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Rapid and sensitive SERS method for determination of Rhodamine B in chili powder with paper-based substrates

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Surface-enhanced Raman spectroscopy (SERS) was used for detecting Rhodamine B (RB) which was always illegally added in chili powder. Based on density functional theory (DFT), the Raman spectrum of RB was calculated and characteristic peaks of RB were assigned to the corresponding vibrational modes. RB was detected utilizing paper-based SERS substrates which were fabricated via liquid/liquid interface-mediated self-assembly technique. Meanwhile, with simple sample pretreatment, the detection limit could reach 10^{-6} g/g for RB in chili powder (5×10^{-7} g/mL in extraction agent). Within the concentration region from 10^{-2} g/g to 10^{-6} g/g, Raman intensity follows certain function relation to the concentration of RB. The recovery of this method ranges from 96.4% to 108.9%, which could afford rapid and reliable quantitative detection of RB in chili powder. Therefore, the proposed SERS method is well suitable for on-site detection and analysis of RB in chili powder.

Introduction

Rhodamine B (RB), molecular formula: $C_{28}H_{31}ClN_2O_3$, is a kind of synthetic dye and has been identified as illegal additive in food by European Food Safety Authority (EFSA).[1] It has been reported that RB has carcinogenicity, reproductive and developmental toxicity, neurotoxicity and chronic toxicity to humans and animals.[2] However, due to its intensive color and simple availability, some counterfeiters stain ordinary corn flour with industrial dye RB to fake chili powder or enhance the natural color of chili powder.[3] On the other hand, chili powder, which is widely used in various foods and food flavoring, is essential to people's life in food seasoning. This is particularly serious incident greatly undermined the confidence of consumers to food safety and the safety of RB has received increasing attention.[4] Hence, monitoring the abuse of RB in chili powder has become an important but tedious task and there is an urgent need to develop a sensitive, fast and convenient method for detecting RB in chili powder.

So far, several analytical methods such as high performance liquid chromatograph (HPLC),[1] spectrofluorimetry,[5] UV–vis-Spectrophotometry,[6] solid phase extraction-high performance liquid chromatography-tandem mass spectrometry (SPE-LC/MS/MS)[7] and Enzyme-linked immunosorbent assays (ELISA)[8], have been developed for the determination of RB in chili powder. However, these methods generally suffer from complex pretreatment, long working time and expensive equipment which make them far from in suit analysis.[9] It is important to develop a much simpler, faster, and cost-effective method involving minimal sample preparation that can possibly be used on spot.

Surface-enhanced Raman spectroscopy (SERS), as an easy, simple and rapid spectroscopic detection technique[10-12], has shown great potential for detection of target samples at trace concentrations down to the single molecule level.[13,14] This technique, with its molecular fingerprint specificity, high sensitivity, narrow spectral bandwidth and water interference, has been widely used in various fields such as food, materials and medicine.[15-17] Meanwhile, SERS technique could employ portable Raman spectrometer, instead of large-scale equipment, which provides rapid detection of food testing on spot.[18,19] In spite of these advantages, SERS application on RB quantification is not widespread and matching research studies have not been reported so far.

In this study, a novel method to detect RB in chili powder with paper-based SERS substrate was developed. Density functional theory (DFT) calculation has been applied to optimize the molecule structure and calculate Raman spectrum of RB. This spectrum has been compared with the experimental spectrum of RB and showed good agreements between the theoretical and experimental results. The detection limit of 5×10^{-8} M for RB in aqueous solution was achieved. In real sample, the limit of detection could also down to 10^{-6} g/g for RB in chili powder. The method based on SERS technique is reliable, simple to operate and with practical significance for on-site measurement of RB in chili powder.

