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Development of a Heat-Induced Surface-Enhanced Raman Scattering Sensing Method for Rapid Detection of Glutathione in Aqueous Solutions

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In this paper, a direct and simple detection method based on surface-enhanced Raman scattering (SERS) named "heat-induced SERS sensing method" is proposed for rapid determination of glutathione in aqueous solutions. It was found that highly enhanced SERS spectra of glutathione can be obtained if the silver colloids adsorbed with the analyte were heated up before the SERS measurement. Besides, it was revealed that silver particles with a size of ~60 nm are suitable for this study and that the SERS intensity is also influenced by the dropped sample volume, drying temperature, buffer concentration, and pH of the solution. It is noted that the thiol group of glutathione has a particularly strong interaction with a silver surface compared with other small biological molecules without a thiol group, validating this method to detect glutathione selectively. Under the optimal conditions, the detection of glutathione can be finished within 5 min, and the detection limit of ca. 50 nM can be reached, which is much better than the reported detection limit of glutathione (~1 μM) by SERS. The enhancement factor of the proposed heat-induced SERS sensing method for the detection of glutathione is about 7.5×10^6. The proposed method holds a specific selectivity toward glutathione, facilitating its rapid detection in practical applications.

Since surface-enhanced Raman scattering (SERS) was discovered about 3 decades ago,[1,2] this technique has attracted more and more interest in a variety of research fields due to its high sensitivity, high selectivity, and abundant molecular information characteristics. It has been generally accepted that enormous SERS signal enhancement arises mainly from an electromagnetic (EM) mechanism[3−6] and that a charge-transfer (CT) mechanism[7−9] has also significant contribution to it. SERS holds various attractive advantages but it still suffers from serious problems, such as reproducibility, substrate stability, and limitation of substrates that exhibit significant localized surface plasmon resonance (LSPR) behavior. Nevertheless, the applications of SERS to surface analysis, biotechnology, forensic science, environmental analysis, and other different disciplines have been matters of keen interest.[10−12]

Among the diverse applications of this promising technique, biological and biomedical applications have always received great attention because of the importance and necessity for human lives. However, these applications have been restricted by poor reproducibility and low enhancement factor of SERS for the detections of biomolecules. To overcome these problems, a number of novel SERS-based Raman label-free methods[13−18] and Raman dye-mediated methods[19−21] have recently been proposed.

Glutathione (γ-L-glutamyl-L-cysteinylglycine; GSH) is the most abundant nonprotein thiol source in most of mammalian tissues.[22,23] It naturally exists both in the reduced form (GSH) and oxidized dimeric form (GSSG). Other than maintaining thiol−disulfide reduction−oxidation potential, glutathione also has many other crucial biological functions, such as repair of the oxidative damage

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of erythrocyte,[24] detoxication,[25] signal transduction,[26] and transportation/elimination of metal ions.[27] Recent studies have also suggested that the level of glutathione in tissues is related to a variety of diseases, such as cancers,[28,29] Alzheimer's disease,[26] Parkinson's disease,[26] HIV,[30] and diseases caused by aging.[23]

Because of the biological importance of glutathione, a number of screening methods have been developed. Separation techniques combined with mass spectrometry, electrochemical systems, fluorescence spectrometry, and UV/vis spectrometry have widely been applied for the detection of glutathione.[31−34] Direct quantitative analysis of glutathione by enzymatic methods,[35] spectroscopic methods,[36] electrochemical systems,[37,38] and mass spectrometry[39] have also been extensively studied. Since glutathione can cause the aggregation of noble metal nanoparticles, detection of glutathione based on such aggregations has also been proposed.[40−42]

Regarding the detection of glutathione by SERS, very few studies were reported so far due to the poor enhancement factor of glutathione.[43,44] Larsson and Lindgren obtained SERS spectra of glutathione aqueous solutions by use of gold-containing chromatographic beads in 2005.[43] It took 30 min to measure a SERS spectrum of the 0.5 mM aqueous solutions of glutathione in that study, so the detection of glutathione by SERS was far away from being a useful analytical method. In our previous study, we developed a reversed reporting agent method for the selective detection of glutathione by SERS technique.[44] This method, however, also could not always solve the problems such as the poor sensitivity and relatively long analysis time.

To overcome the limitations of SERS in the detection of glutathione, a novel SERS method called "heat-induced SERS sensing method" is developed in the present study. This method allows the direct and rapid detection of glutathione in aqueous solutions. A silver colloidal nanoparticle solution is used as a SERS substrate in order to accelerate the screening time and to achieve the higher sensitivity. Prior to each SERS measurement, glutathione mixed with the silver colloidal solution is heated to form a dry film of the SERS substrate layer. By optimizing the detection conditions, including varying dropped sample volume, drying temperature, buffer concentration, and pH of the solution, highly enhanced glutathione SERS spectra can be obtained. Moreover, quantitative analysis of glutathione can also be achieved by correlating the SERS intensity with the concentration of glutathione examined.

