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Diazotization-coupling reaction-based selective determination of nitrite in complex samples using shell-isolated nanoparticle-enhanced Raman spectroscopy

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ABSTRACT

A simple, rapid and selective method based on diazotization-coupling reaction for determination of nitrite ion in complex samples using shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) was developed. Based on diazotization-coupling reaction, nitrite was transformed into azo dye, which has strong SHINERS activity. Subsequently the concentration of nitrite ion can be determined indirectly from the SHINERS of azo dye. The SHINERS active substrate was composed of gold nanoparticle as core with an ultrathin silica shell having pinhole on the surface. Various factors that influence reaction and SHINERS intensity were investigated. Under the optimal conditions, the linearity was observed in the range of 0.5–6.0 mg L^{-1} with good correlation coefficient ($r^2 > 0.9793$). The relative standard deviations (RSDs) for five replicate measurements were less than 14.5%. The limit of detections of the method ($S/N=3$) were 0.07, 0.08 and 0.10 mg L^{-1} at 1137, 1395 and 1432 cm^{-1}, respectively, without sample preconcentration. The selectivity of the proposed method was also tested. The performance of SHINERS to determine the concentration of nitrite in food, biological and environmental samples was evaluated. The results indicate that SHINERS shows great potential as a useful analytical tool for trace analysis of nitrite in real samples. This proposed method provides a practical protocol for determination of compounds with weak Raman response, and can be expanded for the indirect detection of iodate ion, phenols and aromatic amines.

1. Introduction

Raman spectroscopy is one of the important analytical methods especially for its capability for chemical group identification. However, the major limitation of conventional Raman spectroscopy is its low sensitivity due to the low efficiency of inelastic scattering. The discovery of surface-enhanced Raman spectroscopy (SERS) by Fleischmann et al. [1] and Jeanmaire et al. [2], which demonstrated tremendously enhanced Raman scattering has generated renewed interest in the field of Raman spectrometry. In SERS, the Raman vibrational modes of a molecule located in close proximity to a rough noble metal surface are strongly enhanced with enhancement factors of 10^3–10^6. Additionally, when the wavelength of the excitation laser is matching with the absorption of the molecules, the molecules will experience resonance Raman scattering (RRS). SERS combined with RRS is called surface resonance Raman scattering (SERRS), which provides nondestructive and ultrasensitive detection down to a single molecule level [3]. Metal nanoparticles such as Au, Ag and Cu, are widely used as SERS substrates through electromagnetic, chemical, or electronic enhancement [4,5]. For example, SERS has been used for analysis of alkaline phosphatase [6], Bacillus subtilis [7], perchlorate [8], tyrosine [9] and melamine [10]. However, low selectivity and stability of SERS substrates limited the routine application.

In order to improve the selectivity and stability of active substrates, Tian et al. [11,12] developed a new technique called shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS), which consists of a gold nanoparticle with an insulating thin SiO_2 shell (Au/SiO_2) as SERS active substrates. The ultrathin coating keeps the nanoparticle from agglomerating, and protects the SERS-active nanostructure from contacting with whatever is being probed. The selectivity and stability of Au/SiO_2 were improved. Therefore, SHINERS acts as the next generation of advanced spectroscopy [13], which could be the preferred analytical technique for environmental monitoring, life science, food safety and so on. However, as a vibrational spectroscopy technique, SHINERS can not be used to detect atomic cations and anions directly, or other organic molecular species with an extremely low SHINERS cross-section.

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Nitrite is an abundant intermediate in the biological nitrogen cycle and can cause serious hazards to public health [14] and the environment [15]. The nitrogen-based fertilizers used in agriculture have main roles in the production of nitrite in the environment. Nitrite is also usually added to processed meat products as its sodium or potassium salt to retain the red color and flavor of fresh meat [16]. However, high levels of nitrite ion can lead to a wide range of detrimental health effects [14] and ecosystem degradations [15,17]. At present the US EPA considers the maximum contaminant level (MCL) that is allowed for nitrite in drinking water is 1.0 mg L^{-1} [18]. Therefore, the determination of nitrite is assumed to be of vital importance. Several analytical techniques have been proposed for the determination of nitrite ion, such as UV–vis spectrophotometry [19], flow injection analysis [20], high performance liquid chromatography [21], gas chromatography [22], colorimetric assay [23], SERS [24], UV resonance Raman spectroscopy [25], ion chromatography [26] and capillary electrophoresis [27]. Most of these methods are sensitivity, but they also suffer from shortcomings, such as time consuming, expensive and complex operation. So far, because of the weak signals obtained with Raman spectroscopy, nitrite ions have hardly been detected directly by Raman spectroscopy. Kim et al. [24] attempted to detect nitrite ions through diazotization-coupling reaction by SERS. However, Au nanoparticles were immobilized on the inner surface of glass capillary in their work, which needs high requirement of preparation.

In this work, a simple, rapid and selective method for the selective detection of trace nitrite in complex samples using SHINERS was developed based on diazotization-coupling reaction. A key feature of this method is the diazotization-coupling reaction of nitrite with p-nitroaniline in the presence of diphenylamine in acid media providing a colored azo dye, which we used not only to enhance the affinity of the analytes to metal nanoparticles surfaces but also to generate strong SHINERS. The concentration of nitrite ion was determined indirectly from azo dye. Au/SiO_2 nanoparticles with pinhole were used as SHINERS substrate. The performance of SHINERS to determine the concentration of nitrite in food, biological and environmental samples was evaluated. The probe is highly selective due to the specificity of the diazotization-coupling reaction. This proposed method can be expanded for the detection of iodate ion, phenols and aromatic amines. The proposed method is rapid ($\sim$10 min) and highly sensitive (<1.0 mg L^{-1} of nitrite, satisfies the EPA detection limit).

