Simultaneous SERS Detection of Multiple Amino Acids Using ZIF-8@AuNPs as Substrate: Classified with 1D Convolutional Neural Network

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Abstract:
Amino acids found in minor coarse cereals are essential for human growth and development and play a crucial role in efficient and rapid quantitative detection. Surface-enhanced Raman spectroscopy (SERS) enables non-destructive, efficient, and rapid sample detection. Traditional SERS detection efficiency is constrained by the use of a single target. In this study, three different amino acids (cysteine, valine, and tryptophan) were detected simultaneously using a ZIF-8@AuNPs composite substrate. The linear range of detection was $10^{-3}$ to $10^{-1}$ M, with a limit of detection (LOD) of $2.40 \times 10^{-4}$ M, $2.24 \times 10^{-4}$ M, and $1.55 \times 10^{-4}$ M, respectively. Same linear range and LODs were achieved with one-dimensional convolutional neural network method. Furthermore, this substrate enabled the effective detection of amino acids in millet and efficient detection of cysteine in health products. This study presents a novel method for simultaneous detection of multiple analytes.

Keywords: Surface-enhanced Raman spectroscopy, ZIF-8@AuNPs, amino acids, multiple analytes, simultaneous detection
1. Introduction

Amino acids are a class of organic compounds containing amino and carboxyl groups that constitute the basic structural unit of protein and play an irreplaceable role in the human body. For example, tryptophan can promote sleep and relieve tension and anxiety [1]. A lack of sufficient amino acids or insufficient intake of amino acids can lead to various serious diseases that can affect memory, growth, and development. Therefore, it is of utmost importance to determine the levels of certain amino acids in various food [2,3,4]. Commonly used methods for detecting amino acid content are typically time-consuming and require a significant number of detection standards [5,6,7]. However, handling an increasing number of samples is difficult. In addition, the detection costs are relatively high [8]. Therefore, there is an urgent need to develop more efficient and cost-effective detection method to meet the demand.

Surface-enhanced Raman spectroscopy (SERS) is an efficient and rapid detection technique. The analyte signals can be quickly obtained by adsorbing the analyte onto the prepared enhanced substrate surface [8,9]. By utilizing the fingerprint information of the analyte, detection results can be obtained without consuming more standard reagents [10].

The characteristic peaks of a target substance are usually a set of Raman peaks. The vibrations of multiple target substances can influence each other and the fingerprint peaks are highly complex, making them difficult to analyze [11]. Therefore, conventional surface-enhanced Raman spectroscopy typically detects a single target substance simultaneously. Numerous amino acids were involved in their detection. The advantages of using conventional SERS methods [12,13] for improving the detection efficiency are not significant. Therefore, we used a one-dimensional convolutional neural network (1D CNN) [14] to simultaneously classify complex Raman signals.
from multiple amino acids to identify the concentration patterns of amino acids within the Raman peaks [15]. Several achievements have been made in the field of Raman spectroscopy analysis by using [16,17,18] 1D-CNN.

Conventional surface-enhanced Raman substrates are typically designed to adsorb only one type of substrate. Typically, the substrate surface contains only one adsorption moiety [19]. However, multiple amino acids must be adsorbed simultaneously. Therefore, the designed substrate should simultaneously provide multiple adsorption sites. Metal-organic framework materials (MOFs) possess multiple exposed active sites on their surface [20], which promote the amplification of hotspots. The infinite, repetitive, periodic unit structure of MOFs enable homogeneous substrate preparation. In addition, MOFs exhibit characteristics such as large specific surface area, adjustable pore size, and strong adsorption [21]. However, MOFs alone only have weak enhancement effects, even do not exhibit significant enhancement effects. Therefore, a composite substrate was prepared by combining MOFs with precious metals, enabling the superior performance of MOFs and achieving efficient SERS enhancement [22,23]