EXPERIMENTAL SECTION

Materials. Silver nitrate, sodium citrate, hydrochloric acid, sodium hydroxide, glutathione, glycine, alanine, valine, serine, threonine, lysine, arginine, leucine, isoleucine, proline, histidine, aspartic acid, asparigine, glutamic acid, glutamine, cysteine, methionine, phenylalanine, tyrosine, and tryptophan were obtained Wako Co. Ltd. (Osaka, Japan) and were used without further purification. Triply distilled water was used throughout the study.

Preparation of the Silver Nanoparticle Colloidal Solution. The citrate-reduced silver colloidal solution used in this study was synthesized according to the modified Lee and Meisel method.[45,46] In brief, 90 mg of silver nitrate was dissolved in 500 mL of triply distilled water and was refluxed rapidly to boil under vigorous stirring. Ten milliliters of 1% (w/v) sodium citrate aqueous solution was added to the solution right after the boiling commenced and the solution was kept boiling until the designated heating time was reached. The reduced silver nanoparticle colloidal solution was removed from the hot plate and placed in an ice bath immediately to cease the reduction of silver colloids. Absorption spectra of the prepared silver colloidal solutions prepared under different reduction times were recorded by a Shimadzu UV-3101 UV/vis spectrometer to monitor the characteristics of the reduced silver nanoparticle colloidal solutions.

SERS Measurements. All SERS and Raman spectra were measured by a HoloProbe VPT system manufactured by Kaiser Optical Systems (Ann Arbor, MI, USA). Aluminum pan plates (0219-0062, Perkin-Elmer) were used to carrying the analytes mixed silver colloidal solution. To prevent the apparent pH elevation, 50 mL of 10 mM citrate buffer solution (pH = 4.0) was mixed with 50 mL of the prepared silver colloidal solution before the SERS measurement in the whole study unless otherwise specified. The 785 nm line of a NIR laser (Invictus; Kaiser Optical Systems) was used as the excitation source and the spot size of the laser beam at the sample was 10 μm. The laser power was ~15 mW at the sample position, and the exposure time for each SERS measurement was set to be 1 s with 10 accumulations. Field emission scanning electron microscopy (FE-SEM), using a JSM-6700F microscope (JOEL) with an accelerating voltage of 3 kV, was employed to explore the morphologies of silver nanocolloids after different pretreatments.

RESULTS AND DISCUSSION

Characteristics of Prepared Silver Colloidal Nanoparticles. Figure 1A shows absorption spectra of the silver nanoparticle colloidal solutions prepared with different reduction times

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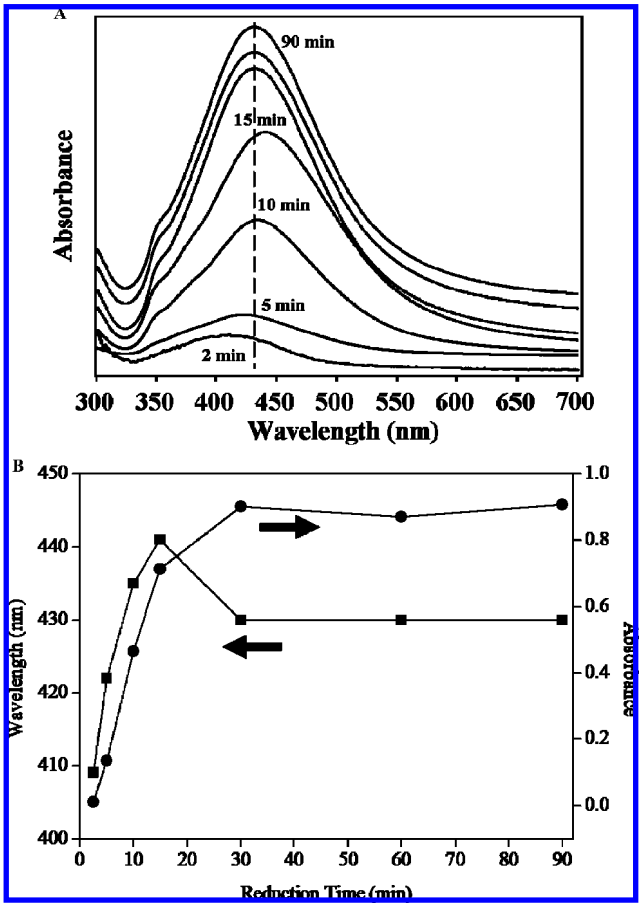