2. Experimental

2.1. Reagent and chemicals

P-phenylenediamine, p-Nitroaniline and o-phenylenediamine were purchased by Shanghai Jingchun Reagent Co., Ltd. (Shanghai, China). Sodium nitrite and sodium carbonate were obtained from Donghai Chemical Factory (Suzhou, China). Diphenylamine, potassium iodate and phenol were brought from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Sulfanilamide and hydroxylammonium chloride were purchased by Damao Chemical Reagent Factory (Tianjin, China). Au colloids (2.94×10^{-4} mol L^{-1}) with diameter $\sim$55 nm, and Au/SiO_2 colloids (2.94×10^{-4} mol L^{-1}) having pinhole which are consisted of the Au nanoparticles cores with diameter of $\sim$55 nm and the silica shell thickness $\sim$1–2 nm, were supplied friendly by Prof. Tian from Xiamen University [11] (Xiamen, China). Au and Au/SiO_2 colloids were concentrated prior to use. All the other chemicals employed were analytical grade and were used as received. Ultrapure water (18.2 MΩ cm^{-1}) was used throughout the study.

Stock nitrite solution (100.0 mg L^{-1}) was prepared by dissolving dried sodium nitrite (at 110 °C for 4 h) in water, and then a pellet of sodium hydroxide and a drop of chloroform were added to make the solution stable by preventing the decomposition of nitrous acid and inhibition of bacterial growth. Sodium carbonate (10%) was prepared by dissolving sodium carbonate in water. Hydroxylammonium chloride (1.0 mol L^{-1}) was prepared by dissolving hydroxylammonium chloride in water. The working solutions were prepared from the stock solution by diluting suitably with water.

The reagents were used in diazotization-coupling reaction.

Reagent A_1: p-Nitroaniline (0.3 g)+12 mol L^{-1} HCl (9 mL) for 100 mL of solution (stored at 4 °C).

Reagent A_2: sulfanilamide (0.9 g)+12 mol L^{-1} HCl (1 mL) for 100 mL of solution (stored at 4 °C).

Reagent B: diphenylamine (0.4 g) was prepared in 100 mL ethanol (stored at 4 °C).

Diazotization-coupling reaction: Nitrite solution+Reagent A +Reagent B (100:1:1, v/v/v).

2.2. Instrumentation

A battery-powered Raman spectrometer (model Inspector Raman, diode laser excitation wavelength $\lambda_{ex}=785$ nm) in the range 200–2200 cm^{-1} was purchased from DeltaNu (Laramie, WY), which was used to demonstrate the feasibility of a field-portable device for spore detection. This system consists of a liquid-N_2-cooled CCD detector (Model Spec-10:400B, Roper Scientific, Trenton, NJ) with a spectral resolution of 8 cm^{-1}, and a data acquisition system (Photometrics, Tucson, AZ). A TG16-WS centrifuge was bought from Xiangyi Laboratory Instrument Co., Ltd (Hunan, China). Cary-100Conc UV–vis spectrophotometer (Varian, American) was employed.

2.3. SHINERS measurement

In a typical experiment for detecting nitrite, a 200 μL of water sample solution containing 2.0 mg L^{-1} nitrite ion in a 2 mL centrifuge tube was spiked with 2.0 μL of Reagent A_1 and Reagent B respectively, and then the mixture solution were placed for 10 min. After the reaction, 3.0 μL of the mixture solution was mixed with 3.0 μL of Au/SiO_2, and then were deposited into a silicon substrate with dimensions of 0.5×0.5 cm^2. The substrate was treated in Piranha solution ($98\%H_2SO_4/30\%H_2O_2=3:1$, v/v; *CAUTION: piranha solution should be handled with great care*) to clean the organic compounds and provide a hydroxylated surface. After being rinsed thoroughly with ultrapure water and dried by N_2 flow gas. A portable Raman spectrometer equipped with wavelength of 785 nm from an NIR diode laser and a power of 60 mW was used for normal Raman spectrometry (NRS) and SHINERS. The typical exposure time for each NRS/SHINERS measurement in this study was 1 s with five accumulations unless specified. NRS/SHINERS were measured at environmental temperature with a portable Raman spectrometer.

2.4. Samples

The tap and lake water were collected from our library and a pond in our campus. Bottled purified water and mineral water were purchased from the local supermarket. Overnight plain boiled water was provided by our laboratory. These samples were analyzed immediately after sampling without any pretreatment process. Saliva was collected from a healthy volunteer and filtered through a 0.45 μm membrane prior to use.

A soil sample was collected from a petrol station. After taken, it was air-dried to constant weight at room temperature and sieved

to particle sizes of about 0.45 mm. Soil (5.0 g) was extracted with 50 mL of potassium chloride (2.0 mol L^{-1}) for 30 min with ultrasonic assistant and then centrifuged at 4000 rpm for 5 min. The extract was filtered through a 0.45 μm membrane prior to use. For the analytical performance assessment, a 100.0 μL standard solution of 100.0 mg L^{-1} was added to 1.0 g of soil to give a spiked level of 10.0 μg g^{-1}.

Pork sausage was bought from the local supermarket and crushed into mash. The pork sausage sample (5.0 g) was weighed. Then 15–20 mL ultrapure water was added and the mixture was homogenized for 15 min in a lab blender. To deproteinize the suspension, 1 mL ferrocyanide solution (106.0 g L^{-1}) and zinc acetate solution (220.0 g L^{-1}) were added respectively. Then the sample solution was diluted directly to 25 mL and shaken for 2 min. The aqueous phase containing nitrite ions was filtered through a 0.45 μm membrane and then analyzed in the same way as the aqueous samples. For the analytical performance assessment, a 100.0 μL standard solution of 100.0 mg L^{-1} was added to 1.0 g of pork sausage to give a spiked level of 10.0 μg g^{-1}.