In this study, ZIF-8, one of the most commonly used MOFs, was used as a template to synthesize a novel SERS substrate via modification with AuNPs (ZIF-8@AuNPs). ZIF-8 is a representative MOF known for its large specific surface area and high stability [23,24], which simultaneously provides large and various adsorption sites [25]. The AuNPs formed enhanced hotspots [26], enabling the effective detection of the Raman signals of amino acids. Subsequently, they were classified using the 1D CNN method [27]. The ZIF-8@AuNPs composite substrate significantly enhances the simultaneous detection efficiency of cysteine, valine, and tryptophan, surpassing the constraints of traditional SERS methods.
2. Experimental

2.1 Materials

Trisodium citrate, chloroauric acid (HAuCl₄·3H₂O), zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O), and 2-methylimidazole (2-MIM) were purchased from Sigma-Aldrich. Cysteine (Cys), tryptophan (Trp), valine (Val), phenylalanine (Phe), glutamic acid (Gln), histidine (His), serine (Ser), and other amino acids were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). Methanol was purchased from Tianjin Tianli Chemical Reagent Co. Ltd. (Tianjin, China). Milli-Q water (18.2 MΩ·cm) was used for all the experiments. Shaanbei Millet was purchased from a local supermarket in Yan’an, China. L-Cysteine capsules were purchased from a nearby drugstore in Yan’an, China.

2.2. Synthesis of ZIF-8@AuNPs

2.2.1 Synthesis of ZIF-8

Following a slight modification of the literature reported [28], 1 mmol (0.2975 g) of Zn(NO₃)₂·6H₂O was dissolved in 20 mL methanol solution. Similarly, varying amounts of 2-MIM (3, 5, and 7 mmol) were dissolved in separate 20 mL methanol solutions. The mixture was then stirred for 15 min to ensure complete dissolution. The methanol solution of 2-methylimidazole was added to the methanol solution of Zn(NO₃)₂·6H₂O and the mixture was thoroughly stirred at room temperature for 24 h, resulting in the formation of a white suspension. The crude product was collected by centrifugation at 8000 rpm for 10 min. The product was washed three times with anhydrous methanol and subsequently dried overnight under vacuum at 60 °C.

2.2.2 Synthesis of AuNPs

AuNPs of various particle sizes were synthesized using the classical trisodium citrate reduction
method [29]. The following procedure was performed: A 100 mL solution of chloroauric acid with a mass fraction of 0.01% was prepared and heated to boiling. Subsequently, different volumes (0.3, 0.5, 0.7, 1, and 2 mL) of trisodium citrate solution with a mass fraction of 1% were accurately added under constant stirring. The mixture was boiled continuously for 15 min and allowed to cool to room temperature before further use.

2.2.3 Synthesis of ZIF-8@AuNPs nanocomposite

ZIF-8 was incorporated into a 4mL gold sol using the solution impregnation method. The mixture was then sonicated for 20 min, incubated for 2 h, centrifuged, and washed three times to obtain the ZIF-8@AuNPs composite materials.

2.3 SERS measurement

Several amino acid molecules, including Cys, Val, and Trp, were used to assess the SERS activity of the ZIF-8@AuNPs composites. A series of amino acid solutions with varying concentrations were prepared. The tested molecules (0.8 mL) were mixed with the composite materials (0.2 ml). Subsequently, 10 μL of the mixture was extracted using a pipette and deposited onto a clean glass sheet for Raman spectroscopy measurements. The selected test parameters were as follows: laser wavelength of 532 nm, grating with a 600 mesh, 100× objective lens, laser power of 10% (9.1 mW), integration time of 15 seconds, and twice integration.

2.4 SERS detection of amino acids in Shaanbei millet and healthcare product.

According to the national standard GB 7650-87 [30], millet is subjected to alkaline hydrolysis. The specific procedure involved a small amount of millet in distilled water to eliminate surface stains, followed by drying at 60 °C and grinding. Subsequently, a 40 mg sample was weighed, followed by the addition of 1mL of 10% potassium hydroxide solution. The resulting mixture was
then incubated at 40 °C for 18 h. Standard samples with varying concentrations were introduced into the treated millet, and 10 μL samples were precisely extracted using a pipette for subsequent SERS testing.

