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A novel surface-enhanced Raman scattering sensor to detect prohibited colorants in food by graphene/silver nanocomposite

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ABSTRACT

A novel surface-enhanced Raman scattering (SERS) sensor made up of a graphene and silver nanocomposite was developed for detecting prohibited colorants in food. This SERS platform exhibited an excellent enrichment effect between the colorant molecules and the graphene and an ability to enhance the Raman spectra of the silver nanoparticles. Detection of different concentrations of each prohibited colorant was carried out by SERS measurements on this novel substrate. In addition, from the SERS spectra of a mixture of four kinds of prohibited colorants, it was possible to easily distinguish each colorant by its characteristic peaks. A control experiment was also performed to compare the SERS spectra obtained using the graphene/silver nanocomposite substrate with spectra obtained using Ag alone as a substrate. The results showed that the SERS nanocomposite was better for detecting prohibited colorants. The proposed method has advantages in terms of providing a simple and rapid method for the sensitive analysis of prohibited additive colorants in food.

1. Introduction

The common association of the color of food with quality makes color one of the most important physical properties of food, and therefore it is given high priority by the food industry. This industry is attempting to adapt industrial food processes to preserve the integrity of compounds used as the basis for acceptable color. It has become normal practice to add colorants to enhance, homogenize, or even change the color of the food to make it more attractive to consumers [1].

Food color comprises both natural and synthetic colorants, and synthetic colorants are a very important class of food additives that are widely used in food which is consumed daily. However, some of these colorants pose a potential risk to human health, especially if they are consumed in excess. For that reason, the use of synthetic colorants in foods is strictly controlled by legislation in many countries. Consequently, ensuring food safety requires accurate and reliable techniques for the determination of synthetic colorants in foods. A large number of analytical methods have been proposed for the identification and determination of various food colorants, such as thin layer chromatography (TLC) [2], adsorptive voltammetry [3], spectrophotometric methods [4], capillary electrophoresis (CE) [5], high-performance liquid chromatography (HPLC) [6–8], and enzyme-linked immunosorbent assay (ELISA) [9]. However, most of these methods require so complex and time-consuming pretreatment process that it is necessary to develop a rapid and simple analytical method to detect prohibited colorants in food.

Since observation of enhanced Raman signals of molecules adsorbed on roughened metal surfaces, Surface-enhanced Raman scattering (SERS) has been demonstrated to be a powerful technique for analytical analyses. Molecules located at the vicinity of the roughened surfaces, owning different spatial field intensity depending on the morphology and size of nanostructures. With the integration of high sensitivity, a unique spectroscopic fingerprint, and nondestructive data acquisition, the SERS technique has become one of the most widely pursued spectroscopic tools for the identification and detection of chemicals and biological sensing [10–18]. Herein, we demonstrate a simple and rapid detection method using SERS to measure prohibited colorants in food. In order to improve its ability to detect colorants, a novel graphene material with silver nanoparticles was introduced into this SERS platform.

Graphene, a zero-band gap semiconductor owing to its two-dimensional (2D) plate-like structure composed of sp^2-bonded carbon atoms, has attracted tremendous attention in recent years due to its unique properties and potential applications [19–21]. Because of its structure, graphene can act as an adsorbent for some benzenoid-form compounds, due to a largely delocalized electron system which forms a strong stacking interaction with the benzene ring [22,23].

In this work, a novel SERS substrate was developed by preparing a graphene/silver (G/Ag) nanoparticle composite material, which could potentially be applied as a method for detecting

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prohibited colorants due to the enrichment effect of graphene and its ability to enhance the Raman spectra of the silver nanoparticles. Detection of different concentrations of each prohibited colorant was carried out by SERS measurements on this novel substrate. In addition, from the SERS spectra of a mixture of four types of prohibited colorants, it was possible to easily distinguish each colorant by its characteristic peaks. A control experiment was also performed to compare the performance of the SERS G/Ag nanocomposite substrate with that of a silver substrate, and the results showed the SERS method using the nanocomposite substrate was better at detecting the prohibited colorants. This SERS platform provides significant advantages in terms of simplicity and rapid, real-time measurements for the sensitive analysis of prohibited additive colorants in food.

