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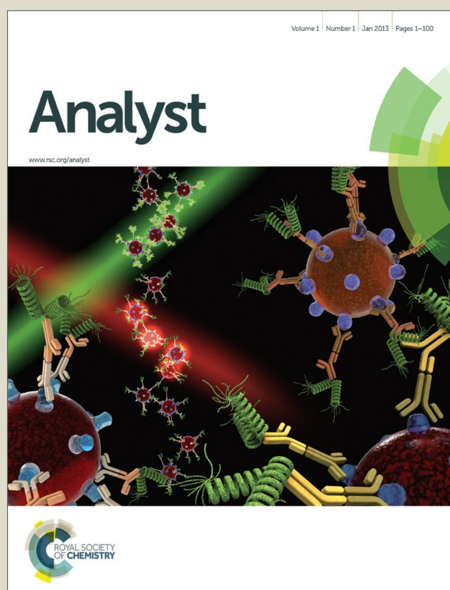

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Free Radical-quenched SERS Probes for Detecting H_2O_2 and Glucose

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To develop a free radical-quenched surface-enhanced Raman scattering (SERS) probe, starch, a linear molecule, was used as a protective layer to coat gold nanoshells (GNSs) as enhancement substrates and then, methylene blue (MB) was absorbed on the starch-coated GNSs as a free radical-responsive element. By detecting the change of the SERS intensity of MB on GNSs, the free radical-quenched SERS probes were used to detect H_2O_2, a low active reactive oxygen species (ROS), which was first converted to free radicals, a high active ROS, to react with MB absorbed on GNSs to quench its SERS. The free radical-quenched SERS probe was also used to detect glucose when existence of glucose oxidase which converted glucose to H_2O_2. The free radical-quenched SERS probe would be a versatile platform for detection of biochemical processes. The integration of optical change of molecules and optical enhancement of nanomaterials provided a way for advanced materials and analytical science.

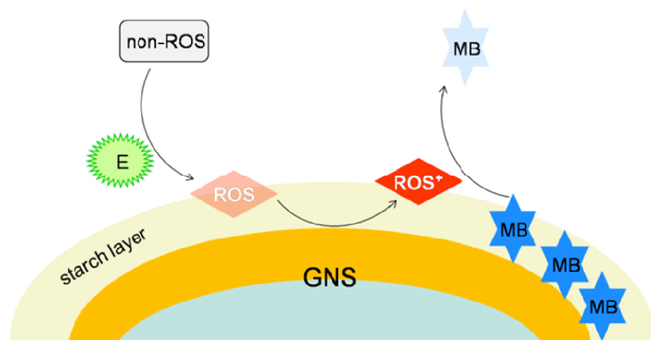
Introduction

The combination of Raman spectroscopy and coin metal nanomaterials promotes the applications of surface-enhanced Raman scattering (SERS) due to a major improvement in the enhancement of Raman signal.[1,2] Ingenious design and fabrication of metal nanomaterials have made SERS become versatile analytical tools. A technique named shell-isolated nanoparticle-enhanced Raman spectroscopy was based on an inert layer-coated gold nanoparticle which endowed the technique with much higher detection sensitivity and vast practical applications.[3] Super-hydrophobic artificial surfaces composed by nanoplasmonic arrays can overcome diffusion limit of highly diluted molecules, and concentrate and detect them at attomolar concentration.[4] In our previous report, gold nanoshells (GNSs) were integrated on acupuncture needles to construct a SERS-active needle which can collect tissue fluids for direct SERS detection with minimal invasion.[5]

Integrating other elements such as colored molecules on subtle metal nanoparticles, new applications have been realized. Due to the giant Raman intensity from their resonance Raman scattering, colored molecules are often integrated on metal nanoparticles to construct Raman-labeled tags in SERS detections.[6-8] As traditional Raman-labeled tags in previous applications, the molecule was only used as a colored molecule to obtain more efficient Raman scattering, and the structure of colored molecules did not change in the whole processes. The tags were labeled by specific receptors (antibody, nucleic acid, etc.) to structure a Raman-labeled probe, and during applications the change in signal intensity is from the number change of the Raman-labeled probes in the detected domains, while the number of colored molecules on a given Raman-labeled probe does not change.[9-13]

