Preparation of Ag NPs @Polyvinyl alcohol/Chitosan as a SERS substrate for Norfloxacin Detection

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In this study, silver nanoparticles (Ag NPs) were decorated on polyvinyl alcohol/chitosan nanofibers (Ag@PVA/PEI) as a SERS substrate for norfloxacin detection by electrospinning and in-situ reduction. Silver ions were anchored on the surface of PVA/CS nanofibers by free amino groups in chitosan framework, and then reduced by ascorbic acid to form AgNPs, which were spherical and uniformly decorated on the surface of PVA/CS membranes. The SERS performance of the prepared substrate was evaluated by using rhodamine 6G (R6G) as a probe. The detection limit was 10^{-6} M. In addition, the Ag@PVA/CS substrate can be used for the identification and detection of norfloxacin. The detection limit was 10^{-5} M. The exceptional SERS performance, sensitive, flexibility, low cost, render the obtained SERS substrate in this study a promising product for detection in practical applications.

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Keywords: SERS, Electrospun nanofibers, Poly(vinyl alcohol), Chitosan, Norfloxacin

1. Introduction

The abuse of antibiotics is a serious threat to food safety, which may lead to serious environmental pollution and human health problems [1]. At present, high- performance liquid chromatography (HPLC), gas chromatography (GC) and liquid chromatography-mass spectrometry (LC-MS) are commonly used into antibiotic residues detection[2]. These methods are accurate, sensitive and widely applicable, but they have disadvantages such as tedious pretreatment, expensive instruments and time-consuming, which limit the application of rapid field test[3]. Since its discovery in 1974, surface enhanced Raman spectroscopy (SERS) has attracted wide attention because of its rich information content and high sensitivity, which can be used as a rapid and non-destructive testing method [4]. In recent years, there are many reports of using SERS technology to detect antibiotic residues. Cheng [5] was synthesized Ag@Au nanoparticles by seed mediated growth. And the detection limit of ofloxacin (OFLX) was 10^{-10} M using this

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SERS substrate. Ma [6] used silver sol membrane as SERS substrate to detect three kinds of antibiotics (chloramphenicol, ciprofloxacin, enrofloxacin). But these SERS substrates are mostly made of metal sol particles, which have the disadvantages of low space utilization, uneven distribution and easy aggregation. In order to solve these problems and meet the requirements of real samples with irregular surface, flexible materials as supports of SERS substrates have been studied, such as silver particles modified filter paper[7], silver particles modified cotton swab[8], silver particles modified aluminum foil [9], etc. Electrospun nanofiber membrane has high specific surface area, high porosity and three-dimensional structure. As a carrier, on the one hand, it can solve the problem of nanoparticle aggregation, on the other hand, it can make more probe molecules combine with nanoparticles to get stronger Raman signal. There are a large number of free amino and hydroxyl groups in the CS framework, which has the characteristics of excellent biocompatibility, biodegradability and non toxicity. CS polymer has become an active platform for the preparation of high efficiency nanofiber membrane probes [11]. The chitosan (CS) was used as a polymer additive in order to uniformly decorate AgNPs onto PVA nanofibers. Sensitive and economical Ag@PVA/CS SERS substrates were prepared by electrospinning and in-situ reduction. The substrate is applied to the detection of norfloxacin, which provides a new method for the detection of biological drugs.

2. Experimental

2.1. Materials: Polyvinyl alcohol (1799, alcoholysis degree 98-99), Chitosan (deacetylation degree ≥ 95%), Sodium dodecyl sulfonate (SDS) (AR), Silver nitrate (AR), Anhydrous ethanol (AR), Rhodamine 6G (AR), Norfloxacin (AR). The above reagents were purchased from Shanghai Aladdin reagent network.