2. Experimental section

2.1. Materials

Silver nitrate (99%, Aldrich, USA), trisodium citrate (99%, Beijing Chemicals, China), allura red (≥ 98%, Fluka, USA), ponceau (≥ 99%, Fluka, USA), amaranth (≥ 98%, Fluka, USA), erythrosine (≥ 98%, Fluka, USA), lemon yellow (≥ 99%, Fluka, USA), sunset yellow (≥ 95%, Fluka, USA), orange II (≥ 98%, Fluka, USA), chrysoidin (for microscopy, Sigma, USA), and graphite powder (spectral requirement, Shanghai Chemicals, China). All the chemicals were used as received unless noted otherwise. All aqueous solutions were prepared with Ultrapure water (> 18 MΩ cm) obtained from a Milli-Q Plus system (Millipore).

2.2. Preparation of graphene oxide (GO)

GO was synthesized from natural graphite powder by a modified Hummers method as originally presented by Kovtyukhova et al. [24]. The detailed preparation process was shown in the Supporting Information.

2.3. Synthesis of the G/Ag nanoparticle composite

An aqueous dispersion of GO was mixed with 40 mL water and 0.036 g $AgNO_3$ and the mixture was brought to the boiling point. A solution of 2% trisodium citrate (4 mL) was then added. The solution was kept boiling for 1 h. The synthesized G/Ag nanoparticle composites were greenish yellow.

2.4. UV–vis measurement

The spectra of samples in the 300–800 nm regions were collected with a UV–vis scanning spectrophotometer (UV-3600, Shimadzu Corporation, Japan) equipped with 1.0 cm quartz cells. The water spectrum was used as the baseline.

2.5. Atomic force microscopy (AFM) measurement

AFM images were obtained in a tapping mode at room temperature (20 °C) with a Digital Instruments Nanoscope IIIA using Si cantilevers purchased from DI and Nanosensor Co., Ltd.

2.6. SERS measurement

Twenty microliters of each sample were added to a modified glass chamber under ambient conditions and then dried; then the SERS measurements were performed. All SERS measurements were taken with a Renishaw 1000 model confocal microscopy Raman spectrometer equipped with a CCD detector and a holographic notch filter. Radiation of 514.5 nm from an air-cooled argon ion laser was used for the SERS excitation with the power of 5 mW at the sample position. The microscope attachment based on a Leica DMLM system with a 50 × objective was used to focus the laser beam onto a spot approximately 1 μm in diameter. The typical accumulation time used in this study was 30 s.

3. Results and discussion

3.1. Characterization of the G/Ag composite

Fig. 1 depicts the proposed protocol for SERS detection of prohibited colorants with the G/Ag composite described in the experimental section. As shown in Fig. 2, the UV–vis spectra of the GO and G/Ag composite dispersions were measured. The spectrum obtained for the GO dispersion (red line) exhibits a maximum at 232 nm (attributed to $\pi-\pi^*$ transitions of aromatic

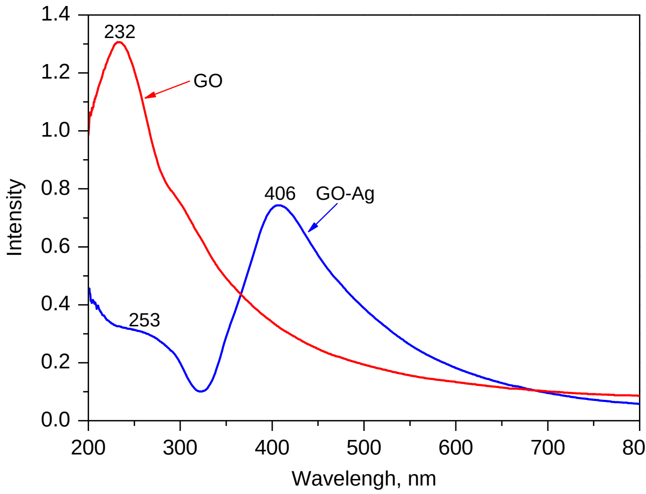

Fig. 2. UV–vis spectra of GO and G/Ag composite dispersions.

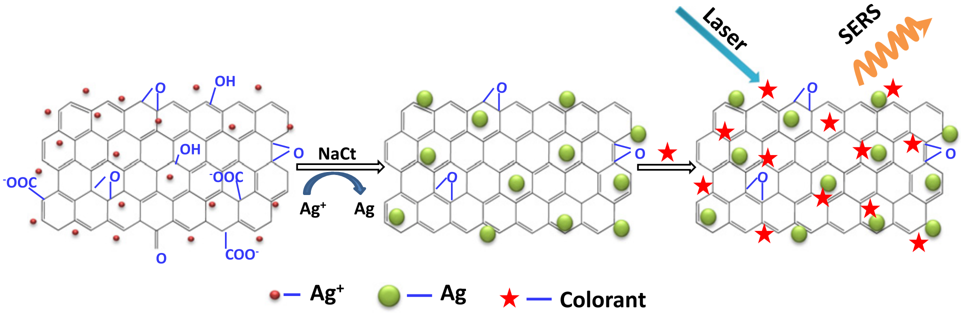

Fig. 1. Schematic illustration of the synthesis of G/Ag composites and SERS detection of prohibited colorants using this substrate.