Hydrogen peroxide (H_2O_2) is a typical free radical of important metabolic products, which plays many roles in the physiological activities. So, it always became an analyte of new analytical methods. In physiological condition, H_2O_2 is a molecule with short half-time, which need the developed methods should fast detect it without pretreatment steps. In previous reports, electrochemical methods have advantages at both detection speed and sensitivity.[14-17] Raman technique is desired for monitoring biological samples in situ and real time because of its application without considering states, temperature, morphology, or size of samples.[18] But H_2O_2 is a molecule with a weak intrinsic Raman activity, and it also has no affinity with SERS substrates such as gold or silver. So it is difficult for directly detecting its SERS signal at low concentration. Now, we developed a SERS method to detect H_2O_2 and attempted to introduce the advantages of SERS into H_2O_2 detection.

Here, a novel SERS probe was reported based on an integration of optical change of labeled molecules (methylene blue, MB) and the optical enhancement of nanomaterials (gold nanoshells, GNSs) for H_2O_2 detection. Unlike previous Raman-labeled probes, in the MB-absorbed GNSs named free radical-quenched SERS probe, colored MB (reduced form) can be converted to colorless form (oxidation form) by free radicals. As shown in Scheme 1, in the free radical-quenched SERS probe, the nanoparticles (GNSs) not only act as a Raman-enhanced substrate but also as a catalytic agent, and the colored molecules (MB) not only as a colored Raman molecule but also as a free radical-responsive element. During applications, high active ROS such as free radicals can directly react with MB to quench the SERS signal of the probe; low active ROS such as H_2O_2 can be catalyzed by GNSs to form high active ones to quench SERS signal of the probes. Thus, the SERS signal of the probes decreased proportional to ROS concentration. Further, the free radical-quenched SERS probe also can be detected the molecules which can be converted into ROS in the system.



Scheme 1 Illustration of mechanism of free radical-quenched SERS probe.

Experimental Section

Materials

The suspensions of silica colloidal particles of 55 nm in radius were from Nissan Chemical Corporation (Japan). Soluble starch, glucose, $HAuCl_4$, H_2O_2, and methylene blue (MB) were from Guoyao Group (China). Glucose oxidase (GOx) was from Shanghai Sangon Co. Ltd. (China). All other reagents used were analytical grade.

Fabrication of free radical-quenched SERS probes

The gold nanoshells (GNSs, [r1, r2]=[55, 85] nm) were fabricated as previous report to as SERS substrates for constructing free radical-quenched SERS probes.[5] Starch was coated on GNSs by adding 2.0 OD of GNSs into an equal volume of 10 g/L starch aqueous solutions incubated at 95 G for 30 min and then, after the temperature of the mixture was decreased to room temperature, it was centrifugated at 3000 rpm for 10 min to collect precipitates. The precipitates redispersed in water were incubated at 95 G for 30 min and then, after the temperature of the mixture was decreased to room temperature, it was centrifuged again to remove free starch, and the precipitates were starch-coated GNSs. 1 ml 2.7 mmol/L of MB was added into 50 ml 2.0 OD of starch-coated GNSs and incubated at 37 G for 30 min and then, the mixture was washed by centrifugation with water for three times to remove free MB, and the precipitates were MB-absorbed and starch-coated GNSs, i.e. free radical-quenched SERS probes.

Characterizations and Measurements

Zeiss ULTRA-plus scanning electron microscopy (SEM) was used to characterize the morphology and size of GNSs and starch-coated GNSs. Transmission electron microscope (TEM) was used to characterize the starch layer of starch-coated GNSs. A UV-vis spectrometer (UV3150, Shimadzu) was used to obtain absorbance spectra. Bruker IR microspectroscopy (Hyperion 1000) was used to obtain IR spectra of samples with reflectance model. The three mixtures (MB and hydrogen peroxide, MB and GNSs, and MB, hydrogen peroxide, and GNSs) were incubated at 20 G for 30 min and then, the absorbance spectra were collected from the supernatant of the mixtures, centrifuged 3000 rpm for 10 min. The absorbance spectra of GNSs in water and starch-coated GNSs in water were collected to show the influence of coated starch on peak position of GNSs. The absorbance spectra of starch-coated GNSs in 0.2 mol/L NaH_2PO_3 aqueous solution and 0.2 mol/L Na_2HPO_3 aqueous solution were collected to show whether they aggregated.