3. Results and discussion

3.1. Principle of the chemical transformation of nitrite to azo dye with high SHINERS activity

The NRS of nitrite is weak and the affinity of nitrite to Au/SiO_2 is also rather weak, resulting in low SHINERS activity. As shown in Fig. 1, no NRS (Fig. 1d) and SHINERS (Fig. 1c) signals of the nitrite were detectable, even at high concentration (1000.0 mg L^{-1}). To solve the above mentioned problem, we proposed a method for detection of nitrite ion based on the diazotization of p-nitroaniline by nitrite in acid media, with the subsequent coupling reaction
to provide a colored azo dye (Fig. 2), which has strong SHINERS (Fig. 1a). Therefore, the concentration of nitrite ion can be determined indirectly from azo dye.

The band was observed at ~1432 cm^{-1} and assigned to the –N═N– stretching vibration [28] of the trans isomer in all samples, indicating the existence of a trans isomer of –N═N– in the azo dye. In addition, the SHINERS spectra of the azo dye was different to the corresponding NRS, indicating there may be

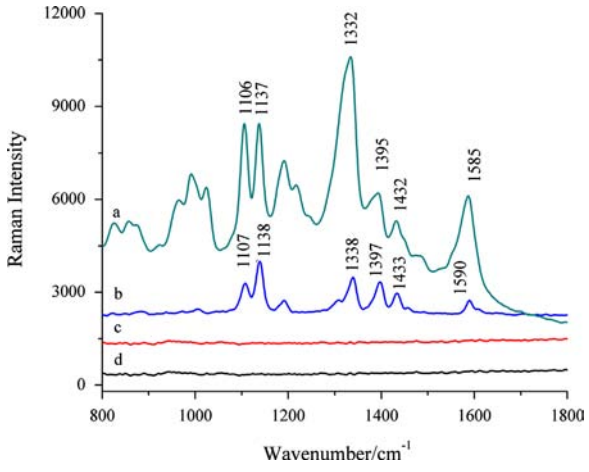

Fig. 1. NRS and SHINERS of nitrite solution and the azo dye derived from the Nitroaniline and diphenylamine. (a) SHINERS of the azo dyes derived from the nitrite (1.0 mg L^{-1}); (b) NRS of the azo dyes derived from the nitrite (1000.0 mg L^{-1}); (c) SHINERS of nitrite solution (1000.0 mg L^{-1}); and (d) NRS of nitrite solution (1000.0 mg L^{-1}); reaction time: 10 min; the concentration of Au/SiO_2 is 1.47×10^{-2} mol L^{-1}.

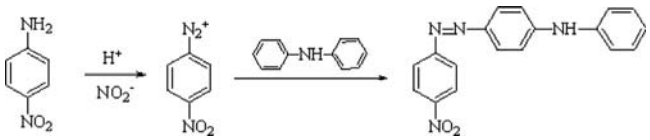

Fig. 2. Scheme demonstrating the composite diazotization-coupling reaction of p-nitroaniline and diphenylamine in acid media.

Table 1
Tentative of assignments of SHINERS Bands of the Azo Dyes[a].

Raman shift (cm^{-1})	Assignments
1106	ν(C–NO_2) with p-nitroaniline
1137	ν(C–N═N) with phenyl rings
1332	ν(C–NO_2) with p-nitroaniline
1395	ν(C–C) within phenyl rings coupling to N═N stretch
1432	ν(N═N) of trans isomers
1585	ν(C–C) within p-nitroaniline and/or phenyl rings

[a] δ denotes in-plane bending; ν, stretching.

chemical adsorption of the azo dye on the Au/SiO_2 surface. Detailed band assignments were listed in Table 1 [29–34].

3.2. Comparison of Au/SiO_2 with Au

One of the most widely used substrates of SERS is Au nanoparticles, which are responsible for high enhancement in numerous applications [35,36]. Compared with Au nanoparticles, Au/SiO_2 nanoparticles have higher sensitivity, better stability and reproducibility. The experimental result shows that Au/SiO_2 nanoparticles are stable for at least 60 d at room temperature. In contrast, obvious agglomeration and adherent phenomenon were observed for Au nanoparticles in the storage or concentration progress (Fig. 3A). The peak area of SHINERS with pinhole is higher than SERS (Fig. 3B) at the same experimental condition because the chemically inert shell protects the SERS-active nanostructure from contacting with whatever is being probed and the pinhole make SERS-active nanostructure contact selectively with target analytes. Compared with SERS of the azo dye derived from 3.0 mg L^{-1} NO_2^- with Au nanoparticles, the peak area of SHINERS with Au/SiO_2 nanoparticles increased 1.4–2.2 times at different Raman shift. Replicate measurements on different area of each sample were made to verify the reproducibility. The RSDs (n=5) for determination of nitrite with Au and Au/SiO_2 nanoparticles as active substrates are 3.6–8.9% and 3.0–7.8% respectively. On the base of above results, it can be concluded that Au/SiO_2 nanoparticles were superior to Au nanoparticles as the active substrate to determine the nitrite in real samples.

3.3. Optimization of reaction and SHINERS intensity conditions

To obtain strong SHINERS, the optimization of reaction and SHINERS enhancement conditions, such as acidity, reaction time, temperature, the concentration of reaction reagents, the concentration of Au/SiO_2 and the effect of NaCl were studied.

3.3.1. Optimization of reaction conditions

The diazotization reaction of p-nitroaniline and nitrite requires a strong acidic condition, however, the coupling reaction of diphenylamine and diazonium salt takes place in the weak acidic or alkaline condition. Sulfuric acid, nitric acid, hydrochloric acid, phosphoric acid and acetic acid were tested, and hydrochloric acid was found to the best one. The effect of the concentration of hydrochloric acid in p-nitroaniline was also studied (Fig. S1).

