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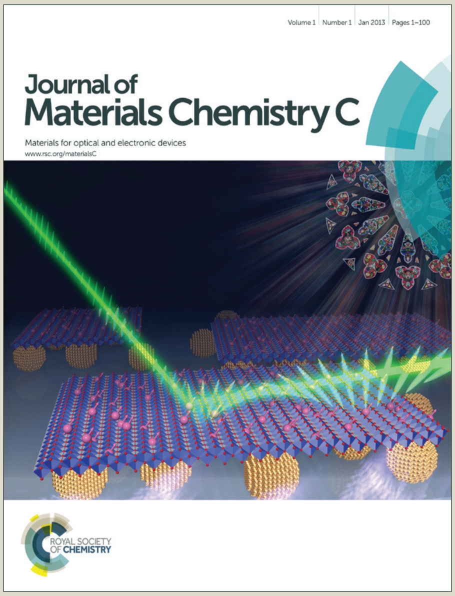

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Quantitative SERS-based detection using Ag-Fe_3O_4 nanocomposites with internal reference

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Abstract

Surface Enhanced Raman Spectroscopy (SERS) is an excellent tool, which provides information about the vibrational modes of molecules. However, quantitative analysis using SERS has remained a challenge, largely because of difficulty in reproducing the SERS substrates. We report a facile synthesis of Ag-Fe_3O_4 nanocomposites, which can be used as SERS substrates in solutions. Quantitative SERS measurements using the Ag-Fe_3O_4 nanocomposites are successfully demonstrated with 4-aminothiophenol and dopamine as analytes. The inclusion of an internal reference greatly improves the reliability and quality of quantification of the analytes. The scheme is versatile, and has the potential for the detection of a wide range of analytes.

Introduction

Surface-enhanced Raman spectroscopy (SERS) is undoubtedly one of the most useful physical chemistry techniques, which provides the information about the vibrational modes of the molecules. The fingerprint information associated with the molecular structure can be used to detect the presence of a variety of analytes. [1-5] The merits of this technique come from the fact that inelastic Raman scattering from the molecule is enhanced by orders of magnitude when the

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molecule is in the vicinity of nanostructured metal (mainly silver and gold) surface. Indeed, SERS has allowed researchers to detect analytes down to single molecular level. [6] However, applications of SERS in quantitative detection are far from well established, largely due to the difficulty in obtaining reproducible SERS substrates and hence the SERS signal intensities. [7-9]

There have been efforts to address the issue of poor quantification using SERS. Isotope edited internal standard (IEIS) method was reported for quantitative SERS measurement. [10-13] While it was an intriguing initiative to resolve the issue, there were some intrinsic drawbacks of the method. Firstly, isotope edited counterpart of the analytes to be detected need to be made available. Secondly, there has to be significant difference in SERS spectra between the analyte and its isotope edited counterpart. In that regard, the fact that most of the molecules tend to have very similar SERS features to those of their isotope edited counterparts greatly limits the choice of analytes. Another reported method to address the quantification issue of SERS was to use self assembled monolayer (SAM) on top of SERS substrates as an internal standard. Such arrangement help to prevent the direct chemisorption of analytes on the SERS substrate, resulting in better reproducibility of the SERS signals. Yet it would likely lead to a decrease in SERS enhancement, due to the spacing between the analyte and the SERS substrates. [14] There have been other studies taking advantage of some Raman probes, of which a few vibrational bands are sensitive to the presence of the analyte. Quantification was carried out by normalizing the SERS intensity of the analyte-sensitive bands versus the bands not sensitive to the analyte. [15-21] Such a strategy relies on the rather unique properties of the Raman probes toward the analytes, somewhat limiting its utility. Considering the challenges encountered in the quantitative SERS measurements, there is ongoing need to explore better alternatives.

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Here we report a quantitative SERS measurement scheme using internal reference based on the Ag-Fe_3O_4 nanocomposites, which are synthesized by a facile and robust method. Polyacrylic acid (PAA) coated Fe_3O_4 nanoparticles were first prepared and silver ions were reduced on the surface of PAA to form the Ag-Fe_3O_4 nanocomposites. PAA simultaneously served as the internal SERS reference. Quantitative measurements of two different analytes, 4-aminothiophenol and dopamine, are shown in this study to demonstrate the detection capability and versatility of this scheme.

Experimental

Materials

Ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous chloride ($FeCl_2 \cdot 6H_2O$), hydrochloric acid, polyacrylic acid (MW 1800), silver nitrate, sodium citrate, sodium borohydride, 4-aminothiophenol (ATP), and dopamine hydrochloride (DA) were obtained from Sigma-Aldrich. Ammonium hydroxide was ordered from Fisher scientific. All chemicals were used as received.

