Developing cysteamine-modified SERS substrate for detection of acidic pigment with weak surface affinity

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A B S T R A C T

In this paper, we developed cysteamine-modified surface-enhanced Raman scattering (SERS) substrate for detecting detect trace amount of acidic pigment that shows weak affinity with gold nanoparticles (Au NPs). To realize sensitive and reproducible detection of pigment with weak affinity, the SERS substrate was prepared by attaching cysteamine (CA) to the Au NPs, the acidic pigment molecule could rapidly reached to the surface of Au NPs because of the formation of multi-hydrogen-bond and electrostatic interaction between the pigment and CA molecule. The proposed method allowed us to detect five kinds of acidic pigment with a limit of 1.0 ppm, which is below the strictest safety limit. Compared with the previous methods, the advantages of the present substrate were its simple substrate preparation, high reproducibility and good universality. Furthermore, the reliable and enough accurate results had been obtained by using of the proposed substrates in the assay of trace pigment in real samples.

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1. Introduction

The color of food products is an essential aspect of promoting appetize and enhancing commercial value. Alongside flavor, texture, and aroma, color is considered to be one of the most important factors to appeal customers. However, most of artificial colorants might lead to some disease like decreased fertility, teratogenesis, diarrhea, suddenness and carcinogenesis, they have been subjected to scrutiny and banned [1]. These fears are exacerbated because artificially colored foods are often marketed to children, and concerns remain about the consumption of artificial colorants. At present, food safety incidents have happened frequently [2]. In order to timely monitor some of the additives used in food for safety of consumers, there is an impendency to exploit quick, accurate, and sensitive analytical methods to detect trace amounts of pigment in food.

Surface-enhanced Raman scattering (SERS) holds great promise in this issue for unique spectroscopic fingerprint and rapid detection. SERS has been considered to be a promising analytical technique for a variety of chemical-related molecules because of its high sensitivity, selectivity and fingerprint characteristics [3–8]. However, there are still some limitations that restrict the SERS technique when the analytes cannot close to the rough noble metal surfaces. Only these analytes which easily approach to substrate could be adsorbed on the substrate surface and provide good signals to meet the ultrasensitive analysis [9–11]. However, acidic pigment molecules -mainly amaranth, sunset yellow, allura red, lemon yellow, acid blue-show weak affinity to gold or silver and it is difficult to directly detect these compounds by SERS. Recently, some reports had made efforts to detect pigment via the fabrication of various Raman-active substrates such as nanoparticle multimers, nanorod arrays, periodic 2D nanostructures and composite materials [12–15]. They made use of the single molecule Raman spectroscopy on the nanoparticle aggregates or at the nanostructural junctions (“hot spots”), chemical enhancement and multi-dimensional plasmonic coupling that enormously maximize SERS resonance experienced by pigment molecule. However, the subnanometer control between two nanostructures has to face the problems with a structural reproducibility and low yield. The most desirable SERS substrates for practically sensing purpose should be repeatable and renewable.

Recently, surface modification on SERS-active substrates are becoming more and more important to achieve the detection of target analytes with small surface affinity or weak Raman activity, compared to traditional direct SERS detection using a bare metal SERS-active substrate [16–21]. The technique relies on functionalized nanoparticles with different media to concentrate analytes close to the substrate surface so that can enhance the Raman signals of resonant molecules [22–24]. Many efforts have been made to amplify the SERS signals of molecules that involving the chelating interaction of diethylenetriaminepentaacetic acid [25], charge-transfer complexing reaction using p-aminobenzenethiol [26], specific recognition between antibodies and aptamers [27,28], as well as electrostatic attraction of ion pairing [29]. The coupling of the localized surface plasmon of plasmonically active nanostructure led to

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surface Raman enhancement. In our group, Zhou had employed \(\alpha,\beta\)-nitroiloacetic acid and Fe(NO\(_3\))\(_3\) modified Au NPs to detect epinephrine in serum [30]. Cao had prepared iron-nitroiloacetic acid functionalized PVP-Au NPs for sensitive detection of catecholamine neurotransmitters [19]. These implied that the ultrahigh Raman scattering of weak affinity molecules is possible by multi surface modification techniques.

The work is aimed to provide a potential rapid and qualitative application platform for monitoring of pigment (Scheme 1). In this paper, cysteamine modified gold nanoparticles (CA-Au NPs) were used for the detection of pigment based on SERS. On the one hand, CA carries a positively charged group -\(\text{NH}_3^+\) which can electrostatically interact with acidic pigments. On the other hand, hydrogen bond is formed between the CA and the pigment molecules, thereby the pigment molecules get closer to the surface of the Au NPs. The sensitivity and reproducibility of CA-modified substrate were evaluated. Our cysteamine-modified SERS sensor holds great promise for reproducible, sensitive and quick detection acidic pigment in various complex fields.

