Fabrication of a network structure SERS substrate and the application in ultra-sensitive crystal violet detection*

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A network structure surface-enhanced Raman scattering (SERS)-active substrate is fabricated by adding Ag sol drop-wise on adhesive tape. Scanning electron microscope (SEM) is employed to characterize the structure of the as-prepared substrate. The substrate shows great SERS enhancement ability and good uniformity by using p-aminothiophenol (PATP) as probe molecules. The detection of crystal violet (CV) in aqueous solution is demonstrated, and the detection limit is as low as 10^{-12} M with the aid of the substrate. The results indicate that the proposed method is a potential approach for the fabrication of SERS substrates.

Surface-enhanced Raman scattering (SERS) was observed by Fleishmann et al firstly in 1974 and explained by Van Duyne et al in 1977[1,2]. About ten years ago, SERS spectrum of a single molecule was detected by Nie and Kneipp et al[3,4], which led SERS to a new stage. Single molecule SERS (SM SERS) is presumed to take place in junctions of aggregated nanoparticles (so-called hot spots), where the Raman enhancement can reach more than 11 orders of magnitude. This enormous enhancement gave a good chance for the routine detection of Raman signal from a single molecule, and made more researchers strive for a new and ultra-sensitive detecting and analyzing technique about chemistry and biology based on SERS.

It has been accepted that two main mechanisms can contribute to the enhancement of SERS, which are the chemical enhancement mechanism and the electromagnetic enhancement mechanism. The electromagnetic enhancement mechanism has been proved to be the dominant mechanism in most situations, which generally produces the enhancement of 10^6 times greater than the normal Raman scattering or even more than 10^{12} times when molecules are adsorbed at hot spots.

Preparation of active-substrates is a crucial problem in SERS application. Ag and Au sols are the most common SERS substrate systems. Since the metal sols are instable for a long period and not suitable for nonaqueous systems, many researchers have made great efforts to assemble metal nanoparticles on solid substrate's surface to break

these limitations. Different materials, such as glass[5,6], quartz, silicon, plasma-treated Teflon, indium-doped SnO$_2$[7] and glassy carbon[8], have been used as the immobilizing substrates, which exhibited the high performance but the complicated preparation. In this paper, adhesive tapes are used as the immobilizing substrates without pretreatment. Ag nanoparticles are dispersed on the surface in monolayer or sub-monolayer for adhesive property of adhesive tapes. The substrate shows the enormous SERS activity by using p-aminothiophenol (PATP) as probe molecules.

Triphenylmethane dyes, such as crystal violet (CV), have been widely used around the world in the aquaculture because of their sensitivity to fungal and parasite infections in fish. But these substances are mutagenic and teratogenic to humans, even cancerogenic, and they are banned in aquaculture now. However, these harmful dyes are still used by some people in the aquaculture. Therefore, it is very important to detect CV in foodstuffs, especially in aquatic products. SERS has been proved as a promising method for rapid and sensitive detection of chemicals and biochemicals. With excellent substrates, the detection limit of SERS can reach the level of 10^{-12} M or even the level of a single molecule. In this paper, the detection limit of CV can be obtained as low as 10^{-12} M with the aid of our prepared substrate. The result shows these substrates can be potential candidates to detect harmful materials in food.

Silver nitrate, trisodium citrate, potassium chloride, CV

and ethanol were obtained from Beijing Chemical Reagent Co. (Beijing, China), and p-aminothiophenol (PATP) was purchased from Acors Chemical Co.. All chemicals were of analytical grade. All aqueous solutions were made with deionized water. The adhesive tapes were common two-side adhesive tapes bought from market.

Ag sol was prepared according to Lee and Meisel's method[9]. Briefly, 36 mg $AgNO_3$ was dissolved into 200 mL deionized water, and then heated up to the boiling point. Then 4 mL of 1% trisodium citrate aqueous solution was added into the boiling solution dropwise with violently stirring. The mixture was kept boiling for another 30 min with stirring, and then cooled naturally. Finally, a green-gray silver colloid was obtained, which was generally stable for several weeks. The sol was diluted to 200 mL for use.

In the preparation of Ag-coated tapes, first, Ag sol was mixed with chloride ions with final concentration of 10^{-2} M for more than 10 h. Then, a 10 μL Ag sol suspension was dropped onto adhesive tape, which was allowed to be dried naturally in air before being washed with water several times.

The obtained substrate was immersed into a 1×10^{-4} M PATP ethanol solution for 30 min, and dried in ambient for SERS measurement. SERS spectra were obtained on a Jobin Yvon (Lab RAM HR800) spectrometer by using a 50× long working distance objective, a 600 lines·mm^{-1} grating and a 632.8 nm laser as excitation source. SEM images were carried out with a Hitachi S-4800 high resolution scanning electron microscopy with an acceleration voltage of 10 kV.