2.2. Sample preparation: 4.8 g PVA and a certain amount of SDS were dissolved in 50 ml deionized water in 98℃ water bath, and stirred continuously for 1 hour. 0.2 g CS was dissolved in 5% acetic acid (10ml). Then the PVA solution was added into it, the electrospinning solution was obtained after mixing for 2 h. The spinning solution was sucked into the syringe, fixed on the micro injection pump, and the spinning equipment was started under 25kV DC high voltage. The nanofibers were collected with tin foil paper, the receiving distance was 15cm, the injection speed was 1ml/h, and the spinning time was 4 hours. 0.005M silver nitrate solution and ascorbic acid solution were prepared. The nanofiber memembranes were immersed in silver nitrate solution and shaked for different times (10min, 20min, 30min), then ascorbic acid solutions were added dropwise and reacted for 30min. The Ag@PVA/CS substrate was prepared.

2.3. Characterization of the Ag@PVA/CS substrate:

The surface morphology of the substrates was characterized by field emission scanning electron microscopy (s4800, Hitachi, Japan). Fourier transform infrared spectroscopy (vertex 70, Brooke, Germany) was used to characterize the molecular structure of the substrates. Raman spectroscopy (DXR, Thermo, USA) was used to characterize the SERS performance. The excitation wavelength was 633nm and the power was 1 mW. Rhodamine 6G and Norfloxacin

solution were selected as the probe molecules to characterize the SERS performance of the substrates.

3. Results and discussions

3.1. Characterization of PVA / CS nanofibers

Fig. 1a shows the SEM images of the PVA/CS nanofibers. Most of the nanofibers have uniform morphology, and the fiber membrane has porous and random orientation, which can absorb more Ag^{+}. The diameter distribution of nanofibers was shown in Fig. 1b. The diameter of nanofibers ranges from 200 nm to 300 nm.

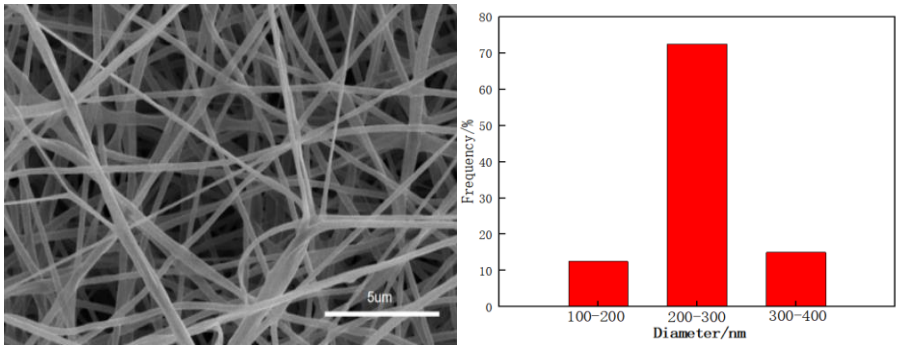

Fig. 1. (a) SEM image of PVA/CS nanofibers; (b) the diameter distribution of nanofibers.

Fig. 2 shows the FTIR spectra of the nanofiber membranes. The characteristic peaks at 3320cm^{-1}, 2940cm^{-1} are assigned to the expansion vibration of O-H and CH2 of PVA respectively. The absorption peak at 1329cm^{-1} is the coupling peak between O-H vibration and C-H vibration (1423cm^{-1}). The characteristic peak at 1558cm^{-1} is assigned to the N-H bending vibration of CS[11]. More Ag^{+} can be adsorbed by CS nanofibers with amine groups, then the AgNPs dispersed on the surface of membrane by in-situ reduction of ascorbic acid.

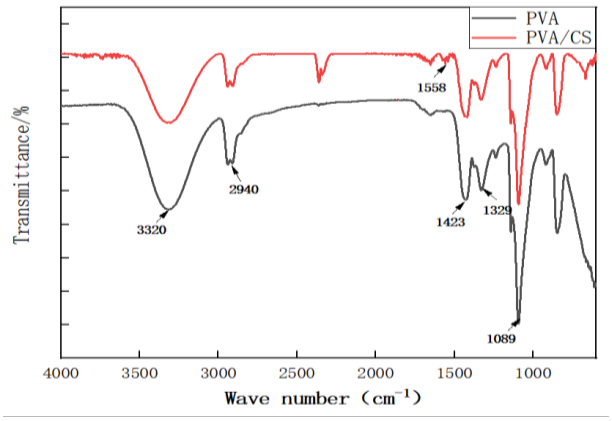

Fig. 2. FTIR spectra of the nanofiber membranes.

