Micelles Prepared with Cinnamaldehyde and Vancomycin by Schiff Base Reaction

Fancui Meng, Quanxin Wang, Zhuangzhuang Qiao, Yan Yao

Abstract—To design a micelle which has a hydrophilic inner shell formed with vancomycin and a hydrophobic outer shell formed with cinnamaldehyde, vancomycin and cinnamaldehyde will be linked with the schiff base reaction, and then a further research on its properties will be conducted. Vancomycin, a good hydrophilic antibiotic drug, is highly effective in the treatment of methicillin resistant Staphylococcus aureus. Cinnamaldehyde, owning the effects of sterilization and antiseptic, has obvious curative effect on fungi and is a hydrophobic drug. When vancomycin was reacted with cinnamaldehyde via schiff base reaction, self-assembled micelles, formed by their hydrophobic differences, were given with not only synergistic bactericidal action but also increasing the cycle time of drugs in vivo. At the same time, its pH response performance enhanced antibacterial properties and drug efficacy of micelles. This study focused on the preparation of micelles modified by cinnamaldehyde.

Index Terms—vancomycin, cinnamaldehyde, schiff base reaction, micelles.

I. INTRODUCTION

Vancomycin has been considered the last resort in the treatment of bacterial infections, which has a profound effect on the treatment of multidrug-resistant bacterial infection, especially the fatal infection of methicillin-resistant staphylococcus aureus [13]. However, the emergence of resistance for vancomycin has attracted worldwide attention, and new effective antibiotics are urgently needed to prevent drug-resistant bacterial infections.

For centuries, plant essential oils have been used against bacterial infections [46]. Due to its complex antibacterial mechanism, there are few reports on the antimicrobial messages of these volatile extracts. But according to many articles, antibacterial mechanism of plant essential oil was explained that oils contain hydrophobic small molecules of terpenoids and phenolic compounds, which can easily penetrate the cell membrane leading to the loss of a proton gradient, destroy the synthesis of adenosine triphosphate (ATP) and influence cell lysis effect, then attain the purpose of antibacterial.

Cinnamaldehyde, a compound derived from cinnamon bark, mainly used in foods, beverages, medical products, cosmetics and perfumes containing cinnamon. the results of antibacterial activity show that it has high potential in antibacterial field [18]. Sanlaead et al. studied the antibacterial activity against ten pathogenic bacteria, strong destructive bacteria and three strains of yeast and the results showed that cinnamaldehyde owns antibacterial activity to all tests.

Along with a development of times and the rapid spread of bacterial infection, we more urgently demand to develop new antibiotics, because the abuse of antibiotics caused a large number of resistant strains to have the rapid development opportunities [10]. The latter is becoming a major problem in modern medicine. Although there are many institutions and researchers searching for new antibiotics, which opportunities to enter the market is quite limited, a lot of research and development expenses are invested to develop a new antibiotics. Retrofitting or deriving existing antibiotics to replace the development of new antimicrobial agents is an effective way and dramatically shorten development time. changing the charge density, solubility, degradation, selectivity and efficiency of these drugs by functional groups are the most common method.

During researching anti-cancer drugs, hydrophilic and hydrophobic anticancer small molecules through chemical bond self-assemble into micelles which increase its effect on tumor tissue penetration. hydrophilic and hydrophobic small molecules system also have the opportunity to apply in the field of antibacterial. So antibacterial micelles, hydrophilic antibiotic vancomycin and hydrophobic cinnamaldehyde connected by schiff base reaction, was formed by self-assembly in the water [11], which can achieve 100 % of drug-loading rate, improve the effect of antibacterial, and possess pH response [12]. The micelle has synergistic effect and multiple antimicrobial effects [13].

As a promising antibacterial system, the self-assembling micelle of hydrophilic and hydrophobic drugs has made a lot of new research progress in recent years [14]. Drug micelle with core-shell structure was formed by hydrophilic and hydrophobic drugs self-assembly in the water, using molecular inter-atomic forces and hydrophilic differences [15]. Hydrophobic core of Drug micelle can increase the solubility of hydrophobic drugs by physical encapsulation. hydrophilic shell can prolong time of micelle in the blood circulation, and can put the hydrophobic drugs assembly to its internal, improve ability to control drug release and make the micelles stability in water medium [16][17]. Moreover, antibacterial drug micelles can effectively inhibit the growth of bacteria and other microorganisms, and the two drugs respectively exert their antibacterial effects, achieving multiple antibacterial effects [18].
Based on the above analysis and summary, hydrophobic cinnamaldehyde modified hydrophilic vancomycin by schiff base reaction and self-assembled into micelles (Van-Cin), a new nano-antibacterial composite materials. The successful synthesis of Van-Cin complex was characterized by 1H NMR, FTIR and UV-Vis. The morphology and structure of Van-Cin micelles were characterized by TEM and laser particle size scattering apparatus (DLS).

**Fig.1.** Self-assembly mechanism of nanoparticles from Van-Cin.

**II. EXPERIMENTAL AND METHODS**

**A. Material**

N,N-Dimethylformamide (DMF), triethylamine (TEA), sodium carbonate (Na2CO3) were purchased from Tianjin ZhiYuan Reagent Co. Ltd. Cinnamaldehyde and vancomycin hydrochloride were purchased from Aladdin. All were used without further purification.