Renishaw Invia microRaman spectroscopy was used to measure SERS spectra at room temperature (~20 G) using a 785 nm excitation laser. The laser was focused onto the sample surface by using a 50× long working distance objective. For monitoring hydrogen peroxide, SERS signals were collected from 1.0 OD of free radical-quenched SERS probes in water containing various concentration of hydrogen peroxide, after incubated at 37 G for 30 min. For monitoring glucose, SERS signals were collected from 1.0 OD of free radical-quenched SERS probes in PBS buffers (0.01 mol/L, pH 5.8) containing various concentration of glucose and 0.1 KU/mL of GOx, after incubated at 37 G for 30 min. Except as otherwise stated, the excitation power and the acquisition time were 1.2 mW and 10 s, respectively. The results were expressed as mean ± a standard deviation (n=3).

Results and Discussion

Fabrication and characterization of GNSs and starch-coated GNSs

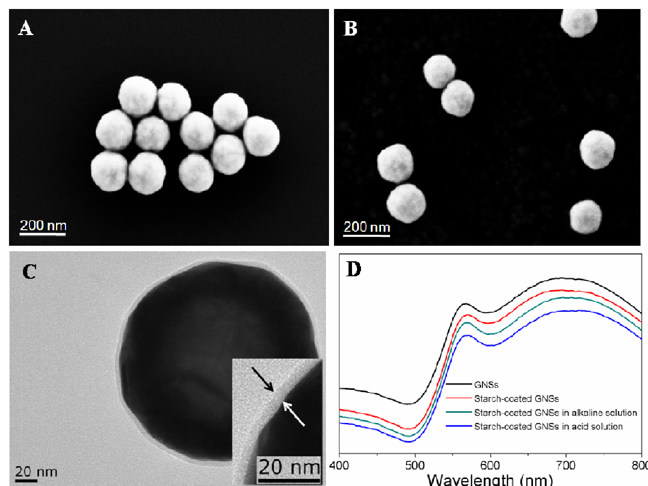


Fig. 1 (A) a SEM image of GNSs, (B) a SEM image of starch-coated GNSs, (C) a TEM image of a starch-coated GNS (the inset shown a 5 nm of starch layer coated on the GNSs), (D) absorbance spectra of GNSs, starch-coated GNSs, starch-coated GNSs in alkaline solution, and starch-coated GNSs in acid solution, respectively.

GNSs, an excellent optics-enhanced material, were hired as a Raman enhancement element as well as a catalytic agent. Methylene blue was absorbed on the GNSs as both a colored Raman molecule and a free radical-quenched element. Starch molecules were coated on GNSs to protect GNSs from aggregation in complex matrices. Fig. 1a and 1b show the SEM images of GNSs and starch-coated GNSs, respectively. From the SEM images, the morphology or size of GNSs and starch-coated GNSs have no difference. Fig. 1c is a typical TEM image of a starch-coated GNS, which shows that a uniform starch layer was successfully coated on the surface of the GNS, and as shown in the inset the thickness of starch layer is about 5 nm. Fig. 1d shows the absorbance spectra of GNSs in water and starch-coated GNSs in water, 0.2 mol/L NaH_2PO_3, and 0.2 mol/L Na_2HPO_3 aqueous solution, respectively. Before and after coating, the peak position of GNSs did not change, while their spectral shape changed (relative intensities in the range of 400 to 600 nm), which

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also suggested indirectly that starch was coated on GNSs. The spectral shape of starch-coated GNSs in alkaline and acid solution almost did not change, suggested that starch as a protective layer can effectively prevent GNSs from aggregation in acidic or alkaline solution.

