Т. 84, № 3 **ЖУРНАЛ ПРИКЛАДНОЙ СПЕКТРОСКОПИИ** МАЙ — ИЮНЬ 2017
V. 84, N 3 **JOURNAL OF APPLIED SPECTROSCOPY** MAY — JUNE 2017

SURFACE-ENHANCED RAMAN SCATTERING FROM SELF-ASSEMBLED FILM OF THIOLATED PEG-MODIFIED GOLD NANOPARTICLES

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Self-assembly of thiolated polyethylene glycol (PEG)-modified Au nanoparticles (NPs) into a closely packed particle film at a water-air interface for the production of reproducible, highly active surface-enhanced Raman scattering (SERS) was developed. Upon modification with thiolated PEG, the Au NPs can sustain severe conditions (e.g., 3 M NaCl solution), and after separation from colloids and drying, they can be redispersed into water, forming colloids again. We found that the thiolated PEG-modified Au NPs, from their concentrated colloids, can spontaneously self assemble into a two-dimensional (2D) closely packed particle film at the water-air interface. With abundant hot spots created and exposed, the 2D particle film produces large, reproducible SERS signal for 4-mercaptobenzoic acid (probe molecule), reaching a detection limit of 1×10^{-11} M. These results offer a useful way for the storage of Au NPs and the fabrication of highly reproducible and active SERS substrate.

Keywords: *Au nanoparticle, thiolated polyethylene glycol, self-assembly nanoparticles, surface-enhanced Raman scattering, metal colloids.*

ГИГАНТСКОЕ КОМБИНАЦИОННОЕ РАССЕЯНИЕ НА САМООРГАНИЗУЮЩЕЙСЯ ПЛЕНКЕ, СОСТОЯЩЕЙ ИЗ ЗОЛОТЫХ НАНОЧАСТИЦ, МОДИФИЦИРОВАННЫХ ТИОЛИРОВАННЫМ ПОЛИЭТИЛЕНГЛИКОЛЕМ

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УДК 535.375.5:620.3:546.59

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(Поступила 13 апреля 2016)

На границе раздела вода–воздух получена пленка, образованная плотноупакованными модифицированными тиолированным полиэтиленгликолем (ТЭГ) наночастицами золота, которые могут использоваться для воспроизводимого гигантского комбинационного рассеяния (ГКР). ТЭГ-модифицированные наночастицы золота могут выдержать жесткие условия (например, 3 М раствор NaCl) и после их отделения от коллоидов и сушки могут вновь диспергироваться в воде, образуя коллоиды. Обнаружено, что ТЭГ-модифицированные наночастицы золота, полученные из их концентрированных коллоидов, могут спонтанно собираться в двумерную плотноупакованную пленку на границе раздела вода–воздух. Частицы 2D-пленки дают большой воспроизводимый сигнал ГКР при использовании 4-меркаптобензойной кислоты в качестве молекулы зонда, достигая предела обнаружения 1×10^{-11} М. На основе этих результатов возможны разработка способа хранения наночастиц золота и изготовление активного субстрата ГКР с высокой воспроизводимостью.

Ключевые слова: *наночастицы золота, тиолированный полиэтиленгликоль, самоорганизующиеся наночастицы, гигантское комбинационное рассеяние, металлические коллоиды.*

Introduction. Surface-enhanced Raman scattering (SERS) is a powerful spectral technique, which allows obtaining vibrational information of molecules adsorbed on noble metal surface [1, 2]. Because of its high sensitivity, high selectivity, and ease of sample pretreatment, SERS has been widely used in diverse fields, such as analytical science [3], material science [4], and biomedicine [5]. Ideally, the SERS substrates should be easy to fabricate; they also should have high spectral sensitivity, reproducibility, and stability [6]. However, for most commonly used SERS substrates (e.g., electrochemically roughened electrodes [7], vacuum deposited metal films [8], and metal colloids [9]), their surfaces are ill-defined, possessing abundant, randomly distributed hot spots (the places where the field created by plasmon is very huge [10–13]). As a result, these substrates usually demonstrate excellent spectral sensitivity but lack good reproducibility.