Experimental

Materials

Silver nitrate ($AgNO_3$), sodium citrate, tetrabutyl ammonium nitrate ($TBA^+NO_3^-$) and Rhodamine B (RB) were obtained from Xilong Chemical Co., Ltd. (Beijing, China), dichloromethane (CH_2Cl_2) was obtained from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China), acetonitrile was obtained from Tianjin Chemical Reagent Co. Ltd. (Tianjin, China), qualitative filter papers (Medium) were obtained from Fushun Civil administration Filter Paper Factory (Fushun, China), chili powder sample was obtained from local food store, deionized water was used for all procedures.

The preparation of paper-based SERS substrate

Silver colloid was prepared according to the Lee and Meisel's method.[20] And the fabrication procedure of the paper-based SERS substrate could refer to our previous work.[21] The simple illustration of the preparation was shown in Figure 1.
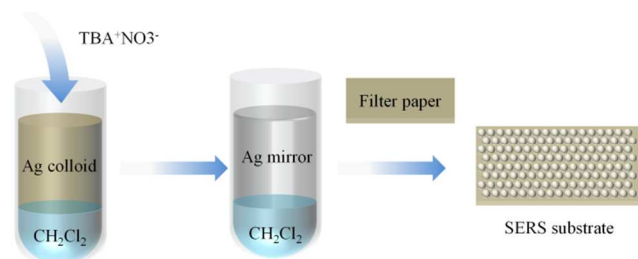

Fig. 1 Preparation of paper-based SERS substrate by liquid-liquid interface self-assembly technique.

Preparation of standard aqueous solution of RB

To prepare the sample standard solution, 0.0479 g RB powder was dissolved into 100 mL deionized water. RB aqueous solutions with concentrations of 5×10^{-6} M、10^{-6} M、5×10^{-7} M、10^{-7} M、5×10^{-8} M were prepared by diluting RB standard solution with different proportions. The blank sample was aqueous solution without RB.

Preparation of spiked chili powder samples containing RB

In the SERS detection of RB in chili powder experiment, the available dried chili was crushed to powder samples. Firstly,

4×10^{-3} g/mL RB aqueous solution was prepared and then the aqueous solution was diluted to different concentrations of 4×10^{-4} g/mL, 4×10^{-5} g/mL, 4×10^{-6} g/mL, and 4×10^{-7} g/mL. Finally, spiked chili powder samples with different concentrations of 10^{-2} g/g、5×10^{-3} g/g、10^{-3} g/g、5×10^{-4} g/g、10^{-4} g/g、5×10^{-5} g/g、10^{-5} g/g、10^{-6} g/g were obtained by adding different volumes of the above-mentioned concentrations of the RB aqueous solution into 1 g chili powder. The blank sample was the chili powder without spiked with RB.

In the recovery experiment, spiked chili powder samples with different concentrations of 2.5×10^{-4} g/g, 2.5×10^{-5} g/g and 2.5×10^{-6} g/g were obtained by mixing 0.625 mL RB aqueous solution of 4×10^{-4} g/mL, 4×10^{-5} g/mL, 4×10^{-6} g/mL with 1 g chili powder respectively.

Pretreatment of spiked chili powder samples

The pretreatment of chili powder combines with centrifugal and ultrasonic treatment. Firstly, 2 mL acetonitrile, which was used as extraction agent, was added into 1g spiked chili powder sample, after shaking for 30 s and ultrasonic extraction for 10 min, 1 mL supernatant was added to the centrifuge tube. Then centrifuging for 2 min, the tube was taken to stand upright and the supernatant would be detected (Fig. 2).[22,23] Therefore, the concentration of RB in the supernatant used in SERS measurements is half of that in spiked chili powder samples. In later parts of this paper, we employ the original concentrations of RB in chili powder to evaluate the ability of SERS method for detecting RB in chili powder. Pretreatment of all samples in this paper followed this method.
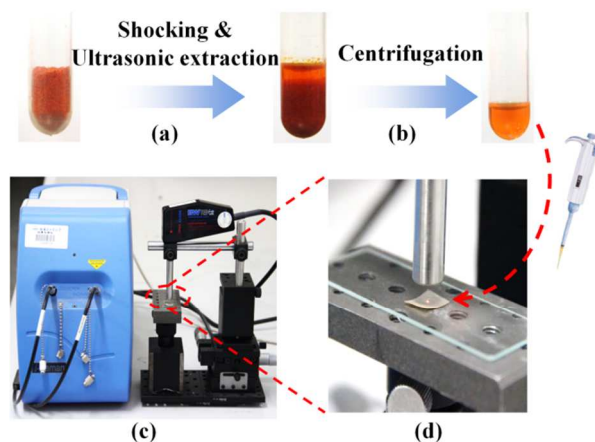