The healthcare products purchased were pretreated according to the literature [31]. The pretreatment procedure was as follows: a capsule (0.4 g) was opened and its contents were ground well. Then, 0.1 g of the powder was weighed accurately and placed in a beaker. Subsequently, 4 mL of 0.1 mol·L⁻¹ HCl solution and 16 mL of ultra-pure water were added to the beaker. The mixture was shaken until fully dissolved and then filtered using a 0.25 μm microporous filtration membrane. The resulting filtrate was collected for testing and set aside.

2.5 Instrumentation

The morphologies of the prepared samples were characterized using a scanning electron microscopy (SEM, JSM-7610F) equipped with an energy-dispersive spectrometer (EDS). The UV–vis spectra were recorded using a UV–vis spectrophotometer (UV-2700) in the wavelength range of 200–700 nm. The morphology of the SERS substrate was observed using a transmission electron microscope (TEM, JEOL F200) operating at 200 kV. X-ray photoelectron spectrometry (XPS) was performed using a Thermo Scientific Escalab Xi X-ray photoelectron spectrometer with an Al Kα source (hv = 1486.68 eV). N₂ adsorption and desorption studies were conducted at 77 K using an ASAP2020 Version 4.03 adsorption apparatus. SERS spectra were collected using a Raman spectrometer (LabRAM Soleil).

2.6 1D CNN

1D CNN is a variant of a neural network, used for processing one sequence data. Unlike the two-dimensional convolution used in traditional image processing, 1D-CNN is primarily used for
processing time series data, text data, or other one-dimensional data. The basic structure of a 1D-CNN is similar to that of a traditional CNN, which including convolutional, pooling, and fully connected layers. The basic workflow of the 1D CNN: 1. Input Layer: Receives one-dimensional sequence data as input. 2. Convolutional Layer: Features were extracted using one-dimensional convolution. The convolutional layer convolves the input data with a set of learnable convolutional kernels to produce a series of new feature maps. Each convolutional kernel captures different local patterns and features. 3. Activation Function: Activation functions are applied after the convolutional layer to introduce nonlinearity and enhance power of the network. 4. Pooling Layer: Reduces the dimensionality of the feature maps through pooling operations, reducing computation and extracting the main features. Common pooling operations include max pooling and average pooling. 5. Fully Connected Layer: The output of the pooling layer is connected to one or more fully connected layers. Linear transformations were performed using weight matrices, and activation functions were applied. Fully connected layers were used to learn high-level representations of the input data and perform classification tasks. 6. Output Layer: Depending on the specific task, the output layer can consist of one or more neurons. For example, in classification tasks, the output layer can contain multiple neurons with softmax activation function. 1D CNN has some advantages in processing one-dimensional sequence data. It can automatically capture local patterns and relationships within a sequence, making it suitable for tasks, such as time series analysis, text classification, and speech recognition. By stacking multiple convolutional layers and pooling layers, 1D CNN can extract features at different levels, enabling a better understanding and processing of complex sequence data.

3. Results and discussion
3.1 Characterization of ZIF-8@AuNPs

The synthesis and detection procedures are illustrated in Scheme 1. The MOFs were synthesized by stirring at room temperature, and AuNPs were subsequently coated inside. As shown in Fig. 1 (a), the surface of the ZIF-8 crystal appears smooth, exhibiting a regular dodecahedral structure with high crystallinity. The average particle size was approximately 50 nm. By interrupting the growth process, the size of ZIF-8 nanoparticles can be controlled to obtain the desired particle diameter. However, the surface ligands still have numerous binding sites, leading to slight aggregation of ZIF-8. The aggregation of ZIF-8 particles encapsulates Au nanoparticles, consequently, the diameter of ZIF-8@AuNPs increased as illustrated in Fig. 1 (b). The corresponding TEM images, shown in Fig. S1, illustrate that the ZIF-8 nanoparticles aggregate in a three-dimensional manner. Moreover, characteristic signals of Au were also observed in the XPS and EDS results, as shown in Figs.1 (c) and (d), respectively.

