Preparation of SERS-active substrates based on graphene oxide/silver nanocomposites for rapid detection of L-Theanine

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A kind of graphene oxide/silver(GO/Ag) nanocomposites with high Surface enhanced Raman scattering (SERS) activity were fabricated via a facile and green liquid phase reduction method. The synthesized materials were characterized in detail using various microscopic and spectroscopic techniques. In this method, the GO sheets worked as a holder which makes silver nanoparticles(AgNPs) aggregate to a particular morphology, and under a suitable dosage of silver ions, well-dispersed AgNPs on the surface of GO were obtained, which could generate more “hot spots” of SERS. Moreover, SERS technique based on the obtained GO/Ag nanocomposites was used as an effective way to detect L-Theanine. The detection limit was estimated to be as low as 10^{-7} M, and a multivariate linear regression model for the concentration of L-Theanine was established. The optimal fitting equation is $Y=5.6765+0.0307X_{1458}-0.0267X_{1251}$.

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1. Introduction

L-Theanine is an essential amino acid that is mainly found in tea, and accounts for over 50% of the total free amino acid in tea.[\(Quan, Bowyer, & Roach, 2011\)](#) The content of L-Theanine has a positive correlation with the quality of tea. Moreover, the L-theanine also has potential effects on biochemistry, pharmacology and health care.[\(Hong-Suk, Seung, Sook-Young, Jeong Ae, Sung-Jun, & Sung, 2008\)](#) Many techniques have been applied to the detection of L-Theanine, such as ninhydrin coloration method,[\(Troll & Cannan, 1953\)](#) automatic amino acid analyzer,[\(Kominz, 1962\)](#) and micellar electrokinetic capillary chromatography,[\(Wang, Warner, & Chem., 1994\)](#) etc. However, these techniques are generally restricted by many obstacles, such as the sample preparation and detection accuracy. Thus, a simple, rapid, and ultrasensitive technique for the identification and detection of L-Theanine is very essential.

Surface enhanced Raman spectroscopy (SERS) technique is widely used in many chemical and biological detection fields due to its excellent performance, such as high sensitivity and great reproducibility.[\(Schlucker, 2014\)](#) Currently, there are two SERS mechanisms that are generally recognized. Electromagnetic mechanism (EM) is based on the enhancement of local electromagnetic field,[\(Yamamoto, Ozaki, & Itoh, 2014\)](#) meanwhile, chemical mechanism (CM) is based on the charge transfer effect between absorbed molecules and substrate.[\(Guerrini & Graham, 2012\)](#) Graphene, which is an ideal two-dimensional monolayer sheet of hexagonal carbon atoms, has been reported that it could be used as a substrate for enhancement of Raman signals of absorbed molecules due to CM.[\(Kavitha, Bramhaiah, John, & Ramachandran, 2015\)](#) However, several issues limit the practical applications of graphene in SERS, such as the aggregation in aqueous solution caused by the strong p-p stacking interaction between graphene sheets, and the chemical inertia originated from the defect-free structure.[\(Neto, Guinea, Peres, Novoselov, Geim, & K., 2007\)](#) Compared to graphene, graphene oxide(GO), an exfoliation of graphite oxide, has great dispersibility in aqueous solution and many reactive sites for decorating organic or inorganic species due to the fact that it contains hydroxyl and epoxy groups in the plane and carboxyl groups at the edge.[\(Dreyer, Sungjin, Bielawski, & Ruoff, 2009\)](#) Furthermore, GO also can be used as SERS substrate due to CM enhancement, but scientists believe that it will not support EM enhancement, because the surface Plasmon on GO is in the terahertz range.[\(Fan, Kanchanapally, & Ray, 2013\)](#) Noble metal nanostructures are excellent in SERS based on EM enhancement,[\(Rajab, Mougin, Derivaz, Josien, Luchnikov, Toufaily, et al., 2015\)](#) so decorating noble metal nanostructures on the surface of GO is an effective method to optimize the SERS performance. Thus, a simple

synthesis to integrate metal nanoparticles with GO is of critical interest. Mehl *et al.* reported a facile method for synthesizing thin films of rGO/ Ag-nanoparticles composites, which was performed with an interfacial system.([Mehl, Oliveira, & Zarbin, 2015](#)) Qian et al. assembled silver nanoparticles onto both sides of GO sheet through covalent interaction.([Qian, Cheng, Zhou, Wu, & Xu, 2013](#)) However, many reported nanocomposites lack dispersion and uniformity. It is still a challenge to exploit a simple approach that can improve the dispersion, uniformity, and stability of GO-based nanocomposites.