Figure 1. (A) Absorption spectra of the citrate-reduced silver colloidal solutions prepared with different reduction times (from bottom to top, 2, 5, 10, 15, 30, 60, and 90 min, respectively). The solution was diluted by 10 times before measuring the spectra, except for the solution prepared with 2 min of reduction time. (B) Correlation between absorption intensity (●) and absorption maximum wavelength (■) of the citrate-reduced silver colloidal solutions and the reduction time. The solution was diluted 10 times before measuring the spectra.

of 2−90 min. The absorption maximum of the silver nanoparticle colloidal solutions changes from 409 to 441 nm with the increase in the boiling time. The change in the absorption maximum corresponds to the variation in the size of silver nanoparticles from ca. 30 to 60 nm.[57] Figure 1B plots the absorption intensity and the absorption maximum versus the boiling time. It is indicated that the absorption maximum of the prepared citrate-reduced silver colloidal solution red-shifted from 409 to 441 nm in the initial 15 min of reduction. When the silver colloidal solution was kept boiling for more than 30 min, the absorption maximum blue-shifted from 441 to 430 nm (Figure 1A,B). This observation is in good agreement with the fact that the size of citrate-reduced silver clusters increases initially as the boiling time increases, but they dissociate into smaller particles as the reduction reaction continues.[47] It can be also observed from Figure 1B that the absorption intensity of silver nanoparticle colloidal solutions increased as the reduction time increased and reached the maximum when the reduction time is longer than 30 min, which can be concluded that the silver nanoparticle concentration increased as the reduction time increased and will approach the maximum value when the reduction time is longer than 30 min.

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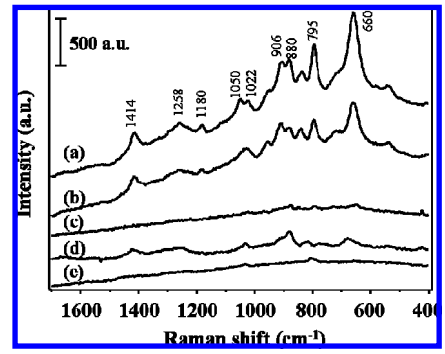

Figure 2. Obtained SERS or Raman spectra of glutathione with different treatments: (from top to bottom) the SERS spectra detected by the heat-induced method (a), the dry-film method (b), without any pretreatment (c), the Raman spectrum of a 0.5 M glutathione aqueous solution without silver colloids (d), and the blank test (e). The concentration of glutathione was 10 μM for SERS detections, silver colloids with 15 min of reduction were used, and the pH of the solution was 4.0.

Detections of Glutathione by SERS with Different Treatments. Since the reported attempts for SERS detections of glutathione proved that the direct detection of glutathione by silver colloids suffered from a poor enhancement factor,[43] different sample pretreatments including a dry film method and a heat-induced method were applied in this study prior to the SERS measurements. In the dry film method, the glutathione mixed silver colloidal solution was dried under room temperature for 90 min, while in the heat-induced method it was dried by heating it at 100 °C for 3 min. Parts a, b, and c of Figure 2 show SERS spectra of 10 μM glutathione obtained with the heat-induced method, the dry film method, and without any pretreatment, respectively. The measurement of glutathione without any pretreatment is obtained by directly measuring the SERS spectra of glutathione mixed with silver colloidal solution dropped in the aluminum pan plates. For comparison, Raman spectra of a 0.5 M glutathione aqueous solution and the blank test of the heat-induced method are also shown in parts d and e of Figure 2, respectively. As can be seen in Figure 2c, the SERS spectrum of glutathione without pretreatment shows a very poor enhancement factor, as reported previously. On the contrary, the enhancement factor is increased dramatically if the dry film method is applied. Although the dry film method improved the enhancement factor significantly, it took more than 90 min to dry the sample at room temperature. On the other hand, when the heating process was applied, the SERS intensity of glutathione was further enhanced and the drying procedure was done in only 3 min. Figure 3 shows the FESEM images of silver nanocolloids without any pretreatment and after different pretreatments. As can be seen in Figure 3A, without any pretreatment, only few silver nanoparticles can be fixed on the aluminum pan. However, when the dry-film method is applied (Figure 3B), more silver nanoparticles were fixed and partial aggregations of the silver nanoparticles are found. In comparison, serious aggregation of the silver nanoparticles was found when the heat-induced method was engaged, and a continuous film was almost formed (Figure 3C). These remarkable differences between the silver nanoparticles with different treatments should be the

