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Ultrastable Substrates for Surface-Enhanced Raman Spectroscopy: Al_2O_3 Overlayers Fabricated by Atomic Layer Deposition Yield Improved Anthrax Biomarker Detection

Xiaoyu Zhang,[†] Jing Zhao,[†] Alyson V. Whitney,[†] Jeffrey W. Elam,[‡] and Richard P. Van Duyne*,[†]

Contribution from the Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, and Energy Systems and Materials Science Divisions, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439

Received June 2, 2006; E-mail: vanduyne@chem.northwestern.edu

Abstract: A new method to stabilize and functionalize surfaces for surface-enhanced Raman spectroscopy (SERS) is demonstrated. Atomic layer deposition (ALD) is used to deposit a sub-1-nm alumina layer on silver film-over-nanosphere (AgFON) substrates. The resulting overlayer maintains and stabilizes the SERS activity of the underlying silver while presenting the surface chemistry of the alumina overlayer, a commonly used polar adsorbent in chromatographic separations. The relative affinity of analytes for alumina-modified AgFON substrates can be determined by their polarity. On the basis of SERS measurements, dipicolinic acid displays the strongest binding to the ALD alumina-modified AgFON among a set of pyridine derivatives with varying polarity. This strong affinity for carboxylate groups makes the SERS substrate an ideal candidate for bacillus spores detection using the dipicolinate biomarker. The SERS signal from extracted dipicolinate was measured over the spore concentration range 10^{-14}–10^{-12} M to determine the saturation binding capacity of the alumina-modified AgFON surface. The adsorption constant was determined to be $K_{spore} = 9.0 \times 10^{13}$ M^{-1}. A 10-s data collection time is capable of achieving a limit of detection of $\sim 1.4 \times 10^3$ spores. The shelf life of prefabricated substrates is at least 9 months prior to use. In comparison to the bare AgFON substrates, the ALD-modified AgFON substrates demonstrate twice the sensitivity with 6 times shorter data acquisition time and 7 times longer temporal stability. ALD expands the palette of available chemical methods to functionalize SERS substrates, which will enable improved and diverse chemical control over the nature of analyte−surface binding for biomedical, homeland security, and environmental applications.

Introduction

Surface-enhanced Raman scattering occurs when a molecule is spatially confined within the zone of enhanced electromagnetic fields generated by excitation of a localized surface plasmon resonance (LSPR).[1] Consequently, the magnitude of the induced dipole moment of the molecule increases, and accordingly, the intensity of the inelastic scattering increases. Surface-enhanced Raman spectroscopy (SERS) has many characteristics that can be exploited in biosensing applications,[2−6] such as sensitivity, selectivity, and no interference from water molecules. However, the acceptance of SERS as a general analytical tool has been hindered by the lack of SERS substrate stability. Generally, SERS activity is affected by the oxidation of silver or the aggregation of noble metal colloidal nanostructures.[7−9] In this work, we demonstrate a simple strategy for dramatically improving the stability of traditional SERS substrates. An ultrathin alumina layer was coated onto silver film-over-nanosphere (AgFON) substrate using atomic layer deposition (ALD). ALD utilizes self-limiting surface reactions to control interfacial thickness and composition with molecular precision.[10] Previous quartz crystal microbalance measurements have demonstrated highly uniform layer-by-layer growth of the ALD alumina on Ag nanoparticles with a growth rate of ~1 Å per deposition.[10] The sub-1-nm thickness is extremely advantageous in preserving sensitivity, because the SERS intensity decays by approximately 1 order of magnitude for each 2.8 nm separation between the surface and the scatterer.[11] The details

[†] Northwestern University.
[‡] Argonne National Laboratory.

