PVA-Coated AgNPs/SiO₂ for Detection of Gentian Violet with High Sensitivity

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Abstract
Improving the detection limit of surface-enhanced Raman scattering (SERS) is a significant issue for the design and application of plasmon substrate. It always suffers from the weak affinity of probe molecules on the nanoscale surface, uncontrollable aggregation behaviors of nanoparticles, and strong fluorescence of the detected substance. In this paper, AgNPs/SiO₂ substrate is prepared by a rapid thermal annealing method with a mean size of 87.9 ± 30.7 nm. A layer of 5–30 nm PVA (polyvinyl alcohol) film is used as the stabilizer of AgNPs. With PVA coating, AgNPs can improve the detection limit of gentian violet by three magnitudes, compared with the one on undecorated AgNPs which is just 10⁻⁵ mol/L. In the sensitive detection using coated AgNPs/SiO₂ substrate, PVA possibly plays three significant roles, affinity increaser between gentian violet and silver nanoparticles, fluorescence inhibitor of gentian violet, and stabilizer of Ag nanoparticles. The results provide a modification method for improving SERS sensitivity of plasmon substrate, which uses convenient and low-cost rapid thermal annealing as the patterning method and PVA as the coater, an advantageous technology for plasmon substrate application into trace detection.

Keywords SERS · PVA modification · Rapid thermal annealing · Gentian violet

Introduction
Noble metallic nanoparticles (NPs) such as Au and Ag exhibit excellent plasmon property compared with their bulk materials. Since the first report about surface-enhanced Raman scattering (SERS) of pyridine adsorbed on roughened silver electrodes in 1975, they attract extensive study attentions due to the great applications in sensing [1, 2], photonics [3], energy harvesting [4], and so on. Metal nanoparticles own the ability to concentrate electromagnetic field into localized concentrated regions near nanoparticle surfaces, which can be used to amplify many optical processes, such as absorption, reflection, and scattering. The plasmon property of NPs is sensitive to their size, geometry, and composites. The colloid and self-assembled nanostructures provide a low-cost and facile preparation process for rapid high-sensing technological application [5]. However, they often suffer from low chemical stability and weak reproducibility, that is, because nanoparticles tend to aggregate due to the high surface-volume ratio, which limits their enhancement performance. Another limitation is the weak adsorbability of probed molecules on NPs surfaces. Great efforts have been made to fabricate effective metal nanoparticles by using colloidal nanoparticle assemblies [6], functionalization of nanoparticles [7, 8], two-dimensional films [9, 10], and metal/polymer composite [11, 12].

It is evaluated that aggregation behaviors of NPs are related to the concentration of saline aqueous media of NPs suspensions. The formation of big clusters can be prevented by lowering the ionic strength [13] via functionalization or top-down methods. The region confining electromagnetic energy generally extends 5–30 nm [14] away from the NP surfaces which requires locating the molecules on or near the NPs surfaces during sensing detection. To increase the affinity of molecules on NPs surfaces, most molecules or metal NPs are functionalized before molecule recognition [12], which cannot overcome the free aggregation. Metal/polymer composite recently gains attention which shows quite advantages at chemical stability, sound affinity, and high
disparity, beneficial for improving detection limits. Gushiken et al. [11] use a nanocomposite consisting gold NPs buried in poly methyl methacrylate (PMMA) as SERS substrate. The use of the PMMA layer ensures a spacing between NPs less than 10 nm and protects NPs from environment influences, guaranteeing the stability of the composite layer. The substrate can concentrate analyte molecules closer to the metal surface, guide molecules to the hot spots, and achieve a 10 μM detection limit [11].

Another applied organic shell is polyvinyl alcohol (PVA), which is a well-known water-soluble acrylic polymer. In this work, PVA is used as the stabilizer of AgNPs which guides molecules to the nanoscale surface features of AgNPs. AgNPs are prepared by using a top-down method—rapid thermal annealing. In this method, a firmly deposited silver thin film is heated at a high annealing temperature for some minutes. The metal film is then rapidly cooled to room temperature, which induces a decrease in the surface energy of thin film resulting in a film structure shrinking formed as uniform AgNPs. With a coating of PVA film, AgNPs/PVA composites are derived and used for SERS measurement of gentian violet. The advantageous SERS effect of composites over pure AgNPs is intercepted based on the morphology analysis and electromagnetic simulations.