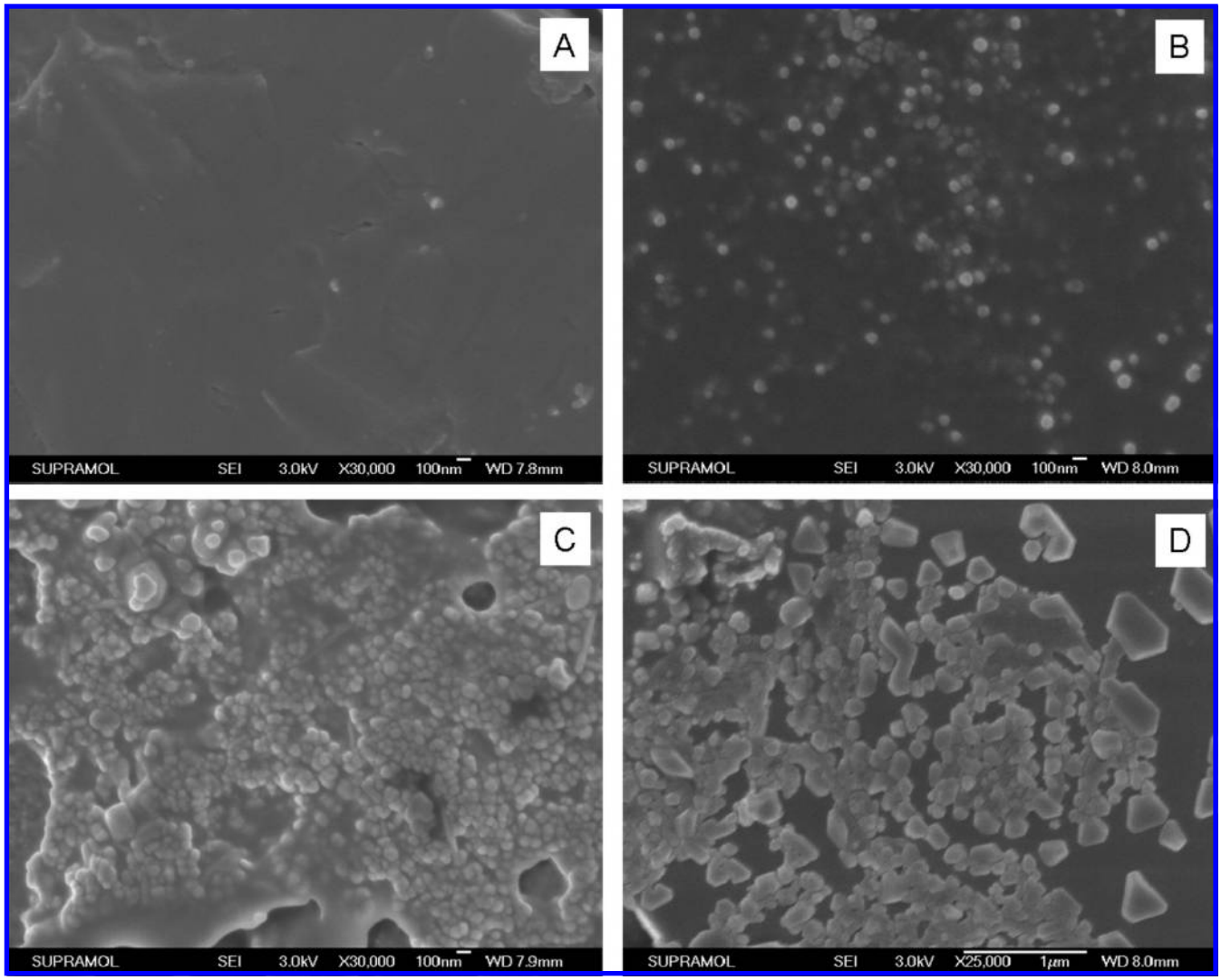

Figure 3. FESEM images of glutathione in the silver colloidal solutions without any pretreatment (A), with the dry-film method (B), with the heat-induced method (C), and the blank test by the heat-induced method (D). The silver colloids with 15 min of reduction were used, the concentration of glutathione in the sample solution is 10 μM, its pH was 4.0, and the sample volume was 30 μL.

reason that the heat-induced method has a much higher enhancement factor than other treatment methods. On the other hand, as mentioned in the previous section, glutathione can cause the aggregation of noble metal nanoparticles. To get a further understanding of the morphology difference, the image of blank test obtained by a heat-induced method was also taken and is displayed in Figure 3D. As can be observed from Figure 3C,D, the addition of glutathione must play an important role in the aggregation of the silver nanoparticles.