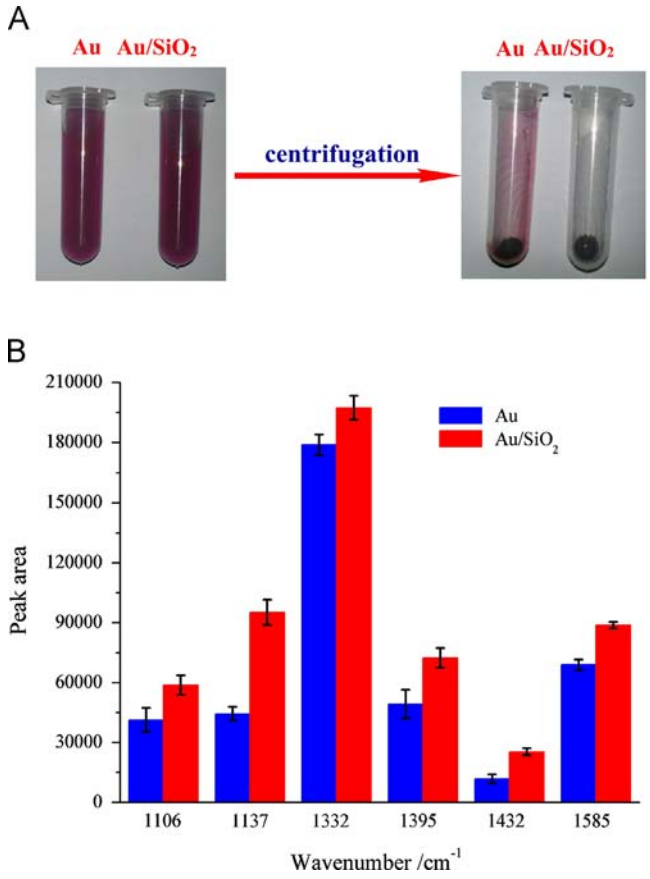

Fig. 3. (A) The photograph of Au and Au/SiO_2 before and after concentration (B) the peak area of SERS and SHINERS of the azo dye deriving from 3.0 mg L^{-1} NO_2^- using Au and Au/SiO_2 as active substrate at different Raman shift; reaction time: 10 min; the concentration of Au and Au/SiO_2 is 1.47×10^{-2} mol L^{-1}.

The SHINERS intensity increased dramatically by increasing hydrochloric acid up to 1.0 mol L^{-1}, and then decreased slowly by keeping on increasing the concentration of hydrochloric acid in p-nitroaniline. It is because that diazotization-coupling reaction requires an acidic condition. On the other hand, the acidic solution would cause the aggregation of Au/SiO_2 sol, which could provide better and higher SHINERS intensity. However, further increase of acid concentration, would result in excessive aggregation of Au/SiO_2 and partial inactivation, and then the intensity of SHINERS decrease. To acquire strong SHINERS intensity, 1.0 mol L^{-1} of hydrochloric acid in p-nitroaniline was used in the subsequent experiment.

Reaction time plays an important role in diazotization-coupling reaction. The effect of reaction time in the range from 3 to 30 min was investigated (Fig. S2). A distinct increased SHINERS intensity was observed up to a reaction time of 10 min and a slight increase occurred with the reaction time above this value. Based on the experiment results, a reaction time of 10 min was used in the following experiment. The effect of temperature on the proposed procedure was studied in the range of 10–40 °C. The SHINERS intensity had no significant change with the increase of temperature. Therefore, the following experiment was carried out at room temperature.

The concentrations of p-nitroaniline and diphenylamine have impact on the formation of azo dye, subsequently influence the SHINERS intensity. Changing the concentration of p-nitroaniline in the range of 0.03–1.5 wt% had no obvious effect on the SHINERS intensity (Fig. S3). Changing the concentration of diphenylamine in the range of 0.04–1.6 wt%, the results showed that the SHINERS intensity increased dramatically by increasing the concentration of diphenylamine up to 0.4 wt% and then it increased slightly at higher concentration (Fig. S4). Therefore, 0.3 wt% p-nitroaniline and 0.4 wt% diphenylamine respectively were applied in the proposed method.

3.3.2. Optimization of SHINERS intensity

3.3.2.1. Effect of the concentration of Au/SiO_2 on SHINERS intensity. The concentrations of Au/SiO_2 from 2.94×10^{-4} to 529×10^{-4} mol L^{-1} were investigated in the SHINERS procedure. The SHINERS intensity increased by increasing the concentration up to 1.47×10^{-2} mol L^{-1} and then decreased at higher concentration (Fig. S5). However, Au/SiO_2 sol aggregated severely with further increase of the concentration of Au/SiO_2 resulting in the decrease of the SHINERS intensity. The above result can be further evidenced by the UV–vis spectroscopy characterization (Fig. S6). The spectrum exhibits a broad absorption peak of Au/SiO_2 at 540 nm. As shown in Fig. S6(B), a new absorbance appeared when Au/SiO_2 mixed with azo dye derived from nitrite, and the new absorbance center was shifted to the red with the increase of the concentration of Au/SiO_2. These results indicated that azo dye induced obviously the aggregation of Au/SiO_2, and the degree of aggregation was increased with increase of the concentration of Au/SiO_2. When the concentration of Au/SiO_2 was 1.47×10^{-2} mol L^{-1}, this may give rise to a large increase electromagnetic enhancement and also could induce so-called specific active sites. To acquire strong SHINERS, 1.47×10^{-2} mol L^{-1} of Au/SiO_2 was used in the subsequent experiment.