**B. Synthesis of Van-Cin complex**

In three bottles, 178.4 mg vancomycin hydrochloride and 20 mL mixture of methanol and DMF (2:1) were added, ultrasonic dissolving. Then, adding suitable TEA in temperature 60 °C[19], the mixture was slowly dropped into 20 mL intense stirring deionized water. After stirring for 30 min, Van-Cin solution was transferred to dialysis bag (MWCO 500), and dialyzed to remove the organic solvent at room temperature for 48 h in deionized water. Van-Cin micelles was kept at 4 °C[20, 21].

**D. Characterization of Van-Cin complex and Van-Cin micelles**

1. An Agilent-NMR-VNMR 400 (400 MHz) was used to record 1H NMR with deuterium dimethyl sulfoxide (DMSO-d6) as the solvent.

2. Infrared spectrogram were obtained on KBr slice with the Nicolet-560 spectrophotometer.

3. Zetasizer analyser (Zetasizer Nano, Zen 3690+MPT2, Malvern, UK, DLS) was used to measure size, zeta potentials, pH responsiveness and stability of the micelle in aqueous solution.

4. The morphology of the micelle was observed by transmission electron microscope (TEM, H-600IV, Japanese Hitachi company).

5. Determination of vancomycin in Van-Cin micelles. A series of vancomycin solution with different concentrations (0.01, 0.00750, 0.0050, 0.0025, 0.001 mg/mL, 3 mL) draw a standard curve, Y = 0.201 X - 0.111, R2 = 0.9900, and then 3 mL absorbance of Van-Cin micelles was measured through the standard curve to calculate content of vancomycin in micelles. Ultraviolet absorption (UV) was tested by Unicam UV500 ultraviolet visible spectrophotometer.

**III. RESULTS AND DISCUSSION**

**A. The 1H NMR and IR spectra of Van-Cin complex**

Through the characterization of 1H NMR and IR, it is proved that Van-Cin complex was successfully prepared. The 1H NMR spectra of raw materials and Van-Cin complex is shown in Fig. 3. In the 1H NMR spectra of Van-Cin complex, the resonance peak at 7.50 ppm (peak a, -CH = CH- CH = N-) is assigned to the protons on the cinnamaldehyde units. The resonance peak at 7.98 ppm (peak a, -CH = CH- CH = N-) is assigned to the protons on the Van-Cin complex units. The presence of these peak proved Van-Cin complex was synthesized successfully.

The IR spectrum of Van-Cin complex, vancomycin and cinnamaldehyde are shown in Fig. 4. Comparing IR spectrums, A stretching vibration at 668 cm⁻¹ corresponding to -CH = CH- is obviously observed at the spectrum of Van-Cin complex. The result can prove that cinnamaldehyde was connected with vancomycin.

**Fig.3.** The 1H NMR of Van-Cin and vancomycin.
B. The TEM and DLS of Van-Cin micelles
The size and morphology of the micelles can be observed directly from the TEM. In Fig. 5 A and B, the morphology of Van-Cin micelles is spherical, and the distribution of micelles is homogeneous, the size and potential of Van-Cin micelles and vancomycin is shown in Fig. 5 C. the size of Van-Cin micelles is about 69.10 nm and unimodal, but vancomycin is triple peak. The result demonstrates Van-Cin micelles was synthesized successfully.

C. The UV spectrogram of Van-Cin micelles
The UV absorption spectrogram of Van-Cin micelles, vancomycin, cinnamaldehyde and mixture (Van+Cin) are shown in Fig. 6. Absorption peak at 280 nm proved the existence of vancomycin in the micelles. Comparing with ultraviolet absorption peak of vancomycin, cinnamaldehyde and Van+Cin, it is found that absorption peak of Van-Cin micelles produces a blue shift, and confirmed to form Van-Cin micelles.

D. The pH responsiveness of Van-Cin micelles
In order to study the pH responsiveness of Van-Cin micelles, the size and potential of micelles under different pH conditions were examined. In Fig 7, Van-Cin micelles is not stable under acid condition, size and potential all show a growth trend. Because C = N bond of Van-Cin micelles occurs disintegration under acid condition and the micelles disintegrate. The phenomenon complies with the requirements of the experimental design, and the pH response can be came ture, using to accurately release the drug in the location of bacteria.

E. The stability analysis of Van-Cin micelles
Before Van-Cin micelles is applied to clinic, it is necessary to ensure its stability when micelles can reach the location of bacteria and then disintegrate to generate the effect of sterilization. To test the stability of Van-Cin micelles, the size distribution of Van-Cin micelles in 18 days (pH = 7.4) were observed and shown in Fig. 8. the result of size distribution proves that the micelles are stable and not prone to collapse.
Micelles Prepared with Cinnamaldehyde and Vancomycin by Schiff Base Reaction

ACKNOWLEDGEMENT
This work has been funded by College of Chemistry and Environmental Protection Engineering, Southwest Minzu University.

REFERENCES