Rapid Detection of Clenbuterol Using Au Nanoparticles Base on Surface-Enhanced Raman Scattering

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***Abstract*—Au nanoparticles (AuNPs) colloid with a good dispersion was synthesized by chemical reduction method and their properties as surface-enhanced Raman scattering substrate were examined. The average particle size obtained is about 80 nm and the UV-vis characteristic absorption peak appears at 528 nm, which is a typical characteristic absorption peak of the AuNPs. The AuNPs colloid was used as substrate to detect Clenbuterol, and the lowest detection limit is 1mg/L. The result shows that the Au nanoparticles have a good surface-enhanced Raman scattering activity.**

Keywords—Surface Enhanced Raman Spectroscopy; Au nanoparticles; Clenbuterol

I. INTRODUCTION

Clenbuterol (CL) is a kind of β-adrenergic agonist, commonly known as "lean meat powder" in China, which can increase lean meat-to-fat ratio by at least 10% by altering the metabolic pathways of animals[1]. The excess of meat which have high CL residues can lead to serious side effects such as increasing of heart rate, headaches, and food poisoning[2]. China has explicitly banned the addition of CL to feed and drinking water, but the CL poisoning incidents still occur sometimes[3]. Thus, it is required to develop an efficient and rapid CL detection method to effectively monitor the safety of livestock products and protect the health of consumers.

The traditional methods for detection of CL are mainly high performance liquid chromatography (HPLC)[4], gas chromatography-mass spectrometry (GC-MS)[5], capillary electrophoresis, and enzyme linked immunoassay (ELISA)[6]. However, these methods need expensive instruments and experienced technicians. Surface Enhanced Raman spectroscopy (SERS), a special surface optical phenomenon at the nanometer scale, has recently used for CL detection[7]. SERS exhibits a high sensitivity for CL detection, improving the reproducibility of SERS intensity measurements and distinguishing different molecules according to different characteristic Raman band.

In this study, AuNPs synthesized by chemical reduction method were successfully used in the SERS detection of CL, including the calculating the theoretical Raman spectrum of CL and identifying the corresponding vibrational modes. The SERS intensity versus Clenbuterol concentration was systematically determined and the limit of detection is obtained, which provide a rapid and easy method for CL detection.

II. EXPERIMENTAL SECTION

A. Materials and reagents

Tetrachloroauric Acid ($HAuCl_4$), sodium citrate ($C_6H_5Na_3O_7$), methanol (≥99.9%) and ethanol (≥99.7%) were obtained from Accelerating Scientific and Industrial Development there by Serving Humanity (China). CL was acquired from purchased from Dr. Ehrenstorfer GmbH and Kwinbon Biotechnology. Deionized water (≥ 18.2 MΩ) was used in all experiments.

B. Preparation of AuNP colloid

The synthesis of AuNPs colloid followed a reduction of $HAuCl_4$ solution with sodium citrate introduced by Turkevich[8]. 4.8 mL 1% $HAuCl_4$ solution was added into 100 mL deionized water and heated to boiling, and then 10 mL 1% Sodium citrate solution was slowly added. The color of solution changed from dark to brown in 10 min. The solution remained under reaction for another 20 min, and then cooled to the room temperature. Before SERS detection, 50 mL of the synthesized gold colloid concentrate into 5 mL to use.

C. SERS measurement

Different concentrations of CL (C=1, 5,10, 50, 100 mg/L) were prepared using methanol in order to determine the SERS intensity versus C calibration curve and the limit of detection of CL.

For CL sample detection, 5 μL drop of colloidal AuNPs was placed on the surface of the silicon substrate and 5 μL CL sample solution was dropt on the AuNPs colloidal. Then the mixture solution was analyzed by a Raman analyzer at a 30 mW laser power and the integral time 10 s. Each sample was measured at least nine random locations.

D. Instrumentation

The optical absorption spectrum of AuNPs was characterized by a UV−vis spectrophotometer. The morphology of the AuNPs was examined using scanning electron microscopy (SEM). SERS measurements were taken with a portable Raman spectrometer (ProRaman-L-785A2, Enwave Optronics, Irvine, CA) with an excitation wavelength of 785 nm, a laser power of 30 mW, and an integration time of

10 s. The average SERS spectra were obtained from nine di□erent locations on the substrate.

