Controlling the orientation of probe molecules on surface-enhanced Raman scattering substrates: A novel strategy to improve sensitivity

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HIGHLIGHTS

- MT-assisted SERS technique was employed to improve the detection sensitivity.
- The surface selection rules of SERS spectra were introduced to improve the LOD.
- The LOD of probe molecules exhibited nearly one order of magnitudes increase.

GRAPHICAL ABSTRACT
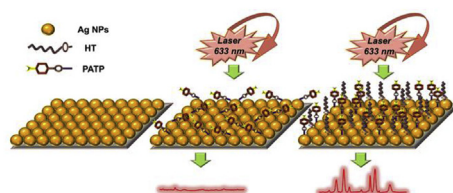
ABSTRACT

Typically, the surface-enhanced Raman scattering (SERS) technique is employed in precious metallic substrates with spontaneously adsorbed probing molecules to acquire signals. Preferred chemical conditions including aggregate status, hydrophilic/hydrophobic surroundings, and smart linkers were created to enable the detection of targets in very low concentrations (lower than 1.0×10^{-7} M). Although the sensitivity of SERS is applicable to certain areas, it is not satisfied in several cases that require obtaining good resolved signals involving extremely few surface molecules. Thus, further improvements in the sensitivity based on existing SERS techniques pose a challenge and is desirable for all aspects of analytical chemistry. In this study, a novel strategy was employed by constructing a molecular template (MT) on the SERS substrates with spontaneously adsorbed probe molecules to improve the detection sensitivity of probe molecules. The proposed MT-assisted SERS technique differed from previous methods as it provides a completely new method for improving the limit of detection (LOD) of SERS by controlling molecular orientations. The surface selection rules of SERS spectra were first introduced as an effective strategy to improve the detection sensitivity, and this was extremely beneficial with respect to analytical applications. The use of the MT-assisted SERS technique indicated that the LOD of probe molecules of p-aminobenzenethiol (8.0×10^{-9} M) and 4-mercaptobenzoic (1.0×10^{-7} M) acids on noble metallic substrates exhibited nearly one order of magnitudes. Hence, the proposed method paves a way to detect the molecules under improved sensitivity at extreme low concentrations. The study corresponded to a proof-of-concept study of MT-assisted SERS for SERS-based applications in ultra-sensitive analysis.

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1. Introduction

Extant research indicates that surface-enhanced Raman scattering (SERS) is a powerful analytical technique and includes advantages such as high sensitivity, high selectivity, trace amount of

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sample required, and no strict requirements for measurement conditions [1,2]. Following its discovery in 1974 [3], SERS has attracted considerable attention due to its advantages of ultra-sensitivity with broad application scopes in materials, environmental, medical, forensics, security, and biological sciences [4–10]. When compared with normal Raman scattering, SERS can enhance the signals that are very weak normally by several order of magnitudes. Additionally, SERS has evolved into a powerful and reliable analytical tool for ultrasensitive detection of analytes [11–13].

Previous studies proposed a series of methods to obtain high quality signals and these included increasing the adsorption capacity of the target molecules on SERS substrates [14], optimizing the shape or aggregate status of nanoparticles (NPs) [15,16], and selecting new hybrid materials with better enhancement effect as substrates [17,18]. However, it is necessary for SERS to satisfy increasingly strict analytical requirements in environmental, biological, food safety, homeland security, and pharmaceutical fields. Hence, in the present study, a proof-of-concept strategy was proposed to improve the SERS sensitivity of probe molecules based on the mechanism of the spectral surface selection rules albeit with a newly-developed protocol of employing molecular templates (MTs). The MTs were composed by a monolayer of long-chain alkanethiols that served as templates to regulate target molecules into preferred orientations by surface-selection-rules. Following the regulation, the target molecules could possess strongly enhanced spectral characteristics. Thus, more robust signals with further enhancements were obtained.

The surface selection rules are defined as the modification of the band intensities of a spectrum due to the proximity of a carrier to a surface [19]. In several cases, the relative vibrational band intensities in the SERS spectra differ from those in the Raman spectrum of the unadsorbed molecules based on the different mechanistic models of SERS and the selection rules [20]. In principle, the surface selection rules for SERS follow the electromagnetic propensity rules. In a rough approximation based on the electromagnetic propensity rules, it could be assumed that the largest Raman enhancement was observed for modes that derived their intensity from the polarizability matrix element α_{ZZ} (with Z perpendicular to the metal surface), which was followed by modes based on α_{XZ} and α_{YZ} [21]. The enhancement of the various Raman active modes was influenced by the magnitude of the local field components parallel and perpendicular to the local surface.