Synthesis of PAA-coated Fe_3O_4 nanoparticles

The synthesis of Fe_3O_4 nanoparticles is similar to what has been reported in the literature. [22, 23] In a typical run, iron salts solution (0.62 g $FeCl_3 \cdot 6H_2O$ and 0.32 g $FeCl_2 \cdot 6H_2O$ in 2 mL 0.6 M HCl) was added to NH_4OH solution (1.8 mL of 30% NH_4OH in 15 mL N_2-purged DI water). The resulting black solution was stirred for 30 s before PAA solution (820 mg of PAA in 5 mL DI water) was added. The resulting suspension was stirred for 2 hr. Nanoparticles were then collected through centrifugation at 4000 rpm for 30 min and washed twice with DI water. PAA-coated Fe_3O_4 nanoparticles were dispersed in water until later use.

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Synthesis of Ag-Fe_3O_4 nanocomposites

Fifty mg of Fe_3O_4 nanoparticles were dispersed in 5 mL of water. 0.8 mL of 0.1 M $AgNO_3$ was added and stirred for 30 minutes. Excess silver ions were removed from the nanoparticles through centrifugation (15000 rpm, 15 min). Nanoparticles were again dispersed in 5 mL DI water. 1 mL sodium citrate solution (0.02 g/mL) and 200 μL of sodium borohydride solution (0.005 g/mL) were subsequently added and stirred for 5 min. The solution turned yellowish black, indicating the formation of Ag-Fe_3O_4 nanocomposites. The nanocomposites were washed twice by water through centrifugation (15000 rpm, 15 min). They were redispersed in DI water in a vial. A magnet was placed on the side of the vial for 8 hr to attract the Ag-Fe_3O_4 nanocomposites before the supernatant was removed by pipette. The same procedures can be repeated multiple times to increase the Ag loading in the Ag-Fe_3O_4 nanocomposites.

TEM

TEM was used to determine the size and morphology of the Ag-Fe_3O_4 nanocomposites. A drop of nanocomposite dispersion was dried on a carbon-coated copper grid at room temperature. TEM images were taken on a Phillips Biotwin 12 transmission electron microscope.

UV-Vis spectra measurement

UV-Vis spectra were recorded by an Ocean Optics USB 4000 spectrophotometer using a 1-cm path-length quartz cell at room temperature.

Raman and FT-IR measurements

Ag-Fe_3O_4 nanocomposites were dispersed thoroughly in DI water. A small amount of the solution was transferred to a quartz capillary tube (I.D: ~1 mm). The tube was then placed on the

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stage of a Renishaw InVia microscope with a 10x objective (NA 0.25) for Raman measurement. Laser intensity at the sample was ~11 mW from the 785 nm line of a diode laser for all measurements. Samples at each concentration were measured 3 times. Each measurement was the average of 5 scans. Exposure time for each scan was 20 sec. FT-IR measurement was performed on an ATR cell (Nicolet 6700 FT-IR, Fisher Thermo Scientific) with sample solution drops dried on the aluminum foil.

Results and discussion

Synthesis of Fe_3O_4 nanoparticles. The synthesis of Fe_3O_4 nanoparticles using PAA as the capping agent has been reported in the literature. [22] The PAA coating on the Fe_3O_4 nanoparticles was supported by the zeta potential and FT-IR measurements. Negative zeta potential (-22.55 mV) is consistent with the carboxyl group of PAA being on the nanoparticle surface. Characteristic peak of the C=O stretching in FT-IR is found at ~1700 cm^{-1}. The peaks at ~1410, ~1260 and ~1150 cm^{-1} correspond to the C-O stretching coupled with O-H bending, and the one at ~1451 cm^{-1} to the CH_2 deformation, all indicating the presence of carboxyl groups, which can only come from PAA. [21] Further evidence of the presence of PAA on the Fe_3O_4 nanoparticle surface is provided from the Raman measurement, where moderate Raman signals of PAA are observed in the Ag-Fe_3O_4 nanocomposites (Figure S1 in the Supporting information).

