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A facile route of microwave to fabricate PVA-coating Ag nanofilm used as NIR-SERS active substrate

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ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) is a very sensitive and selective technique for detecting surface species. Recently, SERS has been increasingly employed in the study of biological macromolecules, from DNA and peptides to whole proteins, and cells. However, visible laser sources usually employed in SERS detections always lead to photochemical reactions as well as intensive fluorescence emission from the biological samples. A way to avoid these questions is the employment of near infrared (NIR) laser excitation; thus, it demands the appropriate designs of NIR-SERS substrates in order to obtain the maximum enhancement of the Raman signals from biological analytes. In this work, we demonstrate the fabrication of a new NIR-SERS substrate of polyvinyl alcohol (PVA) coating Ag nanofilms (PVA-coating Ag nanofilm) using a simple and low-cost microwave strategy. The experimental data show that, the plasmon resonance band of the PVA-coating Ag nanofilm is in the region of 400–900 nm, and the maximum center is at ~780 nm, which matches well with the 785 nm laser excitation employed in this work. With the NIR-SERS detections of hematin and hemoglobin molecules adsorbed on this PVA-coating Ag nanofilm, one can see that the NIR-SERS activity and spectroscopy reproducibility of this NIR-SERS substrate are all perfect. By using of the tested molecule of hematin, the PVA-coating Ag nanofilm shows a high enhancement factor (EF) of ~10^7. As the fabrication process of this NIR-SERS substrate is very simple and inexpensive, this method may be used in large-scale preparation of SERS substrates that have been widely applied in Raman analysis. Especially, this PVA-coating Ag nanofilm can also be served as a novel NIR-SERS substrate in biochemical analysis due to its good NIR characteristics.

1. Introduction

Raman spectroscopy is extremely useful for a vast number of applications in the filed of biochemical analysis in resent years [1,2]. However, the Raman scattering cross section of most biological macromolecules is very small, which generally limits its potential uses [3]. Generally, this inefficient scattering requires longer signal collection times and the use of higher laser power for the acquisition of effective Raman spectra, which may result in damages to the biological samples [4]. Surface-enhanced Raman scattering (SERS) has received more and more attention from researchers all around the world since its discovery in 1974 [5], not only because of its high sensitivity and the small volume of sample needed [6], but also due to the possible wide applicability. It has been reported that single molecule detection is possible by SERS [7], suggesting the enhancement factor of SERS can reach as much as 10^{14}–10^{15} [8,9]. Recently, SERS has been increasingly employed in the study of biological molecules [10,11], from DNA [10,12] and peptides [13,14] to whole proteins [15,16], and cells [17,18]. Thus, the different methods of preparing SERS-active substrates have been extensively explored in order to obtain substrates with high enhancement ability and good spectroscopy reproducibility [19–21]. However, SERS-active substrates prepared by different methods have respective advantages and disadvantages [22]. For example, metal colloids, in general, show strong SERS activity and are easily prepared and manipulated; however, the colloidal aggregation after the addition of analyte will result in instability of the colloid and poor reproducibility of the SERS spectra [22–24]. Ag nanofilms deposited on glass, silicon and AAO template by vacuum evaporation or magnetron sputtering has strong SERS enhancement and stability. However, the metal nanofilms formed by this method require a good-sized apparatus. In addition, this preparation equipments needed are always expensive, which makes it difficult to use widely.

On the other hand, the wavelength of the laser source is another important factor in SERS detections of biological macromolecules. As Puppels et al. [25] reported that a laser wavelength shorter than 514.5 nm is known to enhance photodissociation and cause protein

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degradation even at a low power. However, the sample damages can be avoided by using the laser source of a longer wavelength. In their studies, no paling effects were observed using laser light with wavelength more than 660 nm with the light intensity is up to 127 MW/m^2. Near infrared (NIR) excitation has a basic advantage in SERS detection is that the background will be strongly decreased, and NIR excitation is nonresonant for most molecules, it is allowed to employ high excitation up to saturation without photo-bleaching [7]. However, it demands appropriate designs of suitable substrates for NIR-SERS detection, in order to obtain the maximum enhancement of signals from biological analytes. Thus, a more reliable and low-cost method for obtaining stable, active and biocompatible NIR-SERS substrate for biological macromolecule detections is utterly necessary.

This paper describes the fabrication of NIR-SERS substrate of polyvinyl alcohol (PVA) coating Ag nanofilm on the surface of glass slide using a simple and low-cost microwave strategy. We report here on NIR-SERS effect of hematin and hemoglobin adsorbed on this substrate prepared using the method of microwave heating combined with electrostatic self-assembly. The experimental results show that, the plasmon absorption maximum of the PVA-coating Ag nanofilm is at ~800 nm, which belongs to near-infrared regions. The PVA-coating Ag nanofilms obtained by this simple method have the advantages of large NIR-SERS enhancement and good spectroscopy reproducibility. To our knowledge, few people had employed this facile method to fabricated Ag nanofilm to be used as biocompatible NIR-SERS substrate. As this method does not require complex apparatus or techniques or special reaction conditions, and the fabrication process is very simple and inexpensive, this method might be more amenable to large-scale preparation of this NIR-SERS substrate for biochemical analysis.