To overcome this limitation, one way is to develop structured SERS substrates [14]. With uniform structural characteristics, structured substrates can offer reproducible optical properties, which are highly desired for diverse applications, including SERS. However, their fabrication usually is time-consuming and costly, thereby limiting their applications. Alternatively, self-assembly of NPs is a relatively straightforward way to fabricate a substrate with high SERS activity [15–17]. By choosing suitable surfactants and monodispersed particles as the building blocks, it is possible to fabricate a large-scale monolayer film with high spectral activity, uniformity, and stability from monodisperse Au NPs [18].

Polyethylene glycol (PEG) is a nontoxic, biocompatible linear polymer that has been widely used as drug carrier [19] or as capping agent to stabilize NPs [20]. To improve spectral reproducibility and sensitivity, we, hereby present a strategy that relies on averaging SERS signals from abundant hot spots. The strategy is realized by assembling 2D particle film at a water-air interface from concentrated colloids of thiolated PEG-modified Au NPs. The concentrated colloids used allow self-assembly of NPs into a uniform, void-free particle film. Additionally, the 2D particle film creates and exposes abundant hot spots at the particle-particle junctions, allowing full access to external molecules. Moreover, the PEG chain anchored on the particle surface prevents the analyte molecules from direct contacting with the metal surface, where the huge field created by plasmon is highly sensitive to the subtle structural changes. With these advantages, this hot spot-averaged technique not only can produce strong SERS signals but also is not susceptible to subtle structural changes.

Experimental. Chloroauric acid, sodium citrate, and pyridine, all of analytical grade, were purchased from Sinopharm Chemical Reagent Ltd., Co. (Shanghai, China). α-methoxy-ω-mercapto-poly(ethylene glycol) (thiolated PEG, MW 5000) and 4-mercaptobenzoic acid (4-MBA) were purchased from Jenkem Technology Co., Ltd. (Beijing, China) and Sigma-Aldrich, respectively. All chemicals were used as received without further purification. Ultrapure water, purified by a deionized water system (YZS-RO-S50) and then doubly distilled in a quartz distiller, was used throughout the experiments.

Surface modification of Au NPs with thiolated PEG and their stability test. Au NPs were synthesized by reduction of $HAuCl_4$ with sodium citrate [21]. Their average size was determined to be 55 nm using transmission electron microscopy. To accomplish surface modification, the colloids of pure Au NPs (5 mL) and thiolated PEG solution (30 μL, 2 mM) were mixed in a serum bottle under gentle shaking, followed by standing at room temperature for 12 h. To test the stability of the thiolated PEG-modified Au NPs, NaCl solution was added to their colloids (2 mL) up to a final concentration of 3 M. After that, UV-vis spectra were measured to reveal whether the thiolated PEG-modified Au NPs in the colloids were aggregated or not.

Self-assembly of Au NPs at water-air interface. Self-assembly of thiolated PEG-modified Au NPs into particle film was accomplished at the water-air interface from their concentrated colloids. To this end, the colloids of the thiolated PEG-modified Au NPs (5 mL) were centrifuged at 5500 rpm for 20 min. After removal of the supernatant, residues left were gently sonicated, forming concentrated colloids. Upon natural evaporation, the colloids were further concentrated, reaching a final concentration of 12.5, 16.9, 19.1, 23.9, 31.9, or 71.8 g/L. Alternatively, concentrated colloids can be prepared by redispersing the thiolated PEG-modified Au NPs (separated from their colloids (5 mL) using centrifugation, followed by natural drying) into a small quality of water (<31 μL) again. In concentrated colloids, the thiolated PEG-modified Au NPs spontaneously self-assembled into particle films at the water-air interface, showing metallic luster visible to naked eyes. For SERS tests, probe molecule (4-MBA) was introduced either by mixing with the colloids of the thiolated Au NPs before their concentration or with the small quantity of water that was used to disperse the thiolated PEG-modified Au NPs.

