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Ultra-sensitive lab-on-a-chip detection of Sudan I in food using plasmonics-enhanced diatomaceous thin film

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ABSTRACT

Sudan I is a carcinogenic compound containing an azo group that has been illegally utilized as an adulterant in food products to impart a bright red color to foods. In this paper, we develop a facile lab-on-a-chip device for instant, ultra-sensitive detection of Sudan I from real food samples using plasmonics-enhanced diatomaceous thin film, which can simultaneously perform on-chip separation using thin layer chromatography (TLC) and highly specific sensing using surface-enhanced Raman scattering (SERS) spectroscopy. Diatomite is a kind of nature-created photonic crystal biosilica with periodic pores and was used both as the stationary phase of the TLC plate and photonic crystals to enhance the SERS sensitivity. The on-chip chromatography capability of the TLC plate was verified by isolating Sudan I in a mixture solution containing Rhodamine 6G, while SERS sensing was achieved by spraying gold colloidal nanoparticles into the sensing spot. Such plasmonics-enhanced diatomaceous film can effectively detect Sudan I with more than 10 times improvement of the Raman signal intensity than commercial silica gel TLC plates. We applied this lab-on-a-chip device for real food samples and successfully detected Sudan I in chili sauce and chili oil down to 1 ppm, or 0.5 ng/spot. This on-chip TLC-SERS biosensor based on diatomite biosilica can function as a cost-effective, ultra-sensitive, and reliable technology for screening Sudan I and many other illicit ingredients to enhance food safety.

1. Introduction

In recent decades, there is a growing concern with regards to food safety because foodborne diseases are responsible for the majority of mortality and morbidity worldwide with nearly 30% of the population in industrialized countries suffering from foodborne illness annually (Rahman, Khan, & Oh, 2016; Woh, Thong, Behnke, Lewis, & Zain, 2016). This concern has been highlighted by scandals of illegally adding melamine to dairy products to falsely increase the protein content in China, which affected thousands of children and many of them suffered kidney damages (Chan, Griffiths, & Chan, 2008; Kong & Du, 2011). Each year, nearly 48 million people in the United States suffer from foodborne illnesses, and 128,000 are hospitalized and almost 3000 die from foodborne related diseases (Woh et al., 2016). The consumption of food contaminated with toxic ingredients can result in foodborne illnesses, thus the detection of toxic ingredients in food product is important to enhance food safety.

Sudan I is a carcinogenic compound with an azo group that is commonly used as an industrial dye, such as in dyeing plastics, oil paint, waxes, and printing. Sudan I also has been illegally utilized as an adulterant in food stuffs and cosmetics for imparting a bright red color. In April 2003, Sudan I was detected in chili products made from India and Pakistan by a French agency (Di Donna, Maiuolo, Mazzotti, De Luca, & Sindona, 2004). Sudan dyes can damage DNA, RNA, and some enzymes in the human body. Thus, Sudan dyes have been classified as carcinogenic and mutagenic compounds by International Agency for Research on Cancer (Ahmed Refat et al., 2008; Cheung, Shadi, Xu, & Goodacre, 2010). This makes it illegal to be used as a food additive according to the U.S. Food and Drug Administration (FDA) and European framework (Union, 2005). For practical purposes, instant, ultra-sensitive, and cost-effective detection of Sudan I is of high significance to ensure food safety. Numerous techniques have been employed to identify Sudan I dye in food products, most of which involve the use of chromatographic methods coupled with various detectors (Calbiani et al., 2004; He, Su, Shen, Zeng, & Liu, 2007; Rebane, Leito, Yurchenko, & Herodes, 2010). Several standard procedures of sample preprocessing are indispensable for chromatographic approaches. Sampling is usually

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achieved by solvent extraction based on organic solvents such as acetone, acetonitrile or methanol. This step is, in many cases, followed by an additional cleaning and derivatization, which is time consuming and requires skilled personnel (Ostrea, 1999; Zhang, Wang, Wu, Pei, Chen, & Cui, 2014).

Thin layer chromatography (TLC) is a simple, fast and cost-effective technique for analyte separation, and can play pivotal roles for on-site sensing. Generally, after spotting samples onto the TLC plate and developing in the eluent via capillary force, the separated sample spots are visualized by the optical absorbance or fluorescence and the corresponding components are distinguished by their characteristic colors combined with different retention factor (Rf) values. However, most of the colorimetric methods used for TLC plates lack specificity and sensitivity. Routine spectroscopic technologies like Fourier Transform Infrared Spectroscopy (FT-IR) (Causin et al., 2008), mass spectroscopy (MS) (Wilson, 1999) and Surface-enhanced Raman scattering (SERS) (Zhang, Liu, Liu, Sun, & Wei, 2014) are needed to further characterize the targets. SERS spectroscopy has attained considerable interests as a sensitive and nondestructive detection method for the highly selective and sensitive detection of various analytes (X. Kong et al., 2016; Luo, Huang, Lai, Rasco, & Fan, 2016). The combination of SERS with TLC (TLC-SERS) is very promising in numerous applications due to its high throughput and sensitivity and it requires no complicated sample preprocessing (Lai, Chakravarty, Wang, Lin, & Chen, 2011). Since the pioneering work of TLC-SERS was reported by Zeiss's group (Hezel & Zeiss, 1977), this method has been successfully applied to the separation and identification of analytes from mixture samples. Examples include on-site monitoring of the aromatic pollutants in environmental water samples (Li et al., 2011), rapid identification of harmful pigments in food products, and detecting adulterants in botanical dietary supplements (Lv et al., 2015). The performance of the TLC technology is mainly dependent on the chromatographic materials and eluents. So far, most reported TLC-SERS methods use commercially available TLC plates such as silica gel or cellulose as the stationary phase, which have enabled short separation time and higher resolution. Nevertheless, the sensitivity of SERS spectroscopy from TLC plates is far from sufficient for food safety.