Table 1 tabulates the observed main peak positions and their band assignments in the SERS and Raman of glutathione detected under the different treatments and conditions.[43,48−51] It is noted that most of the bands of glutathione remain at their original positions or just slightly shift after the drying process, even under 100 °C. This observation indicates that the heating process accelerates the drying process while the molecular information of glutathione adsorbed on the silver surfaces is kept.

Effect of Ag Particle Size. To investigate the effect of silver colloid size and to find the best morphology for the SERS detection of glutathione, the silver colloids prepared with different reduction times were used in detecting glutathione with the heat-induced method, the dry film method, and without pretreatment. To monitor the effect of reducing time, a SERS band located at 660 cm^{-1} due to the C−S stretching mode was selected, and the results are plotted in Figure 4. As can be observed in the figure, the SERS intensity of glutathione is poor without the drying process. For the dry film method, the SERS intensity of glutathione increases as the reduction time increases and reaches the maximum value when the reduction time is 60 min. When the heat-induced method is applied, the SERS intensity increases rapidly as the reduction time increases and the SERS intensity reaches the maximum value when the reduction time is 15 min, but it decreases gradually when the reduction time is longer than

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Table 1. Observed Wavenumbers (cm^{-1}) of Raman Bands and Their Assignments in SERS Spectra of Glutathione in Silver Colloids Obtained with Different Methods and Those in a Raman Spectrum of Glutathione of a 0.5 M Aqueous Solution[43,48−51]

Raman	SERS			
0.5 M aqueous solution	no pretreatment	dry-film	heat-induced SERS sensing method	assignments[43,48−51]
2571				S−H stretching
1665				amide I
1417	1393	1393	1414	$-COO^-$ symmetric stretching
1265	1234	1235	1258	amide III
	1115	1115	1125	N−C stretching + C−C stretching
		1053	1053	C−N stretching of Glu
1031	1034	1034	1034	C−N stretching of Gly
	1007	1007	1007	C−C stretching
		905	905	$C-COO^-$ stretching
880	875	880	880	C−C stretching
819	853	838	838	C−CN stretching
	795	795	795	$-COO^-$ bending
765	717	717	717	$-COO^-$ deformation
678	655	660	660	C−S stretching
536		539	539	N−C−C deformation
	236	231	231	Ag−S stretching

15 min. As is shown in Figure 1, the size of silver colloids keeps increasing from that of the initial 15 min of reduction to ~60 nm, but the colloids dissociate into smaller particles as the reduction continues. These results in Figure 1B correspond to the tendency of SERS intensity changes shown in Figure 4. Therefore, when the heat-induced method is utilized for the detection of glutathione, silver colloids with larger size (~60 nm) is appropriate for inducing the enhanced Raman signals. In the following studies, the silver colloids prepared with the reduction time of 15 min in combination with the heat-induced method will be used for exploring other features in the detection of glutathione.

Effects of the Amounts of Silver Colloids and Drying Temperature during the Heat-Induced Process. Since the intense SERS signals are induced by heating the glutathione mixed silver colloidal solution, it is important to drop the proper amounts of silver colloids into the sample holder not only to maintain the minimum screening time but also to keep the high enhancement factor. The amounts of dropped silver colloids can be tuned by either changing the dropped sample volume or varying the concentration of silver colloids in the substrate solution. Figure 5 plots a SERS intensity at 660 cm^{-1} of glutathione versus the dropped sample volume with the fixed silver concentration. It can be seen from Figure 5 that the SERS intensity of glutathione increases as the dropped sample volume increases and approaches the maximum value above 60 μL of the dropped volume. It took less than 5 min to dry the sample if the sample volume is less than 60 μL, but it takes more than 10 min if it is more than 100 μL. Therefore, the sample volume of 60 μL takes advantage of both the high enhancement factor and the short detection time. Effects of different silver colloid concentrations were also examined for the SERS determination of glutathione. The relative silver colloid concentration of the original prepared silver colloidal solution was set as 1.0×, and it was found that the concentration of silver colloid does not have apparent influence on the SERS intensity in the silver colloid concentration range of 0.6−1.8×.

Drying temperature is another important parameter that influences the drying process of the glutathione mixed silver colloidal solution, since it may affect the stability of glutathione adsorbed on the silver surfaces, the drying time, and even the

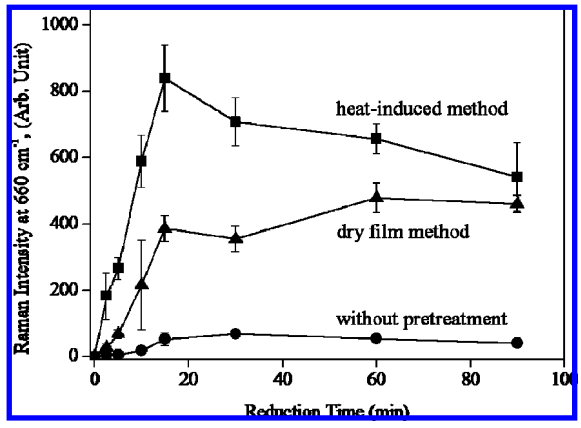

Figure 4. SERS intensity of glutathione in the silver colloidal solutions prepared with different reduction times detected with the heat-induced method (■), the dry-film method (▲), and without pretreatment (●). The concentration of glutathione in the sample solution is 10 μM, its pH was 4.0, and the sample volume was 30 μL.