3.3.2.2. Effect of NaCl on SHINERS intensity. It has been reported that anions play an important role in SERS intensity [37]. The addition of $C1^-$ ions to metal sol resulted in the particles aggregation, which could result in an additional enhancement factor. Furthermore, the presence of Cl^- could help quench fluorescence if the fluorescence quantum yield is larger [38]. To study the effect of NaCl on the SHINERS intensity, NaCl with a final concentration of 0–1.0 mol L^{-1} was added to Au/SiO_2 sol. However, NaCl in the Au/SiO_2 colloid had no significant impact on SHINERS intensity in our work (Fig. S7). The reasons for this phenomenon may be explained as follows. In general, the addition of NaCl may result in Au/SiO_2 particles aggregation, which could provide better and thus higher SHINERS intensity. However, the acidic azo dye solution can result in Au/SiO_2 sol aggregation in our work, so the addition of NaCl have no significant effect in SHINERS intensity. Therefore, no NaCl was used.

3.4. Selectivity

In order to evaluate the selectivity of the proposed method, a variety of cations and anions were investigated by adding known quantities of each ion to a solution containing 2.0 mg L^{-1} of nitrite ion. The results (Table 2) indicated that the investigated anions and cations did not interfere the determination of nitrite by the proposed method,since a variation less than $\pm$15% in the area of 1395 cm^{-1} Raman shift was found. This reveals a higher selectivity for nitrite ion. Such remarkable selectivity should be attributed to

Table 2
Tolerance ratio of various ions to the determination of 2.0 mg L^{-1} nitrite ion.

Ion	Tolerance ratio (ion/nitrite ion)
K^+, NO_3^-, Na^+, Cl^-,	5000
NH_4^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Ba^{2+}	2000
Al^{3+}, Br^-	1000
F^-, CO_3^{2-}, Ni^{2+}, Co^{2+}, CH_3COO^-, Zn^{2+}, Cu^{2+}, PO_4^{3-}	50

both the specificity of the diazotization-coupling reaction and the Au/SiO_2. Firstly, as shown in Fig. 2, the nitrite is transformed selectively to azo dye by diazotization-coupling reaction in acid media. Secondly, the ultrathin coating of Au/SiO_2 protects the SERS-active nanostructure from contacting with whatever is being probed, which improves further the selectivity of this method.

3.5. Evaluation of enhancement factors

The enhancement factors (EFs) were calculated according to the following Eq. (1) [39]:

$$EF = \frac{I_{SHINERS} N_{Raman}}{I_{Raman} N_{SHINERS}} = \frac{I_{SHINERS} C_{Raman}}{I_{Raman} C_{SHINERS}} \quad (1)$$

where $I_{SHINERS}$ and I_{Raman} are the intensity of the same Raman band under SHINERS and NRS conditions, respectively. Both of these data can be directly obtained from the experiment. N_{Raman} and $N_{SHINERS}$ are the number of analyte molecules illuminated by the laser focus spot under NRS and SHINERS conditions respectively. $C_{SHINERS}$ and C_{Raman} are the concentration of nitrite under SHINERS and NRS conditions. In our experiments, supposing that the molecules are dispersed uniformly on the silicon substrate, under the same laser spot and penetration depth, $C_{Raman}/C_{SHINERS}$ is equal to $N_{Raman}/N_{SHINERS}$. During experiments, the Raman measurement settings including the laser power and integration time were kept constant. The Raman peak at 1106/1107, 1137/1138, 1395/1397 and 1432/1433 cm^{-1} (Fig. 1a and b) were selected for determination of the EFs of SHINERS. According to Eq. (1), the EFs were calculated to be 10,000, 4400, 3000 and 4600, respectively.

3.6. Analytical features of the proposed method

Under the optimized experimental conditions, some analytical parameters (i.e., linear equation, linear range and limits of detection) were investigated. As shown in Fig. 4, the linearity was observed in the range of 0.5–6.0 mg L^{-1} for 1137, 1395 and 1432 cm^{-1} with the correlation coefficient (r^2) ranging from 0.9793 to 0.9916. The relative standard deviations (RSDs) were lower than 14.5%. The limit of detection (LOD) is defined as the concentration of the analyte that is required to produce response of three times as large as the standard deviation of the noise level [40]. LODs of the proposed method were 0.07, 0.08 and 0.10 mg L^{-1} at 1137, 1395 and 1432 cm^{-1}, respectively, lower than the MCLs of nitrite (1.0 mg L^{-1}) [5], which was recommended by the U.S. EPA. So, it can be used for rapid monitoring of drinking water quality.

3.7. Determination of nitrite ion in real samples

The proposed method was applied to determine nitrite ion in food, biological and environmental samples. Drinking water including tap water, overnight plain boiled water, bottled purified water and mineral water, lake water, soil, saliva and pork sausage were spiked at 1.0 mg L^{-1} (10.0 μg g^{-1}). As shown in Fig. 5, the soil extract had a low background, which allowed for clear azo dye SHINERS. The recoveries were evaluated by adding known amounts of standard nitrite solution to the samples. For the soil and pork sausage samples, nitrite with the concentration of 10.0 μg g^{-1} was spiked. The recoveries varied from 88.3 to 125.2%, showing the excellent performance of method for the examined samples (Table 3). These results indicated high selectivity of SHINERS for these azo dyes, even in complicated mixtures, and the feasibility of the approach to practical applications. Saliva can be directly determined after filtering without complicated sample pre-treatment. So this simple, rapid and selective method can be used to determine the concentration of the nitrite in saliva, which may have relationships with some diseases.