2. Materials and Methods

2.1. Materials

Polyvinylpyrrolidone (PVP) (MW = 55,000), \(\text{NH}_2\text{OH}\cdot\text{HCl}\) sodium citrate, and hydrogen tetrachloroaurate (HAuCl\(_4\)·4H\(_2\)O) were bought from Shanghai Chemicals Company. Amaranth, lemon yellow, allura red, sunset yellow and acid blue were purchased from Sigma-Aldrich. Cysteamine (CA), Polyamide powder and ammonia aqueous solution were supplied from Aladdin Company. Chemicals were analytical grade in this study. Ultrapure water (18.2 M\(\Omega\) cm) was used in the process of experiment. Beverage was bought from a local supermarket and used directly without any further treatment.

2.2. Experimental Designs

2.2.1. Synthesis of PVP-Au NPs

The synthesis of PVP-Au NPs was by seed growth method. The preparation of Au seed solutions were synthesized via the reduction of HAuCl\(_4\)·4H\(_2\)O. At the beginning, 99 mL of deionized water was poured into the 250 mL round-bottomed flask, which was added to 1 mL of HAuCl\(_4\)·4H\(_2\)O (1% wt). Once the solution was heated to boiling, 4 mL of sodium citrate (1% wt) was added rapidly at the moment. The boiling solution was kept heating for 40 min continuously, and then let it cool. The whole experiment was carried out under vigorous stirring.

The growth procedure for PVP-Au NPs was as follows: 25 mL of seed solution was injected into 100 mL of round bottomed flask, and then 1 mL of sodium citrate (1% wt), 20 mL \(\text{NH}_2\text{OH}\cdot\text{HCl}\) (2.5 mM) and 1 mL PVP solution (1% wt) were added into the solution respectively. 20 mL HAuCl\(_4\) (1% wt) was injected into solution at the speed of 1 mL min\(^{-1}\) by a peristaltic pump under vigorous stirring.

2.2.2. Gold Nanoparticles Surface Modification

For SERS detection of acidic pigment, we have modified the gold nanoparticle surface by CA. The prepared solution of PVP-Au NPs (1 mL) was centrifuged at 7500 rpm for 10 min and washed twice with ultrapure water to remove excess PVP. Then, the CA solution (10\(^{-5}\) M) were mixed with the centrifuged AuNPs and vibrated for 20 min. Finally, the obtained solution was centrifuged at 7500 rpm for 5 min, and the solution sediment in the bottom was left aside to use.

2.2.3. Beverage Sample Treatment

Beverage contained with pigment was used as an experimental sample, and was bought from a local supermarket. In this study, the extraction of pigment from liquid samples was carried out by Polyamide power. Then, Polyamide extraction simplifies the purification steps the pre-treatment steps are as follows. Firstly, 5 mL of the tested solution was extracted in a syringe which equipped with a filter membrane with pore size of 0.45 \(\mu\)m, and mixed with the polyamide powder, then the mixture was squished repeatedly and the waste liquid was extruded. At second, 1 mL of methanol-formic acid (volume ratio 8:2) solution was extracted to the syringe. Thirdly, 5 mL of water was extracted to the syringe and the waste liquid was wiped off after shaking repeatedly, and repeated three times. Finally, 1 mL of ammonia aqueous solution was added to the mixture, and then left the eluting solution. This liquid was ready for use.

2.3. Samples Preparation and SERS Measurements

2.3.1. Samples Preparation

In SERS detection of these five pigment molecules, 1 \(\mu\)L sol of CA modified Au NPs prepared above was dropped on the clean Si wafer, and the SERS functionalized substrate are dried in the air. Then 1 \(\mu\)L of each pigment sample was dropped on the dry functionalized substrate.

2.3.2. SERS Measurement

All samples were analyzed by LabRAM HR800 confocal microscope Raman system (Horiba JobinYvon) with a 633 nm He-Ne laser source. The lasers were focused by a LWD 50/0.5 NA objective lens, the laser spots had a diameter of about 1 \(\mu\)m with the laser powers of approximately 0.8 mW, and the integral time for each spectrum was 5 s.

2.4. Instruments

The scanning electron microscopy images were got by field-emission scanning electron microscope (SIGMA 500). Ultraviolet-
visible (UV–vis) absorption spectra were obtained from Shimadzu UV-2600 spectrophotometer (Japan). X-ray Photoelectron Spectroscopy (XPS) measurements were taken on PHI 5000 VersaProbe Ilv (with a monochromatic Al Kα (1486.6 eV) excitation source). Raman spectra were carried out by LabRAM HR800 confocal microscope Raman system (Horiba JobinYvon) with a 633 nm He-Ne laser source.