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14. ABSTRACT

A new method to stabilize and functionalize surfaces for surface-enhanced Raman spectroscopy (SERS) is demonstrated. Atomic layer deposition (ALD) is used to deposit a sub-1-nm alumina layer on silver film-over-nanosphere (AgFON) substrates. The resulting overlayer maintains and stabilizes the SERS activity of the underlying silver while presenting the surface chemistry of the alumina overlayer, a commonly used polar adsorbent in chromatographic separations. The relative affinity of analytes for alumina-modified AgFON substrates can be determined by their polarity. On the basis of SERS measurements, dipicolinic acid displays the strongest binding to the ALD alumina-modified AgFON among a set of pyridine derivatives with varying polarity. This strong affinity for carboxylate groups makes the SERS substrate an ideal candidate for bacillus spores detection using the dipicolinate biomarker. The SERS signal from extracted dipicolinate was measured over the spore concentration range 10-14-10-12 M to determine the saturation binding capacity of the alumina-modified AgFON surface. The adsorption constant was determined to be Kspore) 9.0 1013 M-1. A 10-s data collection time is capable of achieving a limit of detection of 1.4 103 spores. The shelf life of prefabricated substrates is at least 9 months prior to use. In comparison to the bare AgFON substrates, the ALD-modified AgFON substrates demonstrate twice the sensitivity with 6 times shorter data acquisition time and 7 times longer temporal stability. ALD expands the palette of available chemical methods to functionalize SERS substrates, which will enable improved and diverse chemical control over the nature of analyte-surface binding for biomedical, homeland security, and environmental applications.

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of the intensity decay function are determined by the nanostructure of the underlying silver surface.

The use of ALD alumina presents several advantages. First, compared to conventional overlayer materials, the ultrathin alumina layer is extremely stable to oxidation and high temperature.[12] This helps to maintain the high stability of SERS activity with minimal decrease in signal. Second, alumina is commonly used as a polar adsorbent in chromatographic separations. The relative affinity between Raman scatterers and alumina-modified AgFON substrates can be predicted on the basis of their polar interaction, which has been well established in the chromatography literature. Generally, molecules with strong polarity, such as carboxylic acids, have high affinity to alumina.[13,14] Therefore, this novel SERS substrate is an ideal candidate for the detection of carboxylic acids due to the strong polar interaction. Third, the scope of analytical applications of SERS has been broadened by modifying noble metal surfaces with an analyte-specific affinity coating.[3,6,15] The coatings used range from simple alkanes[15] to complex macrocycles with the common theme of containing a thiol group to anchor the coating to a noble metal substrate. Large partition coefficients on the coating allow analytes to partition closer to the surface.[15] However, the high coverage of the thiolate self-assembled monolayers (SAMs) is thermodynamically unstable.[16] Thermal desorption[17,18] and photooxidation[19−21] of the thiolate molecules result in defects in the coating. In comparison to the previously used thiolate SAMs, ALD alumina enjoys greater molecular thickness control, greater physical and chemical stability, more complete surface coverage, less signal attenuation due to distance effects, and predictable affinity.

In a previous study, the optically optimized AgFON substrate had been applied to quantitatively detect a biomarker for anthrax, calcium dipicolinate (CaDPA), from bacillus spores.[2] A limit of detection (LOD) of ~2550 anthrax spores was achieved on the AgFON sensor with a data acquisition period of 1 min and a laser power of 50 mW. In this study, we have increased the affinity between CaDPA and the sensor surface using ALD alumina, thus increasing the SERS signal intensities. The alumina-modified sensor shows 2-fold improvement in LOD using 6 times shorter data acquisition time. Lifetime testing measurements indicate that the SERS intensity is stable on alumina-coated AgFONs for at least 9 months. These results indicate that this new SERS sensor has the potential to be extremely useful for biomedical, homeland security, and environmental measurements.

To frame the achievements reported herein, this paper addresses (1) the physical characteristics and the surface chemistry behavior of the ALD alumina-modified AgFON surfaces based on SEM, LSPR, and SERS results, and (2) the applicability and efficiency of these substrates as a bacillus spore sensing platform.

Experimental Section

Materials. Ag (99.99%) was purchased from D. F. Goldsmith (Evanston, IL). Glass substrates were 18 mm diameter, No. 2 cover slips from Fisher Scientific (Pittsburgh, PA). Surfactant-free white carboxyl-functionalized polystyrene latex nanospheres with diameters of 590 nm were obtained from Interfacial Dynamics Corp. (Portland, OR). Tungsten vapor deposition boats were purchased from R. D. Mathis (Long Beach, CA). Water (18.2 MΩ·cm) was obtained from an ultrafilter system (Milli-Q, Millipore, Marlborough, MA). All the other chemicals, reagents, and solvents were purchased from Aldrich Chemical (Milwaukee, WI) or Fisher Scientic (Fairlawn, NJ) and used without further purification.