UV-vis spectra and scanning electron microscopy (SEM) images were conducted on a Shimadzu UV-1800 spectrophotometer (using 1-cm quarts cells) and on a Hitachi S-4800 microscope, respectively. SERS spectra were collected on a LabRam-010 Raman spectrometer equipped with a 632.8 nm He–Ne laser as the excitation source. A 50× objective with 8-mm working distance was used to focus the laser beam into a ~2 μm spot at the sample stage and to collect the Raman signals in a back scattering geometry.

Results and discussion. *Modification of the Au NPs with thiolated PEG and their stability test.* The thiolated PEG consists of a long hydrophilic PEG chain and a terminal thiol group. Upon contacting with Au NPs, the thiol group binds Au surface and forms a strong Au-S bond, thereby anchoring PEG chain to the Au surface [22]. When their surface concentration is low, the PEG chains anchored are far away from each other so that their mutual interaction is weak. In such a case, the coiling PEG chain would adopt a mushroom-like configuration. At higher surface concentrations, the PEG chains anchored become denser and will develop into a brush-like configuration [23]. Therefore, the long PEG chains anchored can effectively regulate the interactions amongst particles and thus their stability.

Figure 1 shows the UV-vis spectra of the Au NP colloids, the colloids of the thiolated PEG-modified Au NPs containing 3 M NaCl, and the redispersed colloids of the thiolated PEG-modified Au NPs. The colloids of the thiolated PEG-modified Au NPs containing 3 M NaCl have a nearly identical spectrum to that of pure Au NP colloids, indicating that they can sustain an electrolyte solution with a high concentration. In contrast, pure Au colloids start to destabilize at a NaCl concentration of about 0.008 M [24]. Apparently, the PEG chains anchored on the Au NPs largely improve their stability against electrolyte solution because they offer steric stabilization to the Au NPs [25]. Steric stabilization originates from the mutual interactions among the long chains anchored on particle surfaces, so that the stability of thiolated PEG-modified Au NPs, unlike pure Au colloids, is insensitive to the quantity of electrolyte added.

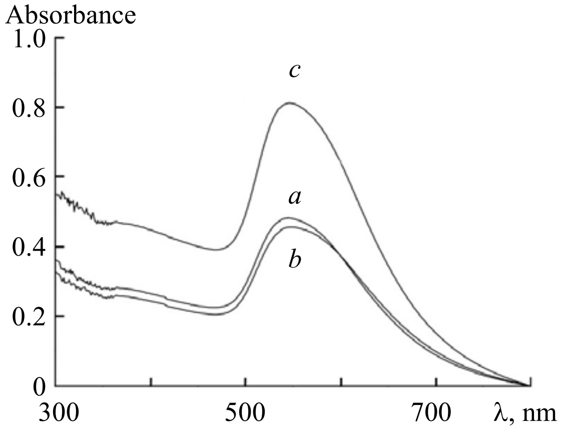

Fig. 1. UV-vis spectra of (a) the pure Au NP colloids, (b) the colloids of the thiolated PEG-modified Au NPs containing 3 M NaCl, and (c) redispersed colloids of the thiolated PEG-modified Au NPs.

To further test their tolerance against severe conditions, the thiolated PEG-modified Au NPs were separated from their colloids by centrifugation, dried in air, and then redispersed into water. Upon drying of the thiolated PEG-modified Au NPs, their redispersed colloids show nearly identical color to that of their parent colloids (optical image not shown), indicating that no severe aggregation occurred after a cycle of separation, drying, and redispersion. This observation is further confirmed by the UV-vis spectra shown in Fig. 1. These results suggest that the thiolated PEG-modified Au NPs can be stored as solids or concentrated colloids and recover into colloids again just by dispersing into water at the terminal spots where they are used.

Self-assembly of Au NPs at the water-air interface. Upon concentration, the thiolated PEG-modified Au NPs in the colloids spontaneously migrate to the water-air interface and self-assemble into a thin particle film therein. Figure 2 shows the normal and enlarged SEM images of the particle film assembled at the water-air interface from the concentrated colloids (23.9 g/L) of the thiolated PEG-modified Au NPs. The Au NPs in the film are closely packed with interparticle spacing of about 8 nm, as is more clearly revealed by the inset image obtained at higher magnification. Apparently, the anchored PEG chains restrain the particles from direct contacting, thereby creating abundant particle-particle gaps (hot spots) in the film. With a 2D architecture, the particle film also sufficiently exposes the hot spots, allowing full access.