Diatomite consists of fossilized remains of diatom, a type of hard-shelled algae. As a kind of natural photonic biosilica from geological deposits, it has a variety of unique properties including a highly porous structure, excellent adsorption capacity, and low cost. In addition, the two dimensional (2-D) periodic pores on diatomite earth with hierarchical nanoscale photonic crystal features can provide additional enhancement for SERS sensing (Gordon, Sinton, Kavanagh, & Brolo, 2008; Ren, Campbell, Rorrer, & Wang, 2014). Herein, we fabricate TLC plates from diatomite as the stationary phase, and combine them with Au NPs to separate and identify Sudan I from real food samples (chili powder and chili sauce). The lab-on-a-chip TLC-SERS device using plasmonics-enhanced diatomaceous film demonstrated in this paper is able to detect Sudan I with nearly 10 times improvement of the intensity of Raman signals than commercially available silica gel TLC plates. It successfully detected Sudan I in chili sauce with ultra-high sensitivity down to 1 ppm.

2. Materials and methods

2.1. Materials and reagents

Tetrachloroauric acid ($HAuCl_4$) was purchased from Alfa Aesar. Trisodium citrate ($Na_3C_6H_5O_7$), cyclohexane, acetone and acetate were purchased from Macron. Diatomite (Celite209) and Sudan I were obtained from Sigma-Aldrich. Rhodamine6G (R6G) was purchased from Tokyo Chemical Industry. The food products (chili powder, chili sauce, chili oil and food oil) were purchased from local supermarkets. The chemical reagents used were of analytical grade. Water used in all experiments was deionized and further purified by a Millipore Synergy UV Unit to a resistivity of ~18.2 MΩ cm.

2.2. Preparation of Au NPs

All glassware used in the Au NP prepare process was cleaned with aqua regia (HNO_3/HCl, 1:3, v/v) followed by washing thoroughly with Milli-Q water. Au NPs with an average diameter of 60 nm were prepared using sodium citrate as the reducing and stabilizing agent according to the literature with minor modification (Grabar, Freeman, Hommer, & Natan, 1995). Briefly, a total of 50 mL of 1 mM chloroauric acid aqueous solution was heated to boil under vigorous stirring. After adding 2.1 mL of 1% trisodium citrate, the pale yellow solution turned fuchsia within several minutes. The colloids were kept under refluxing for another 15 min to ensure complete reduction of Au ions followed by cooling to room temperature.

2.3. Fabrication of diatomite earth TLC plates

The diatomaceous earth plates for TLC were fabricated by spin coating diatomite on glass slides. The diatomite biosilica was dried at 150 °C for 6 h in an oven before spreading on the glass slides. After cooling to room temperature, 11.55 g of diatomaceous earth was first dispersed in 20 mL of 0.5% aqueous solution of carboxymethyl cellulose and then transferred onto the glass slide for spin coating at 1200 rpm for 20 s. The plates were placed in the shade to dry and then annealed at 110 °C for 3 h to improve the adhesion of diatomaceous earth to the glass slides.

2.4. TLC-SERS method

The TLC-SERS method was designed for on-site detection of analytes from mixtures or real food samples as shown in Scheme 1. First, 0.5 μL liquid sample was spotted at 12 mm from the edge of the TLC plate. After drying in air, the TLC plate was kept in the TLC development chamber with mobile phase eluent (Cyclohexane and ethyl acetate v/v = 6:1). After separation of the analytes, the TLC plates were then dried in air. The Rf of the analytes on TLC plates were calculated and marked on the TLC plates so that the analyte spots could be traced even when they are invisible at low concentrations. Then 2 μL concentrated Au NPs were deposited directly three times. A Horiba Jobin Yvon Lab Ram HR800 Raman microscope equipped with a CCD detector was used to acquire the SERS spectra, and a 50× objective lens was used to focus the laser onto the SERS substrates. The excitation wavelength was 785 nm, and the laser spot size was 2 μm in diameter. The confocal pinhole was set to a diameter of 200 μm. SERS mapping images were recorded with a 20 × 20-point array. They were collected using the DuoScan module with a 2.0 μm step size, 0.5 s accumulation time, and collected in the Raman spectral range from 800 cm^{-1} to 1800 cm^{-1}. The acquired data was processed with Horiba LabSpec 5 software. The data were analyzed and processed by OriginPro2016.

2.5. Other instruments

UV–vis absorption spectra were recorded by a NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific) using polystyrene cells of 1 cm optical path. Scanning electron microscopy (SEM) images were acquired on FEI Quanta 600 FEG SEM with 15–30 kV accelerating voltage. The microscopy images were acquired on the Horiba Jobin Yvon Lab Ram HR800 Raman microscope with a 100×