Thin-layer chromatographic (TLC) analyses were performed on aluminum-backed aluminum oxide 60 F-254 neutral with a 0.2 mm layer thickness (type E, E. Merck, Darmstadt, Germany) in 10:1 v/v hexanes:ethyl acetate.

Calcium dipicolinate (CaDPA) was prepared from DPA and calcium hydroxide according to the method of Bailey and co-workers.[22]

Bacillus subtilis spore samples were prepared according to the previously published method.[2] Approximately 1 g of sample was determined to contain 5.6×10^{10} spores by optical microscope measurements (data not shown). The spore suspension was made by dissolving spores in 0.02 M HNO_3 solution and sonicating for 10 min, which effectively extracts CaDPA from spores. This concentration of the HNO_3 solution was selected because of its capability for CaDPA extraction and benign effect on the AgFON SERS activity. The sonication procedure was performed because no SERS signal of CaDPA was observed from the spore solution prior to sonication (data not shown).

AgFON Substrate Fabrication. Glass substrates were pretreated in two steps: (1) piranha etch (***CAUTION***: piranha solution should be handled with great care), in which 3:1 H_2SO_4:30% H_2O_2 at 80 °C for 1 h was used to clean the substrate, and (2) base treatment, in which 5:1:1 H_2O:NH_4OH:30% H_2O_2 with sonication for 1 h was used to render the surface hydrophilic. Approximately 2 μL of the nanosphere suspension (4% solids) was drop-coated onto each substrate and allowed to dry in ambient conditions. The metal films were deposited in a modified Consolidated Vacuum Corp. vapor deposition system with a base pressure of 10^{-6} Torr. The deposition rates for each film (10 Å/s) were measured using a Leybold Inficon XTM/2 quartz crystal microbalance (QCM) (East Syracuse, NY). AgFON substrates were stored in the dark at room temperature prior to use.

Atomic Layer Deposition (ALD). Alumina films were fabricated on the AgFON substrates by ALD. The reactor utilized in these experiments is similar to that used in previous publications.[10] Trimethylaluminum (TMA) and deionized H_2O vapors were alternately pulsed through the reaction chamber, utilizing N_2 as the carrier gas, at a mass flow rate of 360 sccm, a pressure of 1 Torr, and a growth temperature of 50 °C. One complete ALD cycle takes 42 s and includes four steps: (1) TMA reactant exposure time, 1 s; (2) N_2 purge following TMA exposure time, 10 s; (3) H_2O reactant exposure time, 1 s; and (4) N_2 purge following H_2O exposure time, 30 s. Long purge times are necessary at low temperatures to prevent chemical vapor deposition of alumina.[23,24] A previous study indicated nearly ideal layer-by-layer growth of the ALD alumina on Ag surfaces with an average rate ~1 Å/cycle.[10] This result greatly simplifies the interpretation of the

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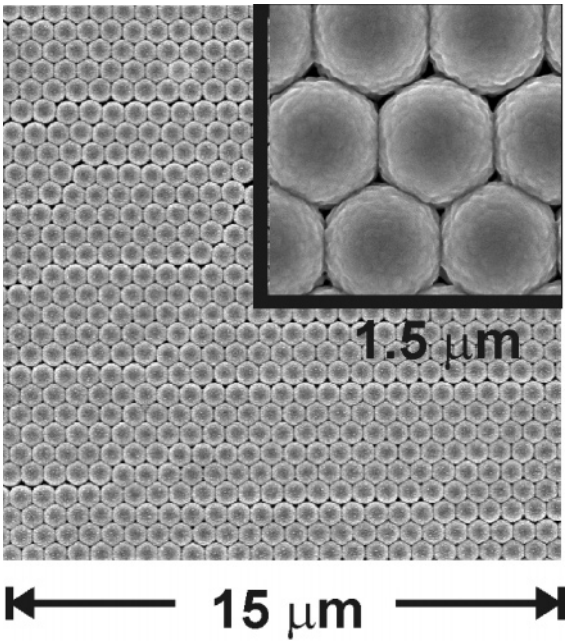

Figure 1. Scanning electron microscope images of alumina-modified AgFON substrates (D = 600 nm, d_m = 200 nm, and two ALD cycles of alumina).

thickness of the alumina overlayers, which can be deduced easily from the number of ALD cycles.

