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Highly water-dispersible silver sulfadiazine decorated with polyvinyl pyrrolidone and its antibacterial activities

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ABSTRACT

Highly water-dispersible silver sulfadiazine (SSD) was prepared by liquid phase method with polyvinyl pyrrolidone (PVP) as a surface modification agent. The structure and morphology of the PVP-modified silver sulfadiazine (P-SSD) were investigated by X-ray powder diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), thermogravimetric analysis (TGA) and Fourier-transform infrared (FT-IR) spectrometry. The produced particles are ginkgo leaf-like architecture with the sizes of micron-nanometer. Due to hydrophilic PVP decorated on the surface, the P-SSD has excellent dispersion in water over a period of 24 h, which is obviously stable by comparison to that of the commercial silver sulfadiazine (C-SSD). In addition, the P-SSD exhibits good antibacterial activities against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*).

1. Introduction

Burn wound is a global public healthy problem, which can injure skin of protective immune system barrier, and subsequently lead to bacterial infections in the wounds [1]. Therefore, antibacterial processes are very important for the burn-wound treatment [2]. So far, silver sulfadiazine (SSD) is regarded as an ideal antibacterial agent for the reason of two-fold action on bacterial growth [3]. The sulfa moiety could prevent bacterial folate absorption and subsequent DNA synthesis, whereas the silver that is released from SSD binds and disrupts the DNA structure, precluding bacterial DNA replication [4–6]. However, due to the intrinsic hydrophobic moiety and large size, the antibacterial performance of SSD is commonly discounted by its poor aqueous solubility [7,8].

In recent decades, nanotechnology has been used to enhance the solubility or dispersion of nanoparticles in media [9,10]. Xie group fabricated ultrasmall water-soluble gold and silver nanoclusters via protein- and peptide-protected route for biomedical applications [11]. Szegedi et al. prepared SSD loaded on nanoporous silica particles with significant enhancement of water solubility, which showed sustained release properties and similar or even better antimicrobial properties than SSD [12]. Among them, surface modification is very popular to control the crystal growth by introducing biomaterials, inorganic materials, polymers and surfactants et al. in the process [13,14]. Poly (N-vinyl-2-pyrrolidone) (PVP) is a water soluble and low cytotoxic organic polymer, which is widely applied in the preparation of hydrophilic nanoparticles [15].

Here, PVP was introduced to synthesize PVP-modified silver sulfadiazine (P-SSD) particle for dual purposes (a) as a steric hindrance to decrease the size of SSD particle down to the micro-nanometer scale, and (b) as modified agent to improve the hydrophily of SSD. Therefore, the resulted SSD particle decorated with PVP is promising to display excellent antibacterial effects against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively.

2. Experimental section

2.1. Materials

Polyvinyl pyrrolidone (PVP, M_w = 111.143), absolute ethanol and ammonium hydroxide ($NH_3 \cdot H_2O$, 28%) were supplied from Luoyang Chemicals (Luoyang, China). Sulfadiazine (SD, M_w = 250.28) was purchased from Aladdin (China), Silver sulfadiazine (SSD, M_w = 367.14) was provided from Gracia Chemical Technology Co. (Chengdu). Silver nitrate ($AgNO_3$) was from Sinopham Chemical Reagent Company. All the chemical reagents were of analytical grade and were used without further purification. Nutrient broth (NB, BR) and nutrient agar (NA, BR) were received from Beijing Aoboxing biotech Company (Beijing, China). Gram-positive *S. aureus* (ATCC 35696), Gram-negative *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 23282) were selected as bacterial strains (China center of industrial culture collection, China).

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2.2. Synthesis of P-SSD

The ginkgo leaf-like P-SSD particles were synthesized by one-pot method in an aqueous solution. In a typical method, 1.0 g of SD and 0.2 g of PVP were firstly dissolved in 15 mL of diluted ammonium hydroxide solution (3 wt.%) in a flask by ultrasonic method. Then, 2 mL of $AgNO_3$ (4 mmol) was dropwised into the flask with constant magnetic stirring. The pH of the solution was adjusted to 9.0 with ammonium hydroxide and the reaction was carried out at room temperature (25 °C). When color of the solution was gradually changed from colorless to white, it indicated the formation of SSD. After 1 h reaction, the suspension was separated by centrifugation and washed with excess amount of ethanol at least 3 times. Finally, P-SSD powder was obtained by vacuum drying at ambient temperature.

2.3. Characterization of P-SSD

The morphology of P-SSD was analyzed with a NOVA Nano SEM 450 scanning electron microscope (SEM; acceleration voltage 5 kV) and a JEOL JEM-2010 transmission electron microscope (TEM; acceleration voltage 200 kV). The crystal structure of P-SSD was analyzed by X'Pert Philips X-ray powder diffraction (XRD; Cu Ka radiation), AVATAR 360 Fourier transform infrared spectrometer (FT-IR), and Seiko EXSTAR 6000 thermal analyzer (TG, N_2 atmosphere scanning rate of 10 °C/min). The antibacterial performance of optical density (OD 600) was collected by UV–Vis spectrophotometer (Oppler, 752 N, China).