Synthesis of Ag-Fe_3O_4 nanocomposites. The positively charged silver ions are electrostatically attracted to the negatively charged surface of the PAA-coated Fe_3O_4 nanoparticles. After removing excess free silver ions from the solution, the silver ions adsorbed on the nanoparticle surface are reduced by the mixture of sodium citrate and sodium borohydride, forming the Ag-Fe_3O_4 nanocomposites. This process can be repeated to increase the Ag portion in the Ag-Fe_3O_4

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nanocomposites (Figure S2 in the Supporting information). There is a small possibility that free Ag nanoparticles may be formed during the synthesis, which would potentially affect the SERS measurements. To this end, we used a magnet to separate the Ag-Fe_3O_4 nanocomposites from any possible free Ag nanoparticles at the end of the washing process (Figure S3 in the Supporting information), ensuring that all SERS substrates in the subsequent SERS measurements are Ag-Fe_3O_4 nanocomposites, not Ag nanoparticles. UV-vis spectra and TEM image of the Ag-Fe_3O_4 nanocomposites are shown in Figure 1. The peak at ~ 400 nm in the spectrum of Ag-Fe_3O_4 nanocomposites represents the surface plasmon of silver.

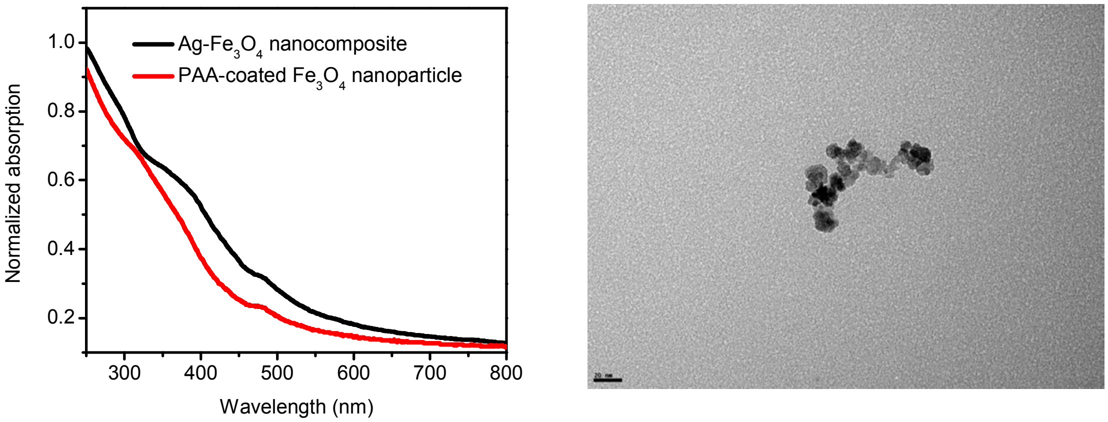

Figure 1. (Left) UV-vis spectra of Fe_3O_4 nanoparticles and Ag-Fe_3O_4 nanocomposites. (Right) TEM of Ag-Fe_3O_4 nanocomposites. Scale bar is 20 nm.

TEM of the Ag-Fe_3O_4 nanocomposites shows that the particles are fairly uniform in size of ~ 30 nm. Dynamic light scattering measurement of the Ag-Fe_3O_4 nanocomposites (Figure S4 in the Supporting information) suggests that slight aggregation of the Ag-Fe_3O_4 nanocomposites may exist. Still, notice that the Ag-Fe_3O_4 nanocomposites are stable for hours after sonication (Figure S5 in the Supporting information), allowing to perform reproducible SERS measurements, which usually take minutes. The detection scheme described herein would work as long as no severe aggregation occurs. In case there is slight precipitation of the Ag-Fe_3O_4 nanocomposites after longer period of storage, they can be redispersed easily under sonication.

Quantitative SERS measurements of ATP. The underlining concept of the detection scheme is that, Ag in the Ag-Fe_3O_4 nanocomposites serves as the substrate to enhance the Raman signals of analytes in the vicinity. Meanwhile, PAA molecules in the Ag-Fe_3O_4 nanocomposites also display moderate SERS signals, which can be used as internal reference to normalize the signals of analytes. The measured SERS signals come from the nanocomposites diffusing into and out of the focused spot under the objective, and thus represent the average intensity for a given solution.