Fig. 2 Illustration of the rapid method for detecting RB in chili powder with SERS. (a) 2mL acetonitrile was added in the spiked chili powder with shocking and ultrasonic extraction. (b) 1mL supernatant was taken out with centrifugation. (c) The sample after pretreatment was detected with the paper-based substrate. (d) The enlarged figure of the detection process.

SERS measurement

Raman spectra were recorded by a portable compact laser Raman Spectrometer BWS415-785H (B&W Tek, Inc.). The excitation wavelength of the laser is 785 nm. The spot size focus laser beam is about 10 μm in diameter. The typical laser power and accumulation time were 150 mW and 5 s unless

otherwise stated. The spectrometer provides Raman spectrum over the range of 68 to 2700 cm^{-1} with a spectral resolution of batter than 3 cm^{-1}. Spectral data were collected by the Bwram 1.01.20 software (B&W Tek, Inc.). Boxcar averaging was used to smooth the raw spectrum, and a baseline correction routine was performed to obtain the final spectrum with the background subtracted.

Quantum chemical calculations and the detection of RB solid powder

Density functional theory (DFT) was performed utilizing Gaussian 09 program package. The molecular structure of RB was optimized and the Raman spectrum of RB was calculated with hybrid density functional B3LYP and basic function set 6-31G. B3LYP when used with the 6-31G provides excellent results.[24]

A small amount of RB powder were taken in the sample bag and the laser probe of portable Raman spectrometer was lay on the surface of the sample for testing. The detection conditions are as follows: laser energy is 15 mW, integration time is 5 s.

Results and discussion

Characterization of the Ag colloid and the paper-based SERS substrate

The average diameter of prepared Ag NPs was determined by the dynamic light scattering measurement (Fig. 3(A)). And the scanning electron image (SEM) analysis was performed to depict the distribution of Ag NPs on paper-based substrates (Fig. 3(B)).

As shown in Fig. 3(A), the sizes of the original synthesized Ag NPs were distributed in two regions: 5-15 nm and 60-120 nm. Particles with the diameter ranging from 5 to 15 nm are silver seeds generated at the beginning of reduction reaction. As the reaction is going on, some seeds gradually grow and finally the diameters range from 60 to 120 nm. While some seeds do not grow to big nanoparticles and keep their original sizes.[25,26] In the SEM image (Fig. 3(B)), Ag NPs deposited on filter paper distributes uniformly, the coverage rate of Ag NPs on filter paper is about 90%, and almost no Ag NPs stacks with each other. The average size of Ag NPs is about 50 ± 4 nm analyzed by Image J software.
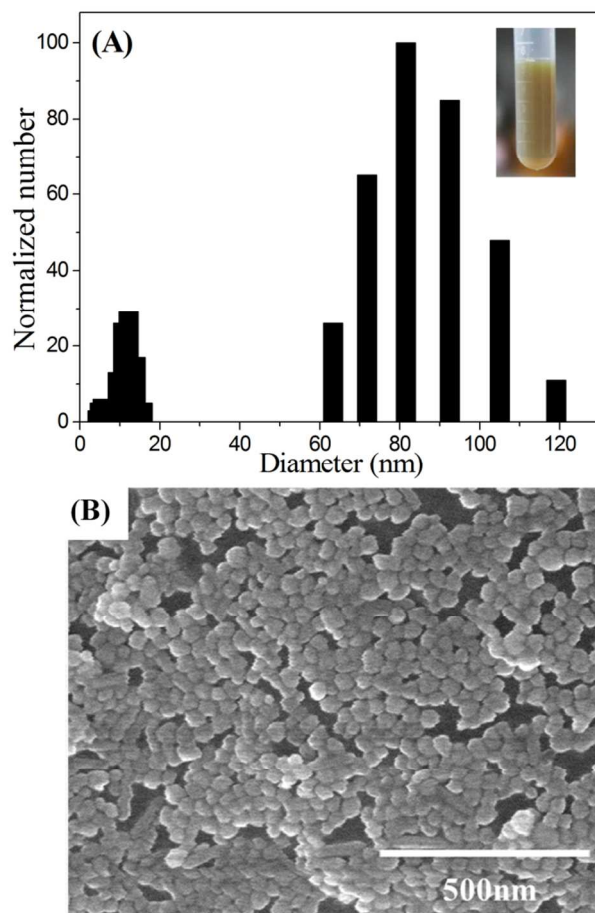

