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Surface-enhanced Raman scattering detection of chemical and biological agents using a portable Raman integrated tunable sensor

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Abstract

This paper describes a compact and rugged Raman integrated tunable sensor coupled with surface-enhanced Raman scattering substrates for the screening of a wide variety of chemical and biological agents for homeland defense applications. The field-deployable instrument, consisting of an 830-nm diode laser for excitation and an avalanche photodiode (APD) for detection, is a fully integrated, tunable, "point-and-shoot" Raman device based on solid-state acousto-optic tunable filter (AOTF) technology. It can provide direct identification of chemical and biological samples in a few seconds under field conditions. This paper illustrates some applications of this portable device for the detection of various compounds of particular interest for homeland defense applications. These include methyl parathion (a nerve agent simulant) and dipicolinic acid (a biomarker for *bacillus endospore*), and other chemical warfare simulants such as dimethyl methylphosphonate, pinacolyl methylphosphonate, diethyl phosphoramidate, and 2-chloroethyl ethylsulfide, which are simulants for sarin (GB), soman (GD), tabun (GA), and sulfur mustard (HD), respectively, and intact bacteria such as *Bacillus globigii*, *Erwinia herbicola*, and *Bacillus thuringiensis*, which are simulants for biological warfare agents.

Keywords: Sensors; Surface-enhanced Raman scattering; Chemical warfare agent; Biological warfare agent; Acousto-optic tunable filter (AOTF)

1. Introduction

In recent years, there has been a growing need for sensitive and selective assay for rapid detection and identification of terrorist threats including chemical and biological warfare (CBW) agents, toxic industrial wastes, and explosives. Numerous advances in current technologies for field-deployable chemical and biological analysis, a vital component of homeland and national security, have evolved [1–5]. However, there is still the need for quick, highly sensitive, robust and dependable technologies that can be readily operable by relatively untrained first responders and homeland security operatives in the field.

Raman spectroscopy is well known for its specificity in chemical and biological analysis, and offers some distinct advantages over other spectroscopic methods for homeland security applications. Following laser irradiation of a sample, the observed Raman shifts are equivalent to the energy changes involved in vibrational transitions of the scattering species and are therefore characteristic of the investigated sample. Technically, a wide variety of chemical and biological molecules can be identified by Raman spectroscopy since they exhibit characteristic vibrational frequencies [6]. Such frequencies, when observed in absorption techniques, occur in the infrared (IR) region of the spectrum. In the Raman technique, the spectrum is in the same spectral region of the exciting laser radiation. Raman spectroscopy provides more detailed vibrational information which is often unavailable or unresolved in fluorescence, UV absorption and reflectance spectroscopies. Raman spectroscopy is also more suitable than IR spectroscopy for field analysis because it does not suffer from the strong IR absorption band of water due to moisture in air or water. Despite such advantages, normal Raman scattering suffers the disadvantage of extremely poor efficiency. Compared to luminescence-based processes, Raman spectroscopy has an inherently small cross-section (e.g. 10^{-30} cm^2 per molecule), thus precluding the possibility of analyte detection at low concentration levels without special enhancement processes. Some modes of signal enhancement have included resonance Raman scattering and nonlinear processes such as coherent anti-Stokes Raman scattering. However, the need for high-power, multiple-wavelength excitation sources has limited the widespread use of these techniques. Because of the aggressive development of SERS substrates and application to a wide range of chemicals, the potential of SERS as a routine analytical technique was recognized by the mid-1980s. Our laboratory

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has been developing SERS-active substrates and investigating their applications for chemical, biological and medical sensing for over two decades [7–17]. More recent reports have cited SERS enhancements from 10^{13} to 10^{15}, thus demonstrating the potential for single-molecule detection with SERS [18–20].

In this paper we describe the construction of a fully integrated portable Raman sensor, and discuss its applications for the detection of methyl parathion and dipicolinic acid, and other chemical warfare simulants such as dimethyl methylphosphonate (DMMP), pinacolyl methylphosphonate (PMP), diethyl phosphoramidate (DEPA), and 2-chloroethyl ethylsulfide (CEES), and biological warfare simulants such as *Bacillus globigii* (BG), *Erwinia herbicola* (EH), and *Bacillus thuringiensis* (BT).

2. Experimental

2.1. Chemicals and materials

Unless stated otherwise, all chemicals, such as dimethyl methylphosphonate, pinacolyl methylphosphonate, diethyl phosphoramidate, 2-chloroethyl ethylsulfide, dipicolinic acid, hydroxylamine hydrochloride, silver nitrate, triethanolamine, potassium hydroxide, sodium hydroxide, sodium citrate, 2-propanol, and methanol, etc. were purchased from Sigma–Aldrich (St. Louis, MO, USA) at the highest purity grade available and used without further purification. *B. globigii*, *E. herbicola*, and *B. thuringiensis* were obtained from the Critical Reagents Program (CRP), the Armed Forces Institute of Pathology (AFIP) and Dugway Proving Grounds (DPG) by Dr. Arpad A. Vass at Oak Ridge National Laboratory (ORNL).