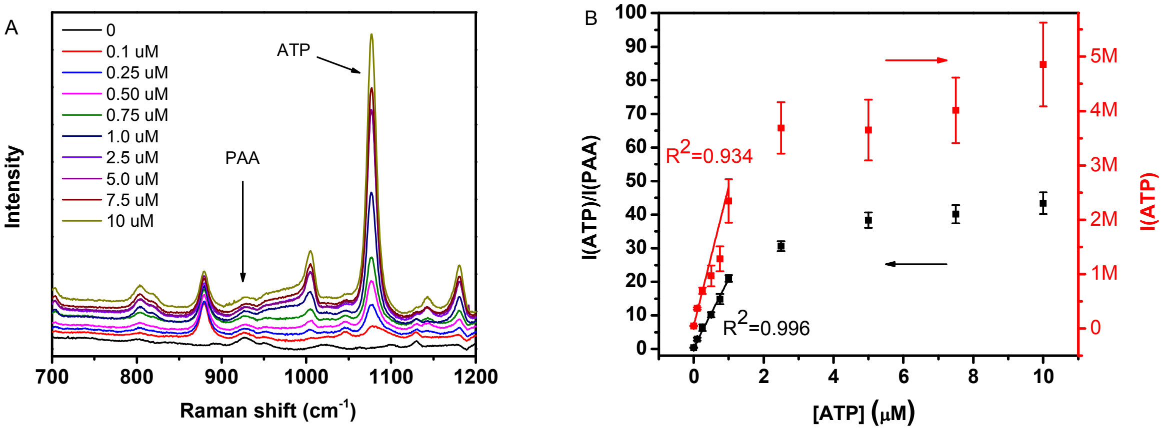

Figure 2. (A) SERS spectra of solutions containing same amount of Ag-Fe_3O_4 nanocomposites and different concentrations of ATP. Baselines are offset for clarity. (B) Plots of I_{ATP} vs. [ATP] and I_{ATP}/I_{PAA} vs. [ATP].

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We first demonstrate the quantitative SERS measurement of ATP using the Ag-Fe_3O_4 nanocomposites. Because of the strong affinity of its thiol group toward the silver surface, ATP would get adsorbed to the silver surface of the Ag-Fe_3O_4 nanocomposites. Results of the SERS measurement on solutions containing different concentrations of ATP and same amount of Ag-Fe_3O_4 nanocomposites are shown in Figure 2A. The peak at 925 cm^{-1} is assigned to PAA [24] and that at 1077 cm^{-1} to ATP. It is observed that the SERS intensity of the 1077 cm^{-1} peak generally increases with the increasing ATP concentration, while that of 925 cm^{-1} only fluctuates slightly among the samples. The advantage of using PAA as the internal reference is illustrated in Figure 2B, where plots of I_{ATP} vs. [ATP] and I_{ATP}/I_{PAA} vs. [ATP] are both included and peak areas are used as intensity. In the region where quantification is possible ([ATP] < 1 μM), I_{ATP}/I_{PAA} vs. [ATP] plot displays a much better linear fit (higher R^2-value), and 0.1 μM of ATP can be readily detected.

It is interesting to note that, at higher concentrations of ATP, partial conversion of ATP into dimercaptoazobenzene may have taken place, as indicated by the appearance of the peak at ~1139 cm^{-1}. This observation is in line with those in some recent reports. [25,26]

Quantitative SERS measurements of dopamine (DA). DA is a well-known neurotransmitter, which plays an important role in the regulation and modulation of brain activities. For instance, Parkinson's disease and Alzheimer's disease are the pathological states associated with the disturbed level of DA. Clinically, it is important to know the concentration level of DA both for treatment of such diseases and for monitoring of the normal functioning of the brain. Thus there is ongoing need to develop rapid and reliable methods to quantitatively detect the DA level in solutions. [27] Here we applied the SERS detection scheme to measure the DA concentration.

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Results of the SERS measurement on solutions containing different concentrations of DA and same amount of the Ag-Fe_3O_4 nanocomposites are shown in Figure 3A. DA in the solution would partition to the nanocomposite surface and display its fingerprint SERS peaks. The peak at 1475 cm^{-1} (C-H bond in aliphatic chain) can be assigned to DA, [27] and is observed to increase with the increasing DA concentrations. The 1130 cm^{-1} peak of PAA is used as the internal reference for quantification. Plots of I_{DA} vs. [DA] and I_{DA}/I_{PAA} vs. [DA] are shown in Figure 3B.

Again, in the region where quantification is possible ([DA] < 0.5 mM), I_{DA}/I_{PAA} vs. [DA] plot displays a much better linear fit, with a detection limit of 10 μM (3σ above blank).