To evaluate the proposed SHINERS method, the samples were also analyzed with the conventional Griess method (UV–vis spectroscopy) [41]. The results of saliva in Table 3 indicated that there was no significant difference (paired t-test, 95% confidence level, $t_{experimental} = 1.89$, $t_{critical} = 2.78$) between the data obtained from both methods when the determination of nitrite ion in the Saliva. The result agreed well with the conventional Griess method, suggesting the

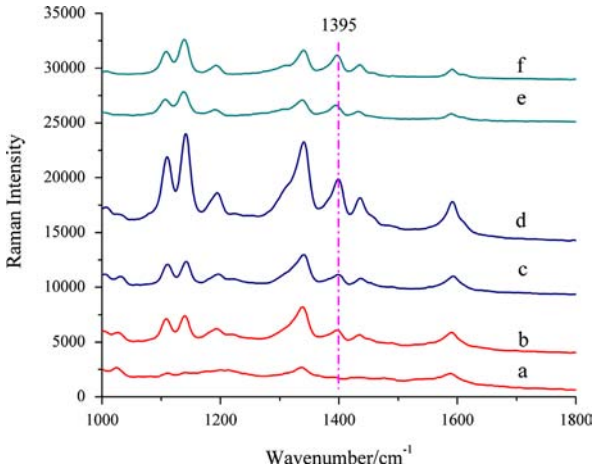

Fig. 5. SHINERS of azo dyes from the extracts of soil (a and b): spiked 0 and 10.0 μg g^{-1}; pork sausage (c and d): sipked 0 and 10.0 μg g^{-1}; and saliva (diluted as 1:1) (e and f): spiked 0 and 1.0 mg L^{-1}.

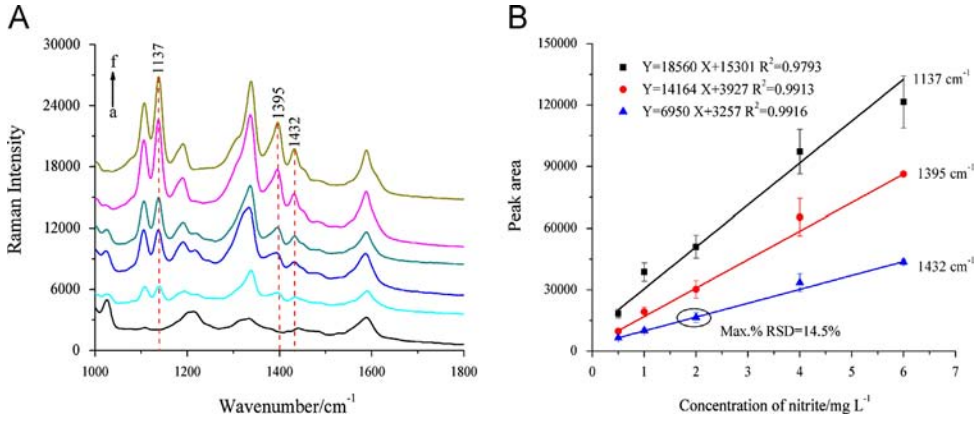

Fig. 4. (A) SHINERS of azo dye derived from the Nitroaniline and diphenylamine with different concentration of nitrite; (a–f): 0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg L^{-1}; (B) Linear plot of the 1137, 1395 and 1432 cm^{-1} area; Error bars represent the standard deviations within five replicated experiments.

Table 3
Determination of nitrite ion in the real samples by the proposed method[a] and Griess method.

Sample	This work			Griess method[b] (mg L^{-1} or μg g^{-1})
	Added (mg L^{-1} or μg g^{-1})	Find[b] (mg L^{-1} or μg g^{-1})	Recovery (%)	
Saliva[c]	0.0	1.9 ± 0.2	–	2.2 ± 0.1
	1.0	2.7 ± 0.2	88.9	
Tap water	0.0	–	–	–
	1.0	1.1 ± 0.1	111.2	
Lake water	0.0	–	–	–
	1.0	1.1 ± 0.1	109.2	
Soil	0.0	–	–	–
	10.0	12.3 ± 1.0	123.4	
Overnight plain boiled water	0.0	–	–	–
	1.0	1.3 ± 0.2	125.2	
Bottled purified water	0.0	–	–	–
	1.0	0.9 ± 0.1	88.3	
Mineral water	0.0	–	–	–
	1.0	1.0 ± 0.1	104.4	
Pork sausage	0.0	7.3 ± 0.8	–	8.1 ± 0.8
	10.0	17.3 ± 2.0	90.7	

[a] Quantitative based on 1395 cm^{-1}.
[b] Average of five determinations ± standard deviation.
[c] Diluted as 1:1 before analysis.

