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

Mengping Huang 1, Shuai Ma 1, Jinrong He 1, Wei Xue 1, Xueyan Hou 1, Yuqi Zhang 1,*, Xiaofeng Liu 2,*, Heping Bai 3 and Ran Li 1,*

1 Yan’an Key Laboratory of Green Chemistry Energy, Key Laboratory of New Energy & New Functional Materials, Shaanxi Key Laboratory of Chemical Reaction Engineering, College of Chemistry and Chemical Engineering, College of Mathematics and Computer Science, Yan’an University, Yan’an 716000, China; hmp0526@163.com (M.H.); ms_skx@163.com (S.M.); hejinrong@yau.edu.cn (J.H.); xuewei@yau.edu.cn (W.X.); xueyan12457@163.com (X.H.)
2 Guangxi Key Laboratory of Urban Water Environment, Baise University, Baise 533000, China
3 Experimental and Practical Education Innovation Center, Beijing Normal University at Zhuhai, Zhuhai 519000, China; baiheping@bnu.edu.cn
*
Correspondence: yqzhang@iccas.ac.cn (Y.Z.); xfliu.1988@163.com (X.L.); lirandeqhd@vip.163.com (R.L.)

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 nondestructive, 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 limits of detection (LODs) of $2.40 \times 10^{-4}$ M, $2.24 \times 10^{-4}$ M, and $1.55 \times 10^{-4}$ M, respectively. Same linear ranges and LODs were achieved with a 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 an 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 foods [2–4]. Commonly used methods for detecting amino acid content are typically time-consuming and require a significant number of detection standards [5–7]. 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 a 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 a prepared enhanced substrate surface [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 are 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 obtained in the field of Raman spectroscopy analysis by using [16–18] 1D CNN.

Conventional single-labelled 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 enables homogeneous substrate preparation. In addition, MOFs exhibit characteristics such as a large specific surface area, adjustable pore size, and strong adsorption [21]. However, MOFs alone only have weak enhancement effects, and do not even 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 [24], which simultaneously provide large and virous 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. Materials and Methods

2.1. Materials

Trisodium citrate, chloroaucric acid (HAuCl₄·3H₂O), zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O), and 2-methylimidazole (2-MIM) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 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 process reported in the literature [28], 1 mmol (0.2975 g) of Zn(NO₃)₂·6H₂O was dissolved in 20 mL of 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 via 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 4 mL 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 s, and integration two times. The low laser power and integration time produced no photothermal conversion in the analytes.

2.4. SERS Detection of Amino Acids in Shaanbei Millet and Healthcare Product

According to the national standard GB 7650-87 [30], ref. [31] 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 1 mL 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 purchased healthcare products were pretreated according to the literature [32]. 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 ultrapure 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 microscope (SEM, JSM-7610F, JEOL, Showima City, Japan) equipped with an energy-dispersive spectrometer (EDS). The UV–vis spectra were recorded using a UV–vis spectrophotometer (UV-2700, Fisher Scientific, Hampton, NH, USA) in the wavelength range of 200–700 nm. The morphology of the SERS substrate was observed using a transmission electron microscope (TEM, JEOL F200, JEOL, Showima City, Japan) operating at 200 kV. X-ray photoelectron spectrometry (XPS) was performed using a Thermo Scientific Escalab Xi X-ray photoelectron spectrometer (Thermo Scientific, Waltham, MA, USA) with an Al Kα source (hv = 1486.68 eV). N\(_2\) 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, HORIBA, Kyoto, Japan).
2.6. One-Dimensional CNN

One-dimensional 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, including convolutional pooling and fully connected layers. The basic workflow of the 1D CNN is as follows: 1. Input Layer: One-dimensional sequence data are received as input. 2. Convolutional Layer: Features are 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 the power of the network. 4. Pooling Layer: The dimensionality of the feature maps are reduced 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 are performed using weight matrices, and activation functions are 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 a soft max-activation function. One-dimensional 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 Figure 1. The MOFs were synthesized via stirring at room temperature, and AuNPs were subsequently coated inside. As shown in Figure 1a, 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 the ZIF-8 nanoparticles can be controlled to obtain the desired particle diameter. However, the surface ligands still possess numerous binding sites, leading to the slight aggregation of ZIF-8. The aggregation of the ZIF-8 particles encapsulates Au nanoparticles. Consequently, the diameter of the ZIF-8@AuNPs increased, as illustrated in Figure 1b. The corresponding TEM images, shown in Figure 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 Figures 1c and 1d, respectively.

The adsorption sites in ZIF-8 ensure the effective adsorption of analytes, and light compromising of the adsorption capacity was 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. Figure 2a 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 Figure 2b 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.
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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 using TEM, as shown in Figure 3a–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 Figure 3e, 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 Figure 3f 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 Figure 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.

![TEM images of AuNPs with different diameters and their UV–Vis spectra](image)

Figure 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).

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 Figure 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 Figure 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 a C=O bond, and the peak of 1349 cm⁻¹ corresponds to the bending vibration of a saturated C–H bond [33]. 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 [31]. In addition, strong Raman characteristic peaks of Trp were exhibited 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 [34].
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. Figure 5a 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 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 Figure 5b. ZIF-8 synthesized at a 1:5 ratio demonstrated superior SERS performance.

![Figure 4. SERS spectra of Cys, Trp, Val, Phe, Gin, His, and Ser.](image)

![Figure 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 quantities of ZIF-8 (c).](image)
The quantity of ZIF-8 used is a critical factor in determining the maximum adsorption capacity of the target molecule. As shown in Figure 5c, 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 the modulating influence of LSPR.

Consequently, AuNPs with a 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 separate quantitative analyses of the three amino acids, and the results are shown in Figure 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 Figure 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 as 2.40 \(\times\) 10\(^{-4}\) M, 2.24 \(\times\) 10\(^{-4}\) M, 1.55 \(\times\) 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 were analyzed to study the reproducibility. The results are shown in Figure S3, and the adequate relative standard deviation (RSD) ranged from 6.41% to 9.40%.

![Figure 6](image)

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

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. The detailed concentration information is listed in Table S1. 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 Figure S4a–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 the 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 Figure 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) [35], the proposed approach not only automates the extraction of spectral features but also facilitates the classification of large and complex nonlinear datasets.

![Figure 7](image_url)

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 Figure S4d. 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% to 102.3%, with an RSD value in 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% to 105.9%, with an RSD ranging from 2.86% to 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.

<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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<tr>
<td></td>
<td>cys</td>
<td>Nd *</td>
<td>5.00 × 10^{-2}</td>
<td>4.80 × 10^{-2}</td>
<td>96.0</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
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<td>104.4</td>
<td>2.86</td>
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</table>

*Nd means not detected.

An L-cysteine-containing healthcare product was chosen, and a standard addition recovery experiment was conducted (Table 2). The L-cysteine content in healthcare products was determined to be $1.13 \times 10^{-2}$ M, with a recovery rate falling within the reasonable range of 97.0–105.3%. Compared to high-performance liquid chromatography, SERS technology exhibits superior efficiency and sensitivity [36,37]. This study presented a viable approach for the detection of complex samples.

<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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</table>

4. Conclusions

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 achieve 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 from $10^{-3}$ to $10^{-1}$ M, with LODs of $2.40 \times 10^{-4}$ M,
2.24 × 10⁻⁴ M, and 1.55 × 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, which provided consistent and satisfactory results. The application of the SERS substrate to real samples, such as Shaanbei Millet and healthcare 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.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14052118/s1.

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