Scanning Electron Microscopy (SEM). SEM images of alumina-coated AgFON were observed with a Hitatchi S-4700-II SEM.

LSPR Reflectance Spectroscopy. Reflectance measurements were carried out using a SD2000 spectrometer coupled to a reflection probe (Ocean Optics, Dunedin, FL) and a halogen lamp (F-O-Lite H, World Precision Instruments, Sarasota, FL). The reflection probe consists of a tight bundle of 13 optical fibers (12 illumination fibers around 1 collection fiber), with a usable wavelength range of 400−900 nm. All reflectance spectra were collected against a mirror-like Ag film over a glass surface as a reference in air.

SERS Apparatus. The macro-Raman system consists of an interference band-pass filter (Coherent, Santa Clara, CA), a 1-in. long-pass dielectric edge filter (Sermrock, Rochester, NY), a single-grating monochromator with the entrance slit set at 100 μm (model VM-505, Acton Research Corp., Acton, MA), a liquid-N_2-cooled CCD detector (model Spec-10:400B, Roper Scientific, Trenton, NJ), and a data acquisition system (Photometrics, Tucson, AZ). A compact diode laser (model RL785, Renishaw plc, Gloucestershire, UK) was used to generate 785 nm. All the measurements were performed in ambient conditions.

Result and Discussion

1. Structure Characterizations of Alumina-Modified AgFON. Figure 1 shows a SEM image of an alumina-modified AgFON substrate with a nanosphere diameter (D) of 590 nm, Ag mass thickness (d_m) of 200 nm, and two ALD cycles of alumina. The low-magnification SEM image (Figure 1) displays a region of hexagonally packed polystyrene spheres. The inset in Figure 1, a high-magnification image, shows that the top of each nanosphere is not smooth but exhibits substructure roughness features 30−50 nm in size.

2. Alumina Thickness Effect on LSPR. Previously, a detailed wavelength-scanned SERS study of benzenethiol adsorbed on Ag nanoparticle arrays revealed that the maximum SERS enhancement factor occurs for excitation wavelengths

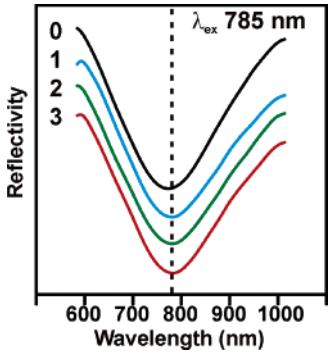

Figure 2. LSPR reflectance spectra of bare AgFON and alumina-modified AgFON substrates with different alumina thickness. The ALD cycles of alumina varied from 1 to 3; D = 600 nm, d_m = 200 nm. The vertical dotted line denotes the laser excitation used in the SERS measurements. All reflectance spectra were collected against a mirror-like Ag film over glass surface as a reference in air.

(λ_{ex}) slightly to the blue of the LSPR maximum wavelength for adsorbate-covered nanoparticle arrays.[25] Additionaly, similar observations have been reported based on plasmon-sampled surface-enhanced Raman excitation spectroscopy.[2,26] This finding yields a general rule for maximizing SERS signals for chemical and biological detection.

The plasmon position of FONs can be tuned by controlling the size of the spheres used in the underlying nanosphere mask. Generally, an increase in polystyrene sphere diameters results in a red shift in LSPR.[2] Here, polystyrene spheres with a diameter of 590 nm were selected to fabricate SERS substrates with the LSPR in the near-infrared (NIR) range.[2] The resulting substrates are optimized for the SERS measurements using NIR laser excitation. NIR laser excitation reduces not only the interference from biological fluorescent background but also the potential for tissue damage due to the low water and tissue absorption of NIR light.