It is still needed to research further mechanism of soluble starch coated on GNSs, but to the best of our knowledge, it is the first report that starch was used as protective layers of gold nanoparticles. Unlike solid protective layers such as silica or polystyrene, linear starch formed a highly hydrated coating layer with excellent permeability, which is essential for environment-responsive probes. Besides, starch is a natural biomolecule with abundant hydroxyl groups, which provides starch-coated nanomaterials with excellent biocompatibility and functionalization sites, becoming a versatile platform for application in biomedicine.

Catalytic effect of starch-coated GNSs and decolor of MB

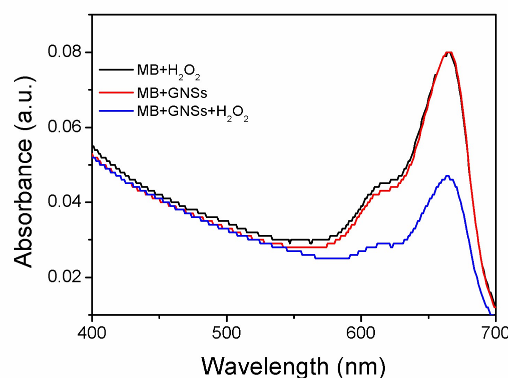

Fig. 2 Absorbance spectra of supernatant of MB and hydrogen peroxide, MB and starch-coated GNSs, and MN, hydrogen peroxide, and starch-coated GNSs, respectively.

In order to investigate catalytic effect of starch-coated GNSs, absorbance spectra of supernatant of three mixtures were obtained at 30 min after mixed, as shown in Fig. 2. The absorbance peak intensity of the two former almost did not change, which suggested that neither hydrogen peroxide nor GNSs can convert reduced MB to oxidation MB. In detected time, the absorbance peak intensity of the third decreased, which suggested that combination of GNSs and hydrogen peroxide can convert MB to oxidation MB. In the mixture of MB, hydrogen peroxide, and GNSs, H_2O_2 was first converted to free radical by GNSs with peroxidase activity and then, the produced free radical decolored MB further. In previous reports, MB was also used as the model of pollutants in researches of free radical catalysis of nanoparticles.[19,20] Here, MB was used as a free radical-responsive element absorbed on GNSs to fabricate a free radical-quenched SERS probe.

IR and surface-enhanced IR (SEIR) spectra of starch and MB

Fig. 3 showed IR spectra and SEIR spectra of starch, MB, respectively. The SEIR spectrum of starch absorbed on GNSs were almost the same as IR of free starch in the range of 4000 to 2000 cm^{-1}, while compared with IR lines of MB, SEIRS lines of MB absorbed on GNSs shifted in the range of 4000 to 2000 cm^{-1}, and the relative intensities of lines in the range also changed. We speculated that the influence of SPR of GNSs on absorbed MB were larger than the absorbed starch. SEIR lines of both starch and MB appeared in the SEIR spectrum of starch-coated GNSs loaded with MB (the free radical-quenched probes), which suggested that MB absorbed on the surface of the starch coated GNSs.

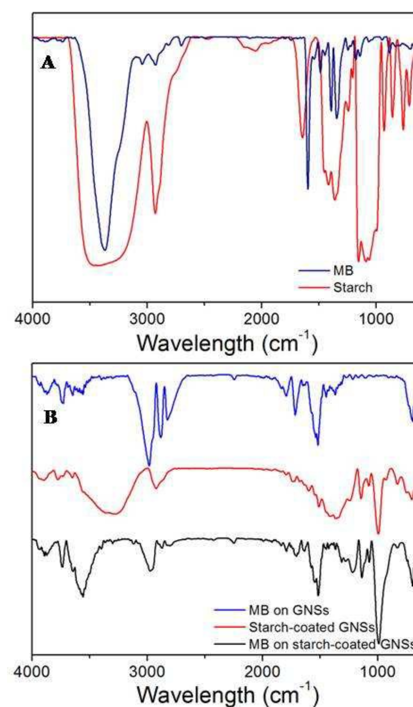

Fig. 3 (A) IR spectra of MB and starch, (B) SEIR spectra of starch and MB.