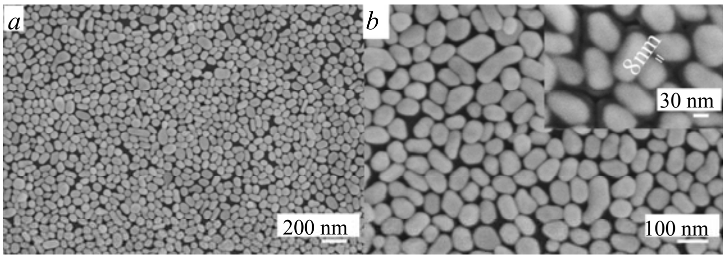

Fig. 2. Normal (a) and enlarged (b) SEM images of the particle film of the thiolated PEG-modified Au NPs assembled at the water-air interface from concentrated Au colloids (23.9 g/L). Inset in image b shows the SEM image obtained at higher magnification (180 k), from which a light shell can be clearly discerned.

PEG modification makes the Au NPs great hydrophilic, thereby largely decreasing their three-phase contact angle at the water-air interface. In such a case, the trapping energy originated from interfacial tension is lower than the thermal energy of the particle. Therefore, the NPs with a very small contact angle can easily escape from the water-air interface, making them inaccessible for subsequent interfacial self-assembly [26, 27]. However, when dilute colloids are concentrated (>160 times), the highly hydrophilic thiolated PEG-modified Au NPs in them can self-assemble into a thin particle film at the water-air interface. The following reasons may account for this self-assembly. Upon concentration of the colloids, the steric interaction among particles is largely increased because their interparticle spacing is very small. As a result, particles are driven to the water-air interface. Moreover, as the solvent evaporates, long PEG chains anchored on particles crosslink with each other, which may further fix and lock the thiolated PEG-modified Au NPs at the water-air interface [28, 29].

SERS test of the Au particle film assembled at water-air interface. To test the SERS behaviors of the particle film, 4-MBA was used as the probe molecule. Figure 3 shows the SERS spectra of 4-MBA obtained from the Au particle film assembled from the concentrated colloids of the thiolated PEG-modified Au NPs