The adsorption sites in ZIF-8 ensure the effective adsorption of analytes, and light compromising of the adsorption capacity caused by the presence of AuNPs. Brunauer-Emmett-Teller (BET) tests were performed and the results verified that AuNPs reduced adsorption capacity. N\textsubscript{2} adsorption and desorption experiments were performed at 77 K and the pore size characterizations of ZIF-8 and ZIF-8@AuNPs were determined. Fig. 2 (a) shows that the BET surface area of ZIF-8 is approximately 1076.79 m\textsuperscript{2}/g, whereas ZIF-8@AuNPs exhibits a BET surface area of approximately 1013.11 m\textsuperscript{2}/g. Moreover, the total pore volume of ZIF-8@AuNPs is measured to be 0.997 cm\textsuperscript{3}/g. Furthermore, the pore size distribution curve depicted in Fig. 2 (b) reveals that the average pore size of ZIF-8 is 5.118 nm, whereas that of ZIF-8@AuNPs is 3.615 nm. The decrease in pore size owing to the presence of AuNPs is also acceptable because the diameter of the
analytes is much smaller than the reduced pore size.

3.2 Size optimization of AuNPs

The size of the AuNPs was carefully selected. Four sizes of AuNPs with various diameters were synthesized and characterized by TEM, as shown in Figs. 3 (a)–(d). The colors of the AuNPs with different diameters were visibly different, and the variation in color is a result of the different localized surface plasmon resonance (LSPR) of the AuNPs. As shown in Fig. 3 (e), the LSPR bands were observed at wavelengths of 537.2, 536.4, 529.0, and 518.2 nm, corresponding to AuNPs with diameters of 50.2, 42.1, 29.3, and 17.4 nm, respectively. The excitation source used was a 532 nm green laser. It was speculated that the AuNPs, with a diameter of 42.1 nm and a LSPR band at 532.0 nm, exhibit the most suitable coupling resonance with the laser, thereby providing better and stronger SERS spectra. The UV-Vis results depicted in Fig. 3 (f) demonstrate a significant reduction in the intensity of the LSPR peak of ZIF-8@AuNPs, whereas the peak position remained unchanged. This finding was attributed to the limited penetration of green light. Moreover, as shown in Fig. S2 despite the larger amount of ZIF-8 compared to AuNPs, it exhibited fewer chemical binding sites with ZIF-8. This finding was consistent with the previous observation, in which ZIF-8 experienced less adsorption site loss.

3.3 Selectivity and assignment on the characteristic SERS band

The SERS spectra of 18 essential amino acids in the human body were acquired to investigate the selectivity of the prepared ZIF-8@AuNPs substrate. As shown in Fig. 4, the ZIF-8@AuNPs substrate demonstrates a selective adsorption capacity for Cys, Val, and Trp, which is attributed to its distinctive pore structure. In addition, only seven SERS spectra are shown in Fig. 4 for conciseness. The SERS spectra of phenylalanine, glutamic acid, histidine, and serine were selected...
randomly from the acquired SERS silent spectra. Val showed strong Raman characteristic peaks at 537, 846, 944, 1349, and 1450 cm\(^{-1}\), among which the peak at 537 cm\(^{-1}\) corresponds to the stretching vibration of C=O bond and the peak of 1349 cm\(^{-1}\) corresponds to the bending vibration of saturated C-H bond [32]. Cys exhibits strong characteristic peaks at 679, 1134, and 1344 cm\(^{-1}\), among which 679 cm\(^{-1}\) and 1344 cm\(^{-1}\) correspond to the out-of-plane bending of -C-S-H and the symmetric stretching vibration of -COO-, respectively [30]. In addition, strong Raman characteristic peaks of Trp at 757, 1010, 1423, and 1542 cm\(^{-1}\), among which the Raman peaks at 757 cm\(^{-1}\) and 1423 cm\(^{-1}\) are attributed to the atomic stretching of C-C and C-N [33].