### Experimental Details

#### Preparation of AgNPs

The high heat-resist quartz (JGS2) is used as the substrate with silica content over 99.999%, which provides an opportunity for the investigation of the macroscopic optical effect of AgNPs. Its size is 10 mm × 10 mm × 2 mm. It is cleaned with alcohol and deionized water ultrasonically before being used. For the preparation of AgNPs, a 7 nm-thick silver film (99.99%) is firstly deposited onto the quartz surface via an ion sputtering coater (MCM-100, SEC Co., Ltd). It is then annealed in a vacuum annealing furnace (RTP-1200, ECO-PIA) under a pressure of 10⁻³ Torr. To obtain high crystallinity of materials and decrease preparation time, the annealing temperature at 900 °C is used which is held on for 5 min before cooling to ambient temperature. The temperature of the furnace rises by 25 °C per second. With the surface energy decreased due to the cooling process, the continuous silver film would transform into nanoparticles, the size and appearance of which are dependent on the film thickness, annealing temperature, and time duration.

To precisely control the thickness of the silver film deposited by ion sputtering coater, the sputtering time is carefully calibrated. There is a proportional relationship of the deposited film thickness d with the voltage V, current I, and sputtering time t as follows: \( d = kIVt \). In this expression, \( k \) is a coefficient, dependent on the kind of sputtered metal and filling gas. When \( t = 31 \text{s} \), \( d \) is measured as 18.822 ± 0.988 nm by using an ellipsometer (M-2000 V, J. A. Woollam Corp.). Thus, to obtain a 7 nm-thick silver film, the needed sputtering time is 12 s.

#### Surface Modification of AgNPs by PVA Coating

The used PVA is analytically pure purchased from Tianjin Damao Chemical Reagent Co., Ltd. In the modification of AgNPs by PVA, the thickness of PVA film coated on AgNPs is a key parameter. That is because the thicker PVA film would keep probes molecules away from the hot spots generated by AgNPs which hardly excites surface-enhanced Raman scattering. In this work, a proper content of PVA solution is made by putting 5 g solid PVA calibrated by a high precision electronic balance (AL204 model, Mettler Toledo) into 300 mL pure water and heated at 95 °C until completely dissolved. The prepared AgNPs substrate is immerged in the PVA solution for 1 min before taken out and dried in air. To guarantee a uniform coating, the substrate is moved by using a dip coater (PB100-BE, Lebo Science). The microscale morphology of AgNPs without and with PVA coating is investigated by using scanning electron microscopy (QUANTA250, FEI).

#### SERS Measurement of Gentian Violet

Analytically pure gentian violet is used as the probed molecules which is purchased from Fuchen Tianjin Chemical Reagent Co., Ltd. Before Raman measurement, gentian violet solutions with a concentration of 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸ mol/L are prepared. The uncoated and coated AgNPs are immersed in gentian violet solution of different concentrations for 2 h, respectively, ensuring the gentian violet molecules totally adsorbed onto AgNPs. SERS measurement of gentian violet is carried on the confocal micro-Raman spectrometer (DXR, Thermo Fisher Scientific Co.) with a laser wavelength at 532 nm and power of 0.1–1 mW. Lower power is used to reduce the laser heating effect on samples. The used collecting time is 15 s. The schematic illustrations of SERS measurement on AgNPs without and with PVA coating are shown in Fig. 1a, b.

#### Results and Discussion

#### Microscale Morphology of AgNPs With and Without PVA Coating

The optical images of the two samples are shown in Fig. 2a. As shown in the SEM images of AgNPs uncoated with PVA (Fig. 2b, d), the mean size of AgNPs is 87.9 ± 30.7 nm. In
comparison, the coated AgNPs have a thin layer of coating on the surfaces (Fig. 2c, e), which makes NPs size increase to 93.7 ± 44.4 nm. Due to the PVA film coating, some AgNPs seem like connected. To evaluate the thickness of PVA coating, a step profiler measurement on AgNPs partly with PVA coating and partly without PVA coating is taken. The measured steps changes from uncoated AgNPs to coated AgNPs (Fig. 2f) show that the thickness of PVA coating is about over the range of 5 to 30 nm.

Figure 2d, e show that there are two kinds of size in AgNPs. Taking uncoated AgNPs for example, the size distribution statistic is analyzed as shown in Fig. 2g. The size distribution is a superposition of two normal single peaks distribution functions with a peak at 100 nm and 50 nm, respectively. It is related to the forming mechanism of annealed AgNPs. Due to the low adhesion energy between the silver film and quartz substrate as well as the high velocity of film sputtering, the silver film grows following the Volmer-Weber mechanism in the ion sputtering process. Silver atoms growing on the substrate surface firstly form as lots of isolate islands which are also called crystal nucleuses. The nucleuses grow up continuously experiencing Oswald mergence, heat sealing, and atomic group migration, then change into trench and network structures. With the secondary and third nucleating in the trench and network structures, the trenches are filled up and become continuous films. When the continuous silver films are given annealing treatment, the primary nucleuses with some network structures dissociate from each other driven by the decreasing surface energy, which results in nanoparticles. The secondary and third nucleuses formed in the trench structures transform into small nanoparticles. The two kinds of nanoparticles can be distinguished in Fig. 2d, e. Due to the two different forming mechanisms, the size of AgNPs distributes following two normal peaks.