3.2. Characterization of Ag@PVA/CS substrate

Fig.3 shows the SEM images of Ag@PVA/CS substrates with different adsorption times of $AgNO_3$ solution (0.05M). The amount of AgNPs is little, as the adsorption time is 10 min (Fig.3a). Because the adsorption time is too short and the amount of adsorbed Ag^+ is less. The adsorption time reach to 20 min (Fig.3b), the amount of spheroidal (the inset of Fig.3b) Ag NPs are significantly increased and dispersed uniformly on the surface of the substrate. On the other hand, after adsorption and reduction, the nanofibers disappear and form a uniform membrane due to the hydrophilicity of PVA/CS. However, the adsorption time reach to 30 min (Fig.3c), the Ag NPs agglomerate obviously.

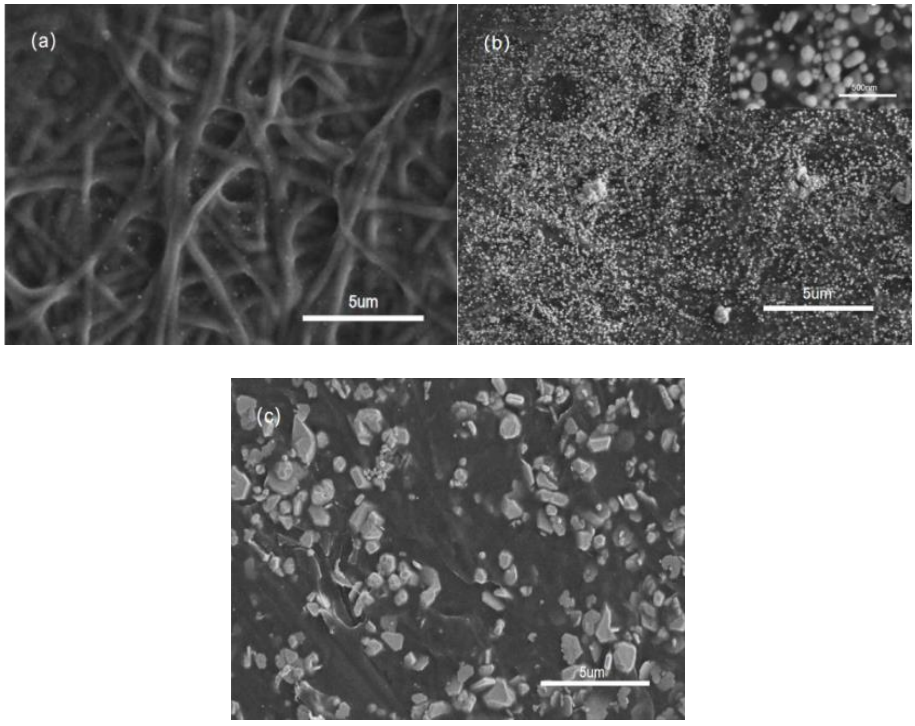

Fig. 3. SEM images of Ag@PVA/CS substrates with different adsorption times:(a) 10min (b)20min (c)30min.

The X-ray diffraction patterns of the substrates are shown in Fig.4. There is a wide diffraction peak between 17° and 23° in both PVA/CS and AgNPs@PVA/CS substrate, corresponding to the (101) plane of PVA semicrystalline structures[12,13]. The peaks at 38.5°, 44.5° and 64.7° correspond to the major characteristic peaks of face-centered-cubic (FCC) silver in AgNPs@PVA/CS substrate, which illustrates that Ag NPS decorated on the substrate.

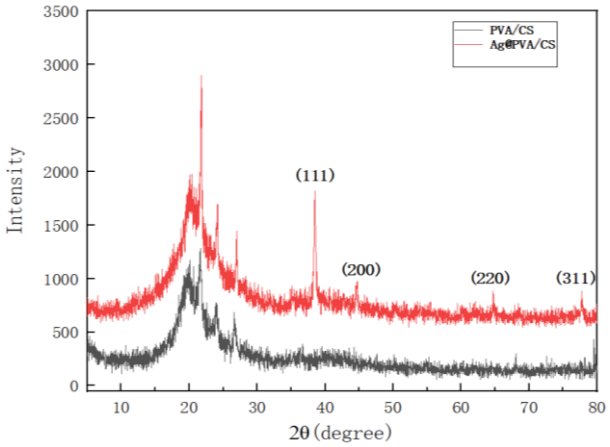

Fig. 4. XRD patterns of PVA/CS and Ag@PVA/CS substrates.

