A Highly Sensitive and Rapid SERS Detection of GSH Based on Hollow Urchin-like Gold Nanoparticles

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\textbf{Abstract.} Glutathione (GSH) plays a key role in many biochemical pathways and the detection of it is of great importance. In this paper, a highly sensitive and rapid detection based on surface-enhanced Raman scattering (SERS) was developed, using the competitive substitution between GSH and \textit{p}-nitrobenzothiol (PNTP) via the Au-S bond on hollow urchin-like gold nanoparticles (HU-GNPs) surface. By Au-S bond, PNTP could easily connect on HU-GNPs surface, where many branches and edges existed and generated a multitude of “hot spots”. Besides, the benzene ring of PNTP also magnified SERS signals. The SERS intensity of PNTP changes was related to the GSH added into the reaction system and there existed linear relationship between SERS intensity and GSH concentrations from 1-10 nM. Owing to its high sensitivity, this method would help to promote the GSH research in biological systems.

\textbf{Introduction}

Glutathione (GSH), as the most abundant intracellular biothiol, plays essential roles in physiology and pathology. At present, a variety of methods have been developed to detect its concentration, such as fluorescence spectroscopy, high performance liquid chromatography (HPLC), electrochemical method, mass spectrometry, etc. However, there still remain some matters unsolved, such as enzyme inactivation, time-consuming, complex detection and high cost.

Surface-enhanced Raman spectroscopy (SERS) has been extensively studied because of the high sensitivity specificity and selectivity [1,2]. SERS can obtain structural information of analyses at the molecular level [3], based on the characteristics of fingerprint recognition [4]. Three-dimensional structures with branches have high surface areas, the Raman signals of analyses, adsorbed on rough metal surfaces, can be enhanced by several orders of magnitude and applied in SERS detection [5]. Compared with fluorescence, IR and other traditional technologies, SERS has many significant advantages, such as its resistance to photo bleaching, high stability and weak Raman signal of water. Therefore, SERS spectroscopy can be widely used in biomedical and chemical analytical science fields, including the detection of protein [6], nucleic acid [7], drugs [8], environmental pollutants [9], and cancer biomarkers [10].

In the present work, we report a simple method, based on SERS, for GSH detection with highly sensitive and rapid detection. HU-GNPs were synthesized with a seed-mediated growth approach. \textit{p}-nitrobenzothiol (PNTP) was used as the SERS signal molecular and could
connect to the gold surface to detect GSH with Au-S bond displacement [11]. This method is simple, fast, economical, and has high sensitivity, selectivity.

Experimental

Materials

Silver nitrate (AgNO$_3$), trisodium citrate (C$_6$H$_5$Na$_3$O$_7$·2H$_2$O), anhydrous ethanol, Chlorauric acid terahydrate (HAuCl$_4$·4H$_2$O), Polyvinyl Pyrrolidone (PVP), dopamine hydrochloride, $p$-nitrothiophenol (PNTP) and arginine (Arg), glutamate (Glu), glycine (Gly), histidine (His), lysine (Lys), tyrosine (Tyr) and glutathione (GSH) were all purchased from Sinopharm Chemical Reagent Co., Ltd. Water used in the experiments was ultrapure water (resistance, 18 MΩ cm$^{-1}$).

Synthesis of Ag Seeds

9 mg AgNO$_3$ was dissolved in 50 mL of water and heated to boil. Then 1.2 mL of trisodium citrate (1 % in mass) was added to initiate the reduction of AgNO$_3$. After 40 min, the collargol was collected and cooled to room temperature.

Synthesis of HU-GNPs

100 μL of Ag seeds were added into 20 mL of water, preheated at 40°C. Five minutes later, 500 μL of 1 % HAuCl$_4$ was added. After intensive mixing, 5 mL of dopamine hydrochloride (12 mM) and 5 mL of 1 % PVP was added, and the color of solution changed to yellow immediately. The product was collected by centrifugation at 5000 rpm for 5 min to remove the excess reagents and redispersed in anhydrous ethanol.

Preparation of SERS Substrate

Based on electrostatic self-assembly, the HU-GNPs substrate was prepared, using APTES functionalized ITO glass slips. The ITO glass slips were washed with Detergent, water, ultrapure water, anhydrous ethanol in turn for several times. Then, the slips were dried at 80°C in the oven. The cleaned ITO glass slips were vertically inserted in 1 wt % APTES ethanol solution for 12 h, and were dried at 100°C. After that, to make the HU-GNPs layer, the amination ITO glass slips were vertically immersed into the colloidal suspension of HU-GNPs under gently stirring overnight. Finally, the substrate was washed with ultrapure water for three times and dried for 30 min at room temperature.
SERS Analysis

HU-GNPs substrates were vertically dipped into 500 µL of 10 µM PNTP for 30 min. Then, the substrates were washed with ultrapure water for three times and were dried for 30 min at room temperature. 10 mL of different concentrations of GSH as well as other biomolecules (Arg, Glu, Gly, His, Lys, Tyr) were added, respectively. Thirty minutes later, the substrates were taken out to be washed with ultrapure water. And then SERS measurements were started on.