As shown in Fig. 2d, e, AgNPs approximately show as spherical crown shape. As is known, silver is a face-centered cubic metal with multi-plane structural crystal particle theoretically. However, the annealing temperature used in this experiment is 900 °C, approaching the silver melting temperature (936 °C) under the experimental pressure of 1 × 10⁻³ Torr. In addition, the melting point of the silver thin film is far below the bulk one. Silver nanoparticles quite probably appear as melt drop at a high annealing temperature. Under the high cooling rate (100 °C/s) in an employed vacuum annealing furnace, the silver nanoparticles retain the spherical crown shape at high temperature when cooled to room temperature.

The Trace Detection of Gentian Violet on Silver Nanoparticles Without and With PVA Coating Showing Marked Fluorescence Quenching Effect

Without PVA coating, the original AgNPs/SiO₂ can detect 10⁻⁵ mol/L gentian violet, as shown in Fig. 3a. Compared with the detection limit of the control sample (10⁻¹ mol/L), the Raman sensitivity is increased by four orders of magnitude, which verifies the SERS effect excited by AgNPs. It is generally accepted that SERS effect mainly origin from electromagnetic enhancement owing to the excitation of surface plasmon, with some contributions from chemical enhancement related to the nature of molecular states and the specific location of adsorbates on the nanoscale surfaces [14]. According to the enhancement theory, the local electric field around AgNPs is simulated to intercept the experimental results by using the finite-difference time-domain (FDTD) method. For a hexagonal array of AgNPs with a diameter of 80 nm and period of 133 nm based on AgNPs SEM images, the calculated absorption curve of AgNPs is shown in Fig. 4a. In the visible range, AgNPs show an absorption peak (black line in Fig. 4a) which is dependent on the permittivity of AgNPs. The light blue color shown in the optical image of the sample (Fig. 2a) is in accord with the absorption peak at 356 nm. The local surface plasmon property of AgNPs can be interpreted by the simulated absorption spectrum and electric field around AgNPs. As shown in Fig. 4b, under the irradiation of 532 nm light, the electric field around the nanoparticle’s surface is strongly enhanced along the polarization direction.
In order to study the detection limit of prepared AgNPs, fluorescence signals of probed molecules are investigated. Gentian violet is a kind of symmetric conjugate molecule with an inelastic and planar structure. Due to the π-electron conjugation, gentian violet molecules show free state fluorescence signal under the laser excitation. Besides, there is also surface-enhanced fluorescence excited by the plasmonic effect of AgNPs, which contributes to background signals. As shown in Fig. 3a, the fluorescence background over the range from 1300 to 1800 cm$^{-1}$ can be investigated from $10^{-3}$ mol/L Raman spectrum of gentian violet on AgNPs. Below this concentration, the fluorescence intensity behaves concentration-dependent. For the concentration reaching $10^{-4}$ and $10^{-5}$ mol/L, the fluorescence background is swamped since it is weaker than the Raman signal. However, when the concentration is as low as $10^{-6}$ mol/L, fluorescence intensity becomes stronger than surface-enhanced Raman scattering and swamps the latter. As is known, SERS substrate may show self-fluorescence quenching property or
enhancing fluorescence property, which is dependent on the competition mechanism between localized electromagnetic field enhancement effect and nonradiative energy transfer to metallic surface from molecules. For absorption resonance enhanced and radiation resonance enhanced cases, the fluorescence intensity would be improved; otherwise, it would be quenched. Recently, it is revealed that the surface-enhanced Raman scattering intensity shows concentration-dependent within a certain range of concentrations. It may be nonlinearly related to the molecules’ concentration [15]. The fluorescence swamping Raman signal at a concentration of $10^{-6}$ mol/L may be related to the competition of luminous mechanism between surface-enhanced Raman scattering and surface-enhanced fluorescence.

To improve the detection limit of SERS, suppressing the fluorescence background is significant. In this work, PVA coating is found to have the ability to well suppress the fluorescence of gentian violet. For AgNPs with PVA coating, the detection level can increase to $10^{-8}$ from $10^{-5}$ mol/L (shown in Fig. 3b), since fluorescence background interference can be soundly suppressed. For the Raman spectrum at $10^{-8}$ mol/L, the characteristic peaks of gentian violet molecules can be investigated clearly over the range from 100 to 1800 cm$^{-1}$, including peaks at 1619 cm$^{-1}$, 1587 cm$^{-1}$, 1535 cm$^{-1}$, 1371 cm$^{-1}$, 1175 cm$^{-1}$, 915 cm$^{-1}$, 806 cm$^{-1}$, 724 cm$^{-1}$, 442 cm$^{-1}$, 421 cm$^{-1}$, and 216 cm$^{-1}$.