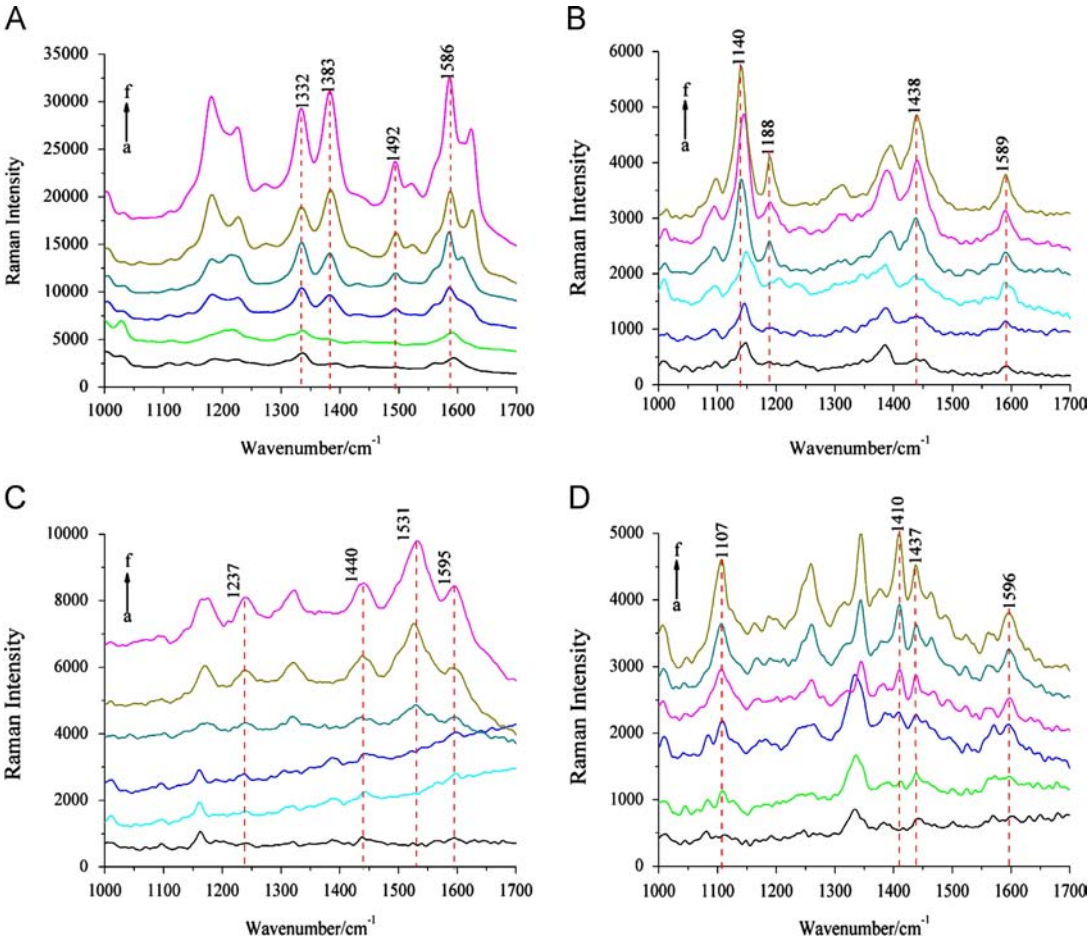

Fig. 6. SHINERS of azo dye derivd from IO_3^- (A), (a–f): 0, 0.01, 0.05, 0.1, 0.5 and 1.0 mmol L^{-1}; phenol (B), (a–f): 0, 0.0005, 0.005, 0.05, 0.1 and 0.2 mmol L^{-1}; p-phenylenediamine (C), (a–f): 0, 0.1, 0.4, 0.8, 2.0 and 3.0 mmol L^{-1}; o-phenylenediamine (D), (a–f): 0, 0.04, 0.08, 0.1, 0.2 and 0.5 mmol L^{-1}; Diazotization-coupling reaction: (A) the volume ratio of IO_3^-, $NH_2OH \cdot HCl$, A_1 and B is 200:2:20:20, (B) the volume ration of NO_2^-, A_2, phenol and Na_2CO_3 is 200:2:2:2, (C) the volume ration of NO_2^-, A_2 and p-phenylenediamine is 200:10:2, (D) the volume ration of NO_2^-, A_1 and o-phenylenediamine is 200:5:2; reaction time: 10 min; the concentration of Au/SiO_2 is 1.47×10^{-2} mol L^{-1}; the volume ratio of azo dye solution and Au/SiO_2 colloid is 1:1; the exposure time are 1 s for (A), (B) and (D), 2 s for (C).

potential application of this simple, rapid and selective method in complex sample.

3.8. Universality

In this proposed method, nitrite was transformed into an azo dye by a diazotization-coupling reaction. Then the concentration was determined indirectly from azo dye. Iodate ion can oxidize hydroxylamine to produce nitrite according to the well-known reaction ($2IO_3^- + 3NH_2OH \rightarrow 3NO_2^- + 3H^+ + 2I^- + 3H_2O$), then the nitrite can produce strongly colored azo dye by the diazotization-coupling reaction (Fig. 6A). Therefore, the concentration of iodate ion was also indirectly determined from azo dye by this proposed method.

In the diazotization-coupling reaction, diazonium ion was firstly produced by the reaction of nitrite with *p*-nitroaniline in acid media. Then azo dye was produced through the coupling reaction between diphenylamine and diazonium ions. The coupling reaction between phenolic or aromatic amine molecules and diazonium ions are universal. To confirm the universality of the approach for versatile phenolic or aromatic amine molecules detection, phenol, *o*-phenylenediamine and *p*-phenylenediamine were investigated. As shown in Fig. 6B–D, SHINERS spectra of the coupling products between diazonium ions based on the reaction of nitrite with A_1 (A_2) in acid media and different coupling reagents were significantly different and easily discerned. Therefore, this proposed method is also useful for their identification and quantification.

4. Conclusions

The experimental results demonstrated clearly that an analytical method based on diazotization-coupling reaction using SHINERS provided high spectral selectivity and identification power for the determination of low concentration of nitrite in complex samples. Before SHINERS measurement, nitrite was changed to azo dye by a diazotization-coupling reaction, which significantly enhanced the SHINERS activity of the analytes. The proposed method is simple, rapid, less sample consumption ($\sim$3 μL) and highly selective. The analytical procedure was very simple by mixing the nitrite, Pauly's reagent, and Au/SiO_2 to create strong Raman response. This method was accomplished within 10 min. The promising technique with its advantages of simplicity, rapidness, universality, high sensitivity and selectivity, has great potential in application of nitrite detection in environmental safety control and exploring the relationship between the nitrite content in saliva and the certain diseases or the diagnosis of the disease. In future work, we will apply this proposed method to determinate iodate ion, phenols and aromatic amines.

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Appendix A. Supplementry material.