3. Results and Discussion

3.1. Characterization of CA Modified Au NPs

Our detection was based on the assumption that the pigment molecules show weak affinity with Au NPs so that unable approach to the SERS substrate surface to resonate together, while in the presence of CA, pigment molecules get closer to the NPs surface and can be rapidly captured by the SERS sensor. On the one hand, CA bears positively charged groups $-\text{NH}_3^+$, which could interact with the acidic pigment molecules, on the other hand, multi hydrogen bonds could be formed between the pigment and the CA molecule. As a result, it provided a significant enhancement of the Raman signal intensity by several orders of magnitude.

We chose CA as Raman tag due to its small Raman scattering cross section and fast-labeled. After the addition of CA to prepared PVP-Au NPs, the surface was modified by CA through the Au$\text{S}$ covalent bond (as shown in Fig. 1(A)) and CA solution follows a rapid kinetics to reach the saturation within a few minutes [31]. As shown in Fig. 1(B), the CA-Au NPs were close-packed with a large area of monolayer distribution which because of the highly uniform of PVP-Au NPs [19,30,32], and could act as ideal SERS active substrates with excellent uniformity and high sensitivity. As the cysteamine ($10^{-3}$ M) adding into colloids PVP-Au NPs colloids, the color of colloids changed to aubergine (No.2) from wine-red (No.1), which indicated the efficient aggregation of Au NPs owing to the presence of $\text{N-H-N}$ bonds, The dimer was not precipitate but a stable state. From UV–visible spectra (Fig. 1(C)) we could see that new absorption peak occurred that signifies the presence of efficient aggregation of Au NPs owing to the presence of hydrogen bonding ($\text{N-H-N}$), illustrating that the attachment of the probing CA with the Au NPs. And our XPS measurements (as shown in Fig. 1(D)) showed that when CA was added to PVP-coated Au surface, the sulfur signal appears at 163 eV in the spectra, which was attributed to CA, because PVP does not contain sulfur, and the peak is assigned to Au$\text{S}$ bond [33–35]. In addition, we observed no shift in the nitrogen peak that signifies no nitrogen combined with Au NPs (Fig. 1(E)). The XPS spectra suggested that PVP was exchanged with CA, and the CA molecules were strongly adsorbed on the Au NP surface through Au$\text{S}$ bond. After ligand exchange, as shown in Fig. S2, there were some differences in the resonances of the background signals of the SERS substrates. All our data indicated that PVP was successfully exchanged with CA.

Fig. 1. (A) Schematic illustration of surface ligand exchange between PVP and CA on Au NPs. (B) SEM image of CA-Au NPs. Inset shows higher magnification image. (C) UV–visible spectra of PVP-Au NPs (black) and CA-Au NPs (red). X-ray photoelectron spectroscopy (XPS) results for high-resolution S 2p (D) and N 1 s (E) of CA and CA-Au NPs.
3.2. SERS Detection of Pigment Molecules

This pigment detection was mainly established based on the formation of hydrogen bond between pigment and CA. Typically, it is very difficult to directly detect pigment by using PVP-Au NPs because of the steric hindrance, as shown by the Raman spectra in the right side of Fig. 2(A). While PVP as surfactant, the pigment molecules were demonstrated the weak Raman resonances due to the steric hindrance. As presented in Fig. 2(B), in the presence of CA, the strong Raman signals are clearly observed. The signal-on effect is due to the formation of hydrogen bond, which induces the pigment molecule gets closer to the NPs surface. The CA was used as a pigment recognition element and the Raman tag on the Au NPs provided an amplified signal. Thus, the resonances brought the high electromagnetic (EM) field enhancement for sensitive detection of pigment.

In order to consider the effect of SERS detection about pigment molecules, a series of tests to prove the sensitivity and reproducibility of the functionalized substrate were performed. As shown in Fig. 3(A), take amaranth as an example, the characteristic bands of amaranth molecules could be clearly identified, it showed an extremely low LOD of 1 ppm. In SERS detection process, the performance of the substrate is important for the reliability of the experimental results and the RSD values of amaranth peak ascribed to 1344 cm$^{-1}$ and 1362 cm$^{-1}$ were lower than 15% with the concentration of 5 ppm according to the SERS spectra in Fig. 3(C) and (D). In order to further verify the uniformity of the substrate, SERS mapping with the area of 20 μm × 20 μm for 1362 cm$^{-1}$ band of amaranth (5 ppm) had been performed to demonstrate the uniform of SERS substrates. As presented in Fig. S3, this functionalized substrate showed good reproducibility and thus can realize the reliable detection of pigment molecules. To test the universality and sensitivity of the substrate to the other four kinds of pigments, the signal sensing results for SERS detection was shown in Figs. 4 and S4. Furthermore, in terms of SERS detection, an ideal SERS active substrate for sensing applications should induce stable properties. Thus, the stability of SERS substrate had been evaluated. As shown in Fig. S5, the SERS spectra of amaranth demonstrated slighter alteration within 5 days, therefore, cysteamine modified Au NPs in this report can be used as the stable SERS substrate for detection of acidic pigment. Hence, the data above showed the functionalized substrate with high reproducibility and good universality.