III. RESULTS AND DISCUSSION

A. Characterization of Au nanoparticles

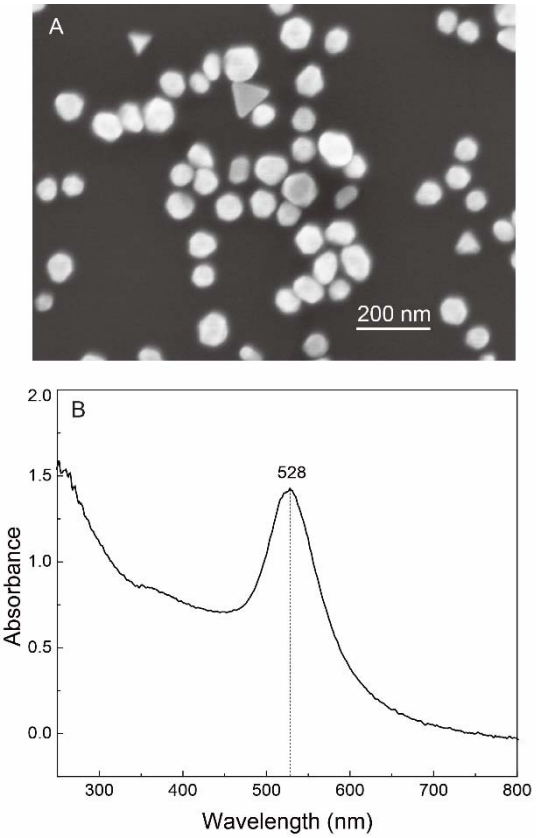

Fig. 1. (A) Top view SEM image of the AuNPs, (B) UV-Vis absorption spectrum of AuNPs.

During the synthesis experiment, when the sodium citrate solution was added into $HAuCl_4$, the color of the multiple solution quickly turned dark. With the heating time increasing, it turned purple and finally turned red. When the color was stable in red, the AuNPs colloid solution has been completed. Fig. 1A is a SEM image of Au nanoparticles prepared by sodium citrate aqueous solution system. As can be seen from the SEM image, the Au nanoparticles have uniform size distribution with an average size of 80 nm. The shapes of AuNPs colloidal are spherical and have a good dispersion. Numerous studies have shown that, AuNPs with such morphologies have a better SERS performance, which is beneficial to the further application of gold nanoparticles.

Fig. 1B shows the UV-Vis absorption spectrum of Au nanoparticles. UV-vis absorption spectroscopy is a common method for the investigation of AuNPs colloidal because it has completely different responses to metal particles in highly dispersed and associated states. By analyzing the spectrum, important information such as particle size and shape can be obtained. The wavelength and shape of absorption peak are closely related to the size, shape, and dispersion state of the particles. The absorption peak appearing near the wavelength of 528 nm can be regarded as the plasmonic resonance absorption peak of the Au nanoparticles.

B. DFT-calculated and experimental Raman and SERS spectrum of CL

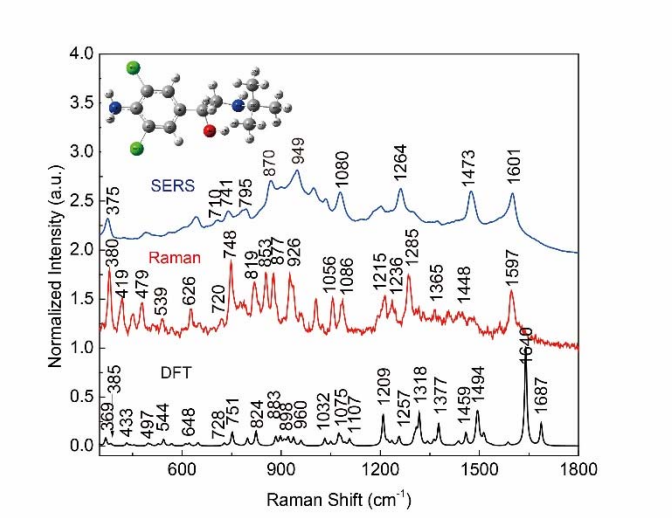

Fig. 2. Raman spectrum calculated by DFT (black) and the experimental Raman (red) and SERS (blue) spectrum of CL. Spectra were normalized to the most intensive peaks and offset for clarification.

In order to identify the corresponding vibrational modes of the CL, the Gaussian 09 W DFT software based on Becke's three-parameter exchange function (B3) was used to calculate the theoretical Raman spectrum of CL. The B3LYP function and a modest 6-311g (d) basis set were used to optimize the molecular structure of CL. Fig. 2 shows the comparison of the Raman spectrum of CL calculated by DFT, Raman spectrum of the CL and the SERS spectrum of 100 mg/L CL.