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References

[1] M. Fleischmann, P.J. Hendra, A.J. McQuillan, Chem. Phys. Lett. 26 (1974) 163–166.
[2] D.L. Jeanmaire, R.P.V. Duyne, J. Electroanal. Chem. 84 (1977) 1–20.
[3] S.M. Nie, S.R. Emory, Science 275 (1997) 1102–1106.
[4] C.L. Haynes, A.D. McFarland, R.P.V. Duyne, Anal. Chem. 77 (2005) 338–346.
[5] K. Kneipp, A.S. Haka, H. Kneipp, K. Badizadean, N. Yoshizzwa, C. Boone K.E. Shaffe-peltier, J.T. Motz, R.R. Dasari, M.S. Feld, Appl. Spectrosc. 56 (2002) 150–154.
[6] C.M. Ruan, W. Wang, B.H. Gu, Anal. Chem. 78 (2006) 3379–3384.
[7] H.W. Cheng, Y.Y. Chen, X.X. Lin, S.Y. Huan, H.L. Wu, G.L. Shen, R.Q. Yu, Anal. Chim. Acta 707 (2011) 155–163.
[8] C.M Ruan, W. Wang, B.H. Gu, Anal. Chim. Acta 567 (2006) 114–120.
[9] M.L. Cheng, B.C. Tsai, J. Yang, Anal. Chim. Acta 708 (2011) 89–96.
[10] L.C. Mecker, K.M. Tyner, J.F. Kauffman, S. Arzhantsev, D.J. Mans C.M. Gryniewicz-Ruzicka, Anal. Chim. Acta 733 (2012) 48–55.
[11] J.F. Li, Y.F. Huang, Y. Ding, Z.L. Yang, S.B. Li, X.S. Zhou, F.R. Fan, W. Zhang Z.Y. Zhou, D.Y. Wu, B. Ren, Z.L. Wang, Z.Q. Tian, Nature 464 (2010) 392–395.
[12] J.F. Li, S.Y. Ding, Z.L. Yang, M.L. Bai, J.R. Anema, X. Wang, A. Wang, D.Y. Wu, B. Ren, S.M. Hou, T. Wandlowski, Z.Q. Tian, J. Am. Chem. Soc. 133 (2011) 15922–15925.
[13] D. Graham, Angew Chem. Int. Ed. 49 (2010) 9325–9327.
[14] D. Ralt, Medical Hypotheses 73 (2009) 794–796.
[15] J.C. Chung, S.S. Chou, D.F. Hwang, Food Addit. Contam. 21 (2004) 317–322.
[16] T.R. Paixao, J.L. Cardoso, M. Bertotti, Talanta 71 (2007) 186–191.
[17] J.D. Brender, J.M. Olive, M. Felkner, L. Suarez, W. Marckwardt, K.A. Hendricks, Epidemiology 15 (2004) 330–336.
[18] US EPA, Drinking Water Standards and Health Advisories, EPA 822-R-04-005, U.S. Environmental Protection Agency, Washington, DC, 2004.
[19] M.N. Abbas, G.A. Mostafa, Anal. Chim. Acta 410 (2000) 185–192.
[20] C.E. Lopez Pasquali, P. Fernandez Hernando, J.S.Durand Alegria, Anal. Chim. Acta 600 (2007) 177–182.
[21] L.J. He, K.G. Zhang, C.J. Wang, X.L. Luo, S.S. Zhang, J. Chromatogr. A 1218 (2011) 3595–3600.
[22] M. Akyuz, S. Ata, Talanta 79 (2009) 900–904.
[23] W.L. Daniel, M.S. Han, J.S. Lee, C.A. Mirkin, J. Am. Chem. Soc. 123 (2009) 6362–6363.
[24] K. Kim, K.L. Kim, K.S. Shin, Analyst 137 (2012) 3836–3840.
[25] A. Lanoul, T. Coleman, S.A. Asher, Anal. Chem. 74 (2002) 1458–1461.
[26] K. Ito, Y. Takayama, N. Makabe, R. Mitsui, T. Hirokawa, J. Chromatogr. A 1083 (2005) 63–67.
[27] E. Morcos, N.P. Wiklund, Electrophoresis 22 (2001) 2763–2768.
[28] N. Xiao, C.X. Yu, Anal. Chem. 82 (2010) 3659–3663.
[29] N. Biswas, S. Umapathy, J. Phys. Chem. A 101 (1997) 5555–5566.
[30] C.M. Stuart, R.R. Frontiera, R.A. Mathies, J. Phys. Chem. A 111 (2007) 12072–12080.
[31] Y.Q. Wu, B. Zhao, W.Q. Xu, B.F. Li, Y.M. Jung, Y. Ozaki, Langmuir 15 (1999) 4625–4629.
[32] L.S. Jiao, L. Niu, J. Shen, T.Y. You, S.J. Dong, A. Ivaska, Electrochem. Commun. 7 (2005) 219–222.
[33] X.X. Han, P. Pienpinijtham, B. Zhao, Y. Ozaki, Anal.Chem. 83 (2011) 8582–8588.
[34] X. Kang, S.K. Sharma, G.T. Taylor, D.W. Muenow, Appl. Spectrosc. 46 (1992) 819–826.
[35] X.X. Han, Y. Kitahama, T. Itoh, C.X. Wang, B. Zhao, Y. Ozaki, Anal. Chem. 81 (2009) 3350–3355.
[36] X.X. Han, L. Chen, J. Guo, B. Zhao, Y. Ozaki, Anal. Chem. 82 (2010) 4102–4106.
[37] P. Hildebrandt, M. Stockburger, J. Phys. Chem. 88 (1984) 5935–5944.
[38] D.A. Weitz, S. Gareff, J.L. Gersten, A. Nitzan, J. Chem. Phys. 78 (1983) 5324–5338.
[39] M.L. Zhang, X. Fan, H.W. Zhou, M.W. Shao, J.A. Zapien, N.B. Wong, S.T. Lee, J. Phys. Chem. C 114 (2010) 1969–1975.
[40] D. Macdougall, B. Warren, Anal. Chem. 52 (1980) 2242–2249.
[41] Official Methods of Analysis, 16th ed., Gaithersburg: Association of Official analytical chemists, AOAC 1997.