3.3. SERS performance

R6G is typically used as a probe molecular and in this study used to determine the SERS performance of the prepared Ag@PVA/CS substrates. First, the PVA/CS substrate and Ag@PVA/CS with different adsorption times were tested, as shown in Fig. 5 R6G signals are detected in Ag@PVA/CS substrates except pure PVA/CS substrate (0 min). The bands at respectively 610 cm^{-1}, 769 cm^{-1} and 1182 cm^{-1} are assigned to the C-C-C ring in-plane, out-of-plane bending and C-H stretching in-plane vibrations of R6G molecule. The other Raman bands at 1309 cm^{-1}, 1362 cm^{-1},and 1650 $cm^{-1}$are generally associated with the tretching vibrations of C-C bond [14]. The peak at about 610 cm^{-1} is used to stand for the SERS intensity. we can reveal that the SERS activity of Ag@PVA/CS substrates enhances with increasing of adsorption time, but decreases with further increase to 30min. If the adsorption time is too short, too few Ag NPs to form enough "hot spots". If the adsorption time is too long, the nanoparticles increase and aggregate, which affects the SERS performance.

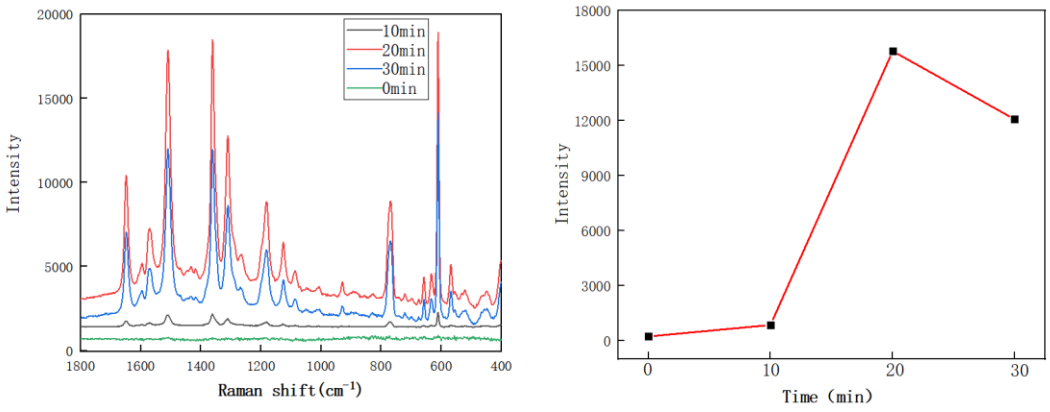

Fig. 5. (a) SERS spectra of Ag@PVA/CS substrates with different adsorption times (b)peak intensity of 610 cm^{-1} in different SERS spectra in (a).

Fig. 6 shows the SERS spectra of different R6G concentrations (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M) adsorbed on the Ag@PVA/CS substrates. As the concentration of R6G becomes lower, the peak values are weaker. The SERS substrate was able to detect R6G at a low concentration of 10^{-6} M, which indicates that the flexible SERS substrate has high sensitivity and application prospect.