Fig. 3 The distribution of particle size of Ag nanoparticles (A) and SEM image of the paper-based substrate, the magnification is 50K (B). The inset photo in (A) shows the prepared Ag colloid.

Theoretical Raman and solid powder Raman spectra of RB

DFT method is useful for calculating the vibration spectrum of molecular and its efficiency has been proven by large number of studies.[27,28] The optimized structure of RB, the observed Raman spectrum of RB solid powder along with the DFT calculated spectrum of RB molecule were shown in Fig. 4.
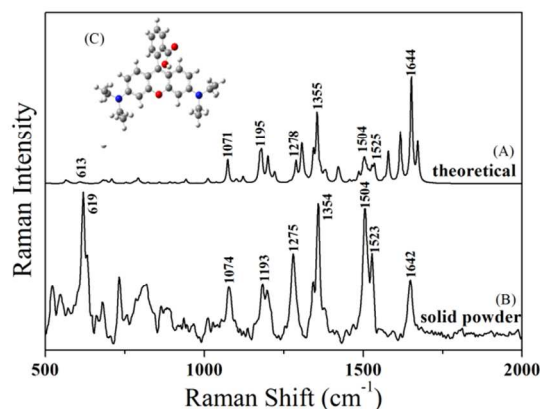

Fig. 4 (A): Theoretical Raman spectrum of RB; (B): Raman spectrum of RB solid powder; (C): Molecular structure of RB.

As can be seen, the predicted spectrum has similar profile with the experimental result except some discrepancies in the peaks position. This may be due to that the theoretical calculation is attributed to an isolated molecule in the gas phase whereas the experimental detection is attributed to RB molecules in the solid phase.[29] The Raman band located at 619 cm^{-1} is attributed to the xanthene ring puckering mode.[30] The most intense bands in the region from 1100 to 1700 cm^{-1} on the Raman spectrum of RB were related to C-C, C-H and C=C.[31] A detailed assignment of the vibrational peaks was given in Table 1:

Table 1 Comparison of theoretical and experimental RB peaks positions

DFT（cm[-1]）	Solid Powder（cm[-1]）	Assignment
613	619	xanthene ring puckering
1195	1193	C-C bridge-bands stretching & aromatic C-H bending
1278	1275	
1355	1354	aromatic C-C bending
1504	1504	aromatic C-H bending
1525	1523	
1644	1642	aromatic C-C bending & C=C stretching