2.2. Preparation of surface-enhanced Raman scattering substrates

2.2.1. Silver nanoparticle island films

A 9-nm mass thickness of silver was deposited on a cleaned glass surface prepared by a physical vapor deposition (PVD) method using an electron beam evaporation system (CVE 301 EB, Coke Vacuum Products, Norwalk, CT, USA). Prior to deposition, glass slides were soaked in saturated KOH solution in 2-propanol overnight, then they were rinsed with distilled water several times and air-dried. A 10 Å chromium layer was deposited on the glass surface before silver deposition to stabilize the silver layer on the glass surface. The deposition rate and PVD chamber pressure were 0.15 Å s^{-1} and 10^{-6} Torr (1 Torr = 133.3 Pa), respectively.

2.2.2. Silver colloids

Silver particles with an average diameter in the region of 23 nm (as examined by TEM measurements) were prepared according to a method reported by Leopold and Lendl [21]. Briefly, 10 mL of $AgNO_3$ aqueous solution (10 mM) were added to 90 mL of a hydroxylamine hydrochloride solution (1.67 mM) containing 3.33 mM sodium hydroxide, and the solution was kept stirring for ∼30 min.

2.2.3. Silver-oxide thin films and silver nanoclusters

Uniform and specularly reflective silver-oxide films with different thickness were produced on glass substrates using methods reported previously [22]. Briefly, 1 g of solid $AgNO_3$ was dissolved in 5 mL of deionized water in a beaker. Then, triethanolamine solution was added gradually, with constant stirring, until the initially formed precipitate was dissolved. More deionized water was added to make a total volume of 80 mL. Precleaned glass slides were vertically placed into the beaker and the bath was brought to and kept at 45–50 °C on a hot plate. After various periods of time, the coated slides were removed from the bath, thoroughly rinsed with deionized water and air-dried. Illumination of silver-oxide samples with blue and UV mercury lamp excitation produces Ag_n nanoclusters that range in size from $n = 2$ to 8 atoms with strong size- and geometry-dependent fluorescence [23,24].

2.3. Instrument

Atomic force microscopy (AFM) was used to determine the surface roughness parameters of the different SERS substrates. AFM measurements were performed with an Explorer system and SPMLabNT software Version 5.01 Explorer AFM (Thermomicroscopes, Sunnyvale, CA, USA). The AFM Explorer system consists of a v-type cantilever, a CCD camera, laser,

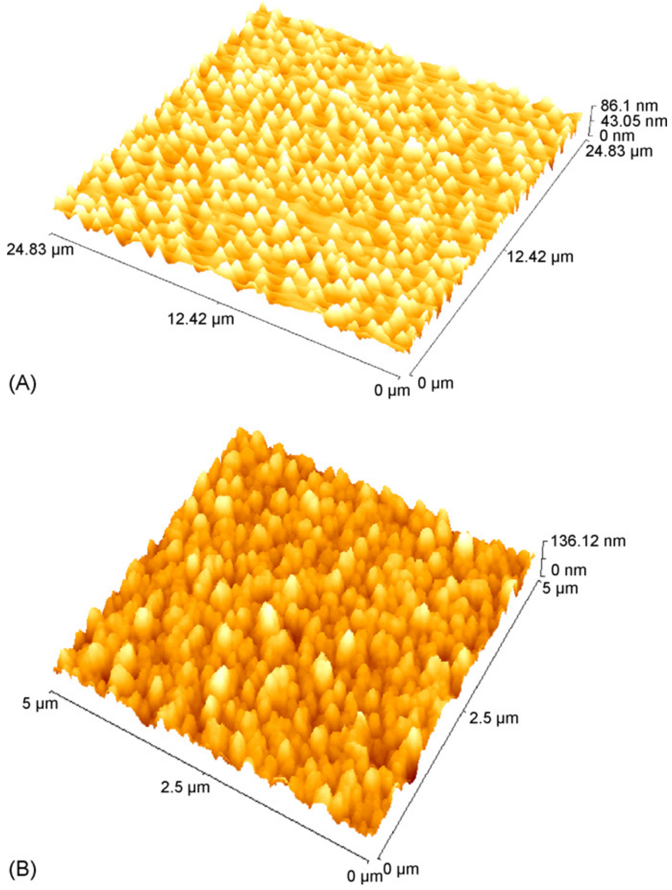

Fig. 1. AFM images of (A) silver nanoparticle island films prepared by physical vapor deposition, and (B) silver oxide films/silver nanoclusters grown on glass slides by a chemical-bath-deposition technique after UV irradiation for 5 min.