Dendritic Silver Microstructures as Highly Sensitive SERS Platform for the Detection of Trace Urea

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Abstract. In this communication, Surface-Enhanced Raman Scattering (SERS) coupled with Ag dendrites has been firstly used for the detection of limited urea. Dendritic silver microparticles are immobilized on indium doped tin oxide (ITO) surface via replacement reaction, and the formation mechanism could be attributed to electroless deposition (ELD) and diffusion-limited aggregation (DLA), respectively. The plot of the average Raman intensity measured at 1004 cm⁻¹ as a function of urea concentration with R² value of 0.999 indicates silver dendrites exhibit a satisfactory and consistent performance with an analytical urea enhancement. The limit of detection for urea could reach the physiological range of urea.

1. Introduction

Urea [CO(NH₂)₂], an end product of nitrogen metabolism and a normal constituent of milk, is widely distributed in nature and our daily life [1, 2]. The normal level of urea in serum is from 0.15 to 0.4 mg/ml [3]. Supra-physiological concentration of urea in alcoholic liquid may increase oxidative stress in cells and generate ethyl carbamate, a known carcinogen that has been reported [4]. Therefore, trace urea detection and its estimation have great significance in clinical analyses and food processing technology.

Although there are a variety of techniques that can be used for the detection of urea, the most analytically attractive ones are those enable real-time monitoring possibilities [5, 6]. Raman methods are well known to be particularly sensitive for the detection of nitrogen-containing compounds, and moreover, extensive studies have also demonstrated that silver microstructures show excellent SERS behaviors. Besides the components, the surface morphologies of silver microstructure are also important to their Raman performance [7, 8]. Due to their high surface-area-to-volume ratio, dendritic silver microstructures always exhibit outstanding electromagnetic and chemical enhancement, which endow them with superior SERS activity and sensitivity. For example, Song et al. [⁹] found remarkable
enhancement in the SERS spectrum of 4-pyridinethiol (PySH) adsorbed on the minute silver dendrites. Albert et al. [10] observed that silver dendrites were highly active in detection of analytes with aromatic rings and thiol groups. Although dendritic silver microstructures exhibit such good performances, none of them has been applied in the detection of trace urea.

Herein, we demonstrate the synthesis of dendritic silver microstructure on ITO glass by galvanic replacement from pre-deposited flower-like copper. The obtained dendritic silver microstructure is used as a SERS substrate in the detection of urea, on which the detection limits and standard deviation are also carefully investigated.

2. Material and Methods
All the chemical reagents are analytical grade and used without further purification. The typical synthetic process is described as follows. Firstly, an ITO is rinsed with acetone, ammonia and ethanol under an ultrasonic condition, respectively. After drying at the room temperature, Cu is electrochemically deposited on the cleaned ITO glass, using 10ml of electrolyte solution containing 0.1ml of 0.1M CuCl$_2$ and 1mM of cetyltrimethylammonium bromide (CTAB) as the electrolyte. Thereafter, the ITO glass coated by copper film is immersed in 0.1M ascorbic acid (AA) for 5min, and then soaked into the 0.1M AgNO$_3$, where Ag is spontaneously grown on the ITO surface.

Scanning electron microscopic (SEM) images of the prepared material are taken on a FEI Inspect SEM, and the color pictures are gathered from NIR enhanced deep depletion CCD (STD). For detection of urea solution, the SERS measurement is performed on Renishaw in Via Micro-Raman spectroscopy system using a 532nm wavelength laser set to 1mW. Typically, spectra are measured with 15s exposure time. The wavenumbers are calibrated using a silicon wafer with the spectrum centered at 521cm$^{-1}$. Urea solution for Raman detection is prepared in concentrations of 0.2, 0.5, 1, 5, and 10mg/ml in deionized water, and all of measurements on the analyte are repeated three times.

3. Results and Discussion

Figure 1. Experimental schematic for the formation of silver dendrites on the ITO substrate.

Figure 1a represents the electrodeposition process for fabricating copper film on ITO surface. It is worth noting that the relative high over-potential promotes the growth process of flowerlike copper film. Under higher over-potential condition, large amount of OH$^-$ is produced during the water electrolysis to form precursor Cu (OH)$_n$$^{2-}$. Then, the Cu (OH)$_n$$^{2-}$ anions company with the cationic CTA$^+$ would produce Cu (OH)$_4$$^{2-}$-CTA$^+$ ion pairs, which may also accelerate the movement of copper ions toward the cathode [11]. Figure 1b and figure 1c depict the schematic of copper film treated with
AA immersed in silver nitrate solution. Upon immersing the as-prepared ITO substrate in 0.1M AgNO$_3$ solution, replacement reaction occurs simultaneously at all Cu-AgNO$_3$ solution interfaces. Figure 1d indicates the dendritic silver structure obtained via galvanic replacement reaction. The growth of Ag is extremely fast, and Ag particles with dendritic morphologies could fully cover the ITO surface in less than one minute. It should be mentioned that the reaction between copper oxide and AA should be carried out completely, so that the resultant metal copper can instruct the growth of Ag microstructure during the replacement reaction. The typical mechanism of the above procedure of copper with silver nitrate can be described as equation (1):

$$Cu + AgNO_3 \rightarrow Ag + Cu(NO_3)_2$$  \hspace{1cm} (1)