Figure 2. (A-B) The SEM and TEM image of the representatives of HU-GNPs. (C) The SEM image of the representative of SERS substrate made of HU-GNPs. (D) The XRD pattern characterizations of HU-GNPs. (E) The SERS spectra of HU-GNPs substrate placed after different time (0 d, 5 d, 10 d, 20 d and 30 d). (F) The SERS spectra of 100 points of HU-GNPs substrate. The Raman spectra were recorded under experimental condition (785 nm excitation wavelength, 10 s integration time).

Results and Discussion

HU-GNPs and SERS Substrate Characterization

HU-GNPs were synthesized through a seed-mediated growth method. As shown in Figure 2A and 2B, the morphology and structure of the as-prepared HU-GNPs were characterized by SEM and TEM. It has been reported that HU-GNPs have rough surface with sharp edges and branches, which generated ‘hot spots’ and could greatly enhance the surrounding electric field strength. The crystal structure and the phase composition were characterized by XRD. Figure 2D represented four peaks, corresponding to the diffractions from the (111), (200), (220), and (311) planes.

HU-GNPs substrate was prepared based on the self-assembly method, which could provide substrates with good uniformity and stability (Figure 2C). In the experiment, the substrate was tested after placing for different time, from 0 days to 30 days, and as shown in the results that the nanoparticles and substrate had good stability (Figure 2E). Besides, 100 points were randomly selected for SERS detection. The results showed that the homogeneity of the substrate was excellent (Figure 2F). In the experiment, PNTP was used as the Raman signal molecule.
PNTP Absorption Processes

There are three typical bands of \( p \)-nitrothiophenol (PNTP) at 328, 1340, and 1570 cm\(^{-1}\), of which the peak at 1340 cm\(^{-1}\) is attributed to the -NO\(_2\) stretching mode and used as reference peak in the following experiment. In order to study the adsorption process of PTNP on HU-GNPs, the incubation concentration and incubation time were researched and discussed. Figure 3A showed different SERS spectra of HU-GNPs substrate adsorbed with different concentrations of PNTP. Along with the increase of PNTP concentration, the intensity also increased (Figure 3B). When the concentration of PNTP reached at 10 \( \mu \)M, the SERS intensity tended to be balanced, indicating that the adsorption state of HU-GNPs was saturated.

Figure 3C showed the effect of SERS intensity with different adsorption time of PNTP. The SERS intensity of PNTP grew incrementally over the adsorption time in the beginning, and then tended to be stable. It could be seen that when the adsorption time of PNTP was 15 minutes, the SERS signal intensity became balanced (Figure 3D). Thus, to ensure the stability of the PNTP adsorption on the HU-GNPs surface, the adsorption time was set at 15 minutes.

![Figure 3](image1.png)

**Figure 3.** (A) SERS spectral changes of PNTP upon the addition of PNTP with different concentrations. (B) The changing curve of spectra peak intensity at 1340 cm\(^{-1}\). (C) SERS spectra recorded for PNTP adsorption on HU-GNPs surface in an aqueous solution. (D) Plots of increase in the band intensity at 1340 cm\(^{-1}\) with the adsorption time.

Displacement Processes Detection Mediated by GSH

Figure 4 shows the SERS spectra changes after adding GSH at different concentrations. As can be seen in Figure 4A, with the increase of GSH concentrations, SERS signal strength decreased at 1340 cm\(^{-1}\). The results confirmed that there existed competition between GSH and PNTP over the adsorption on HU-GNPs surface.

Besides, when the GSH concentration was higher than 10 nM, the SERS intensity tended to change non-significantly. The illustration showed the linear relationship between SERS intensity and GSH concentrations from 1-10 nM. The results showed that this work constructed a highly sensitive SERS sensor for detecting GSH with hollow urchin-like gold nanoparticles.

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Figure 4. (A) SERS spectral changes of PNTP upon the addition of GSH with different concentrations. (B) Plots of spectra peak intensity at 1340 cm$^{-1}$ against the GSH concentrations from 0 nM to 100 nM. Inset: Linear fitting curve of PNTP with the GSH addition (concentrations from 1 nM to 10 nM).

Selectivity of the Propose Method

In order to study the selectivity and specificity of this system, selective experiments were designed to evaluate the SERS probe. Arginine (Arg), glutamate (Glu), glycine (Gly), histidine (His), lysine (Lys) and tyrosine (Tyr) were used in check tests. As shown in Figure 5, under the same experimental conditions, these substances did not interfere with the replacement reaction. That was to say, the SERS probe constructed in this experiment possessed good selectivity and was insusceptible to other interference.

Figure 5. Selectivity of the SERS substrate in the presence of Arg, Glu, Gly, His, Lys and Tyr.
Summary

According to the principle of competitive substitution via the Au-S bond, the rapid SERS detection of GSH with high sensitivity was established. In the above, the conditions in the process of SERS probe construction were optimized, such as the adsorption of PNTP onto HU-GNPs surface. Besides, the competitive substitution of GSH and PNTP was discussed, and the linear relationship between SERS signal intensity and GSH concentrations was established. Compared with others, this method possessed a higher selectivity and a lower detection limit for GSH, and could be used in complex systems for further GSH research, which contributed to the comprehension of its function and effect in biological systems.

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References