C=C bonds) and a shoulder at about 300 nm (ascribed to $n-\pi^*$ transitions of C=O bonds). Compared with the GO dispersion after the reduction reaction (blue line), the aromatic C=C bonds show a red shift to 253 nm, indicating the restoration of a $\pi-$conjugation network within GS [25]. Also, there was a new adsorption peak at 406 nm, which was assigned to the formation of the silver nanoparticles. The above phenomena proved that the G/Ag composite was synthesized by citrate reduction.

Fig. 3A and B show the AFM images of the G/Ag composite nanoparticles, which were synthesized by citrate reduction. The tapping mode AFM image in Fig. 3A clearly shows the formation of nanoparticles with a spherical shape. As shown in Fig. 3B, many small silver nanoparticles aggregate to form a larger nanoparticle, as seen in Fig. 3A. The size of the aggregated particle in Fig 3A is about 250 nm and the height of the nanoparticles in Fig. 3B is about 20 nm, which corresponds to the adsorption peak at 406 nm of the UV–vis spectrum in Fig. 2 [26]. The nanometer-scale surface roughness of the particles can provide several hot spots on a single particle, which is useful for significantly increasing the SERS enhancement ability [27,28].

3.2. Determination of prohibited colorants

The high signal-to-noise ratio of the SERS spectra shown in Fig. 4A, B, C and D suggest an excellent Raman enhancement effect by the G/Ag composite nanoparticle substrate. We then investigated the ability of the SERS substrate to detect prohibited colorants. To promote the homogeneity of the SERS response, we recorded SERS spectra from three randomly selected locations on the substrate surface.

Fig. 4A, B, C and D show the SERS spectra using the G/Ag composite substrate for different concentrations of four types of prohibited colorants: amaranth, erythrosine, lemon yellow, and sunset yellow. In each spectrum, the characteristic Raman peak for a prohibited colorant, which is listed in Table 1, is marked by a dashed line according to the concentration of colorant. Moreover, it is labeled by its wavenumber, which shows a corresponding relationship whose characteristic peaks have been listed in Table 1. Furthermore, it was found that the detection limit of the four types of prohibited colorants measured was 10^{-5}, 10^{-7}, 10^{-5}, and 10^{-5} M.

3.3. Control SERS experiment comparing G/Ag composite and Ag substrate

To confirm the SERS enhancement effect by the G/Ag composite substrate as compared with the Ag-only substrate, a control experiment was performed by taking the SERS measurement of 10^{-5} M erythrosine colorant using the two different substrates. Fig. 5 shows the comparison of the SERS spectra of the erythrosine colorant using both the G/Ag and the Ag-only substrates. The enhanced Raman peaks have been marked by asterisks according to the Raman spectrum of an erythrosine solid. Fig. 5 shows that the characteristic Raman peaks were enhanced more by using the G/Ag SERS substrate than they were by the use of the Ag substrate alone. Due to the excellent adsorbent effect of graphene and enhanced effect due to the silver nanoparticles, this novel SERS substrate appears to have a strong potential application for detection of prohibited colorants in food.

3.4. SERS detection of four types of prohibited colorant systems

SERS detection of each colorant was successfully performed utilizing the novel model as shown in Fig. 4A–D. The SERS