Fig.1 shows the SEM images of the as-prepared substrate, from which we know the dominant morphology of the particles is spherical with average size of about 50 nm, and others are a few nanorods. The substrate is of a network structure, because AgNPs come into chains firstly due to the effect of chloride ions, and then the chains are connected into branched aggregates as depicted in Ref.[10]. More hot spots were formed in the substrate due to the network structure, which is responsible for huge Raman signal enhancement[11,12].

To verify the SERS activity of the substrate, PATP is selected as the probe molecule. We estimate the average enhancement factor (EF) as[14]:
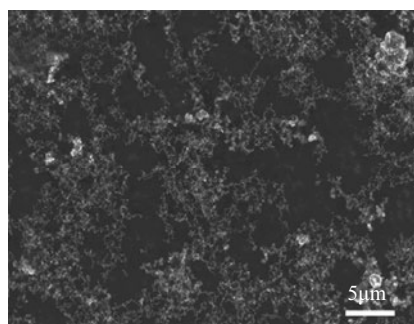

(a) Large area
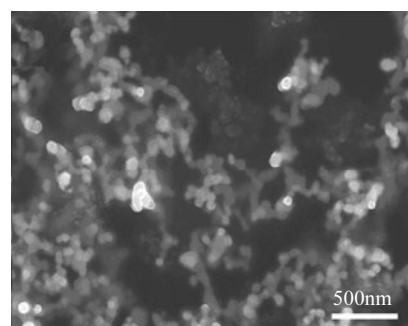
(b) Expanded view

Fig.1 SEM images of the prepared substrate

$$EF=I_{SERS}/N_{surf}/N_{bulk}/I_{norm} ,\qquad(1)$$

where I_{SERS} and I_{norm} are the measured vibration intensities in the SERS and normal Raman spectra, respectively, and N_{bulk} and N_{surf} are the numbers of the probe molecules in the laser illumination volume in the bulk sample and adsorbed on the SERS substrates, respectively. We select the vibration mode around 1079 cm^{-1} to evaluate the EF as shown in Fig.2. According to objective and laser used in our experiments, the laser spot area at the sample's surface is estimated as 1.2 μm^2, and the penetration depth of light in the PATP is about 15 μm$^{[15]}$. The beam of laser is supposed to be columned. Thus, N_{bulk} is calculated as 1.0×10^{11} considering the density of PATP. As shown in SEM images, the particles can be seen as sphere with average diameter of 50 nm. The surface area of a particle is about 7.8×10^{-3} μm^2, and the surface area occupied by one adsorbed PATP molecule is about 2.0×10^{-7} μm$^{2[16]}$. One particle occupying area on tape surface is regarded as 2.5×10^{-3} μm^2. Considering that the AgNPs disperse in monolayer, the density of particles on the tape surface is about 480 μm^{-2}. The total number of adsorbed molecules in the laser spot can be calculated as 9.5×10^6, assuming that PATP molecules adsorbed on the top 50% area of particle are contributed to SERS.
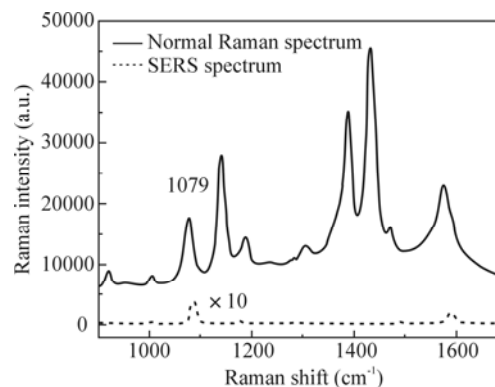
Fig.2 Normal Raman spectrum of solid PATP and SERS spectrum of PATP adsorbed on the as-prepared substrate with laser power of 0.3 mW and integration time of 10 s

According to the results of the SERS and normal Ra-

man measurements, *EF* of the vibration mode at 1079 cm^{-1} is obtained as 6.2×10^5 for the as-prepared substrate. As mentioned above, AgNPs are closely aggregated to produce active SERS effect[17]. Besides, huge electromagnetic fields are confined around the highly-packed nanoparticles, resulting in obvious Raman enhancement[18].

We collect the SERS signal optionally from different sites on prepared substrate and the intensity of bands varies mildly except for molecules on special spots, which is enhanced by about ten times enormously. These obviously enhanced spots appear only a few times, the special site of producing enormous enhancement is rare and with heterogeneous structure[19,20], and their frames are understood in future. SERS was measured again after samples were kept more than a month in water, and the intensity does not decrease markedly.