3.4 Optimization of ZIF-8@AuNPs

AuNPs exhibit significant enhancement of the Raman signal, primarily attributable to their unique LSPR, which typically varies according to the size of the AuNPs. Moreover, the chemical bonding between ZIF-8 and AuNPs within the ZIF-8@AuNPs composite can alter the original LSPR of the AuNPs. The impact on the sizes of AuNPs in the ZIF-8@AuNPs was thoroughly investigated. Fig. 5(a) illustrates the size effect of AuNPs in ZIF-8@AuNPs which agrees with that of bulk AuNPs. Notably, AuNPs with a diameter of 42.1 nm exhibit stronger SERS signals. Zn ions exhibit multiple coordination modes owing to their outer electronic structure. A straightforward means of influencing the coordination mode and consequential structural attributes of MOFs involves meticulous adjustment of the quantity of ligands employed in experimental procedures.

Particle sizes of ZIF-8 were varied by manipulating of the zinc nitrate and 2-MIM ratio (1:3, 1:5, and 1:7), using Val as the probe molecule. The results are shown in Fig. 5(b). ZIF-8 synthesized at a 1:5 ratio demonstrated superior SERS performance.

The quantity of ZIF-8 used is a critical factor in determining the maximum adsorption capacity of
the target molecule. However, an increased quantity is not inherently advantageous, as the consensus postulates that the more distantly positioned the target molecules are from the LSPR center, which diminished the influence of LSPR on their SERS signals. The quantity of ZIF-8 additionally modulates the thickness of the external MOF layer encompassing AuNPs, consequently impacting the SERS intensity of the adsorbed molecules. As shown in Fig. 5(c), discernible SERS signals were observed for ZIF-8 quantities of 1, 5, 8, and 10 mg. This observation highlights the delicate equilibrium between the adsorption capacity and modulating influence of LSPR.

Consequently, AuNPs with diameter of 42.1 nm were selected. The ligand solution ratio for the preparation of ZIF-8 was 1:5, and a composite substrate with a loading capacity of 5 mg was formulated for ensuing discussions concerning the SERS performance of ZIF-8@AuNPs.

3.5 SERS performance

The three amino acids of Val, Cys, and Trp, can be quantitatively analyzed separately, which is the basis for quantitatively analyzing their mixture. Therefore, we first conducted a separate quantitative analysis of the three amino acids, and the results are shown in Fig. 6. The bending vibration of the C-H bond of Val at 1450 cm$^{-1}$, out-of-plane bending vibration of C-S-H bond of Cys at 679 cm$^{-1}$ and atomic stretching of the C-C bond of Trp at 757 cm$^{-1}$ were taken as the quantitative characteristic peaks. As shown in Fig. 6, the linear range for all three amino acids was 10$^{-1}$–10$^{-3}$ M. The linear relationships were satisfactory with correlation coefficients ($R^2$) of 0.9996 for Val, 0.9806 for Cys, and 0.9929 for Trp. LODs were calculated 2.40×10$^{-4}$ M, 2.24×10$^{-4}$ M, 1.55×10$^{-4}$ M for Cys, Val and Trp, respectively. Error bars represent the variance of seven data points associated with each concentration-intensity pair. The consistent width of the error bars
suggests the uniform stability of the SERS substrate. Additionally, SERS intensities of 13 random sites to study the reproducibility. The results are shown in Fig. S3, the adequate relative standard deviation (RSD) ranged from 6.41% to 9.40%.

In the context of composite samples, adherence to the principles of permutations and combinations dictates that the quantity of standard concentration data sets is contingent on the product of standard concentration data points for each amino acid. Here, the base denotes the number of standard concentration data points pertaining to each amino acid and the exponent signifies the number of distinct amino acid types. In this study, three distinct amino acids were tested at discrete concentrations of $10^{-1}$, $10^{-2}$, and $10^{-3}$ M, yielding 27 unique sample sets. To mitigate the potential stochastic occurrences and systematic errors inherent in the instrumentation, a meticulous collection of 60 spectral data points was undertaken for each amalgamated sample set, culminating in the acquisition of 1620 spectral diagrams. All the characteristic SERS spectra of the 27 mixed samples are presented in Fig. S4 (a)-(c), discerning spectra of mixed samples with varying concentrations poses a challenge through conventional spectral analysis methods. Therefore, a strategy employing 1D CNN was introduced for quantitative analysis of three distinct types of amino acids at various concentrations.