Self-assembled monolayers have attracted considerable attention over the last two decades due to their special physical and chemical properties [22]. Alkanethiols can bond to gold (and other metal) surfaces via the formation of thiolate-metal bonds and assemble into well-packed layers via van der Waals forces between the tail groups. Applications of alkanethiols include molecular recognition [23,24], vapor sensing [25], optical systems [26], microcontact printing [27], and molecular electronics [28]. In the present study, SAMs were applied for the first time to improve the limit of detection (LOD) of SERS by optimizing the molecular orientations using 1-hexanethiol self-assembled monolayers.

In the study, a new concept was created following the surface selection rules to tune the orientation of target molecules by using a novel MT technique. In general chemical conditions, the orientations of analyte molecules are random at extremely low concentrations when the molecules are combined with SERS substrates. Surface selection rules indicate that these orientations are detrimental in obtaining improved SERS signals. The proposed MT technique was potentially capable of solving this issue. A series of molecular aisles were built on SERS substrates by using MTs. When the molecules accessed the molecular aisles, they preferred to be oriented perpendicular to the substrates due to the steric hindrance effect. This method could acquire ordered surface

molecular structures and better SERS signals. The MT-assisted SERS can potentially be used as an advanced ultra-sensitive analytical tool in pharmaceutical, environmental, forensic, biological, material, and surface sciences.

2. Experimental section

2.1. Chemicals

Trisodium citrate dihydrate (HOC(COONa)(CH$_2$COONa)$_2 \cdot 2$H$_2$O, 99.0%), silver nitrate (AgNO$_3$, 99.8%), poly(-diallyldimethylammonium chloride) (PDDA) (molecular weight 200,000−350,000, 20 wt % in aqueous solution), 1-hexanethiol (HT), *p*-aminobenzenethiol (PATP), and 4-mercaptobenzoic acid (4-MBA) were purchased from Sigma−Aldrich Chemical Co., Inc. Other chemicals (anhydrous ethanol, acetone, chloroform, H$_2$SO$_4$ 98%, and H$_2$O$_2$ 30%) were all reagent grade and obtained from Beijing Chemical Plant. All chemicals were used without further purification. Ultrapure water (>18 MΩ) purified with a Milli-Q plus system (Millipore Co.) was exclusively used in all aqueous solutions and rinsing procedures.

2.2. Preparation of SERS substrates

2.2.1. Pretreatment of glass slides
Glass slides (with dimensions of 1.5 cm × 1.5 cm) were cleaned by sonicating the slides separately in solutions in the order of water, ethanol, acetone, chloroform, acetone, ethanol, and water, with each slide sonicated in each solution for 2 min successively. The slides were then immersed in a fresh piranha solution (30% H$_2$O$_2$/98% H$_2$SO$_4$, 3:7 v/v) at 80 °C for 20 min. After cooling, the slides were repeatedly rinsed with water and dried by nitrogen stream. All the steps that followed used the same washing and drying processes as those detailed in this section.

2.2.2. Preparation and deposition of Ag NPs
Ag NPs were prepared according to a process specified in a previous study [29]. A typical synthesis involved dissolving 36 mg of AgNO$_3$ in 200 mL of water in a flask. This aqueous solution was then heated to boiling temperature under stirring and reflux. Additionally, 4 mL of trisodium citrate solution (1%, w/v) was added into the flask and allowed to react for 1 h. The Ag NPs were then obtained. The Ag NPs can be stored for long periods of time. (couple of weeks at room temperature).

The Ag NPs were deposited on the glass slides through the layer-by-layer self-assembly technique. The glass slides were first immersed in a 0.5 wt % PDDA aqueous solution for 20 min. The glass slides were then removed and soaked in Ag NP solutions for 24 h. With respect to each step, the substrates were washed and dried step by step when the soaking solution was changed. Finally, the SERS substrates of Ag NP films were obtained and stored in a vacuum chamber for future usage.

2.2.3. Construction of probing molecule and MT hybrid monolayers
Initially, the as-prepared Ag NP substrates were soaked in a mixed ethanol solution of 1.0×10^{-5} M HT and PATP at different concentrations ranging from 1.0×10^{-3} to 1.0×10^{-9} M for 6 h. The substrates were then removed, rinsed by ethanol, and dried by nitrogen stream. A PATP−MT system on the Ag NP films was generated and could be used directly for SERS detection. A PATP system without MT molecules on the Ag NP films was prepared as a reference in the same experimental conditions. The other probing molecule MT systems could also be prepared through a process similar to the PATP−MT system.