Herein, we propose a simple and facile method for fabricating graphene oxide/silver (GO/Ag) nanocomposites with high SERS activity and large uniformity. In this method, the Ag ions were reduced to Ag nanoparticles(AgNPs) on the surface of GO by using ascorbic acid molecules as reductant, and the size, density, distribution of the AgNPs can be greatly adjusted by varying the reaction parameters. Furthermore, it was found that the GO/Ag nanocomposites prepared with different sizes and density of AgNPs show distinct SERS properties. The GO/Ag nanocomposites with high AgNPs loading amount, controllable particle size, and appropriate inter-particle gap can generate more “hot spots” of SERS due to the strong Localized Surface Plasmon Resonance (LSPR) effect of AgNPs, and the fact that GO provides additional enhancement of Raman signals through covalent interaction. What's more, the prepared GO/Ag nanocomposites can be used as active substrate for SERS detection of L-Theanine, and the detection limit was estimated to be around 10^{-7} M. The multivariate linear regression model for the concentration of L-Theanine was also established based on the result of Principal Component Analysis (PCA).

2. Experimental

2.1 Materials

All the chemicals used in this work, including graphite powder, sulfuric acid(H_2SO_4), potassium permanganate($KMnO_4$), hydrogen peroxide(H_2O_2), hydrochloric acid(HCl), silver nitrate ($AgNO_3$), ascorbic acid, Rhodamine 6G (R6G), L-Theanine, were analytical reagents without further purification. Ultrapure water (18.3 M Ω) was used for all solution preparations.

2.2 Preparation of graphene oxide

Graphene oxide (GO) was prepared by a modified hummers method. First, 50 ml of H_2SO_4 was added to a beaker, containing 0.8 g of graphite powder and 4 g of KMnO_4 , and then stirred by a glass stirring rod for 2 min. The mixture was maintained under vigorous magnetic stirring for 4 h. Then, 4 ml of H_2O_2 solution (30%) was added slowly to the system, followed by the addition of 100 ml ultrapure water. In the next step, the mixture was vibrated in the ultrasonicator for 30 min. Afterwards, the solid product was separated by centrifugation, and washed repeatedly with HCl solution (10%) and ultrapure water until it was free of impurities. Finally, the solid product was dispersed in 20 ml of ultrapure water by ultrasonic dispersion.

2.3 Preparation of SERS substrates based on GO/Ag nanocomposites

Fabrication of GO/Ag nanocomposites: 4 ml of AgNO_3 solution (0.2M) was added to a beaker, containing 20 ml of GO dispersion (1 mg/ml), stirred magnetically for 3h at 25°C . Then, 2 ml of ascorbic acid solution (0.5 M) was quickly added to the mixed solution, and the mixture was maintained under vigorous magnetic stirring for 1 h. The reaction product was separated by centrifugation and washed repeatedly with ultrapure water until it was free of impurities. Finally, the nanocomposite was dispersed in 20 ml of ultrapure water.

Preparation of SERS substrate: 2 ml of GO/Ag nanocomposites dispersion was placed on a Si wafer (0.5×0.5 cm), and then the Si wafer was dried at 40°C in the vacuum oven for 8 h, in order to deposit GO/Ag nanocomposites film on the Si wafer.

2.4 Measurements of SERS

The prepared SERS substrate was put into an aqueous solution of R6G (10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M) for 2 h, and then measured by Raman spectrometry. The acquisition time was 3 s. The same procedure was also performed using neat Si wafer substrate (control). The standard solutions of L-Theanine were prepared at different concentrations ranging from 10^{-7} M to 10^{-2} M, and the experiments were carried out with the same procedure as described in the above step. In addition, ten different points on each substrate were selected for SERS measurement, in order to calculate the average Raman spectrum.