Raman and SERS spectra of starch and MB

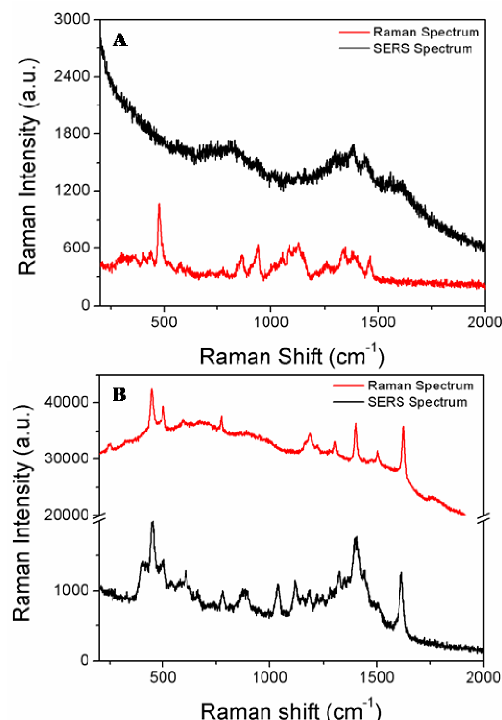

Fig. 4 (A) A Raman spectrum of starch powder (The excitation power and the acquisition time were 12 mW and 10 s, respectively) and a SERS spectrum of starch coated on GNSs (1.0 OD of starch-

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coated GNSs, the excitation power and the acquisition time were 12 mW and 10 s, respectively); (B) A Raman spectrum of MB (2.7 mmol/L MB aqueous solution, the excitation power and the acquisition time were 60 mW and 10 s, respectively)and a SERS spectrum of MB absorbed on starch -coated GNS (1.0 OD of free radical-quenched SERS probes, the excitation power and the acquisition time were 1.2 mW and 10 s, respectively).

To structure a SERS probe, the used coating molecule should have no disturbance to the labeled molecule. Fig. 4A shows a Raman spectrum of starch power and a SERS spectrum of starch-coated GNSs. Starch has it typical Raman lines, while it does not provide a typical SERS lines on GNSs, which showed that starch was a desirable coating reagent having no typical SERS lines to disturb the SERS spectra of labeled molecules. As shown in Fig. 4B, compared with starch, MB has a strong intrinsic Raman activity, which was a desirable candidate of Raman label molecules. When the acquisition time is equal and the used excitation power was 50 times lower than that in the detection of Raman spectrum of MB, all of typical SERS lines of MB appeared in the SERS spectrum of 1 OD of free radical-quenched SERS probes and did not be overlaped or disturbed by those of coated starch. The results suggested that the starch was a desirable coating reagent for MB-absorbed SERS probes.

Free Radical-quenched SERS Probes for Detecting H_2O_2 and Glucose

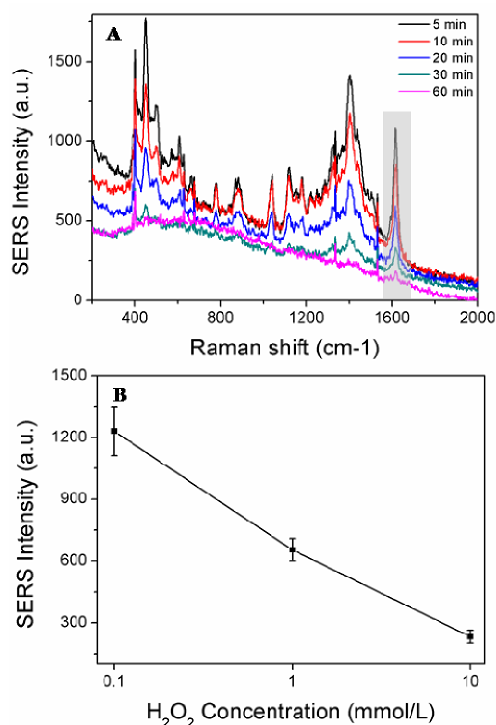

Fig. 5 (A) the evolution of SERS spectra of free radical-quenched SERS probe in10 mmol/L H_2O_2 solution (1.0 OD of free radical-quenched SERS probes in the system, the excitation power and the acquisition time were 1.2 mW and 10 s, respectively); (B) A plot of the SERS intensity of free radical-quenched SERS probe versus H_2O_2 concentrations.