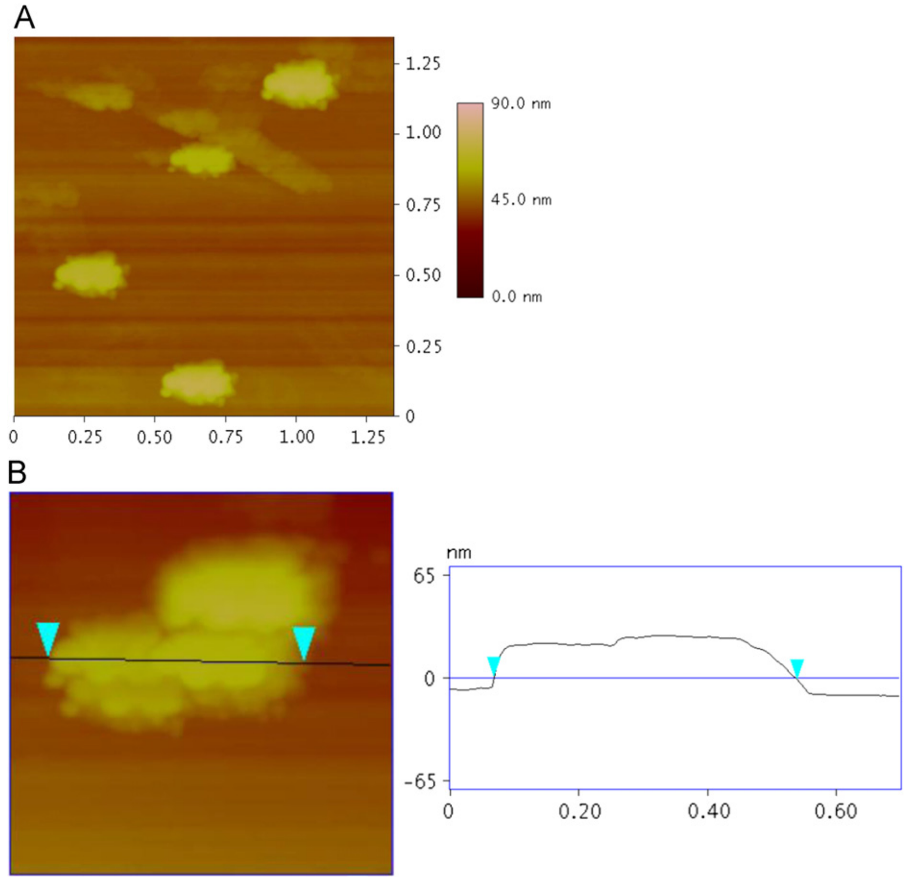

Fig. 3. (A) AFM photograph of G/Ag composite nanoparticles reduced by citrate. (B) Enlarged AFM photograph of (A).