After randomizing the data order, a training dataset comprising 70% of the total data (1120 spectra) and a validation dataset comprising 30% (486 spectra) were established. Meticulous analysis of the confusion matrix, delineating distinct amino acid types and concentrations (as shown in the Fig. 7), revealed sporadic misclassifications within the extensive dataset. This observation signifies the congruence between the predictive outputs of the model and the actual values. The computed recognition accuracy of the model was 97.35% coupled with a data recall
rate of 98.75%, underscoring the robustness of the method. In contrast to traditional Principal Component Analysis (PCA) [34], the proposed approach not only automates the extraction of spectral features but also facilitates the classification of large and complex nonlinear datasets.

To substantiate the reliability of this analytical modeling methodology, the dataset was subjected to additional shuffling, and repetitive verification experiments were performed using a five-fold cross-analysis validation approach. The specific operational intricacies are shown in Fig. S4(d). The primary procedure entailed partitioning the complete dataset into five equitably sized segments, with each iteration involving modeling on four data subsets (80%) and validating on one subset (20%). The resulting accuracies for each iteration were 98.44%, 96.25%, 98.44%, 97.50%, and 97.81%, yielding an average recall rate of 97.61%. These commendable experimental outcomes significantly enhanced the reliability of the findings, thus imparting statistical significance to the dataset. Compared with the traditional PCA dimensionality reduction method, the introduced approach markedly elevates the scientific and analytical precision of the data, providing a novel perspective for the analysis of complex components in subsequent studies.

3.6 Applications on real samples

Shaanbei millet samples purchased from the northern Shaanxi local market were processed in accordance with the national standard GB 7650-87. The results obtained from spiked solutions with different concentrations of Val, Cys, and Trp are listed in Table 1. The analysis revealed a Trp concentration of $1.68 \times 10^{-3}$ M in millet, whereas Cys and Val were not detected. The detection of these three amino acids in millet resulted in recoveries within a reasonable range. The recoveries for Cys ranged from 96.0%–102.3% with an RSD value between the range of 7.82%–9.95%. The Trp recoveries were within the range of 95.2%–110.0%, with RSD values in the range...
of 5.17–9.04%. The Val recoveries ranged from 98.4%–105.9%, with an RSD ranging from 2.86%–6.01%. These results indicate good homogeneity and accuracy of the substrate, confirming the applicability of the prepared ZIF-8@AuNPs substrate for real sample detection.

An L-cysteine-containing healthcare product was chosen, and a standard addition recovery experiment was conducted (Table 2). The L-cysteine content in health care products was determined to be $1.13 \times 10^{-2}$ M, with a recovery rate falling within the reasonable range of 97.0% to 105.3%. Compared to high-performance liquid chromatography, SERS technology exhibits superior efficiency and sensitivity [35,36]. This study presented a viable approach for the detection of complex samples.

4. Conclusion

In this study, the developed SERS substrate of ZIF-8@AuNPs demonstrated superior performance, by incorporating the advantages of MOFs and AuNPs. Optimization studies, including the size selection of AuNPs and tuning of ZIF-8 quantity, underscored the delicate balance needed to achieving optimal SERS performance. Three different amino acids (Cys, Val, and Trp) were detected simultaneously using the ZIF-8@AuNPs composite substrate. The linear range of detection was $10^{-3}$ to $10^{-1}$ M, with LODs of $2.40 \times 10^{-4}$ M, $2.24 \times 10^{-4}$ M, and $1.55 \times 10^{-4}$ M, respectively. The developed 1D CNN model exhibited high accuracy and reliability for classifying amino acids in complex mixtures. The method was further validated through cross-analysis experiments, provided consistent and satisfactory results. The application of the SERS substrate to real samples, such as Shaanbei Millet and health care products, demonstrated its practical utility with recoveries within reasonable ranges. This study contributes to the advancement of efficient amino acid detection methods and highlights the potential of combining
SERS with advanced computational techniques for complex sample analysis. The developed approach opens new avenues for exploring the intricate components in various food analytical studies.
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Author Contributions

Huang Mengping, performed the experiments, data analysis and original draft writing.

Xue Wei, Shuai Ma, and He Jinrong, Ai Ganggang, Sha Yelong Hou Xueyan, and Bai Heping performed data analysis.