Since AgFONs are not optically transparent, the reflectivity minimum (λ_{min}) was used to locate the LSPR positions. Figure 2 shows the LSPR reflectance spectra of AgFON coated with 0−3 ALD cycles. For reference, the vertical dotted line in Figure 2 denotes the 785 nm laser excitation used in the following SERS measurements. The LSPR λ_{min} positions of AgFON coated with 0−3 ALD cycles remain close to the SERS excitation wavelength, ranging between 774 and 783 nm, and are not greatly affected by coating alumina. This result agrees with a more detailed and rigorous study on NSL-produced Ag nanoparticles with alumina layers.[10]

3. Competitive Adsorption on Alumina-Modified AgFON. In the analysis of biologically, industrially, and environmentally relevant samples, multiple analytes with similar molecular structures are often in competition for the SERS surface. In that situation, the detection selectivity is improved when the affinity between the target analyte and the sensing platform is optimized. Central to the work reported here is our ability to predict the relative affinity of different molecules on an alumina-modified AgFON substrate. As a proof-of-concept experiment, we examine the competitive adsorption of dipicolinic acid (**a**), diacetylpyridine (**b**), and dimethoxypyridine (**c**) onto an alumina-modified AgFON surface at room temperature (Figure 3A).

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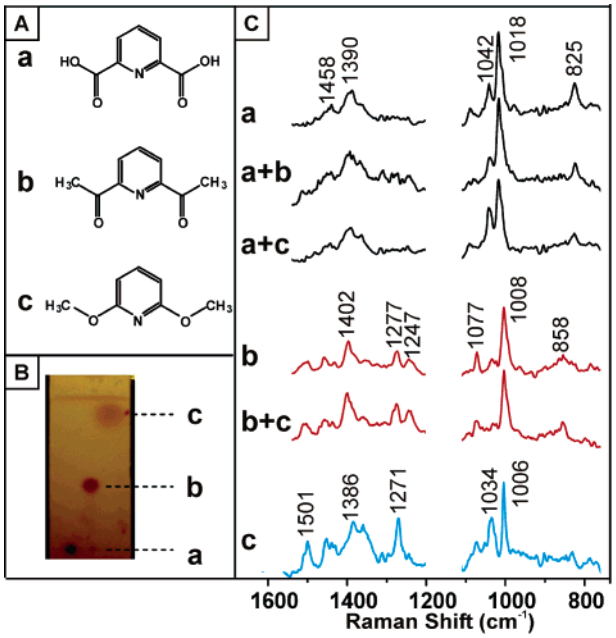

Figure 3. (A) Notations of dipicolinic acid (**a**), diacetylpyridine (**b**), and dimethoxypyridine (**c**). (B) TLC chromatogram showing the separation of **a**, **b**, and **c**. Eluent, 10:1 hexanes:ethyl acetate. Spots were detected by placing the TLC plate in iodine vapor. (C) SERS spectra of 2 mM **a**, **b**, and **c** on alumina-modified AgFON substrates. Reference spectra of individual pyridine derivatives are shown along with the spectra of the equimolar mixtures. Laser excitation, 785 nm; laser power, 50 mW; acquisition time, 30 s; $D = 590$ nm; $d_m = 200$ nm; and two ALD cycles of alumina. The gap in spectral coverage results from changing the grating in the spectrometer.

These pyridine derivative analytes serve as a model system because they provide the unambiguous SERS spectral signatures important to differentiate between the relative adsorption affinities observed during competitive adsorption experiments. On the basis of a thin-layer chromatography (TLC) experiment (Figure 3B), the relative affinity between each analyte and alumina is determined to be **a** > **b**> **c**. Therefore, we expect that the SERS spectra of mixed analytes on the alumina-modified AgFON substrates would be dominated by the strongest adsorbate **a**. To verify this hypothesis, a series of SERS measurement were made. We produced the SERS samples by incubating the alumina-modified AgFON substrates in 2 mM analyte in ethanol solutions for 5−6 h. Adsorption of each individual analyte produces a specific SERS signature (Figure 3C). It is worth noting that high signal-to-noise SERS spectra were obtained on alumina-modified AgFON substrates, even though the adsorbates were not in direct contact with the silver surface. The spectra can be compared with those from equimolar mixtures of pairs of **a**+**b**, **a**+**c** and **b**+**c**. For mixtures that contain **a**, the spectra are dominated by peaks characteristic of **a** at 1458, 1390, 1042, and 1018 cm^{-1}. Similarly, the spectrum for **b**+**c** combinations is dominated by signatures of **b**. From these results, it is clear that **a** has the highest adsorption affinity on alumina-modified AgFON surfaces and **c** has the lowest, which is consistent with anticipation in light of the TLC results. The high affinity of dipicolinic acid has numerous practical implications. As an example, alumina-modified AgFON surfaces are ideal sensing platforms for calcium dipicolinate, a well-known biomarker for bacillus spores,[22] because the increase in affinity improves the ultimate LOD.

