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Fabrication of hydrogels with nanoparticles as surface-enhanced Raman scattered (SERS) substrates and their application in Raman imaging

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Abstract

A polyvinyl alcohol–silver nanoparticle (PVA–Ag NP) hydrogel was fabricated using a simple and facile method; it was used as a substrate for surface-enhanced Raman scattering (SERS). The Ag⁺ ions dispersed uniformly in the PVA network were reduced by NaBH₄; this process was termed dipping method. Compared with conventional SERS substrates e.g., colloidal suspensions, the prepared PVA–Ag NP hydrogel has prominent Raman enhancement effects for crystal violet (CV), and the detection limit was 10⁻¹² M. It could also help detect rhodamine B, methylene blue, and 4-mercaptobenzoic acid. In addition to SERS, the reliable 3D porous structure was utilized in the Raman imaging of CV at 1621 cm⁻¹ in a nude mouse.

1. Introduction

In recent years, surface-enhanced Raman scattering (SERS) has been the most effective spectral analysis method because of its high sensitivity and ability to provide detailed molecular structure information of analytes [1–4]. In the past few years, SERS has been widely used in many fields such as chemical analysis [5, 6], environmental protection [7, 8], food detection [9, 10], and biomedical research [11–14]. Classical noble metals (Ag, Au and Cu) are the most common SERS-active materials [15]. The Raman signal of adsorbents on nanostructured SERS-active metal surfaces can be effectively increased by several orders of magnitude [16]. Numerous studies have attempted to design and synthesize substrates with SERS performance [17, 18]. However, developing SERS substrates that exhibit high stability and good reproducibility for easy practical applications remains a challenge. Therefore, a simple strategy must be determined to synthesize an efficient and sensitive SERS substrate to explore the applications of SERS.

The development of nanoparticle (NP)-embedded polymer materials for SERS substrates has drawn considerable attention in terms of reliability for analytical applications [19–22]. According to previous works, SERS substrates containing silver nanoparticles exhibit excellent SERS properties and high enhancement efficiency for RhB [23–25], CV [26], MB [27], and 4-MBA [28] probe molecules. Hydrogels are ideal candidate carrier systems for a series of metallic NPs or nanorods [29]. The 3D network structure of a hydrogel enables it to combine with metal NPs and helps accelerate assembly, control the diameter and morphology, and prevent the agglomeration of metal NPs [30]. Furthermore, it exhibits an effective adsorption capacity for water-soluble pollutants because of its superior water uptake ability [31], thus finding applications in Raman spectroscopy as a SERS substrate. Various studies have attempted to synthesize hydrogel composites with properties such as antifreeze, high strength, self-healing, and conductivity [32, 33]. Hydrogel composites with SERS properties can be applied to track hydrogel drug release and improve monitoring sensitivity. Hence, it is desirable to synthesize hydrogel composites with excellent sensitivity, stability, and reproducibility for use as SERS substrates. In this regard, this study mainly focuses on the preparation of nanoparticle-loaded hydrogels with SERS properties utilizing the three-dimensional (3D) porous structure of polyvinyl alcohol (PVA) [34, 35].

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In this study, we report a simple and facile process for synthesizing a PVA–Ag NP hydrogel as a reliable SERS substrate (as shown in figure 1). First, a PVA hydrogel containing AgNO₃ was prepared using the conventional freezing–thawing method [36]. Second, the PVA–Ag NP hydrogel was fabricated through a dipping method with NaBH₄ as the reducing agent. The reduction process was controlled by diffusing the reducing agent in the gel matrix, and the PVA network helped enhance the stability of the Ag NPs. We applied the PVA–Ag NP hydrogel to SERS analysis, and the resulting PVA–Ag NP hydrogel was found to have superior SERS sensitivity, stability, and reproducibility for crystal violet (CV) and Rhodamine B (RhB). We demonstrated that a synthetic SERS substrate exhibits excellent results for four target molecules because of simple adsorption of the probe molecules. The gel can also be used for rapid Raman imaging; we performed Raman imaging of CV at 1621 cm⁻¹ in a nude mouse; the distribution of the target molecules was clear, and the damage to the animal was negligible. Therefore, the PVA–Ag NP composite gel substrate is expected to have broad application prospects in detecting food additives and heavy metals and in biological diagnosis.