2.5 Characterization

The microstructure analysis was carried out on a Zeiss SUPRA 55 SAPPHIRE scanning electron microscopy (SEM). The X-ray diffraction (XRD) patterns were recorded on a Bruker D2 PHASER X-ray

diffractometer at room temperature. The Raman spectra were recorded by the confocal microscope Raman spectrometer system (Horiba, LabRAM HR) using a 532 nm laser excitation source with 25 mW power.

3. Results and discussion

3.1 Characterization of the GO/Ag nanocomposites

Fig. 1 SEM images of (A)GO and (B)GO/Ag nanocomposites; (C) XRD pattern of GO/Ag nanocomposites; (D) Raman spectra of (a)GO and (b)GO/Ag nanocomposites

The morphology and microstructure of the obtained GO/Ag nanocomposites were examined by the SEM. **Fig.1(A)** shows the SEM image of neat GO sheet. It can be seen that the surface of neat GO sheet is corrugate and clean. **Fig.1(B)** exhibits a representative SEM image of GO/Ag nanocomposites. It can be observed that the GO sheet is modified by a large amount of silver nanoparticles(AgNPs) about 10–15 nm in diameter, which are homogeneously dispersed and less agglomerated. Furthermore, as shown in **Fig.1(C)**, the successful preparation of GO/Ag nanocomposites was confirmed by the XRD measurement. In the XRD pattern, four typical diffraction peaks, corresponding to the (111), (200), (220), and (311) crystalline planes of silver present at $2\theta = 38.1^\circ$, 44.3° , 64.4° , and 77.5° , respectively. Moreover, two diffraction peaks corresponding to the (111) and (220) crystalline planes of silicon present at $2\theta = 28.4^\circ$ and 47.3° (derived from the silicon substrate). However, no obvious diffraction peaks of GO are observed, indicating the surfaces of GO are modified by AgNPs and the stacking of GO may be disordered.

Fig. 1(D) shows the Raman spectra of neat GO sheets and GO/Ag nanocomposites. For neat GO sheets, two prominent peaks present at 1344 cm^{-1} and 1568 cm^{-1} , which are corresponding to the D and G bands as reported.([Wei, Yih Hong, Srikanth, Qi, Tianxi, & Xing Yi, 2014](#)) The G band is related to the graphitic hexagon-pinch mode, and the D band corresponds to the defects in GO.([Liu, Li, Zou, Qian, Jin, Li, et al., 2013](#)) For GO/Ag nanocomposites, the D and G bands are also present, but the intensities ratio of the D and G bands obviously increases in comparison with the neat GO at same test conditions, indicating the reduction of GO.([Zhou & Qiao, 2014](#)) In addition, an apparent enhancing effect of the Raman signals of GO can be observed after the decoration with AgNPs due to the **Plasmon** resonance effect of AgNPs.([Kai-Chih & Dong-Hwang, 2014](#)) These results further

indicate that the AgNPs were attached on the GO surface after the in-situ reduction process, and the electronic properties of GO have been adjusted by the AgNPs.

Fig. 2 SEM images of GO/Ag nanocomposites prepared with different volume of AgNO₃ solution: (A) 1 ml, (B) 2 ml, (C) 6 ml (keeping the volume of ascorbic acid is 2 ml)

The performance of SERS substrate depends largely on the size and density of metal nanoparticles. [\(Ding, Xie, Liu, Wang, & Xu, 2015\)](#) In order to obtain a better SERS performance, a series of GO/Ag nanocomposites which were synthesized with different reaction condition (the volume of AgNO₃ solution) were produced. As shown in **Fig.2**, the presence of AgNPs is evident in all GO/Ag nanocomposites, but the sizes, distribution and aggregation of AgNPs in different samples are distinct. When the volume of AgNO₃ solution is 1 ml, a small amount of AgNPs about 15-20 nm in diameter are observed on the surface of GO. A significant increase of AgNPs loading amount is observed when the amount of AgNO₃ solution increased to 2 ml, but the particles sizes do not change obviously. However, as the amount of AgNO₃ solution increase to 4 ml, the surface coverage increases dramatically, but the particles sizes decrease obviously, as shown in **Fig.1(B)**. This phenomenon may be attributed to the fast reduction rate due to high concentration of Ag ions in the system. Further increasing the amount of AgNO₃ solution to 6 ml, the surface coverage decrease, and agglomerated particles with diameters of 20-60 nm are observed, which may be from the aggregative growth of the small particles during the reduction process.