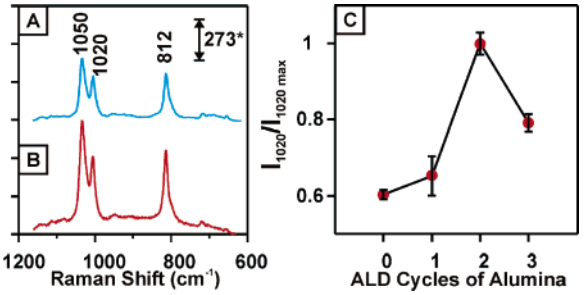

Figure 4. (A) SERS spectrum of 2×10^{-5} M CaDPA in 0.2 μL of 0.02 M HNO_3 on alumina-modified AgFON substrate (two ALD cycles of alumina). (B) SERS spectrum of CaDPA on bare AgFON substrate. (C) The alumina thickness effect on the SERS intensity. The normalized spectral intensities at 1020 cm^{-1}, I_{1020}, were plotted versus ALD cycles. I_{1020} was taken from SERS spectra that correspond to varying alumina thickness on the AgFON substrates. Each data point represents the average value from three SERS samples. Error bars show the standard deviations. Laser excitation, 785 nm; laser power, 50 mW; acquisition time, 10 s; $D = 600$ nm; and $d_m = 200$ nm. * denotes adu/(s·mW).

4. Bacillus Spore Detection Using Alumina-Modified AgFON. In our previous study, CaDPA was efficiently extracted from spores by sonication in 0.02 M HNO_3, deposited onto AgFON substrates, and then detected by SERS.[2] In this work, we follow the same extraction protocol but use ALD alumina-modified AgFON sensors. The results presented below detail a substantial advance toward the development of a practical SERS-based anthrax detection device. The performance of the SERS sensor in terms of sensitivity and temporal stability will be discussed.

4.1. Effect of Alumina Thickness on SERS Detection for CaDPA. CaDPA was selected as the probe molecule to test the effect of alumina thickness on SERS detection for bacillus spores. Figure 4A demonstrates a SERS spectrum of 2×10^{-5} M CaDPA on a AgFON substrate ($D = 590$ nm, $d_m = 200$ nm). The SERS bands at 1020 and 812 cm^{-1} are associated with CaDPA, in agreement with the previous Raman studies. The peak at 1050 cm^{-1} is from the symmetrical stretching vibration of NO_3^- from nitric acid, which is used as an internal standard to reduce the sample-to-sample deviations. For comparison, a parallel SERS experiment was conducted on an alumina-modified AgFON substrate ($D = 590$ nm, $d_m = 200$ nm, two ALD cycles). The SERS spectrum obtained on the modified substrate has the same spectral patterns but higher intensities. A plot of the normalized SERS intensity at 1020 cm^{-1} as a function of ALD cycles is shown in Figure 4C. Each data point represents the average intensity at 1020 cm^{-1} from three samples, with the standard deviation shown by the error bars. Interestingly, the SERS intensity of CaDPA achieves a maximum when the AgFON substrates are modified with two ALD cycles of alumina. The intensity then decreases with further addition of alumina.