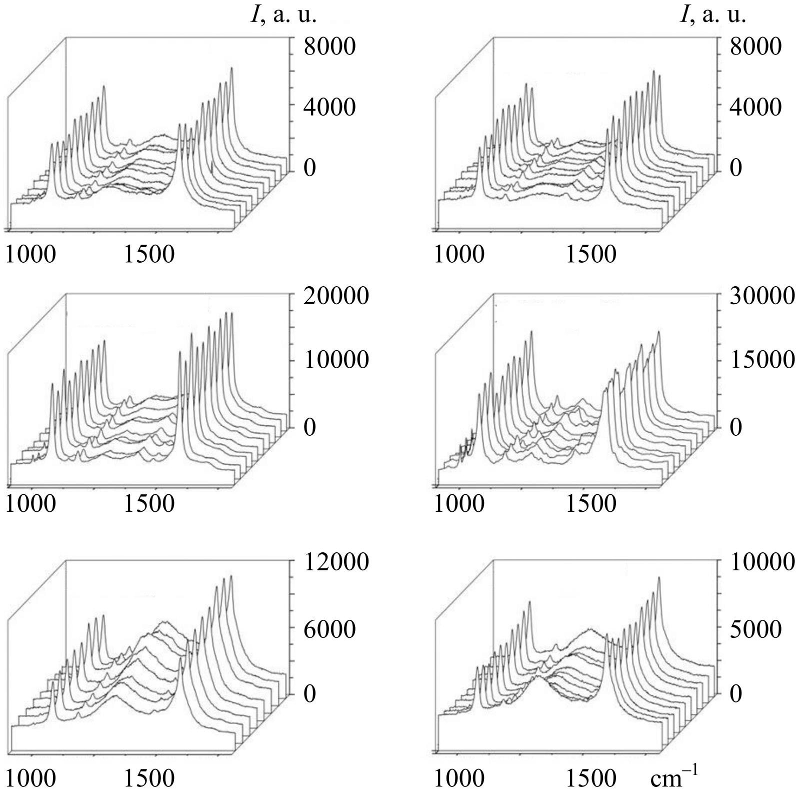

Fig. 3. SERS spectral sequences of 1×10^{-5} M 4-MBA obtained from different locations across the particle film of the thiolated PEG-modified Au NPs assembled at water-air interface from concentrated Au colloids with different concentrations (12.5, 16.9, 19.1, 23.9, 31.9, and 71.8 g/L). Accumulation time 4 s.

with different concentrations. To reveal spectral reproducibility, a series of spectra was measured from different spots across the surface of each particle film. Accordingly, the relative standard deviations (RSDs) were calculated using the intensity of the peak at 1067 $\mathrm{cm^{-1}}$. With increasing colloidal concentration from 12.5, 16.9, 19.1, 23.9, 31.9, to 71.8 g/L, the SERS intensity gradually increases and then slightly decreases. Meanwhile, the RSDs achieved on each particle film are 5.45, 6.86, 2.55, 3.15, 9.8, and 10%, respectively. Initially, the spectral sensitivity and reproducibility are improved because the assembled particle film becomes more uniform and the particles closer with more thiolated PEG-modified Au NPs available at the water-air interface. They both deteriorate slightly on the particle films assembled from the concentrated colloids with a concentration higher than 23.9 g/L. This deterioration happens because the particle film may stack up, resulting in the formation of multilayer films. Figure 4 shows the SERS spectra of 4-MBA obtained from the particle film assembled from the concentrated Au colloids (23.9 g/L) with optimized SERS activity. When the concentration of 4-MBA is decreased, the SERS signal from impurities becomes apparent. However, owing to the high SERS activity of the particle film, the main peaks from 4-MBA can still be discerned. The detection limit achieved on the particle film can be as low as 1×10^{-11} M.

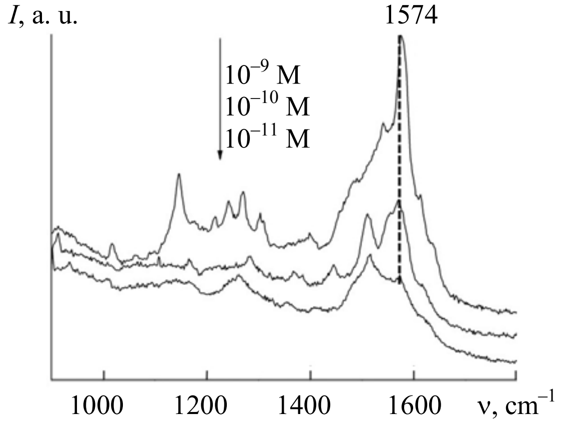

Fig. 4. Concentration-dependent SERS spectra of 4-MBA obtained from the particle film of the thiolated PEG-modified Au NPs assembled at the water-air interface from concentrated Au colloids (23.9 g/L). Accumulation time 30 s.

Conclusion. We have demonstrated that thiolated PEG-modified Au NPs possess excellent stability and can sustain severe conditions (e.g., 3 M NaCl). Upon formation of concentrated colloids, the thiolated PEG-modified Au NPs can self-assemble into closely packed 2D particle film at the water-air interface, thereby creating and exposing abundant hot spots at the particle-particle junctions. Because the SERS signals are averaged from abundant hot spots, they can be very strong and highly reproducible. These results thus suggest a concentration method for the storage of Au NPs and the fabrication of highly active, reproducible SERS substrate, which may be useful for reliable detection of analyte at terminal spots.

Acknowledgment. This work was financially supported by the National Natural Science Foundation of China (Grant Nos. J1210040 and 21673070).

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