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Surface-enhanced Raman spectroscopy based on conical holed enhancing substrates

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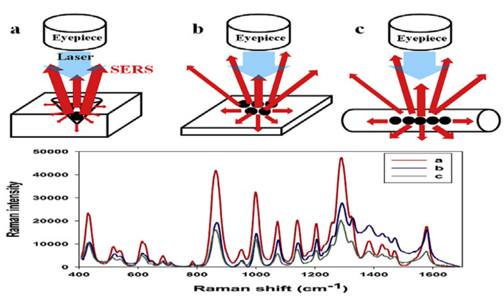
HIGHLIGHTS

- A novel conical holed SERS enhancing substrate was designed and manufactured.
- The optimal conical holed glass substrates can produce stronger SERS signal.
- The novel substrates can overcome the shortcomings of both dry and wet methods.
- The novel substrates coupled with MEM_{SERS} can realize quantitative SERS assays.

GRAPHICAL ABSTRACT



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ABSTRACT

In this contribution, surface-enhanced Raman spectroscopy (SERS) based on conical holed glass substrates deposited with silver colloids was reported for the first time. It combines the advantages of both dry SERS assays based on plane films deposited with silver colloids and wet SERS assays utilizing cuvettes or capillary tubes. Compared with plane glass substrates deposited with silver colloids, the conical holed glass substrates deposited with silver colloids exhibited five-to ten-folds of increase in the rate of signal enhancement, due to the internal multiple reflections of both the excitation laser beam and the Raman scattering photons within conical holes. The application of conical holed glass substrates could also yield significantly stronger and more reproducible SERS signals than SERS assays utilizing capillary tubes to sample the mixture of silver colloids and the solution of the analyte of interest. The conical holed glass substrates in combination with the multiplicative effects model for surface-enhanced Raman spectroscopy (MEM_{SERS}) achieved quite sensitive and precise quantification of 6-mercaptopurine in complex plasma samples with an average relative prediction error of about 4% and a limit of detection of about 0.02 μM using a portable *i*-Raman 785H spectrometer. It is reasonable to expect that SERS technique based on conical holed enhancing substrates in combination with MEM_{SERS} model can be developed and extended to other application areas such as drug detection, environmental monitoring, and clinic analysis, etc.

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) is a surface-sensitive technique deriving from localized surface plasmon resonance in nano-structured metals which give rise to huge

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electromagnetic fields at rough metal surfaces [1] or nanostructures [2]. There are two primary ways to perform in vitro SERS assays , i.e. dry method and wet method. Dry method is to drop droplets of a sample solution onto SERS enhancing substrates such as chemically deposited silver or gold films [3–6], and directly focus the excitation laser beam on the sample stain area after the evaporation of solvent to acquire SERS spectrum. Deposited metallic films can be mass-produced with good reproducibility, and are highly convenient and suitable for assaying large amounts of samples. However, due to the localized heating problem caused by the excitation laser beam, SERS assays based on deposited metallic films are generally performed with relatively lower laser power level for excitation [7], which might result in relatively lower detection sensitivity. Alternatively, SERS spectrum can be obtained by wet method in which the mixture of a sample and noble metal nanocolloids is kept in a small cuvette or a capillary tube and the excitation laser beam is focused inside the cuvette or tube trough the walls of the cuvette or tube [8–11]. The presence of solvents can absorb most of the heat generated by the excitation laser beam. A relatively higher laser power level can then be employed for excitation to increase the detection sensitivity. Compared with the dry SERS assays based on deposited metallic films, the wet SERS assays based on cuvettes or capillary tubes are less convenient and reproducible.

Recently, Larkin et al. [12] proposed to utilize cylindrical-conical holes to improve backscattering fourier transform Raman sampling in pharmaceutical tablets. The cylindrical-conical hole-based measuring technique can cause internal multiple scattering and hence resulted in a 3.4- to 5.6- fold enhancement of Raman signals when it is applied to backscattering geometry. In the present work, the above simple but effective idea has been further developed and extended to conical hole-based surface-enhanced Raman spectroscopy, with a view to combined the advantages of both dry SERS assays based on deposited metallic films and wet SERS assays based on cuvettes or capillary tubes and at the same time to avoid their disadvantages. In the conical hole-based surface-enhanced Raman spectroscopy, conical holed glass substrates deposited with silver colloids are used as SERS enhancing substrates.