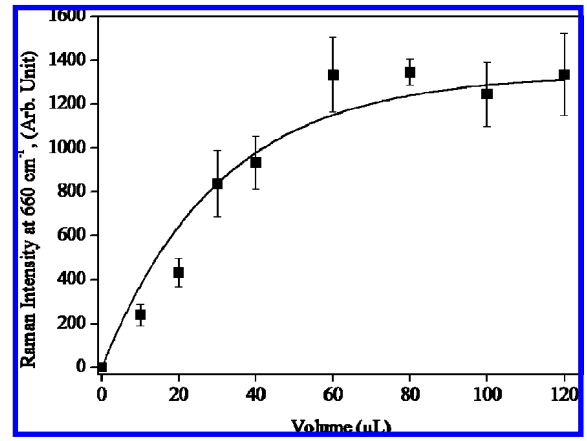

Figure 5. SERS intensity of glutathione detected by using different sample volumes. The concentration of glutathione was 10 μM, silver colloids with 15 min of reduction were used, pH was 4.0, and sample was dried at 100 °C.

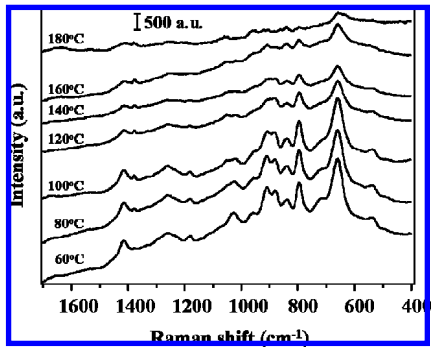

Figure 6. SERS spectra of glutathione obtained with different drying temperatures: (from top to bottom) 180, 160, 140, 120, 100, 80, and 60 °C. Silver colloid with the reduction time of 15 min was used. The glutathione concentration was 10 μM, the sample volume was 60 μL, and the pH of the solution was 4.0.

morphology of silver aggregates after the drying process. To study the effect of the applied temperature, the sample was dried by heating at a temperature from 60 to 180 °C prior to the SERS detection. As can be seen in Figure 6, intense SERS signals can be observed if the drying temperature is between 60 and 100 °C, and they are reduced apparently when it is higher than 120 °C. The possible reason for this observation is that the morphology of silver colloids aggregates is no more suitable above 120 °C for inducing the intense SERS signal. The other possible reason may be the lower stability of glutathione adsorbed on the surfaces of silver colloidal aggregates at higher temperatures.

pH Effects in the SERS Detection of Glutathione. Glutathione has four acid dissociation constants and their pKs are 2.05 for −COOH of glutamic acid, 3.40 for −COOH of glycine, 8.72 for −SH, and 9.49 for the amino group, respectively,[52,53] making the structure of glutathione sensitive to the pH variation. Moreover, since the citrate-reduced silver colloids are surrounded by negatively charged citrate ions, surface charges of silver particles are also affected by the pH of the solution. Therefore, the SERS intensity of adsorbed glutathione should depend on the pH. To examine the pH effects in the SERS detection of glutathione, the pH of the silver substrate solutions was adjusted by the addition of a HCl or NaOH solution before the addition of glutathione solution. The SERS intensity of glutathione detected under different pHs is plotted in Figure 7. According to Figure 7, the highest enhancement factor of glutathione is obtained when the pH of the silver colloidal solution is around 4.0.

Since the silver particles used in this study are negatively charged, the positively charged form and zwitterionic form of glutathione (i.e, pH < 8.7) are much easier to adsorb on the silver surfaces. However, silver aggregates may be destroyed under strong acidic conditions, so that the SERS signal of glutathione is almost absent when the pH is lower than 2.5. The other reason is that glutathione causes the aggregation of silver colloids when the pH is around 4.0 and that no aggregation would occur in neutral or higher pH.[42]

Citrate Buffer Concentration Effect. To further confirm that the observed SERS spectra arise from the adsorption of glutathione, SERS spectra were measured by use of silver colloidal solutions mixed with citrate buffer solutions with different concentrations. Since the surface charges of silver colloids vary with the concentration of citrate buffer solutions, the SERS signal