2. Experimental

2.1. Chemicals and materials
AgNO₃ and NaBH₄ were purchased from Tianjin Guangfu Fine Chemical Research Institute. Methylene blue (MB), 4-mercaptobenzoic (4-MBA), crystal violet (CV), and rhodamine B (RhB) were obtained from Tianjin Yuanli Chemical Co. Ltd (Tianjin, China). PVA (PVA-1799) was purchased from Shanghai Macklin Biochemical Co. Ltd (Shanghai, China). All reagents were of analytical grade and used as received without further purification. Distilled water was used throughout the experiment for solution preparation.

2.2. Instrumentation
The morphology of the PVA–Ag NP hydrogels was characterized by field emission scanning electron microscopy (FE-SEM) (JEM-2100F, Japan) and transmission electron microscopy (TEM) (FEI Tecnai G2F20, USA) at a maximum accelerated voltage of 200 kV. Raman scattering was performed on a Renishaw InVia Reflex confocal microscopy Raman Spectrometer with a 1024 CCD detector, using 532 and 633 nm laser sources. Different dyes have certain selectivity for Raman excitation light source; MB and CV exhibit Raman enhanced performance under 633 nm excitation light, while RhB and 4-MBA are more suitable for Raman detection under 532 nm excitation light. The laser power was in the range of 7–10 mW. The SERS spectra were collected at 50× objective with an exposure time of 2 s; three sets of measurements were recorded. The spot size of the laser was 1 μm in diameter. Raman imaging of CV on the skin of the mouse was performed with a 633 nm laser and 5× objective; the laser power was 6.5 mW, and the exposure time was 20 s per picture. The Raman-scattered light was focused through a slit (width = 65 μm) with a spot size diameter of 250 μm and dispersed using a diffraction grating (1200 grooves per millimeter) onto a 576 CCD detector.
2.3. Preparation of PVA–Ag NP hydrogel

In this study, the chemicals were of analytical purity. The PVA hydrogel containing AgNO₃ was prepared using the conventional freezing–thawing method. We dissolved 1.0 g of PVA powder in 9 ml of distilled water at 90 °C. The synthesized PVA hydrogel solution was then cooled to room temperature, and 2 ml of a specific concentration of AgNO₃ solution (ranging from 20 to 100 mM) was added to it. Next, the mixed solution was stirred for 30 min in darkness and transferred to a centrifuge for 2 min (400 rpm) to remove air bubbles. Finally, 1 ml of the above solution was then transferred to an injector with a volume of 1 ml. The injector was placed in a refrigerator to freeze at −18 °C for 12 h and thawed for 2 h at room temperature. This freeze–thaw cycle was performed repeatedly four times to obtain a product with a certain 3D network structure. The PVA–Ag NP hydrogel was fabricated using the dipping method with NaBH₄ as the reducing agent. Briefly, 3 mm thick hydrogels were cut and dipped into a beaker containing 10 ml of a certain concentration of NaBH₄ solution (CNaBH₄/CAgNO₃ = 3:1). The beaker was placed in a 4 °C water bath for 12 h to reduce Ag ions into Ag NPs. The final gel sample was recorded as PVA–xAg and soaked in distilled water for one day. The water was changed every half a day to remove excess NaBH₄ and free Ag NPs. Finally, the sample was stored in a refrigerator for future use.

2.4. Swelling measurements

The prepared PVA–Ag NP gel sample was freeze dried and recorded as W₀. The sample was soaked in excess deionized water at 25 °C. The sample was taken out every 10 min, and the water on the surface of the sample was blotted on a filter paper to weigh the sample. This was recorded as Wᵣ, and the same operation was repeated four times. When the weight of the sample remained unchanged, the soaking time interval was set to 30 min, and the operation was repeated thrice; the soaking time interval was further extended to 1 h, and the operation was repeated thrice; this was repeated until condensation. The sample reached swelling equilibrium. The swelling ratio (Sᵣ) of the gel sample can be calculated using equation (1), and the swelling ratio/time curve can be drawn.

