave the highest Raman intensities at both typical peaks, which was used for further SERS analysis.

Next, the function of 1 mM CTAB in 0.1 M HCl solution was investigated. The spectra of 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs in 0.1 M HCl solution with and without 1 mM CTAB are

shown in Figure S2. After comparing the two spectra, an identified peak at the wavelength at 1330 cm^{-1} was observed (Figure S2b), which was assigned to the vibration of nitrite ions (Kang et al. 2013; Kim et al. 2012). The new peak was supposedly due to the absorbance of NO_2^- on the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. Moreover, the SERS intensities at the three typical peaks significantly decreased, which could be explained as follows: the absorbance of nitrite ions reduced the capping of 4-ATP molecules on the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. Thus, the CTAB in HCl solution was able to reduce the absorbance of nitrite ions, as well as avoid the aggregation of the $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs as stated in previous literature (Liu et al. 2015; Xiao and Yu 2010). The optimizations of incubation time and HCl concentration are shown in Figure S4. The incubation of 5 min and HCl concentration of 0.1 M were used for further experiments.

Furthermore, previous studies indicated that O_2 and H_2O vapor played an extremely important role in the formation of azo bonds (Liu et al. 2015; Xu et al. 2013). After air-drying, these azo bonds can be formed on 4-ATP capped AuNPs without the addition of nitrite ions (Xu et al. 2013). In order to investigate the effect of air-drying on the azo bond formation, the spectra of four different treatment types were collected after the air-drying of $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs (Figure S3). Due to the shortened distance between nitrite ions and $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs after air-drying, a peak representing the vibration of nitrite ions was observed (Figure S3b, d). In addition, air-drying caused the three typical peaks of azo bonds to appear even in the absence of nitrite ions (Figure S3c). Thus, the detection of nitrite ions using 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs needs to be conducted in aqueous conditions.

3.5 Analytical Performance for Nitrite Ions detection

Under the optimized conditions and experimental parameters, the sensitivity and dynamic range of this proposed method were evaluated using the NO_2^- -triggered azo bonds formed on 4-

ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs as an indicator. The spectra of azo bonds towards different concentration of nitrite ions are shown in Figure 5a. With the increasing concentrations of nitrite ions, the three typical peaks of azo bonds at the wavelength of 1436, 1391, and 1140 cm^{-1} were gradually observed. These three typical peaks towards nitrite ion concentrations were highlighted in Figure 5b. And, their intensities were normalized using the intensity at the wavelength of 1080 cm^{-1} for better comparison. The normalized intensities at the three peaks were plotted towards the concentration of nitrite ions, respectively (Figure 3c-e). All the calibration plots displayed a good linear relationship between the normalized intensity and the NO_2^- concentration in the range from 10 μM to 100 μM . As previously reported method, the mean of the control replicates (without addition of nitrite ions) plus 3 times their standard deviation was used to calculate the limit of detection (LOD) (Connelly et al. 2015). The LODs using this proposed method were as low as 15.63, 13.69, and 17.77 μM at the three typical peaks, respectively. All the obtained LOD were well below the U.S. EPA recommended maximum contamination levels in drinking water (71.4 μM).

Figure 5. (a) SERS spectra of 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs with various concentration of nitrite ions. (b) Highlighted SERS spectra range of 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs with various concentrations around the wavelength of 1436, 1391, and 1140 cm^{-1} . (c-e) Normalized SERS intensities were plotted with NO_2^- concentration at the wavelength of 1436, 1391, and 1140 cm^{-1} . (e) SERS spectra of 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs against competing ions. (f) Normalized intensities at the wavelength of 1436, 1391, and 1140 cm^{-1} towards competing ions. (Competing ions: blank, Cl^- , CH_3COO^- , PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- , CO_3^{2-} , HCO_3^- , $\text{S}_2\text{O}_3^{2-}$, SO_4^{2-} , NO_3^- , NO_2^-).