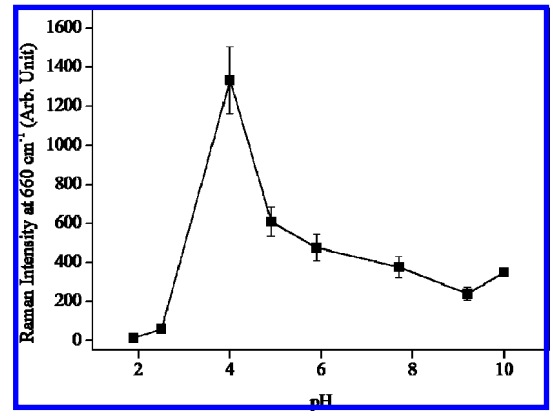

Figure 7. SERS intensity of glutathione obtained under different pHs. The concentration of glutathione was 10 μM, the dropped sample volume was 60 μL, the silver colloid with the reduction time of 15 min was used, and the sample was dried at 100 °C.

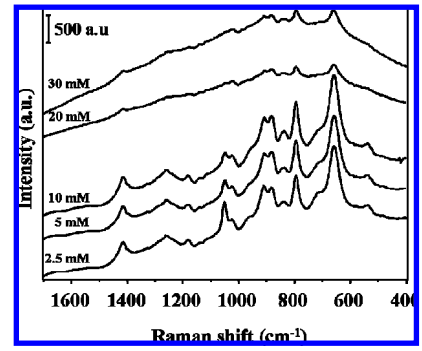

Figure 8. SERS spectra of glutathione obtained with different concentrations of citrate buffer solutions. The concentration of glutathione was 10 μM, the dropped sample volume was 60 μL, the silver colloid with the reduction time of 15 min was used, the pH was 4.0, and the sample was dried at 100 °C.

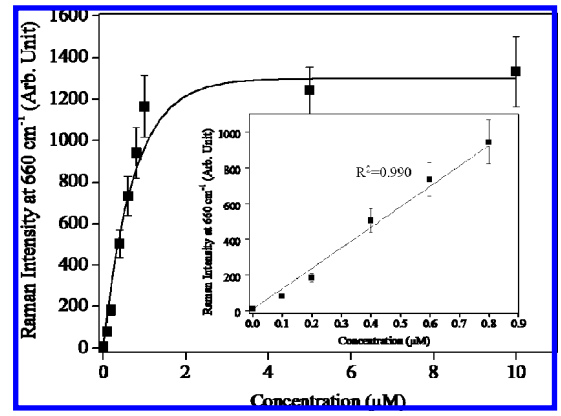

Figure 9. The concentration profile of glutathione detected by the heat-induced SERS method. The silver colloid prepared with the reduction time of 15 min was used, the dropped sample volume was 60 μL, the pH was 4.0, and the sample was dried at 100 °C. The inset shows the enlarged figure for the concentration range of 100−800 nM.

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Table 2. SERS Intensities of a COO^- Symmetric Stretching Band at 1410 cm^{-1} for Various Amino Acids (10 μM) and Glutathione Observed by the Heat-Induced SERS Method Proposed in This Study (in arbitrary units)

samples	SERS intensity of an $-COO^-$ symmetric stretching band at 1410 cm^{-1} (au)	samples	SERS intensity of an $-COO^-$ symmetric stretching band at 1410 cm^{-1} (au)
glutathione	399.2 ± 49.6	lysine	4.3 ± 2.9
cysteine	300.2 ± 40.2	aspartic acid	7.1 ± 4.6
methionine	26.7 ± 8.6	asparigine	2.6 ± 2.1
glycine	6.2 ± 3.9	glutamic acid	5.6 ± 3.8
alanine	3.4 ± 3.2	glutamine	1.5 ± 2.0
valine	1.55 ± 4.0	tryptophan	36.9 ± 10.2
serine	2.0 ± 2.7	phenylalanine	3.9 ± 2.9
threonine	2.1 ± 2.1	tyrosine	2.2 ± 2.4
leucine	1.7 ± 2.5	proline	4.8 ± 5.3
isoleucine	2.2 ± 1.8	histidine	2.4 ± 4.1
arginine	2.0 ± 3.2		

of glutathione would also be affected if the SERS spectra are contributed by the adsorption of glutathione by silver colloidal particles. Figure 8 shows the SERS spectra of glutathione under the different concentrations of citrate buffer solutions. It is noted that the SERS intensity of glutathione is relatively higher when the concentration of citrate buffer is less than 10 mM. This observation is in good agreement with the assumption that the intense SERS signal comes from the adsorption of glutathione on the silver surfaces. When the citrate concentration is higher, glutathione molecules cannot adsorb on the surface of silver nanoparticles nor cause the aggregation easily. On the other hand, when the citrate buffer concentration is lower, the adsorption of glutathione on the silver surface is much easier, inducing the aggregation, which leads to an intense SERS signal.