2. Materials and methods

2.1. Reagents and preparation of hemoglobin

Silver nitrate (99.8%), sulfuric acid (98%), sodium citrate (SC, 99.0%), polyvinyl alcohol (PVA, 99.0%), Hematin (Sigma, 99%), Silver poles (>99.9%), and glass slide (with positive charges on the surface, 1.2 mm thick) were all of AR grade and used as received. All other chemical products, unless specified, were of reagent grade, and highly purified water, of resistivity greater than 18.0 MΩ cm, was used throughout the experiments.

To obtain hemoglobin, human blood samples obtained from healthy volunteers were placed in glass tubes containing 200 μL of 3.2% SC aqueous solution act as anticoagulant. The plasma and white cells were removed after a low-speed centrifugation (2000 rpm, at 4 °C). The red cells were washed in PBS (phosphate buffered saline) 3 times and then were lysed by adding deionized water in a volume ratio of 20:1 (water/packed cells) [26]. Then, the dilute hemoglobin solution with the concentration of ~1.5% (mass fraction) was centrifuged at 6000 rpm for 15 min to remove cell debris and stored at 0 °C until needed.

2.2. Preparation of colloidal Ag nanoparticles by microwave heating

To obtain the colloidal nanoparticles, here we employed a simple method of microwave heating. Firstly, 30 mg silver nitrate and 10 mg sodium citrate were added in 200 mL deionized water, stirred adequately. Then, this mixture was put into the microwave oven and heated by using microwave (2450 MHz, 1300 W) for 10 min. Then, colloidal Ag nanoparticles were obtained. Based on electrophoresis experimental, it was found that these Ag nanoparticles prepared by this simple method were adsorbed with negative charges.

2.3. Preparation of NIR-SERS substrate of PVA-coating Ag nanofilm

A mixture of 200 mL contained 30 mg silver nitrate and 100 mg PVA was prepared with the highly purified water. The mixture was fully heated and stirred to make the PVA dissolved enough. Then, this mixture was cooled at home temperature (22 °C). Subsequently, 10 mg sodium citrate was added in this mixture, stirred adequately. Then, the reaction mixture of 200 mL contained 30 mg silver nitrate, 100 mg PVA and 10 mg sodium citrate were obtained.

PVA-coating Ag nanofilms were prepared by the method of microwave heating combined with electrostatic self-assembly. Firstly, glass slides (with positive charges on the surface) were ultrasonically cleaned in acetone, ethanol and deionized water, respectively. Then, these cleaned glass slides were washed with deionized water for 3 times and dried in the atmosphere of high purity N_2 (99.99%). Secondly, these positively charged glass slides were dipped into the reaction mixture obtained above, and then they were put into the microwave oven and heated by microwave (2450 MHz, 1300 W) for 20 min. Then, the PVA-coating Ag nanofilms were grown on the surfaces of the glass slides.

2.4. Experimental equipments and spectroscopy detections

UV–vis absorption spectra of hematin, colloidal Ag nanoparticles and PVA-coating Ag nanofilms were recorded using a UV-3101 spectrophotometer (Shimaduz Co., Japan) with a spectral resolution of 2 nm and a slot width of 0.5 nm. Transmission electron microscopy (TEM) image of colloidal Ag nanoparticles prepared using the method of microwave heating was obtained on an H-600 transmission electron microscope (HITACHI Co., Japan) at 100 kV. The scanning electron microscopy (SEM) images of PVA-coating Ag nanofilms were obtained with a scanning electron microscope system (JSM-6700F mode, JEOL Co., Japan) operating at 30 kV. Additionally, the thickness of the nanofilms was analyzed by an ellipsometer (ELLIP-SR-I, WoBang Co., China), which is 14.9061 ± 0.3642 μm. According to our SEM images, we also estimated the thickness of PVA which is about 18.421 ± 3.039 nm. All Raman and NIR-SERS spectra were recorded by using a portable Raman spectrometer (R-3000^{TM}, Ocean Optics Co., USA) with 785-nm radiation from a 65-mW air-cooled diode laser. The laser light was vertically projected onto the samples with a resultant beam intensity of 10^3 W cm^{-2}, and the integration time was 16 s.

For the NIR-SERS detection of hematin, hematin sample with the concentration of 10^{-3} M was prepared with highly purified water firstly. Then it was diluted into the concentrations 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M. Before spectral collection, each diluted hematin samples of 50 μL was dropped onto the surfaces of the PVA-coating Ag nanofilms with a diameter of 1 cm, respectively. Similarly, 50 μL of 1.5% (mass fraction) hemoglobin aqueous solution was treated with the same processes. Lastly, NIR-SERS spectra of diluted hematin and hemoglobin adsorbed on PVA-coating Ag nanofilms were recorded. On the other hand, the diluted samples of hematin (10^{-5} M) and hemoglobin (1.5%, mass fraction) of 60 μL were dropped into Ag colloid of 1 mL, respectively. After stirred adequately, NIR-SERS detections for these samples were performed. For the UV–vis absorption spectrum detection of hematin solution, the reference we adopted was 3 mL highly purified water and the sample was 3.0 mL hematin solution (10^{-4} M, pH 13.0). Lastly, the method of Gauss-fitted analysis was employed to analyze these UV–vis absorption spectra with the software of Origin 8.0.