To further verify SERS activity and sensitivity of the prepared substrates in ultra-low analyte concentration, the as-prepared substrates were immersed into PATP solution with concentration of 10^{-10} M and 10^{-11} M for 30 min, respectively. As shown in Fig.3, a strong signal can be observed when PATP concentration is 10^{-10} M, and the detection limit of these substrates can reach as low as 10^{-11} M, which illuminates that the substrate prepared by this approach has very high sensitivity to SERS. Therefore, it is considered that it is one kind of potential method in making an optical SERS sensor due to the high sensitivity, stability and facile fabrication.
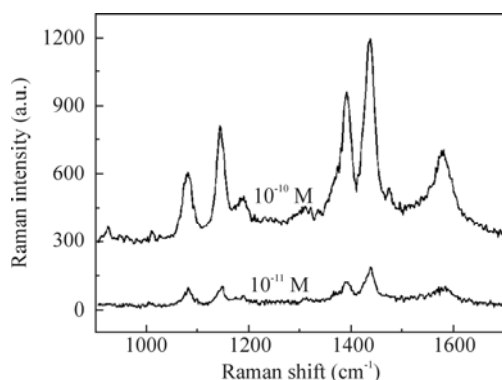

Fig.3 SERS spectra of 10^{-11} M and 10^{-10} M PATP solution on the as-prepared substrate with laser power of 0.3 mW and integration time of 10 s

As demonstrated above, the substrate is considered as a potential candidate in molecular detection. To access the application of the as-prepared substrate in food safety examination, we apply the substrate to detect CV in aqueous solution. The stock solution of CV was prepared and diluted in the range of 10^{-7}–10^{-12} M. The substrates were immersed into the CV solution with concentrations of 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M and 10^{-12} M for 1 h before SERS measurement, respectively. Fig.4 shows the SERS spectra of substrates adsorbed with various concentrations of CV. The characteristic bands of CV are distinguishable even the concentration of CV

as low as 10^{-12} M. Band around 1177 cm^{-1}, assigned to be the in-plane vibration of ring C-H[21,22], is selected to depict the relationship between concentration and peak intensity. As shown in Fig.5, the intensities of bands are increased monotonously with the dyes molecule concentration. The concentration is saturated when it reaches as high as 10^{-8} M. The result shows that we can implement the trace detection of CV based on SERS.
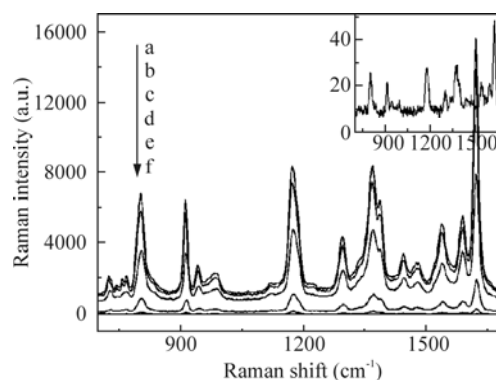

Fig.4 SERS spectra of CV with various concentrations of (a) 10^{-7} M, (b) 10^{-8} M, (c) 10^{-9} M, (d) 10^{-10} M, (e) 10^{-11} M and (f) 10^{-12} M, laser power of 3 mW, integration time of 1 s and removed baselines (The insert is the expanded SERS spectrum of 10^{-12} M CV.)
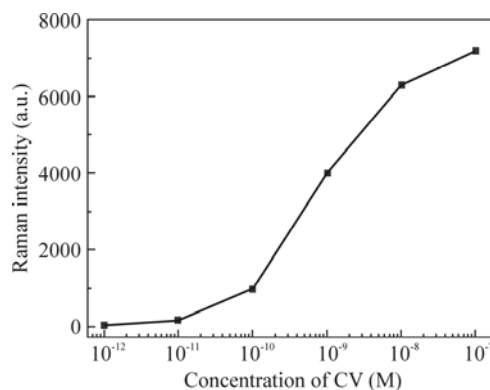

Fig.5 The relationship between peak intensity (bands around 1177 cm^{-1}) and the concentration of CV

We prepared a new kind of network SERS-active substrate by a simple method, which shows great SERS enhancement ability verified by PATP. The *EF* value of the substrate is up to 6.2×10^5. AgNPs with small gap produce giant Raman enhancement due to strong electromagnetic coupling. At the same time, substrates made by this way are uniform and stable. The low concentration detection of CV in aqueous solution is achieved based on the substrate, which shows that this method is a potential approach for the fabrication of high activity SERS substrate.

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