Liu Xiaofeng, Li Ran and Zhang Yuqi designed and supervised the project and revised manuscripts.

All authors discussed the results and commented on the manuscript.
Notes

The authors declare no competing financial interest.
References


Fig. 4
Fig. 5

(a) [Graph showing Raman spectra with peaks labeled.]
(b) [Graph showing Raman spectra with peaks labeled.]
(c) [Graph showing Raman spectra with peaks labeled.]
Fig. 6

(a) Raman Shift (cm$^{-1}$) vs. Intensity (a.u.)

(b) I = 47497.46C + 1409.78
R$^2$ = 0.9996

(c) Raman Shift (cm$^{-1}$) vs. Intensity (a.u.)

(d) I = 101832.50C + 1875.78
R$^2$ = 0.9806

(e) Raman Shift (cm$^{-1}$) vs. Intensity (a.u.)

(f) I = 105164.19C + 2908.52
R$^2$ = 0.9929
|   | 00 | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 00| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 01| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 02| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 03| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 04| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 05| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 06| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 07| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 08| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 09| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 10| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 11| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 12| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 13| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 14| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 15| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 16| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 17| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 18| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 19| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 21| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 22| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 23| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 24| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 25| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 26| 15 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
Figure captions

Sketch 1. Schematic illustration of the preparation process for ZIF-8@AuNPs

Fig. 1. SEM images of ZIF-8 (a), ZIF-8@AuNPs (b), XPS spectrum of ZIF-8@AuNPs (c) and EDS spectrum of ZIF-8@AuNPs (d).

Fig. 2. N\textsubscript{2} sorption isotherms at 77 K of ZIF-8 and ZIF-8@Au. (a) The pore size distribution map of ZIF-8 and ZIF-8@Au. (b)

Fig. 3 TEM images of AuNPs with different diameters (a-d), and their UV-Vis spectra with corresponding optical photos (e). UV–vis absorption spectra of AuNPs, ZIF-8 and ZIF-8@AuNPs (f).

Fig. 4 SERS spectra of Cys, Trp, Val, Phe, Gin, His, and Ser.

Fig. 5 SERS spectra of Val with different sizes of AuNPs (a). SERS spectra of Val with different sizes of ZIF-8 (b). SERS spectra of Val with different quantity of ZIF-8 (c).

Fig. 6 SERS spectra of with various concentrations of Val (a), The relationship between concentration of Val and SERS intensity (b). SERS spectra of with various concentrations of Cys (c), The relationship between concentration of Cys and SERS intensity (d). SERS spectra of with various concentrations of Trp (e), The relationship between concentration of Trp and SERS intensity (f).

Fig. 7 The confusion matrix produced by our proposed 1D CNN method delineates the quantitative analysis of composite concentrations involving Val, Cys, and Trp. The green background designates the serial numbers of datasets associated with mixtures featuring diverse concentration levels.
### Table 1: Detection of Val, Cys and Trp in Shaanbei Millet

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amino acid</th>
<th>Detected(M)</th>
<th>Spiked(M)</th>
<th>Found(M)</th>
<th>Recovery(%)</th>
<th>RSD(%)</th>
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</thead>
<tbody>
<tr>
<td>cys</td>
<td>Nd*</td>
<td>5.00×10⁻²</td>
<td>4.80×10⁻²</td>
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<tr>
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<td>9.95</td>
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<tr>
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<td></td>
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<td>6.87×10⁻³</td>
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<tr>
<td></td>
<td></td>
<td>5.00×10⁻²</td>
<td>4.92×10⁻²</td>
<td>98.4</td>
<td>6.01</td>
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<td>1.00×10⁻²</td>
<td>1.06×10⁻²</td>
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*Nd means not detected
Table 2 Detection of L-Cysteine in healthcare product

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<th>Sample</th>
<th>Amino acid</th>
<th>Detected(M)</th>
<th>Spiked(M)</th>
<th>Found(M)</th>
<th>Recovery(%)</th>
<th>RSD(%)</th>
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<tbody>
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<td>L-Cysteine capsule</td>
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