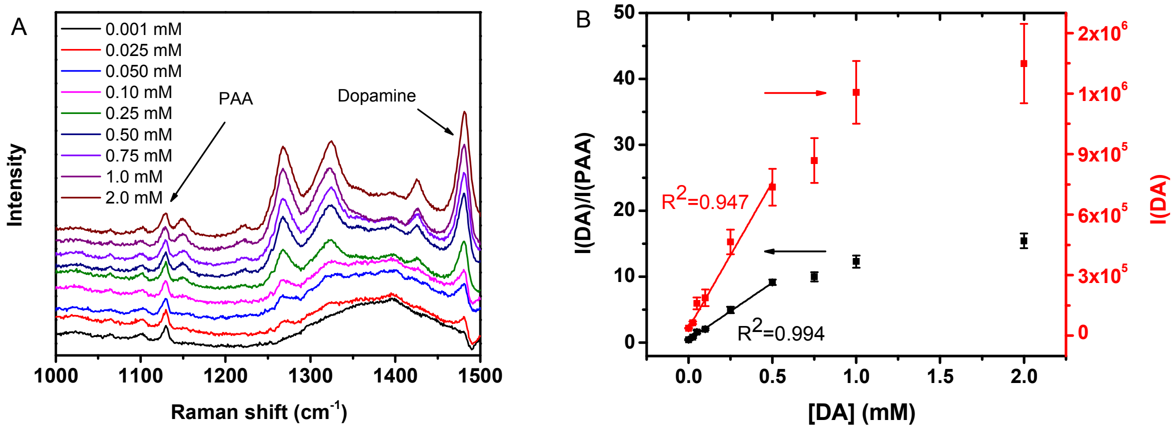

Figure 3. (A) SERS spectra of solutions containing same amount of Ag-Fe_3O_4 nanocomposites and different concentrations of DA. Baselines are offset for clarity. (B) Plots of I_{DA} vs. [DA] and I_{DA}/I_{PAA} vs. [DA].

Note that in both cases the SERS signals of the analytes approach saturation at higher concentrations of analytes, which is reasonable considering that the adsorption of analytes onto the Ag-Fe_3O_4 nanocomposites follows the Langmuir model. The dynamic range of detection is directly related to the amount of Ag-Fe_3O_4 nanocomposites used in the mixture, and can be improved by using more Ag-Fe_3O_4 nanocomposites. The sensitivity of the detection is dependent on the amount of Ag-Fe_3O_4 nanocomposites used, and the interaction between Ag-Fe_3O_4 nanocomposites and the analyte. The stronger the affinity of the analyte (such as ATP) towards the Ag-Fe_3O_4 nanocomposites, the higher the sensitivity of detection. In that regard, one would expect that the detection limit for DA can be improved by functionalizing the surface of Ag-Fe_3O_4 nanocomposites to attract DA more efficiently. [27] Notice that the SERS measurements are done in solution of well dispersed nanoparticles, and should be expected to be more reproducible and representative of the sample than the measurements done on solid substrates.

We shall point out that PAA plays multiple roles in this SERS-based detection scheme. It serves as the capping agent to stabilize Fe_3O_4 nanoparticles, and simultaneously attract silver ions, which subsequently are reduced into Ag to act as the SERS substrates. PAA also serves as the internal reference for quantitative SERS measurements. It is possible that other polymers or molecules than PAA can be used in the same manner. We expect that the scheme can be adapted for the detection of other analytes. Furthermore, there exists a large body of gold- and/or silver-coated core-shell Fe_3O_4 nanoparticles in the literature, [28-30] which may all be potentially adapted for this type of SERS applications, when an internal Raman probe is introduced into those nanostructures.

Conclusions

We report a facile synthesis of Ag-Fe_3O_4 nanocomposites, which can be used as SERS substrates. Quantitative SERS measurements using the Ag-Fe_3O_4 nanocomposites are successfully demonstrated with 4-aminothiophenol and dopamine as analytes. The inclusion of

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an internal reference, polyacrylic acid in this study, greatly improves the reliability and quality of the linear fitting for quantification of the analyte concentrations. The scheme is versatile, and has the potential for the detection of a wide range of analytes.

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Quantitative SERS-based detection using Ag-Fe_3O_4 nanocomposites with internal reference

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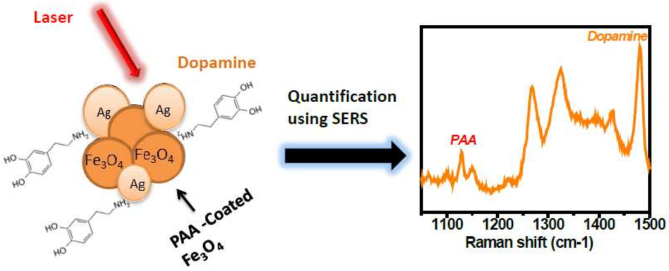

We report a method for quantitative SERS measurements using Ag-Fe_3O_4 nanocomposites with an internal reference for detecting different analytes.

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