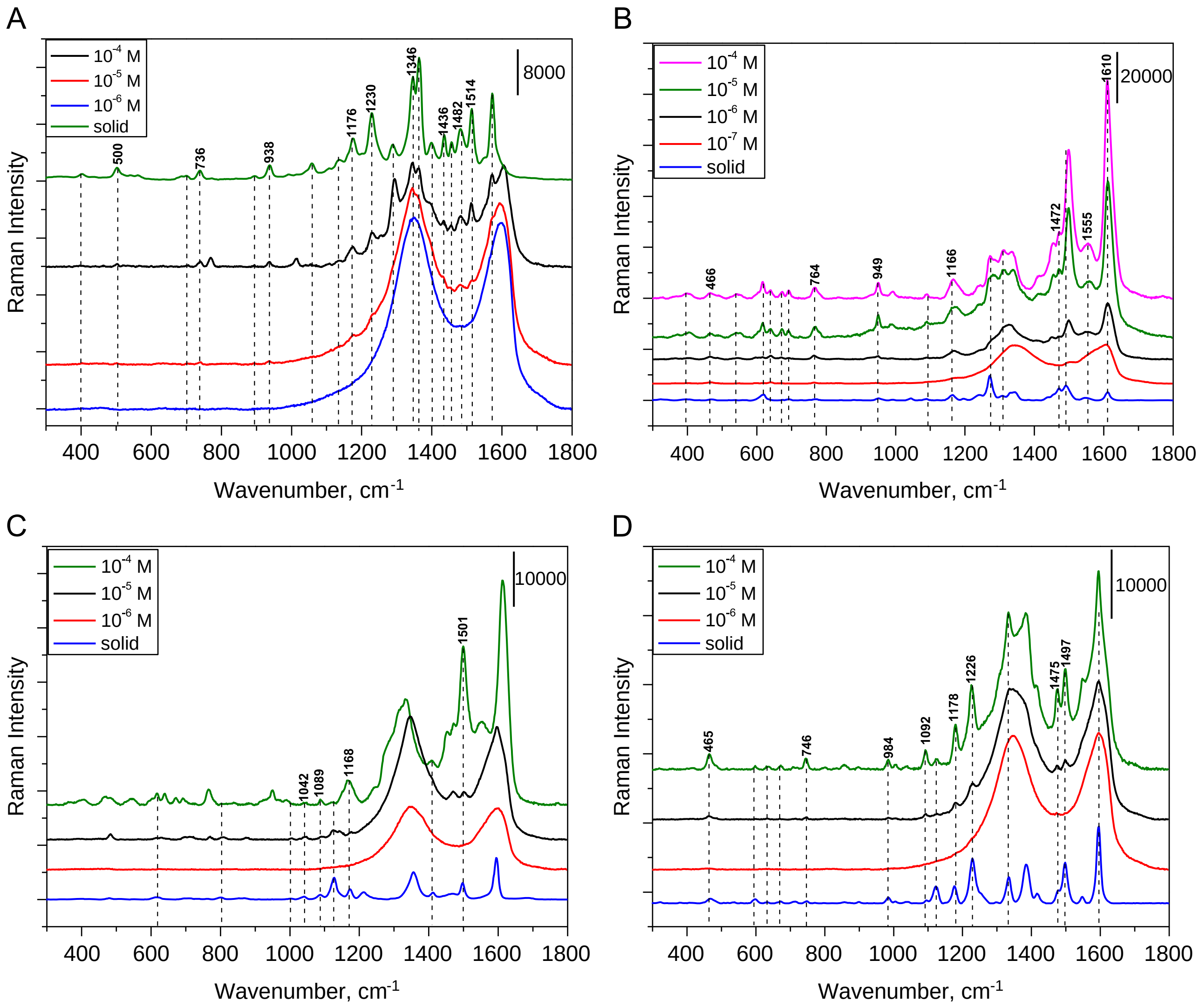

Fig. 4. SERS spectra of amaranth (A); erythrosine (B); lemon yellow (C); sunset yellow (D) at concentrations (a)–(d) 1×10^{-4}, 1×10^{-5}, 1×10^{-6}, and 1×10^{-7} M, respectively. The entire characteristic Raman peak in the prohibited colorant solid is been marked by dashed line, according to the different concentrations of colorant. The peaks are also labeled by wavenumbers, which show an evident corresponding relationship.

Table 1
Characteristic SERS peaks of components in color system mixtures.

Characteristic SERS peaks/cm^{-1}							
Red color system				Yellow color system			
Allura red	Ponceau	Amaranth	Erythrosine	Lemon yellow	Sunset yellow	Orange II	Chrysoidin
490	493	894	690	400	734	1094	734
636	1436	938	1088	614	1094	1122	966
750	1570	1058	1344	1002	1178	1226	992
1128		1134	1470	1412	1228	1334	1120
1182		1346	1546	1498	1256	1478	1150
1226		1364		1594	1300	1498	1258
1496		1436			1334	1546	1332
		1572			1452	1594	1380
					1480		1594
					1498		1620
					1552		
					1594		

qualitative analyses of more complicated systems containing red and yellow colorant systems were also measured. Fig. 6A and B show that the SERS spectra of red and yellow prohibited colorant

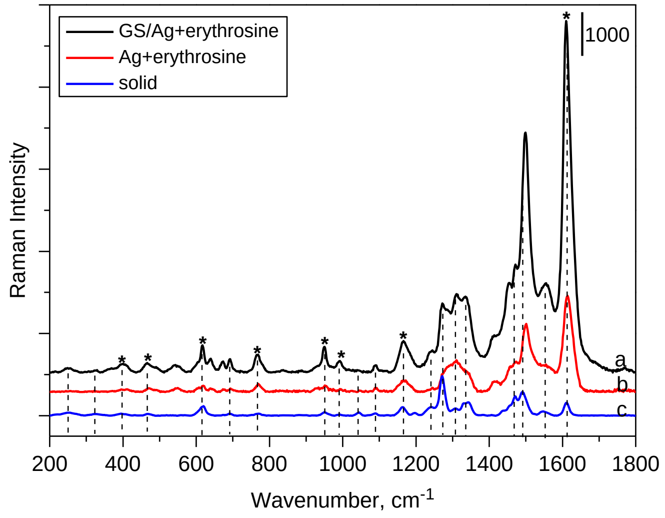

Fig. 5. SERS spectra of 1×10^{-5} M erythrosine colorant determined with SERS substrate by (a) G/Ag and (b) Ag nanoparticles, and (c) Raman spectrum of erythrosine solid.