According to the results in Fig. 2, hydrogen peroxide itself cannot decolor MB. Only when touched GNSs, can hydrogen peroxide be catalyzed to produce free radical, which converted reduced MB to oxidation ones. The reduced MB has a positive-charged quaternary ammonium group, absorbed on GNSs to obtaining strong SERS signal, and after converted to oxidation ones (losing resonance Raman scattering), its charged group was replaced by a neutral tertiary ammonium group, which would de-absorb from GNSs (losing chemical enhancement) and the collecting SERS signal also decreased. Thus, the combination of GNSs and hydrogen peroxide converts MB to oxidation ones, that is, hydrogen peroxide, a low active ROS, can quench the SERS signal of the free radical-quenched SERS probe. As shown in Fig. 5A, 10 mmol/L of hydrogen peroxide almost completely quenched the SERS signal of the free radical-quenched SERS probe in 60 min, which also suggested that the hydrated starch layer had excellent permeability for the diffusion of hydrogen peroxide. According to the result in Fig.4A, SERS spectra were collected at 30 min after the free radical-quenched SERS probe mixed with hydrogen peroxide, and the SERS intensity at 1615 cm^{-1} labeled with gray area was selected as a quantitative indicator. As shown in fig. 5B, the free radical-quenched SERS probe can detect hydrogen peroxide in the range of 0.1-10 mmol/L. By varying incubation time of the mixture of free radical-quenched SERS probes and hydrogen peroxide, the detected concentration of hydrogen peroxide would be lower or higher than the above detected range.

In body, many biomolecules can be decomposed by corresponding oxidase, such as glucose oxidase, L-amino acid oxidase, xanthine oxidase, and pyruvate oxidase. In the metabolic process of the molecules, H_2O_2 is a common product, and the substrates and the oxidases would be detected indirectly by detecting the product. Thus, the free radical-quenched SERS probe also can be used to indirectly monitor biomolecules or enzymes of the biochemical processes. Glucose oxidase (GOx) can catalyze glucose to hydrogen peroxide and gluconic acid. Here, the reaction was used as a model of biochemical processes to assess the free radical-quenched SERS probe in detection of biomolecules. The free radical-quenched SERS probe was used to monitor glucose concentration because the produced amount of H_2O_2 in the biochemical process is proportional to glucose amount in the solution. As shown in Fig. 6, glucose concentrations were indirectly detected by collecting the SERS signals of the probes in the range of 0.1-100 mmol/L. In the same way, if glucose oxidase was replaced by L-amino acid oxidase, xanthine oxidase, or pyruvate oxidase, the corresponding SERS data can indicate indirectly the amount of L-amino acid, xanthine, pyruvatem or their oxidases.

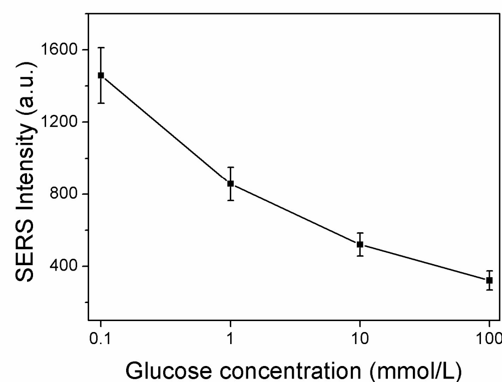

Fig. 6 A plot of the SERS intensity of free radical-quenched SERS probe versus glucose concentrations.

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Conclusions

In summary, starch was used to coat GNSs forming highly hydrated protective layer, which provided excellent permeability essential for environment-responsive probes. MB was absorbed on starch-coated GNSs as a free radical-responsive element to fabricate a free radical-quenched SERS probe. By detecting the change in the SERS signal of MB on GNSs, the free radical-quenched SERS probes can be used to detect H_2O_2, a low active ROS, which should be first converted to free radicals by GNSs. The free radical-quenched SERS probe was also used to monitor biomolecules in biochemical processes. The free radical-quenched probe would be a versatile platform for SERS detection of biochemical processes or molecules in the processes. The integration of optical change of molecules and optical enhancement of nanomaterials provided a way for advanced materials and analytical methods.

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