The mechanism of PVA coating quenching the gentian violet fluorescence has not been revealed. The absorption curve considering 20 nm PVA coating is also calculated as shown in Fig. 4a (red dash line). The absorption peak of AgNPs is not changed after coated with PVA, which can be validated by the light blue color of PVA-coated color in the optical image (Fig. 2a). The calculated electric field distribution around PVA-coated AgNPs also shows less changes compared with that of pure AgNPs (Fig. 4b). It reveals that the plasmon resonance property of AgNPs may not be affected obviously by PVA coating, though the permittivity of which is different from air. There may be several causes for gentian violet fluorescence quenched by PVA. (1) Compared with the quartz substrate surface with a few hydroxyl
groups, there are quantities of active groups on the surface of PVA thin film. It benefits for improving the affinity of label molecules on the PVA surface and concentrating molecules closer to the silver nanoparticle’s surfaces. The capacity of PVA coating adsorbing gentian violet molecules is stronger compared with silver nanoparticles, contributing some electromagnetic and chemical enhancement effect [16, 17]. (2) The interaction between PVA and the symmetric conjugation structure of gentian violet molecules may quench the fluorescence [18]. (3) The separation of gentian violet molecules from the surfaces of silver nanoparticles by PVA coating, which makes gentian violet molecules away from the electromagnetic hot spots resulting in quenching the irradiance of the surface-enhanced fluorescence. Besides, PVA coating provides a protection for AgNPs prevented from oxidation and ensures a steady electric field enhancement effect, which contributes to the increased detection limit.

The Interference in Surface-Enhanced Raman Scattering of Gentian Violet from Raman Scattering of PVA Coating and Quartz

In the surface-enhanced Raman scattering of gentian violet, there are mixed with Raman signals of quartz substrate and PVA coating. Raman intensity of quartz is weak in the case of 10⁻⁶ mol/L solution under the laser power of 0.1 mW. It is swamped by the Raman spectrum of gentian violet with a concentration over 10⁻⁶ mol/L. For the solution with a concentration at 10⁻⁷ to 10⁻⁸ mol/L, it becomes stronger and can compare with surface-enhanced Raman scattering of gentian violet when the laser power of 1 mW is used. On the other hand, the Raman signal of thin PVA coating is quite weak without the surface-enhanced effect. It can compare with the Raman spectrum of gentian violet until the concentration of solution reaches 10⁻⁸ mol/L, and laser power is over 1 mW. Due to the interference from the Raman spectrum of quartz and PVA coating, the surface-enhanced Raman spectrum of 10⁻⁸ mol/L gentian violet shows Si–O vibration mold of quartz at 400 cm⁻¹, 800 cm⁻¹, and 1333 cm⁻¹ and CH₂ stretching vibration mold of PVA coating at 2912 cm⁻¹. In spite of this, the characteristic bands of gentian violet at 10⁻⁸ mol/L can still be detected, confirming the practicable trace detection of gentian violet by using AgNPs with PVA coating.

Conclusion

The SERS effect of AgNPs on quartz substrate is investigated which is prepared by a rapid thermal annealing method. The mean diameter of AgNPs is 87.9±30.7 nm when annealed for 5 min at 900 °C. Its high detection level for gentian violet reaches 10⁻⁵ mol/L, which is limited by the fluorescence interference. A thin layer of PVA coating film on AgNPs is found to be able to increase the level to 10⁻⁸ mol/L. It is revealed that the fluorescence interference is well quenched by PVA film which stabilizes AgNPs and benefits for the affinity of gentian violet on the substrate surface. The coverage of prepared substrate could be extended to a broader range such as rhodamine, malachite green, and heterocyclic compounds like pyridine and phosmet. It provides a convenient method for preparing an efficient SERS substrate, which helps plasmon substrate application into trace detection.

Author Contribution Material preparation, data collection, and analysis were performed by Yanqing Wang and Yanru Xu. The first draft of the manuscript was written by Yanqing Wang and all authors commented on previous versions of the manuscript. Yanru Xu and Yanqing Wang wrote the main manuscript text. Yanqing Wang prepared Figs. 2 and 3, and Yanru Xu and Zhiyong Jia prepared Figs. 1 and 4. All authors read and approved the final manuscript.

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Data Availability The data that support the findings of this study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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