\[
Sᵣ(\%) = \frac{(Wᵣ - W₀)}{W₀} \times 100\%
\] (1)

2.5. SERS measurements

First, to determine the optimal PVA–Ag hydrogel for use as the SERS substrate, we used three hydrogel substrates with different silver contents and a blank control group to obtain SERS spectra with RhB at a concentration of 10⁻⁵ M. For the SERS spectra, a series of probe molecule solutions with different concentrations were prepared. The RhB, CV, and MB solutions were all aqueous solutions; however, 4-MBA was first dissolved in 5 ml of ethanol and then diluted with distilled water to the required concentration. The PVA–Ag NP hydrogels were then immersed in these solutions for 1 h. The swollen hydrogel was removed and dried at room temperature for 2 h to ensure that the volume of the swollen hydrogel returned to the original condition. The SERS spectra of the hydrogels captured by these analytes were collected. The spectral curves were baseline-corrected.

2.6. Raman imaging in vivo

For the Raman imaging, the dried PVA–50Ag NP hydrogel was immersed in an excess amount of CV (10⁻⁵ M) solution at 25 °C to attain full swelling saturation. Thereafter, the PVA–50Ag NP hydrogel containing CV was dissolved in distilled water at 90 °C, and the obtained 50 µl sol was injected into the subcutaneous tissue of a nude mouse. For comparison, a 50 µl pure 10⁻⁵ M CV solution was injected into the upper area of the nude mouse. The Raman images were then obtained.

3. Results and discussion

3.1. Morphology and structure

As shown in figures 2(a)–(c), the Ag NPs are spherical, size in the range 10–100 nm. At 100 mM AgNO₃, the particle size increases, and the Ag NPs aggregate. The micromorphology of the freeze-dried PVA–Ag NP hydrogel can be observed through SEM, as shown in figures 2(d)–(f). The hydrogel has a relatively uniform porous structure, which significantly affects the absorption and dispersion of probe molecules.

3.2. Swelling ratio study

The swelling rate of the gel quantitatively indicates the looseness of the internal structure of the gel network, which is an indicator of the structure of the gel network. A higher index indicates a looser interior gel structure [37]. Figure 3 shows that the hydrogel reaches swelling equilibrium within 100 min and that the water
absorption gradually increases with an increase in AgNO₃ concentration (Ag content). Figure clearly shows that the rate of swelling of the hydrogel increases with the concentration of silver ions during the initial phase, and it takes a longer time to reach the swelling equilibrium. The slope of the swelling curve for pure PVA gels is the smallest, and the time required to reach equilibrium is less than 50 min. The slope of the swelling curve for the gels with more adsorbed silver ions increased with the increasing silver nitrate dosage. In summary, the swelling rate of PVA-50Ag is relatively fast, and the time required to reach the swelling equilibrium is shorter; this gel is more suitable for sample processing and facilitates the rapid adsorption of probe molecules. Moreover, PVA-50Ag showed a higher detection limit when evaluated using SERS (figure 4). The reason can be explained as follows. With an increase in the AgNO₃ concentration, the number of silver ions inside the gel structure increases, thereby increasing the osmotic pressure of the internal network and enhancing the relaxation of the network chain. The swelling rate of the PVA–Ag NP hydrogel increases owing to the increases in the amount of silver ions and osmotic pressure [38]. The enhancement in the water sorption capacity is conducive to the adsorption of probe molecules in the SERS test.

Figure 2. TEM images of (a) PVA–20Ag, (b) PVA–50Ag, (c) PVA–100Ag sol, and SEM images of (d) PVA–20Ag, (e) PVA–50Ag, and (f) PVA–100Ag hydrogel.

Figure 3. Swelling ratio of PVA–AgNP hydrogels at 25 °C as a function of time.
3.3. SERS performance

Figure 4 shows that at the beginning, the SERS detection limit of RhB increases in proportion to the concentration of AgNO$_3$, and when the concentration of AgNO$_3$ reaches approximately 50 mM, the detection limit reaches a maximum of $10^{-7}$ mM. These analytical results show that the loading of Ag NPs has an important influence on the Raman enhancement.