In sum, the GO sheet worked as a kind of holder which makes AgNPs aggregate to a particular morphology, and the size, agglomeration and distribution of AgNPs on GO surfaces are strongly influenced by the initial amount of AgNO₃.

Fig. 3 (A) SERS spectra of R6G (10⁻³ M) adsorbed on GO(a) and GO/Ag nanocomposites prepared with different volume of AgNO₃ solution: (b) 1 ml, (c) 2 ml, (d) 4 ml, (e) 6 ml; (B) SERS spectra of R6G with different concentration adsorbed on GO/Ag nanocomposites (the volume of AgNO₃ solution is 4 ml): (a) 10⁻⁴, (b) 10⁻⁵, (c) 10⁻⁶, (d) 10⁻⁷, (e) 10⁻⁸, (f) 10⁻⁹ M

To investigate the SERS effect, all of these GO/Ag nanocomposites were used as SERS substrates for sensing R6G. **Fig. 3(A)** shows the SERS spectra of R6G molecules (10⁻³ M) adsorbed on neat GO sheet and GO/Ag nanocomposites prepared with different volume of AgNO₃ solution. Obviously, the SERS performances of these GO/Ag nanocomposites are better than the neat GO. In addition, there are significant differences in SERS ability, the GO/Ag nanocomposites prepared with 4 ml AgNO₃ solution exhibit the highest SERS ability especially.

Typical Raman spectra of R6G with GO-SERS, G-SERS (Ag) in Fig. 3. First, for GO-SERS(Ag), the spectral

features are the same as one another, with clear peaks at 612, 635, 660, 771, 1181, 1313, 1361, 1511, 1648 cm^{-1} .

The peaks at around 1570 cm^{-1} is the G-band monolayer graphene, respectively. The relative intensity of the graphene peak as compared to R6G is different in GO/Ag-SERS (this variability is reproducible from sample to sample in our parallel measurements); this may be because of a different chemical contribution of silver. In contrast, Raman spectra enhanced by SERS(Ag) show several additional peaks compared to the GO-SERS results. Among these additional peaks, such as at 937, 1112, 1403, 1471, 1531 cm^{-1} in GO-SERS(Ag), some of them may be from contamination of surface carbons, some are new emerging bands possibly due to the modified surface selection rules (because of alteration of the symmetry of the vibrational modes under changed configurations), while others remain difficult to assign precisely. ([Dutta, Ray, Sarkar, Pradhan, Negishi, & Pal, 2013](#); [Xu, Ling, Xiao, Dresselhaus, Kong, Xu, et al., 2012](#))

Theoretical calculation and experiments have shown that the giant SERS signals generally deriving from the molecules located on particular nanogaps that are often known as “hot spots”. ([Rycenga, Xia, Moran, Zhou, Qin, Li, et al., 2011](#)) Such “hot spots” support strongly electromagnetic SERS enhancement. When there appears a small amount of AgNPs on GO surfaces, as shown in **Fig. 2(A)**, the average size of the gaps between neighbouring AgNPs is relative large. For the large gap, it cannot generate giant electromagnetic enhancement under laser excitation, ([Camargo, Cobley, Matthew, & Younan, 2009](#)) which contributes to the lower SERS intensity. With the increases of AgNPs loading amount, as shown in **Fig. 2(B)** and **Fig. 1(B)**, the number of gaps between neighbouring AgNPs increases accordingly, and the average size of gaps between neighbouring AgNPs decreases significantly. In this case, the electromagnetic coupling between neighbouring AgNPs is extremely strong, which results in a significant increase of “hot spots”. However, when the AgNPs become agglomerate, as shown in **Fig. 2(C)**, the number of gaps between neighbouring AgNPs will reduce, which results in a decrease of “hot spots”, the intensity of SERS signal presents the drop. Meanwhile, the GO has a certain degree of SERS ability due to chemical mechanism. Thus, it can be concluded that both electromagnetic and chemical mechanisms are involved in the amplification of SERS signal, but the electromagnetic enhancement from AgNPs is dominant. Moreover, the GO/Ag nanocomposites with high AgNPs loading amount, controllable particle size, and appropriate interparticle gap are optimal for SERS-active substrate.