SERS of RB with different concentrations in aqueous solutions

A drop (about 15 μL) of RB aqueous solution with different concentrations was dropped on paper-based SERS substrates and the corresponding spectra were collected (Fig. 5). In Figure 5, characteristic peaks at 1355 cm^{-1}, 1504 cm^{-1}, 1644 cm^{-1} are relatively obvious on SERS spectra of RB aqueous solutions. As a result, the three characteristic peaks are selected to characterize RB. The characteristic peaks can still be identified in Raman spectrum down to the concentration of 5×10^{-8} M, so the detection limit of RB aqueous solution can reach 5×10^{-8} M.
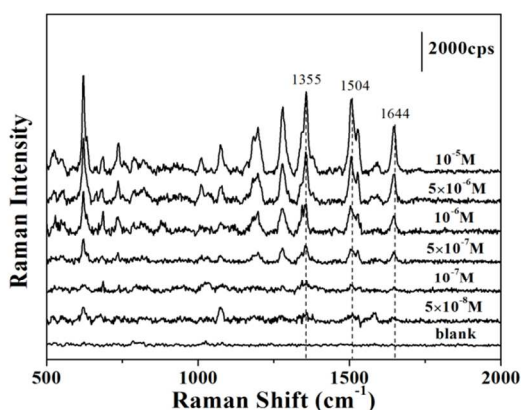

Fig. 5 SERS spectra of RB aqueous solution with different concentrations

SERS detection of RB in chili powder

A drop (about 15 μL) of spiked sample after pretreatment with different concentrations was dropped on paper-based SERS substrates and the spectra were presented in Figure 6.
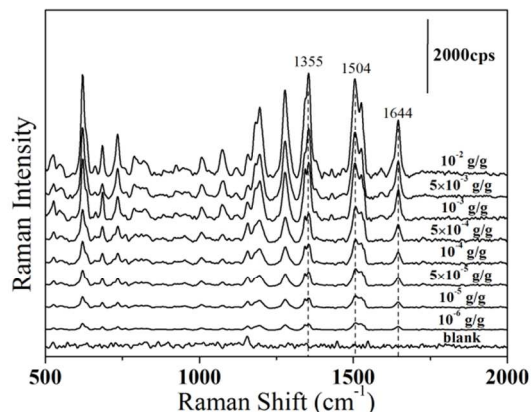

Fig. 6 SERS spectra of RB in chili powder with different concentrations

In Figure 6, the blank sample after pretreatment has no significant peaks at 1355 cm^{-1}, 1504 cm^{-1}, 1644 cm^{-1}, indicating that chili powder and other substances involved in the pretreatment process would not generate interface for detecting RB in chili powder. Meanwhile, when the concentration of RB in chili powder is high, the signal of Raman peaks at 1355 cm^{-1}, 1504 cm^{-1}, 1644 cm^{-1} is particularly obvious; however, with concentrations decreasing, the intensity of signal also declines. The limit of detection was found to be 10^{-6} g/g (5×10^{-7} g/mL in extraction agent), which enables the detection and analysis of RB in chili powder and has great practical value for regulating the safety of chili powder.

Quantitative analysis of RB in chili powder

For quantitative analysis, the peak with the property of uniqueness should be selected to ensure the accuracy. It is obvious that the peak located at 1644 cm^{-1} is suited for quantitative assessment of RB. As the concentration of RB increases, the relative SERS intensity also goes up (Fig. 7). The function relationship of Raman intensity and RB concentrations in the sample was fitted.
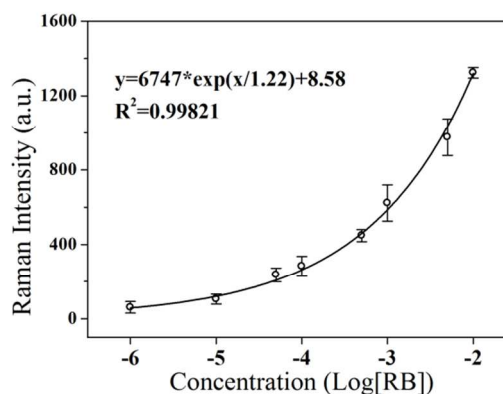

Fig. 7 The relationship between the Raman intensity at peak of 1644 cm^{-1} and RB concentrations in chili powder