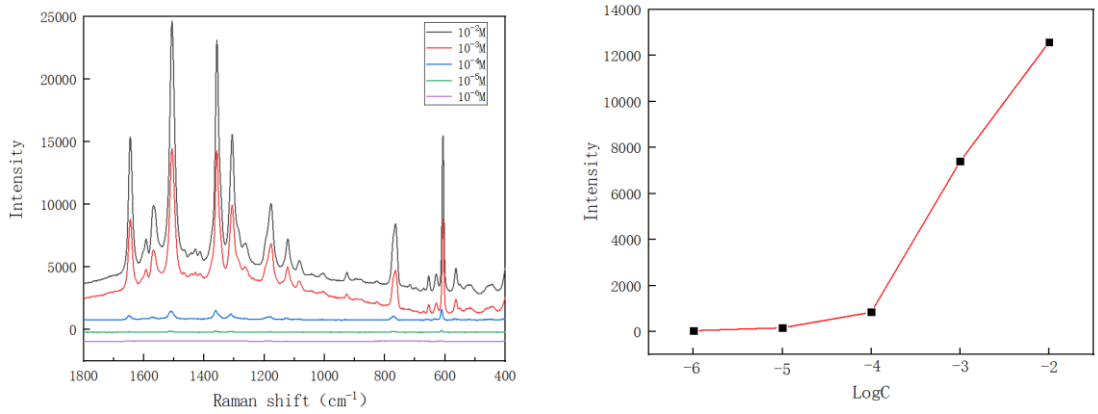

Fig. 6. (a) SERS spectra of Ag@PVA/CS substrates with different R6G concentrations (b)peak intensity of 610 cm^{-1} in different SERS spectra in (a).

In order to highlight the advantage of the prepared Ag@PVA/CS substrates. The SERS spectra of different norfloxacin concentrations adsorbed on the substrates were measured as shown in Fig.7. The band at 1625 cm^{-1} is assigned to the C=O stretching vibration, 1541 cm^{-1}, 1469 cm^{-1} are assigned to quinoline ring deformation vibration, 1389cm^{-1} is assigned to O-C-O in-plane bending vibration, 1294 cm^{-1} is assigned to C-F stretching vibration, 770 cm^{-1}, 746 cm^{-1} are assigned to quinoline ring breathing vibration[15]. It is clearly that the raman characteristics of analyte still can be distinguished at levels as low as 10^{-5} M. The SERS substrate can achieve a fast determination of norfloxacin residue.

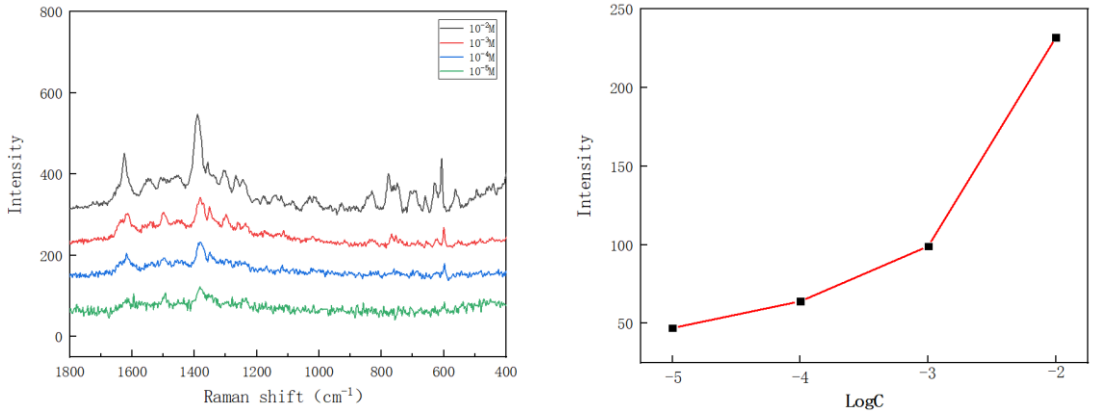

Fig. 7. (a) SERS spectra of Ag@PVA/CS substrates with different norfloxacin concentrations(b)peak intensity of 1389 cm^{-1} in different SERS spectra in (a).

4. Conclusions

In summary, sensitive and economical Ag@PVA/CS SERS substrates were prepared by electrospinning and in situ reduction. Silver ions adsorbed on nanofibers were decorated on the substrates after in situ reduction, which can form more “hot spots”. The SERS performance of the prepared substrate was evaluated by using rhodamine 6G as a probe. The detection limit was 10^{-6}M. The as-prepared SERS substrates were successfully applied in the analysis of norfloxacin. The detection limit of norfloxacin is 10^{-5}M. The prepared substrate has great development potential in sensitive, in-situ and on-site detection.

Acknowledgements

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