3.3. Application in Real Sample

The use of pigment including amaranth, allura red, lemon yellow, sunset yellow and acid blue is related with health, therefore, it is encouraging to develop a method to detect and distinguish the pigment from mixture sample. It is known that drink is difficult to analyze because the direct SERS signal acquirement of pigment from the SERS substrates cannot avoid the interference of other substance, as the numerous molecules in drink can also excite SERS signal on the substrate. Before the analysis, the extraction of pigment from the complex matrices is important. In this study, the extraction of pigment from liquid samples was carried out by Polyamide power (as shown in Fig. 5(A)), which the amide bond (—CO—NH—) can combine with the sulfonic acid group and hydroxyl of acidic pigment molecules to form strong hydrogen bond and achieve absorption. Under alkaline condition, the ability of hydrogen bond was weakened, so that the pigment was eluted (Fig. 5(B)). The entire pretreatment process won’t exceed 10 min. But before the process of extraction, it’s important to consider the interference of ammonia aqueous solution for SERS detection of pigment in real sample. The SERS spectra of ammonia aqueous solution has been collected by using cysteamine modified substrate to demonstrate the possible interference. As shown in Fig. S6, the SERS signal of ammonia aqueous solution is very weak. Also, the SERS performances of five kinds of pigment molecules mixed with ammonia aqueous solution were detected. All results indicated that ammonia aqueous solution has slighter effect on sensitive detection of pigment and won’t influence detection of real sample. From the SERS spectra of pigment in beverage sample, we could observe the characteristic peaks of pigment in different flavors of drinks could be clearly identified (Fig. 5(C)).

As presented in Fig. 6, there is little difference among the SERS spectra of amaranth, allura red, lemon yellow, sunset yellow and acid blue.
owing to their similar molecular structures, while the Raman shift and related intensities of characteristic bands had slightly fluctuated. Therefore, our data convincingly showed that the pigment molecules in real sample could be successfully distinguished by SERS method.

Attractively, our approach was generically applicable to gold nanoparticles of other surfactants. For example, CA can also be modified with gold nanorods of CTAB as surfactant. As presented in Fig. S7 a large scale of gold nanorods was prepared and there is slight blue

Fig. 3. (A) SERS spectra of amaranth at different concentrations of 20 ppm, 15 ppm, 10 ppm, 5 ppm and 1 ppm detected. (B) Thirty SERS spectra of 5 ppm amaranth collected randomly from 30 spots of the self-assembled Au NP arrays. The intensities of the main vibrations of 5 ppm amaranth at 1344 cm\(^{-1}\) (C) and 1362 cm\(^{-1}\) (D) from the spectra given in (B).

Fig. 4. SERS spectra for detection of allura red (5 ppm) (A), sunset yellow (5 ppm) (B), lemon yellow (5 ppm) (C) and acid blue (1 ppm) (D) respectively. The dotted line represents 633 nm laser for SERS detection.
shift occurred in UV–visible spectra because hydrogen bonds interaction between gold nanorods themselves. When the pigment was detected using gold nanorods with CTAB (CA-Au NRs) as surfactant, a relatively weak SERS signal were detected (Fig. S8(A)). To amplify the weak SERS signal, CA was modified on the surface of the nanorods in the same procedure as described above. Similarly, as shown in Fig. S8(B), SERS intensity reached several orders of magnitude enhancement.

4. Conclusions

In summary, we developed CA modified SERS substrate for detecting trace amount of five kinds of pigment that show weak affinity with Au NPs, which be sensitively detected on the basis of the formation of multi hydrogen bonds between CA and pigment molecules. It should be noted that the present detection method for the pigment had the advantages of high reproducibility, efficiency and good universality.
Compared with preparation of new materials and two-dimensional structure, the proposed method not only avoided complex fabricated processes, but also ensured high stability and reproducibility of the substrate. Furthermore, it was obvious that the present active SERS substrate had demonstrated the advantages of reproducibility, sensitivity for quick detection of acidic pigment, without any complicated instrumentation. Meanwhile, it could be used for detecting pigment in real sample. All the results indicated that the present method provided a promising platform for fabricating fast and economical SERS substrate to detect illegal additives in food.

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Appendix A. Supplementary data

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References