2.4. Antibacterial tests

To investigate the antibacterial activity of P-SSD, Gram-positive *S. aureus*, Gram-negative *P. aeruginosa* and *E. coli* were selected as indicators. All disks and materials were sterilized in an autoclave before experiments. The minimum inhibition concentration (MIC) and the minimal bactericidal concentration (MBC) methods were applied to assess the antibacterial activity of the P-SSD. In MIC test, the serial P-SSD solutions with different concentrations were dispersed in sterilized tubes with 2 mL broth media by a twofold serial dilution method. Then 20 μL bacteria suspensions (10^8 CFU/mL) were added into the serial tubes, respectively. Meanwhile, the commercial silver sulfadiazine (C-SSD) sample was chosen as a comparative material and performed the similar process. Finally, the tubes were incubated at 37 °C for 24 h and the lowest concentration**s** of samples that inhibited visible growth of bacteria by turbidimetric method were signed as the MIC values.

MBC is defined as the minimum concentration of the sample required to kill 99.9% bacteria after a defined period of incubation [16]. In MBC test, the nutrient agar was spread onto a Petri plate, and then the invisible bacterial suspensions with different concentration**s** of samples taken out from the tubes were coated on the agar plates. Final agar plates with bacterial suspension were incubated at 37 °C for 24 h. The number of survival colonies was counted to get the MBC of the P-SSD. For comparison, serial C-SSD bacterial broth suspensions were performed as well.

The antibacterial activity of P-SSD sample against tested strains was also measured by cup diffusion method. The nutrient agar was first spread onto a Petri plate, and then 100 μL bacteria suspension was coated on the agar plate. Subsequently, oxford cups were placed on an agar plate, and 50 μL sample solutions with different concentrations were added into the cup hole. Meanwhile, 0.9% of sterilized saline was selected as contrast sample dropped into the central cup. After 24 h of incubation at 37 °C, the inhibition rings were measured to evaluate the antibacterial effect.

Antibacterial performance was also evaluated by the bacterial growth kinetics in the broth media. P-SSD was first dispersed in beef broth to yield a series of broth suspensions with concentrations of 3.91 μg/mL, 7.81 μg/mL, 15.62 μg/mL, 31.25 μg/mL, 62.5 μg/mL and 125 μg/mL, respectively. 2 mL of a certain concentration of sample solutions were mixed with 2 mL of sterilized broth media in 10 mL culture tubes. For comparison purpose, 2 mL of deionized water was mixed with 2 mL of sterilized broth media in 10 mL culture tube in the control test. Subsequently, 40 μL of bacterial suspensions were added into these tubes and incubated at 37 °C within 48 h. Finally, these tubes were withdrawn at set time intervals to measure the optical density (OD) at 600 nm. The parameter of optical density (OD600) was performed by UV–Vis spectrophotometer.

3. Results and discussion

3.1. XRD analysis

Fig. 1 shows the XRD patterns of both C-SSD and P-SSD. It is clear that both samples display similar diffraction peaks. In the patterns, the mainly characteristic peaks located at 8.8°, 10.2° and 18.5° are ascribed to (002), (011) and (020) planes of SSD [17,18], which indicates the intrinsic structure of P-SSD. In the further study, the characteristic diffraction peaks of sliver at 38.1°, 44.3°, 64.4°, 77.4° and 81.5° aren't found in the pattern [19]. Therefore, PVP didn't reduce SSD to Ag in the process.

3.2. Morphology analysis

Fig. 2 gives the SEM and TEM images of C-SSD and P-SSD. The C-SSD sample displays irregular firewood shape, with rough surface and the sizes in the micrometer range (Fig. 2a). Interestingly, when a certain amount of PVP was introduced, the ginkgo leaf-like P-SSD was obtained (Fig. 2b). P-SSD particle was composed of many radically growing nanosticks, with an average length of 10 μm and width of 100–200 nm (Fig. 2c). Additionally, the surface of P-SSD stick is smooth and the edge of P-SSD contains two parts (Fig. 2d). The inner deep gray part indicates SSD subject, and outer light gray part reveals PVP modified layer, which indicated that PVP was covered on the surface of SSD. Possibly due to the part of crystal growth active sites of SSD adsorbed, the uniform growth of SSD was inhibited, while the oriented growth was developed. Finally, the ginkgo leaf-like P-SSD was produced.

3.3. FTIR spectra

The FTIR spectra of both PVP and P-SSD are exhibited in Fig. 3a. For PVP, the peak at 1659 cm^{-1} can be assigned to the C═O group, the peaks at about 2945 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of $-CH_2-$ chains, and the band at 1296 cm^{-1} originates from the stretching vibration absorption of C–N group [20,21]. For pure SSD [22–24], the peak at 1590 cm^{-1} is assigned to

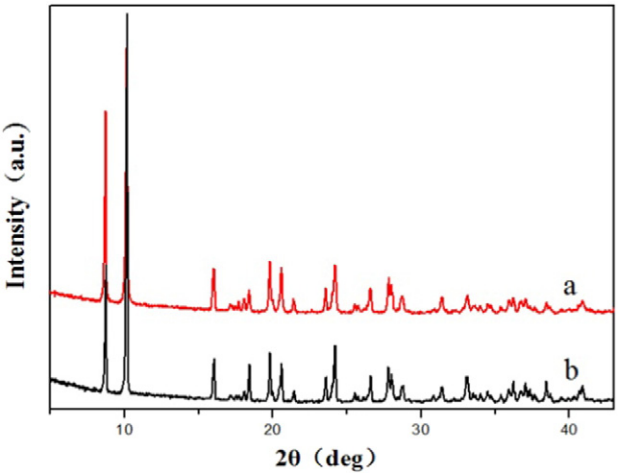

Fig. 1. XRD patterns of (a) P-SSD and (b) C-SSD.