This result requires special consideration, given that the surface-enhanced Raman spectral intensities decrease with the addition of spacers (e.g., a polymer film, cold-condensed molecular layer, or thiolate SAM) between the Raman scatterer and the metal surface.[27−29] The SERS intensity is essentially dependent on both the electromagnetic field and the number of

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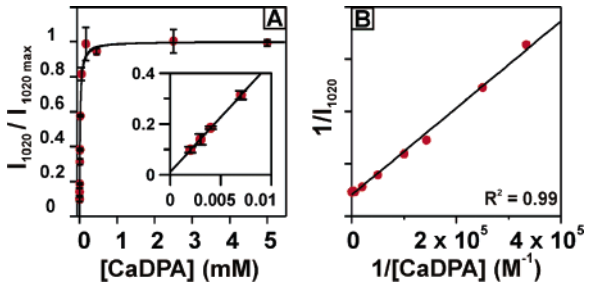

Figure 5. (A) Adsorption isotherm for CaDPA onto alumina-modified AgFON substrates. I_{1020} was taken from SERS spectra that correspond to varying CaDPA concentrations in 0.2 μL of 0.02 M HNO_3 on alumina-modified AgFON substrates. Laser excitation, 785 nm; laser power, 50 mW; acquisition time, 10 s; D = 600 nm; d_m = 200 nm; and two ALD cycles of alumina. The inset shows the linear range that is used to determine the LOD. Each data point represents the average value from three SERS spectra. Error bars show the standard deviations. (B) Adsorption data of CaDPA fit with the linear form of the Langmuir model, eq 2. The slope and intercept values are used to calculate the adsorption constant.

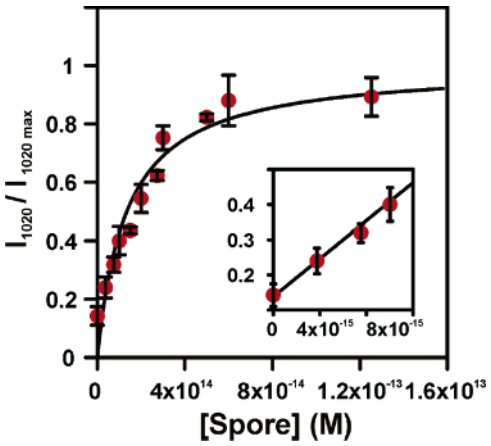

Figure 6. Adsorption isotherm for *B. subtilis* spore suspension on alumina-modified AgFON substrates. I_{1020} was taken from SERS spectra that correspond to varying spore concentrations in 0.2 μL of 0.02 M HNO_3 on the substrates. The inset shows the linear range that is used to determine the LOD. Each data point represents the average value from three SERS samples. Error bars show the standard deviations. Laser excitation, 785 nm; laser power, 50 mW; acquisition time, 10 s; D = 600 nm; d_m = 200 nm; and two ALD cycles of alumina.

adsorbed molecules.[1] According to previous SERS distance dependence studies, the electromagnetic field decays rapidly when the distance between the scatterers and the silver nanostructures increases. The origin of the increase in overall SERS intensity (Figure 4C) likely comes from an increase in the number of scattering molecules. In other words, the adsorption affinity of CaDPA to alumina is anticipated to be greater than that of CaDPA to silver. To have a clearer picture of this affinity difference, the adsorption constants must be measured.

4.2. Adsorption Isotherm and LOD for CaDPA on Alumina-Modified AgFON Substrates. The quantitative relationship between SERS signal intensity and CaDPA concentration is demonstrated in Figure 5. At low concentrations, the peak intensity increases linearly with concentration. In this study, the LOD is defined as the concentration of CaDPA for which the strongest SERS signal of CaDPA at 1020 cm^{-1} is equal to 3 times the background SERS signal for a 10-s acquisition period and 50 mW laser power. The background signal refers to the SERS intensity from a sample with a CaDPA concentration equal to zero, which is calculated to be the intercept of the low-concentration end of the adsorption isotherm (Figure 5A). The LOD for CaDPA, evaluated by extrapolation of the linear concentration range of the adsorption isotherms (Figure 5A inset), is found to be 1.9×10^{-6} M (in 0.2 μL of 0.02 M HNO_3).

