SERS-active Ag Nanostars Substrates for Sensitive Detection of Ethyl Carbamate in Wine

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A simple and sensitive surface-enhanced Raman scattering (SERS) method for the detection of ethyl carbamate (EC) is reported in this work. Star-shaped silver nanostars (Ag NSs) were used as a novel SERS substrate. In comparison to other plasmonic nanoparticles (NPs), including Au NPs, Au NSs and Ag NPs, Ag NSs exhibit best SERS activity. Raman signal of EC at a trace level can be enhanced by several orders of magnitude with the help of Ag NSs. The Raman intensity of EC increased linearly with an increase of the EC concentration in the range from $5 \times 10^{-9}$ mol L$^{-1}$ to $1.0 \times 10^{-4}$ mol L$^{-1}$ with detection limit (LOD) of $1.37 \times 10^{-9}$ mol L$^{-1}$ ($S/N = 3$). The developed SERS approach also has the advantages of being simple, fast and requiring less amount of the sample. It could serve as a useful technology for the rapid determination of EC in both alcoholic beverages and fermented food.

Keywords SERS, surface-enhanced Raman scattering, ethyl carbamate, silver nanostars, detection

(Received February 25, 2016; Accepted March 3, 2016; Published July 10, 2016)

Experimental

Chemical and materials

Silver nitrate (AgNO$_3$, 99%), trisodium citrate dihydrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O, 99%), chloroauric acid (HAuCl$_4$·4H$_2$O, 99%), poly(vinyl alcohol) (PVA, 99%), sodium borohydride (NaBH$_4$, 96%), ascorbic acid (C$_6$H$_8$O$_6$, 99%), sodium hydroxide (NaOH, 96%), absolute
alcohol, 65% nitric acid, 37% hydrochloric acid and Tween-20 (C₃₆H₇₁O₁₃Na₃) were purchased from Sinopharm Chemical Reagent Co., Ltd. Hydroxylamine solution (HA, 50%, v/v in water) was purchased from Beijing InnoChem Science & Technology Co., Ltd. Silicon slides (5 × 5 mm) were purchased from Suzhou Crystalline silicon Electronics Technology Co., Ltd. Ultrapure water (18.2 MΩ•cm) was produced using a Millipore water purification system. The alcoholic beverage (Red Star Wine, a wine made from cereal crop, 56% vol) used in this work was purchased from a local supermarket. All glassware were cleaned by nitric acid, and rinsed thoroughly by ultrapure water before use.

Apparatus

Absorption spectra were measured on a TU-1901 double beams UV-vis spectrophotometer with a quartz cuvette of 1 cm optical length (PERSEE, China). The morphology of the NPs was characterized by electron microscopy (SEM) (Japan), operated at an accelerating voltage of 15 kV. SERS spectra were measured with a Raman microscope (in Via, Renishaw, Britain), using a ×50 objective.

Synthesis of Ag NSs, Ag NPs, Au NSs, Au NPs

Ag NSs were synthesized according to the literature, the procedures were as follows: an amount of 500 μL 6.0 × 10⁻² mol L⁻¹ HAuCl₄ under stirring for 5 min. Then, an aliquot of 500 μL 5.0 × 10⁻² mol L⁻¹ NaOH solution was added into the mixture under vigorous stirring, and the final pH of the solution was adjusted to 5.8. Subsequently, an amount of 100 μL 4.13 × 10⁻² mol L⁻¹ trisodium citrate dihydrate was added into the mixture. The color of the solution changed from colorless to grass green. After 48 h, the Ag NSs solution was centrifuged at 6000 rpm for 10 min, and the precipitates were then dispersed in ultrapure water for subsequent use.