6-Mercaptopurine is an immunosuppressive drug [13] used to treat leukemia, pediatric non-Hodgkin's lymphoma, polycythemia vera, psoriatic arthritis, and inflammatory bowel disease. It has also demonstrated some in vitro effectiveness against mycobacterium paratuberculosis [14]. Lin et al. reported the application of high performance liquid chromatography (HPLC) to the determination of 6-mercaptopurine in the plasma of renal transplant patients [15]. In the present contribution, the proposed surface-enhanced Raman spectroscopy based on conical holed glass substrates coupled with multiplicative effects model [16,17] has been applied to the quantification of 6-Mercaptopurine in plasma samples.

2. Experimental

2.1. Reagents and chemicals

4-Mercaptopyridine (96%), 4-mercaptobenzoic acid (90%), 6-mercaptopurine monohydrate (99.5%), (3-aminopropyl) triethoxysilane (APTMS, 98%) and silver nitrate (99.9%) were purchased from Aladdin. Sodium citrate dehydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) was obtained from Sigma–Aldrich Chemical Reagent Co., Ltd. (Shanghai, China). Concentrated HCl, HNO_3 and H_2SO_4 were purchased from Chemical Industry Institute (Zhuzhou, China), and 30% H_2O_2 was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Plasma samples were supplied by the City Blood Center of Changsha (Changsha, China). Ultrapure water (18.2 MΩ cm^{-1}) used throughout the paper was produced by an Aquapro water system (Aquapro, Chongqing, China).

2.2. Preparation of Ag nanoparticles

Ag nanoparticles were prepared by citrate reduction of $AgNO_3$ according to the method proposed by Lee and Meisel [18]. All glasswares were immersed in aqua regia ($HCl:HNO_3 = 3:1$ v/v) for about 8 h, and then were rinsed with ultrapure water. Under vigorous stirring, 2 mL 1% (m/v) trisodium citrate solution was quickly added to a boiling solution of 18 mg $AgNO_3$ in 100 mL of ultrapure water. The mixture was refluxed for 1 h, and then cooled to room temperature. The solution of Ag nanoparticles obtained was stored at 4 °C in a dark bottle.

2.3. Derivatization of glass substrates

Fig. 1 showed schematic of conical holed and cylindrical-conical holed glass substrates adopted in this contribution, which were designed by the present authors and manufactured by Weida Quartz Products Co., Ltd (Lianyungang, China). The specific diameters and depths of conical and cylindrical-conical holes investigated were listed in Table S-1 (Supporting Information). The glass substrates were cleaned in a mixture of $H_2SO_4:H_2O_2$ (4:1 v/v) for 10 min at 60 °C. After that, the glass substrates were successively sonicated in ultrapure water and absolute ethanol, and then air dried. The cleaned substrates were submerged into 2% APTMS for derivatization. After 24 h of derivatization, the substrates were rinsed with absolute ethanol to remove unbound monomer from the surface. Prior to the deposition of silver colloids, the substrates were rinsed with ultrapure water, and then immersed in vials containing silver colloids for 12 h. Finally, the substrates deposited with silver colloids were rinsed with ultrapure water and stored in ultrapure water until use [3,4].

2.4. Optimization of the depth and diameter of conical and cylindrical-conical holes

4-Mercaptopyridine was used to scrutinize the SERS enhancing rates of glass substrates with different diameters and depths of conical and cylindrical-conical holes. Stock solution of 4-mercaptopyridine (13 mM) was prepared in ethanol and stored at

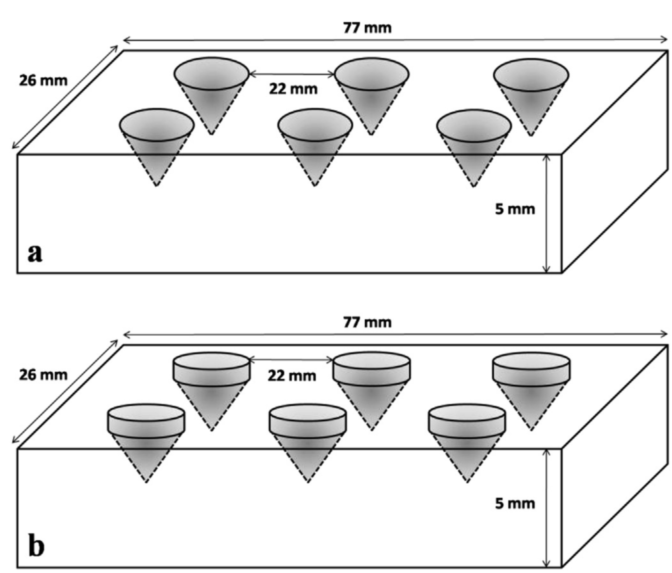

Fig. 1. Schematic of conical (a) and cylindrical-conical (b) holed glass substrates.