Linearity, Detection Limit, And Enhancement Factor in the SERS Detection of Glutathione. To investigate the linear range of the heat-induced method in the SERS detection of glutathione, different concentrations of glutathione were examined under the optimal conditions, i.e. the size of ~60 nm of silver nanocolloids, 60 μL of sample volume, 100 °C of drying temperature, 10 mM of citrate buffer, and pH of 4.0. Figure 9 plots the SERS intensity at 660 cm^{-1} versus the concentration of glutathione. As can be seen in the plot, the SERS signal increases rapidly as the concentration of glutathione increases and approaches the maximum value when the concentration is higher than 1 μM. This observation is consisted with the behavior of surface adsorption.[54,55] In this isotherm, the rate of change in the surface coverage of silver surfaces due to adsorption is proportional to the concentration of the glutathione and the number of vacant sites of silver colloids. It may be deduced from the observation that there is a strong interaction between silver nanoparticles and glutathione based on the fact that almost all the active sites on the silver surfaces are occupied by glutathione molecules, even if the concentration of glutathione is as low as 1 μM. Since the adsorption of glutathione fits the Langmuir isotherm, the linear relationship can be obtained under the condition that the concentration of glutathione is low enough. The linear regression in the different concentration ranges were calculated, the results showed that the regression coefficient (R^2) is higher than 0.99 in the concentration range of 100−800 nM (see the inset of Figure 9). The detection limit of glutathione by the heat-induced SERS method was estimated on the basis of 3 times the blank test standard deviation. It was found that the detection limit is 50 nM when the intensity of C−S stretching vibration band at 660 cm^{-1} is selected.

As for the enhancement factor (EF) for glutathione, it was calculated by the equation[56]

$$EF = (I_{SERS}/I_{Raman})(N_{neat}/N_{ads}) \quad (1)$$

where I_{SERS} is the band intensity of the selected band at 660 cm^{-1} obtained by SERS, I_{Raman} is the corresponding band intensity of the concentrated aqueous solution sample, N_{neat} and N_{ads} are the numbers of molecules in the cross section of the laser beam from the concentrated sample and that of adsorbed molecules in the cross section of laser beam, respectively. The cross sections of the laser beam are identical for the Raman and SERS measurements because we used the same Raman spectrometer. Thus, N_{neat} and N_{ads} may be replaced by the concentrations of the neat and SERS samples, respectively. The calculated enhancement factor was 7.5×10^6 for the SERS detection of glutathione under optimized conditions.

Selectivity of the Heat-Induced SERS Method. To examine the selectivity of the proposed heat-induced SERS method, SERS spectra were measured for 20 kinds of amino acids, such as cysteine, methionine, tryptophan, and histidine, using the heat-induced SERS sensing method under the same experimental conditions. In order to reflect the real affinities between the silver surfaces and the amino acids, the intensity of a band at around 1410 cm^{-1} due to the $-COO^-$ symmetric stretching mode was used to investigate the selectivity. Table 2 compares the selectivity of the 20 kinds of amino acids and glutathione. As can be seen in Table 2, the heat-induced SERS method shows specific selectivity for glutathione and cysteine. It is very likely that this specific selectivity is caused by the strong interaction between the thiol groups and the silver nanoparticles.

CONCLUSION

In this study, we have developed a novel heat-induced SERS method for the detection of glutathione in aqueous solutions. This method can overcome the low enhancement factor and low reproducibility limitation of SERS for the detection of biomolecules. By drying a glutathione mixed silver colloidal solution with

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proper temperature prior to SERS measurement, an intense SERS spectrum of glutathione can be observed and the whole assay can be finished within 5 min. The experimental results have indicated that the citrate-reduced silver colloids with the size of ca. 60 nm are more suitable for inducing the intense SERS signals of glutathione. Moreover, it has been found that the enhancement factor is also affected by the dropped sample volume, the given temperature in the drying process, the pH of the sample, and the concentration of buffer solution added. Under the optimal conditions, the regression coefficient for the determination of glutathione in the range of 100−800 nM is higher than 0.99. The detection limit of this method for glutathione is ca. 50 nM, and the enhancement factor is 7.5×10^6. Moreover, the heat-induced SERS method provides specific selectivity toward glutathione and molecules with thiol groups. Since the surprisingly high enhancement factor has been observed for the direct detection of biomolecules in this study, the further investigations and applications of the heat-induced SERS method are in progress.

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