The most characteristic peaks of the experimental SERS spectrum match with that of theoretical SERS spectrum. The slightly difference between the experimental SERS spectrum and the DFT-calculated spectrum owing to the base group setting of DFT cannot match the experiment conditions perfectly. The SERS characteristic peaks of CL are found at $\Delta\upsilon$ = 375, 710, 741, 870, 949, 1080, 1264, 1473 and 1601 cm^{-1}. The peak at $\Delta\upsilon$ =1264 cm^{-1} was attributed to the C-N stretching and C=C ring symmetric stretching; the $\Delta\upsilon$ =1473 cm^{-1} peak results from the C-H rocking; the $\Delta\upsilon$ =1601 cm^{-1} peak corresponds to the C=C-Cl ring symmetric stretching[9].

C. SERS performance of AuNPs for CL

In order to determine the detection limit of CL, the SERS detection of the CL molecule at different concentrations from 1-100 mg/L was performed using the synthesized AuNPs colloid. Fig. 2A is the SERS spectra of CL in methanol of various concentrations. As we can see, the dominant peaks at $\Delta\upsilon$ = 1264, 1473 and 1601 cm^{-1} were found in the control sample. For the spectra of CL, the intensity of that three characteristic peaks become weaker as the decrease of CL concentration. We plot the peak intensity at $\Delta\upsilon$ = 1473 cm^{-1} in Fig. 2B to quantify the SERS peak intensity and CL concentration. The result exhibits a linear relationship of intensity versus concentration in the range of 1-100 mg/L

(I_{1473}= 9.1+ 431*logC*) with the R^2 = 0.99. Based on these results, the lowest concentration can be detected as 1 mg/L with the 3σ method (σ is the mean square root of the noise signal, which was determined by standard deviation of the spectral intensity at a spectral region: 1700–1800 cm^{-1}). Thus, the LOD for CL was approximately 1 mg/L using the AuNP colloid.

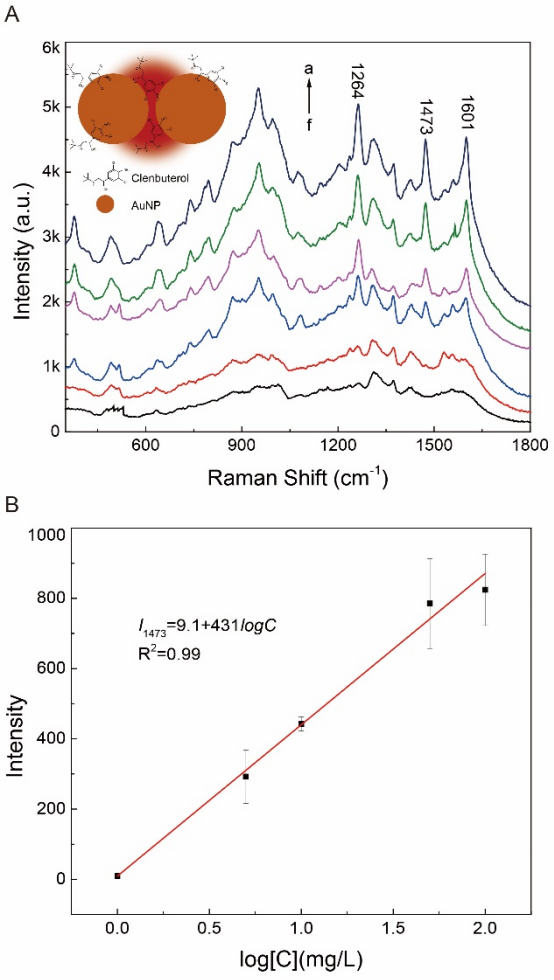

Fig. 3. (A) SERS spectra of Clenbuterol in methanol of various concentrations on AuNPs substrate: (a) 100 mg/L, (b) 50 mg/L, (c) 10 mg/L, (d) 5 mg/L, (e) 1 mg/L, (f) control line. (B) the corresponding intensity of the characteristic peak at 1473 cm^{-1}.

IV. Conclusions

Au nanoparticles prepared through chemical reduction method were studied in ultraviolet-visible absorption spectroscopy, particle size analysis, morphology characterization. The results show that the Au nanoparticles have excellent SERS enhancement performance and can be used in CL detection. The limit of detection can reach 1 mg/L which provide a sensitive and reliable new method for on-site detection of CL.

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