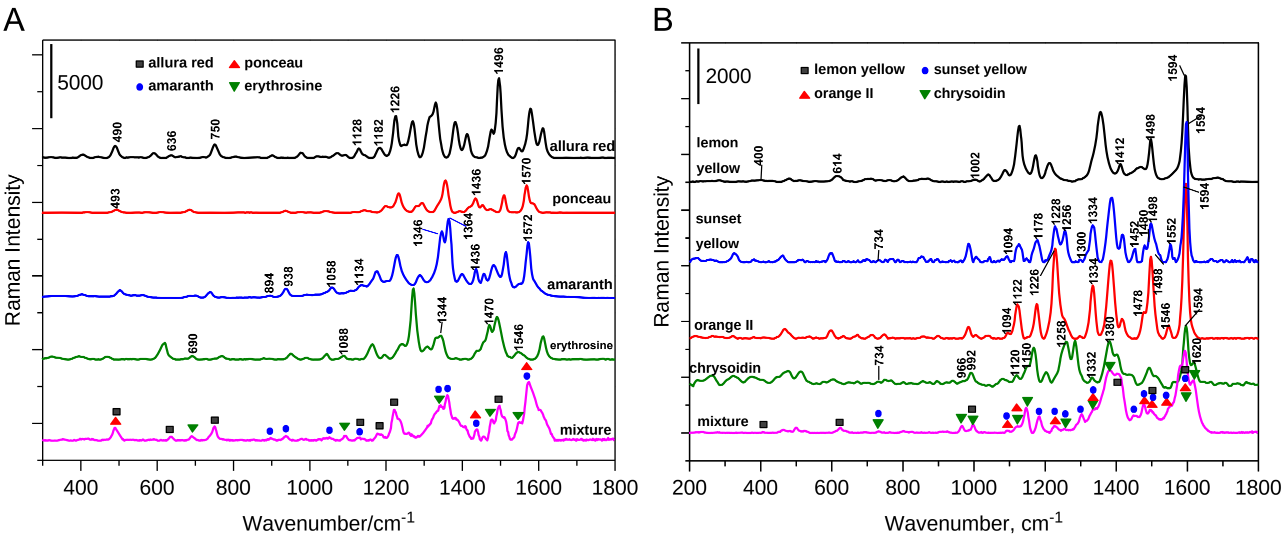

Fig. 6. (A) SERS spectra of 10^{-4} M red prohibited colorant systems (including allura red, ponceau, amaranth and erythrosine; each of these colorants was 10^{-4} M) by utilizing the substrate of G/Ag composite; (B) SERS spectra of 10^{-4} M yellow prohibited colorant systems (including lemon yellow, sunset yellow, orange II and chrysoidin, and each of these colorants was 10^{-4} M) by utilizing the G/Ag composite substrate. Different symbols represent characteristic SERS wavenumbers for each colorant in the mixture.

systems were achieved by utilizing this G/Ag composite substrate. To promote homogeneity of the SERS response, all the SERS spectra shown in Fig. 6A and B were randomly selected from three different locations. These SERS spectra were measured by exposing the G/Ag composite to 10^{-4} M of four kinds of red and yellow prohibited colorant solutions. The red color system was made up of allura red, ponceau, amaranth, and erythrosine. The yellow prohibited colorant system was made up of lemon yellow, sunset yellow, orange II, and chrysoidin. Fig. 6A and B show the different characteristic wavenumbers for each colorant contained in the mixture; these have been labeled by different symbols in the SERS spectrum. Characteristic SERS peaks corresponding to the color system mixtures are listed in Table 1. It is noticeable that obvious SERS features from all the colorants analyzed appear in the spectra of colorant mixtures, where the peaks of each colorant are labeled with different symbols. In the case of the complicated system containing the four types of colorant molecules of two systems, this novel SERS model showed good pre-concentration detection abilities for these red and yellow colorant systems.

4. Conclusions

In this work, a novel SERS substrate was designed to characterize prohibited colorants. This G/Ag SERS model exhibited potential application for detecting prohibited colorants including and showed an excellent wonderful enrichment effect between colorant molecules and graphene, as well as excellent Raman enhancement effect due to the silver nanoparticles. This SERS platform was applied to the detection of four types of prohibited colorants, and two-color systems made up of four types of colorants can be distinguished from one another, based on their characteristic peaks. The proposed method possesses significant advantages in terms of simplicity and rapidity for the sensitive analysis of prohibited colorants. All the obtained SERS spectra suggest that the method can be used as an effective method in detecting qualitatively and quantitatively prohibited additive colorants in food.

Acknowledgments

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Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.07.080.

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