Figure 1d indicates the dendritic silver structure obtained via galvanic replacement reaction. The growth of Ag is extremely fast, and Ag particles with dendritic morphologies could fully cover the ITO surface in less than one minute. It should be mentioned that the reaction between copper oxide and AA should be carried out completely, so that the resultant metal copper can instruct the growth of Ag microstructure during the replacement reaction. The typical mechanism of the above procedure of copper with silver nitrate can be described as equation (1):

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Figure 2. Optical micrographs of replacement reaction between Cu and AgNO$_3$ at different reaction stages.

Figure 2a shows the optical micrograph of copper film electrodeposited on the ITO substrate. It exhibits a fractal character, and some copper oxide branches decorated at the edge of flowerlike structure could be observed in the inset of figure 2a. The SEM images at two different magnifications are shown in figure 2b and figure 2c, exhibiting uniform silver dendritic microstructure with silver nanoparticles around them. The individual dendrite length is about 5–10μm and is composed of symmetrical branches. After the ELD process, the former space interface between two copper flowers is occupied by silver nanoparticles. Energy dispersive spectrum (EDS) further confirms that there is no copper residual on ITO glass.

To shed light on the formation mechanism of these dendritic silver microstructures, their growth process has been examined by extending the reaction time (figure 2d). As the reaction proceeds, small silver grains may have sufficient time to relax and minimize the energy. After 10min reaction, the products convert their shapes from dendritic to compact hexagonal. Morphological transition during the formation of silver microstructures by ELD and DLA methods has proven to be a powerful tool for understanding range of dendritic and fractal growth theory, which is in good agreement with the previous literature [12]. For our current substrate, dendritic branching growth enables facile coverage of the substrate with silver nanoparticles while avoiding growth of a thicker layer or increasing nucleation density.
In order to evaluate the sensitivity of detecting urea by SERS, a series of concentrations of urea (0.2, 0.5, 1, 5, and 10mg/ml) are deposited on the spots of silver dendrites and analyzed by Raman spectroscopy. When urea molecule is in close proximity to a dendritic silver surface that supports regions of heightened electromagnetic field intensity, giant enhancement of nitrogen moiety at about 1000 cm⁻¹ could be unambiguously observed in figure 3. As the surface plasmon and electromagnetic enhancement is produced from the interaction between urea and the "hot spots", the SERS and Raman characteristic band of the symmetrical C-N stretch transfer from 1016cm⁻¹ to 1004cm⁻¹. Besides the main bands at 1004cm⁻¹, the Raman spectrum of urea (0.2~10mg/ml) shows another five Raman bands (528cm⁻¹, 1158 cm⁻¹, 1535 cm⁻¹, 1600 cm⁻¹, 1670 cm⁻¹). These bands may be attributed to the interaction between the analyte molecule and metal microparticles, which, according to previous literature reports [13-15], could be assigned to N-C-O bending, NH2 rocking and NH2 bending vibrations, respectively.

From Figure 4, it can be seen that the correlation of the urea 1004cm⁻¹ band intensity with different urea concentration (0.2~10mg/ml). Where error bars are drawn taking into account the measurement accuracies and reproducibility of the SERS-substrate. Relationship between the peak height in SERS spectra and concentrations of the sample is examined. The SERS-urea concentration curve is nearly flat between 0.2 and 10mg/ml with a R² of 0.999, which indicates that the substrate exhibits perfect performance in trace urea detection. The results suggest that the as-prepared substrate in our study is capable of identifying and detecting the urea well among the physiological concentration in urine, which can be used to monitor kidney activity of a patient. In addition, the reproducibility of the Raman intensity is another interesting parameter that can be easily estimated since 3 parallel tests are performed for each concentration. Calculating the standard deviation, it appears that the substrate has very reproducible SERS ability since their error bars indicate two standard deviations are very short.

4. Conclusions
In summary, the fabrication of a SERS based silver dendrites for the detection of urea in an aqueous situation is presented. The results suggest that dendritic silver microparticles are capable of detecting urea well among the typical concentrations in physiological condition, as well as the potential of application in biosensor of urea. We believe this fabrication of dendritic silver microparticles on ITO substrates can be appealing as SERS platform for detection of trace chemicals and toxicants, and we plan to test these hypotheses in a future study.
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