For the synthesis of Au NSs, the procedures were according to the literature: an amount of 10 mL 1.0 × 10⁻³ mol L⁻¹ AgNO₃ and 200 μL 0.1 mol L⁻¹ ascorbic acid were quickly added to the mixture under moderate stirring. The pH of the solution was adjusted to 3 by adding an appropriate amount of 1.0 mol L⁻¹ HCl. An aliquot of 1.5 mL 1% trisodium citrate dihydrate was quickly added into the above solution under vigorous stirring. The mixtures were heated for 15 min until the color turned from light yellow to wine red. The solution was gradually cooled to room temperature and stored at 4°C. An aliquot of 400 μL the synthesized Au seeds solution was added dropwise in 40 mL of 2.5 × 10⁻⁴ mol L⁻¹ HauCl₄ under moderate stirring. The pH of the solution was adjusted to 3 by adding an appropriate amount of 1.0 mol L⁻¹ HCl. An amount of 400 μL 1.0 × 10⁻³ mol L⁻¹ AgNO₃ and 200 μL 0.1 mol L⁻¹ ascorbic acid were quickly added to the mixture simultaneously. The color of the mixture rapidly changed from pink to blue, and finally to blackish green in 30 s. The Au NSs were centrifuged at 6000 rpm for 10 min, and dispersed into 0.05% Tween-20 for subsequent use.

Also, 20 nm Au NPs were also prepared by sodium citrate reduction. HauCl₄ was reduced to Au NPs in the presence of 1% trisodium citrate dehydrate. Then, 20 nm Ag NPs were also synthesized by the reduction of AgNO₃ through sodium borohydride. NPs were centrifuged at 10000 rpm and 7000 rpm for 10 min, respectively, and dispersed in ultrapure water.

SERS signal dependent EC detection

A alcoholic beverage samples along with standard-addition samples were analyzed with the SERS method. After an aliquot of 20 μL of SERS substrate was dropped on the silicon slide, then 10 μL of a sample solution was dropped onto the dried SERS substrate, the measurement was taken after the sample solution was dried. A 785-nm line laser was used as the excitation source. The laser power for the sample was set to 0.5%. A spectral range from 300 to 2000 cm⁻¹ was recorded with a resolution of 1 cm⁻¹. All of the Raman spectra were recorded and processed with a LabSpec 5.0 from Renishaw, and were exported to Origin 8.5 for further evaluation.

Results and Discussion

Synthesis and characterization of Ag NSs

The synthesized Ag NSs exhibited a uniform size and morphology. As illustrated in SEM images (Fig. 1), Ag NSs show a star-shaped morphology with several branches and protuberances. The average diameters of Ag NSs were in the range of 400 - 500 nm. Ag NSs exhibited an obvious SPR peak at 390 nm (Fig. 2). The different numbers of branches enabled Ag NSs to have different vertex angles, resulting in the formation of intense background absorption.

SERS activity of EC on Ag NSs substrate

Solid EC exhibits several characteristic Raman bands (Fig. 3A). The characteristic Raman bands for EC molecule in the range from 300 to 2000 cm⁻¹ are 396, 512, 672, 854, 996, 1076, 1127, 1150, 1273, 1346, 1440, 1457, 1622, 1688 cm⁻¹, attributing to carboxyl group, C–C, C–H, N–H stretching and deformation vibrations. Among them, 996 cm⁻¹, 1440 cm⁻¹ are attributed to C=C symmetric stretching vibration, C–H side swing vibration, C=C symmetric stretching vibration, the stretching vibration of –CH₂. These two bands are often used for quantitative analysis in SERS. The SERS activity of Ag NSs was exploited using 1.0 × 10⁻³ mol L⁻¹ EC as Raman labels. In particular,
the characteristic band of 996 cm⁻¹ in the Raman spectrum shifted to 1030 cm⁻¹ in the SERS spectrum, owing to the absorption of the EC molecule on the surface of the SERS substrates. According to a density functional theory study, the band at 1030 cm⁻¹ was assigned to \( \nu_{\text{s(CC)}} + \omega_{\text{(CH)}} + \delta_{\text{(NH)}} + \nu_{\text{s(OC)}} + \delta_{\text{(CH2)}} \), which implies that the EC might attach to the surface of Ag NS via amide group. As illustrated in Fig 3B, a strong SERS signal of EC on the surface of Ag NSs was observed. In comparison to spherical-shaped NPs (Au NPs and Ag NPs), star-shaped morphology endows Ag NSs with higher EM fields around the sharp tips, thus resulting in a larger LSPR, which is advantageous to SERS. The SERS activity of EC on the Ag NSs substrate is stronger than that on the Au NSs substrate. This is because Ag possesses the best plasmonic enhancement at visible frequencies, and has a broader EM region of activity. The EM region of Ag ranges from blue to the near-IR region, yet the EM region of Au appears only in the red near-IR region. Ag NSs featured with favorable SERS activity can serve as an excellent candidate SERS substrate for detecting EC. The Raman peak at 1030 cm⁻¹, shows the strongest Raman intensity, and is selected as a characteristic band for quantitative analysis.