To verify the reliability and reasonableness of this detection method, the recovery was estimated by spiking RB with known concentrations to chili powder at three levels: $2.5×10^{-4}$ g/g, $2.5×10^{-5}$ g/g and $2.5×10^{-6}$ g/g. Three replicate samples at all concentrations were measured according to the established pretreatment and detection process. According to the measured Raman intensity at the peak of 1644 cm^{-1} and the fitting function curve, the concentrations of RB in the samples were calculated. Meanwhile, the known spiked concentrations were divided by the calculated concentrations. Thus, the recovery with the relative standard deviation (RSD) of this detection method[32,33] were obtained (Table 2).

Table 2 Recovery of RB concentrations in chili powder; each calculated concentration and recovery is the average of calculating from the Raman intensity of 3 SERS spectra.

Spiked concentrations （g/g）		Calculated Concentrations （g/g）	Recovery （%）	RSD （%）
$2.5×10^{-4}$	1	$2.612×10^{-4}$	104.4	
	2	$2.424×10^{-4}$	96.9	3.848
	3	$2.563×10^{-4}$	102.5	
	Average	**$2.533×10^{-4}$**	**101.3**	
$2.5×10^{-5}$	1	$2.724×10^{-5}$	108.9	
	2	$2.601×10^{-5}$	104.0	2.391
	3	$2.692×10^{-5}$	107.7	
	Average	**$2.672×10^{-5}$**	**106.8**	
$2.5×10^{-6}$	1	$2.528×10^{-6}$	101.1	
	2	$2.542×10^{-6}$	101.7	2.911
	3	$2.410×10^{-6}$	96.4	
	Average	**$2.493×10^{-6}$**	**99.7**	

In Table 2, the recovery of this method for detecting RB in chili powder was calculated to be 96.4%~108.9% and RSDs were all less than 5%, demonstrating that this method is reliable and accurate and can be used for quantitative analysis of RB in chili powder. In addition, this method is convenient, simple and fast, which has great significance for on-site detecting of RB in chili powder. Moreover, the effective and simple extraction techniques and excellent SERS substrates could greatly improve the capability of SERS methods for characterization of chemical contaminants in food.

Conclusions

In summary, the theoretical SERS spectrum of RB was calculated with the help of DFT. Paper-based SERS substrate was fabricated and employed to detect RB. The Raman spectra of RB aqueous solutions were collected and the detection limit could reach $5×10^{-8}$ M. Based on the centrifugal and ultrasonic treatment methods, a feasible sample pretreatment was developed to extract RB in chili powder and the whole procedure only took about 10 min, with the detection limit of 10^{-6} g/g ($5×10^{-7}$ g/mL in extraction agent). In addition, at the concentration ranging from 10^{-2} g/g to 10^{-6} g/g, the Raman intensity of peaks with concentrations of RB in chili powder follows a certain function and the recovery of this detection method ranges from 96.4% to 108.9%. It indicates that this method has excellent reliability and accuracy and enables quantitative analysis for the concentrations of RB in chili powder. On the whole, this method is easy to operate, fast and efficient, which is immensely promising and has unique advantages for on-site detection and identification of RB in chili powder.

Notes and references

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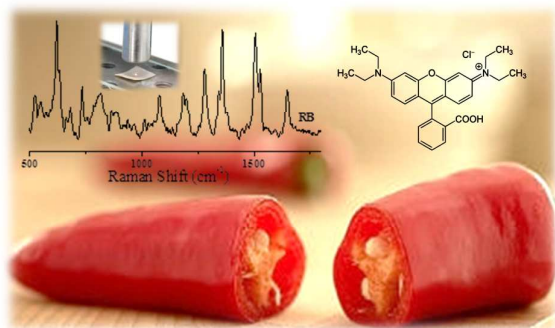
A rapid and reliable approach based on SERS for quantitatively detecting

RB in chili powder using paper-based substrate was developed.