To further reveal the SERS ability of the GO/Ag nanocomposites, as shown in **Fig. 3(B)**, SERS spectra of R6G solution with different concentrations were measured by using the GO/Ag nanocomposites (the volume of AgNO₃ solution is 4 ml) as substrates. As the concentration of R6G decreases, the SERS signal intensity decreases accordingly, but it can still be distinguished even the concentration of R6G is reduced to 10⁻⁸ M, which indicates that the detection limit for R6G is estimated to be around 10⁻⁸ M under this experimental conditions. Such detection sensitivity of the GO/Ag nanocomposites is higher than most pure Ag nanostructures that reported in early articles. ([Hakonen, Andersson, Stenbaek Schmidt, Rindzevicius, & Kall, 2015](#))

3.2 SERS detection of L-Theanine

Fig.4(A) shows the molecular structure of L-Theanine. L-Theanine molecule that mainly contains functional group or chemical bond of -CH₃, -CH₂, -CONH, -NH₃, -COO⁻, C-C, C-H, C-N, and C=O, etc. It is well known that each functional group or chemical bond in L-Theanine molecule has its corresponding vibration frequency. However, the vibration frequency of each functional group or chemical bond is normally affected by many other factors, such as the other functional group, chemical bond, and external environment. ([Chen, Xi, Chen, Li, Feng, Lei, et al., 2011](#)) **Fig.4(B)** is the normal Raman spectrum of pure L-Theanine powder, as it shows that the peaks presenting at 1458 cm⁻¹, 1251 cm⁻¹, 1189 cm⁻¹, and 1416 cm⁻¹, respectively, which is consist to Chen et al report. ([Chen, et al., 2011](#)) And **Fig.4(C)** shows the SERS spectra of L-Theanine molecules adsorbed on the GO/Ag nanocomposites. Obviously, there are significant differences between the two spectrums, indicating there are strong interactions between L-Theanine molecules and GO/Ag nanocomposites. Analyse the influence of GO/Ag nanocomposites on Raman scattering of L-Theanine molecules, the Raman shifts and relative intensities of the characteristic peaks at the two spectrums were summarized, and the possible vibrational assignments of these peaks were identified. The result as shown in **Table 1**.

Fig. 4 (A) The molecular structure of L-Theanine; (B) Normal Raman spectrum of L-Theanine; (C) SERS spectra of L-Theanine with different concentration adsorbed on GO/Ag nanocomposites(the volume of AgNO₃ solution is 4 ml): (a) 10⁻², (b) 10⁻³, (c) 10⁻⁴, (d) 10⁻⁵, (e) 10⁻⁶, (f)10⁻⁷ M

Table 1 The main peaks of L-Theanine Raman spectrum and SERS spectrum within the range of 300~1700cm⁻¹ and their possible vibrational assignment

As is shown in **Fig. 4(C)**, as the concentration of L-Theanine decreases, the SERS intensity decreases accordingly. However, the SERS signal of L-Theanine still can be distinctly observed even as the concentration is reduced to 10^{-7} M, indicating the detection limit is estimated to be around 10^{-7} M. In addition, it also can be concluded that the SERS intensity has significant correlation with the concentration of L-Theanine. There are twelve characteristic peaks presenting at the SERS spectra of L-Theanine solution. The multivariate statistical analysis increases the computational complexity that there are too many variables. In order to improve the accuracy and simplicity, the Principal Component Analysis (PCA) method was used for data processing. Based on the result of PCA, a multivariate linear regression model could be established by using several specific variables. Therefore, the multivariate linear regression models for the concentration of L-Theanine were established by the variables with larger coefficient as the result of PCA. The variables are the peaks presenting at 1458 cm^{-1} , 1251 cm^{-1} , 1189 cm^{-1} , and 1416 cm^{-1} , respectively. Thus, four kinds of multivariate linear regression models are listed as follows:

1. $Y = \beta_0 + \beta_1 X_{1458} + \beta_2 X_{1251} + \beta_3 X_{1189} + \beta_4 X_{1416}$
2. $Y = \beta_0 + \beta_1 X_{1458} + \beta_2 X_{1251} + \beta_3 X_{1189}$
3. $Y = \beta_0 + \beta_1 X_{1458} + \beta_2 X_{1251}$
4. $Y = \beta_0 + \beta_1 X_{1458}$

In these models, Y represents the $-\log C$ (C is the concentration of L-Theanine, and its unit is M), and X_n represents the SERS intensities at $n\text{ cm}^{-1}$. There are three statistics testing the fitting degree of these regression models, namely coefficient of determination (R^2), F value, and the probability (P) corresponding to the F value. By comparing the fitting degree of the four regression models, it can be found that the third model is optimal. The optimal fitting equation is listed as follow:

$$Y = 5.6765 + 0.0307 X_{1458} - 0.0267 X_{1251}$$

For this regression model, the coefficient of determination (R^2) is as high as 0.9899. **Fig. 5** shows the result of the error analysis. These results further reveal that this SERS strategy based on GO/Ag nanocomposites has higher accuracy and more practical potential for the rapid detection of L-Theanine.

Fig. 5 The results of the error analysis

4. Conclusions

In conclusion, the GO/Ag nanocomposites with high SERS activity and large uniformity have been successfully synthesized. Size, density, and distribution of the AgNPs on GO surface can be greatly adjusted by varying the volume of AgNO₃ solution, and the GO/Ag nanocomposites with different sizes and densities of AgNPs exhibit distinct SERS properties. The optimized GO/Ag nanocomposites are prepared with 4 ml AgNO₃. In addition, the prepared GO/Ag nanocomposites can be served as an effective platform for SERS detection of L-Theanine. The detection limit is estimated to be around 10⁻⁷ M, and the optimal fitting equation is $Y=5.6765+0.0307X_{1458}-0.0267X_{1251}$. This work provides an experimental and theoretical basis for the application of SERS technique in rapid identification and quantitative detection of L-Theanine.

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Table list:

Table 1 The main peaks of L-Theanine Raman spectrum and SERS spectrum within the range of 300~1700cm⁻¹ and their possible vibrational assignment.

Table 1

Raman spectra (cm ⁻¹)	SERS spectra (cm ⁻¹)	Assignment
320 s	-	Skeleton, CH ₃
369 w	366 m	Skeleton, NH ₃
420 w	426 m	Skeleton, Amide V, CH ₂
479 s	476 m	Skeleton, Amide V
550 m	-	Amide V
655 m	-	CCN, COO ⁻ , CH ₂
825 w	-	CC, CN, CH ₂ , COO ⁻
897 vs	899 s	Skeleton, NH ₃ , CH
935 s	-	CC, CN, CH ₂
999 m	-	CC, CN, CH
1032 m	1026 w	CC, CN, NH ₃ , CH
1088 m	1069 m	CC, CN, CH ₃ , CH ₂
1149 s	1120 m	CH ₂ , NH ₃ , CH, CC
1202 w	1189 m	CH ₂ , CCN, CH
1245 w	1251 s	CH
1310 vs	-	CH, CH ₂ , CC, COO ⁻
1355 vs	1387 s	CH, CH ₂ , CC, COO ⁻
1427 m	1416 w	CH ₃
1452 s	1458 w	CH ₂ , CH ₃
1520 w	-	NH ₃
1580 w	-	COO ⁻ , NH ₃
1646 vs	-	CH ₂

vs=very strong; s=strong; m=middle; w=weak

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5