To demonstrate the specificity of this developed method for NO_2^- detection, common ions at the concentration of 500 μM were chosen as competing ion solutions, including Cl^- , CH_3COO^- , PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- , CO_3^{2-} , HCO_3^- , $\text{S}_2\text{O}_3^{2-}$, SO_4^{2-} , and NO_3^- . These competing ion solutions were added into 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs for further detection. Meanwhile, Mill-Q water without any ions as negative control and nitrite ion solution at the concentration of 50 μM as positive control were measured. After incubating for 5 minutes and washing three times, the spectra against the competing ions were recorded (Figure 5f). The normalized intensities at the three typical peaks towards the competing ion solutions were plotted (Figure 5g). As shown, the SERS signal from three typical azo bond peaks at the wavelength of 1436, 1391, and 1140 cm^{-1} were observed only in the presence of nitrite ions, even where its concentration was 10 times lower than that of competing ions. Thus, these results revealed that our proposed method had a good specificity toward NO_2^- detection.

3.6 Application for Nitrite Ions detection in Pond Water, Urine Solution, or Pickle Brine

To further investigate the practicality of this proposed method for the NO_2^- detection, pond water, urine solution, and pickle brine were chosen as models for environmental, biological, and food samples, respectively. The pond water, urine solution, and pickle brine were spiked with various concentrations of nitrite ions were measured using 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. Further, the obtained SERS spectra with various concentrations of nitrite ions in these pond water, urine solution, and pickle brine are shown (Figure S5), respectively. The three typical peaks of azo bonds were not identified in the control spectra, indicating that no nitrite ions were detected in all pond water, urine solution, and pickle brine (without addition of nitrite ions). These results also demonstrated that the pond water, urine solution, and pickle brine were suitable models for environmental, biological, and food samples, respectively. As shown in

Figure 6, the normalized intensities at the wavelength of 1436, 1391, and 1040 cm^{-1} were plotted towards the concentration of nitrite ions in pond water, urine solution, and pickle brine, respectively. Good linearity between normalized intensities at the three typical peaks and the NO_2^- concentration from 10 μM to 100 μM were also obtained in all pond water, urine solution and pickle brine samples. As analyzed from these experimental data using above-mentioned method, the LOD at the three typical peaks were as low as 15.58, 17.37, and 17.36 μM for pond water samples, 11.01, 20.60, and 17.96 μM for urine solution, and 16.48, 21.39, and 14.02 μM for pickle brine. These LOD met the detection limits required by U.S. EPA laws, strongly demonstrating that our proposed nitrite NO_2^- method has the potential for real sample detection.

Figure 6. Normalized intensities of 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs against various concentrations of nitrite ions at the wavelength of 1436, 1391, and 1140 cm^{-1} in (a) pond water, (b) urine solution, and (c) pickle brine, respectively.

4. Conclusions

In summary, we have demonstrated a SERS-based nanoplatform for the sensitive and selective detection of nitrite ions using 4-ATP capped $\text{Fe}_3\text{O}_4@\text{SiO}_2/\text{Au}$ MNPs. Under acidic condition, based on the azo bonds triggered by nitrite ions on the 4-ATP modified bifunctional nanocomposites, the concentration-dependent SERS signals displayed an excellent linear range from 10 μM to 100 μM . Of particular significance, the novel sensor allowed for the detection of NO_2^- concentration as low as 15.63, 13.69, and 17.77 μM at the three typical peaks. In addition to the high sensitivity, the specificity to detect nitrite ions had been demonstrated against competing ions. Remarkably, we further confirmed that our proposed method was able to detect nitrite ions in pond water, urine solution, and pickle brine, indicating that this technique was a facile, accurate, sensitive, specific, reliable, and pragmatic analytical tool for sensing nitrite ions.

ACKNOWLEDGMENT

Financial supports were provided by Center for Hierarchical Manufacturing at University of Massachusetts, a National Science Foundation Nanoscale Science and Engineering center (CMMI-1025020), and USDA National Institute of Food and Agriculture (USDA 2013-02037).

Supporting Information. Additional figures on the control experiments, experimental condition optimizations, and SERS spectra for NO_2^- detection in pond water, urine solution, and pickle brine.

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