**Table 1** Determination of EC in the Red Star Wine samples (n = 3)

| Sample | Added/mol L⁻¹ | Found/mol L⁻¹ | Recovery, % |
|--------|---------------|---------------|-------------|
| 1      | 0             | 0             | 0           |
| 2      | \(1.0 \times 10^{-8}\) | \(1.076 \times 10^{-8}\) | 107.6       |
| 3      | \(1.0 \times 10^{-7}\) | \(4.840 \times 10^{-8}\) | 96.8        |
| 4      | \(1.0 \times 10^{-6}\) | \(1.036 \times 10^{-7}\) | 103.6       |
| 5      | \(5.0 \times 10^{-7}\) | \(5.171 \times 10^{-7}\) | 103.4       |
| 6      | \(1.0 \times 10^{-5}\) | \(0.985 \times 10^{-6}\) | 98.5        |
| 7      | \(1.0 \times 10^{-4}\) | \(1.025 \times 10^{-5}\) | 102.5       |

the range of \(1.0 \times 10^{-3}\) mol L⁻¹ and \(1.0 \times 10^{-2}\) mol L⁻¹ were prepared and separately dropped on the surface of Ag NSs substrates. The obtained SERS spectra showed no significant shifts. As shown in Fig. 4B, a linear relationship was made by plotting the peak height at 1030 cm⁻¹ and the logarithm of the EC concentration, in the range from \(5 \times 10^{-9}\) to \(1.0 \times 10^{-4}\) mol L⁻¹. The linear-regression equation was \(y = 27401 + 2842 \log C\), with a correlation coefficient of 0.9913. The LOD was calculated to be \(1.37 \times 10^{-9}\) mol L⁻¹, based on the three-time standard deviations of the baseline noises, which was lower than the maximum permitted level of EC in alcoholic beverages.
The commercial alcoholic beverage was analyzed by the developed Raman method. The sample matrices had no influence on SERS spectra. As shown in Table 1, the recoveries ranged from 96.8 to 107.6%, illustrating the good performance of the developed Raman sensors. Ag NSs served as SERS substrates, and could be applied to the determination of EC in practical samples.

Conclusions

In summary, Ag NSs featured with star-shaped protuberances were used in this work as SERS substrates to detect EC in wine. Ag NSs exhibited a strong SERS signal in the presence of EC, attributing to the best plasmonic enhancement of Ag at visible frequencies and the unique morphology. The application of Ag NSs serving as SERS substrates has the advantages of being simple, fast, requiring less quantity of sample and having a lower limit of detection. This approach provides a useful method for the rapid determination of EC in alcoholic beverages and fermented food. Furthermore, as an excellent SERS substrate, the Ag NSs prepared in this work may be used to quantify other chemicals that are otherwise difficult to be detected.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 21175060) and the Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, China (No. JDSJ2013-02).

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