As shown in figure 5(A), the three types of PVA–Ag hydrogel substrates with different Ag NPs have different Raman enhancement effects on RhB at $10^{-5}$ M concentration. The PVA–100Ag and PVA–50Ag hydrogel substrates outperform the PVA–20Ag hydrogel substrate in the SERS test. In terms of resolution, the PVA–50Ag hydrogel substrate is the best. At $10^{-5}$ M concentration, the SERS peaks at 624 and 1648 cm$^{-1}$ for RhB are clearly visible. The corresponding curve of PVA-50Ag in figure 5(A) at 1300–1400 cm$^{-1}$ multiple peaks are clearly visible and easy to distinguish and no broadening of peaks. Not only the intensity, but also the distinguishable fingerprint peaks are clearer compared with other substrates; PVA-50Ag can suppress the fluorescence more effectively, and therefore some characteristic fingerprint peaks can be detected. Based on the above analysis, the PVA–50Ag hydrogel was chosen as the optimal SERS substrate for detecting the three other molecules.

To evaluate the sensitivity of the PVA–50Ag hydrogel as a SERS substrate, CV, MB, and 4-MBA were selected as probe molecules to obtain SERS spectra corresponding to their concentrations from low to high on a Renishaw Laser Raman Spectrometer. Figure 5(B) shows the SERS spectra of CV solution with different concentrations. The peak at 808 cm$^{-1}$ represents the out-of-plane ring C-H bending and those at 915 and 1178 cm$^{-1}$ represent ring skeletal vibration and N-phenyl stretching, respectively. The peaks at 1443, 1536, 1588, and 1620 cm$^{-1}$ can be attributed to ring C-C stretching.

The observed peaks in the Raman spectrum of CV are consistent with those obtained on other Ag NP SERS substrates [39]. The intensity of the corresponding peaks increased with increasing CV concentration. There is no obvious peak in the spectrum when the concentration of CV is $10^{-12}$ M; however, although the signal of the spectrum is weak, the characteristic peak of CV can be clearly observed for a concentration of $10^{-12}$ M.

Therefore, we can conclude that the intensity of the characteristic peak increases with an increase in the CV concentration, and the detection limit of CV is as low as $10^{-12}$ M. The more obvious peaks at 1398 and 1622 cm$^{-1}$ are characteristic of MB, and the detection limit of MB can reach up to $10^{-6}$ M for the same reason, as seen in figure 5(C). In figure 5(D), 1080 and 1589 cm$^{-1}$ are the characteristic peaks of 4-MBA, and the detection limit of 4-MBA is $10^{-7}$ M. Clearly, the PVA–Ag NP gel has good SERS activity and universality. By comparing and analyzing the molecular structures of CV, MB, and 4-MBA, we find that the molecular structure of CV is more symmetrical and that the Raman signals are more sensitive to such complexes in the presence of Au or Ag nanoparticles [40]. Therefore, the amplification effect for CV detection is more obvious, resulting in a lower detection limit for CV compared with the other two.

3.4. Raman imaging in vivo

Figure 6(A) reveals that $10^{-5}$ M of CV mixed in the PVA–50Ag sol can be in vivo detected, with pure CV (black line) exhibiting no SERS signal. Figure 6(B) shows the Raman image for the peak of 1621 cm$^{-1}$, and the brighter area (approximately 250 μm) clearly indicate the mapping of CV. These results confirm that the distribution of the target molecules can be distinguished, making this a viable and practical technique for the sensitive detection of target tissues and cells [41, 42].
4. Conclusions

In summary, we demonstrated a facile fabrication method for PVA–Ag hydrogel substrates, providing a new inexpensive method for the practical synthesis of SERS substrates. The Ag NPs increased in size with increasing AgNO₃ concentration and were uniformly dispersed in the entire substrate owing to the PVA network. Improving the water sorption capacity was found to be conducive to the adsorption of probe molecules in SERS tests. Compared with conventional SERS substrates, the synthetic PVA–Ag NP hydrogel has superior SERS...
sensitivity and stability for dyes such as CV, RhB, MB, and 4-MBA. The enhanced SERS signal may be attributed to the increased number of Ag hotspots. Furthermore, we performed Raman imaging on the distribution of target molecules in a nude mouse, thus demonstrating the potential of this method for biological diagnosis.

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