At higher CaDPA concentrations, the response saturates as the adsorption sites on the alumina-modified AgFON substrate become fully occupied. Saturation occurs when CaDPA concentrations exceed $\sim 1.5 \times 10^{-4}$ M (in 0.2 μL of 0.02 M HNO_3). To determine the adsorption capacity of extracted CaDPA on an alumina-modified AgFON, the Langmuir adsorption isotherm was used to fit the data:

$$\theta = \frac{I_{1020}}{I_{1020,\max}} = \frac{K_{\mathrm{CaDPA}}[\mathrm{CaDPA}]}{1 + K_{\mathrm{CaDPA}}[\mathrm{CaDPA}]} \quad (1)$$

$$\frac{1}{I_{1020}} = \frac{1}{K_{\mathrm{CaDPA}} I_{1020,\max}} \frac{1}{[\mathrm{CaDPA}]} + \frac{1}{I_{1020,\max}} \quad (2)$$

where θ is the coverage of CaDPA on the alumina-modified AgFON, $I_{1020,max}$ is the maximum SERS signal intensity at 1020 cm^{-1} when all the SERS active sites on alumina-modified AgFON are occupied by CaDPA, [CaDPA] is the concentration of CaDPA (M), and K_{CaDPA} is the adsorption constant of CaDPA to alumina-modified AgFON (M^{-1}). From eq 2, K_{CaDPA} is calculated from the ratio between the intercept and the slope. Slope and intercept analyses of the linear fit (Figure 5B) lead to the value of the adsorption constant $K_{CaDPA} = 4.9 \times 10^4$ M^{-1}.

Previous SERS studies on bare AgFON substrates indicated that the adsorption constant for CaDPA was 9.0×10^3 M^{-1} and the LOD was 3.1×10^{-6} M (laser excitation, 750 nm; laser power, 50 mW; acquisition time, 60 s).[2] The affinity between CaDPA and alumina-modified AgFON is ~5 times stronger than that of bare AgFON. As a result of the change in surface chemistry, the addition of alumina improves the LOD of CaDPA.

4.3. Adsorption Isotherm and LOD for Bacillus Spores on Alumina-Modified AgFON Substrates. The SERS spectra of a bacillus spore suspension are dominated by CaDPA on alumina-modified AgFON. Illustrated in Figure 6, the parallel studies of SERS intensities at 1020 cm^{-1} versus spore concentrations indicate that the LOD is 1.4×10^3 spores in 0.2 μL of 0.02 M HNO_3 and the adsorption constant for CaDPA extracted from spores, K_{spore}, is 9.0×10^{13} M^{-1}. In contrast, the adsorption constant was 1.7×10^{13} M^{-1} for extracted CaDPA on bare AgFON surface and the LOD was 2.6×10^3 spores (laser excitation, 750 nm; laser power, 50 mW; acquisition time, 60 s).[2]

4.4. Temporal Stability of Alumina-Modified AgFON. An ideal sensor should be stable for long periods of time and require infrequent maintenance. In this work, the temporal stability of alumina-modified AgFON substrates (D = 590 nm, d_m = 200 nm, two ALD cycles) was studied over a period of 9 months. The alumina-modified AgFON substrates were stored in Petri dishes in the dark prior to use. SERS spectra of 3.0×10^{-14} M spores (3.6×10^3 spores in 0.2 μL of 0.02 M HNO_3) were captured on alumina-modified AgFON substrates of different ages. Figure 7 shows a representative SERS spectrum on a 9-month-old alumina-modified AgFON substrate prior to use. The intensity ratios between the strongest CaDPA peak at 1020 cm^{-1} and the NO_3^- peak at 1050 cm^{-1} (I_{1020}/I_{1050}) were

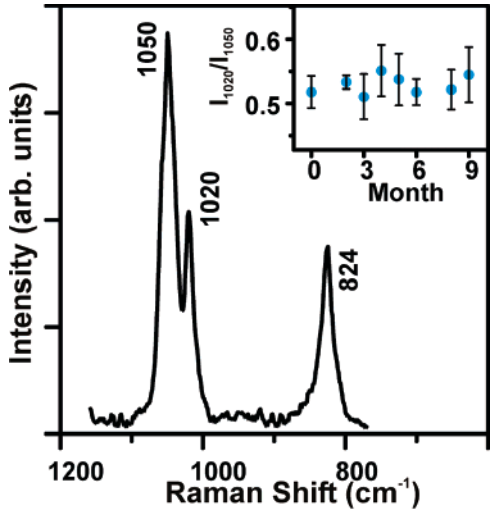

Figure 7. SERS spectrum of 3.0×10^{-14} M spores (3.6×10^3 spores in 0.2 μL of 0.02 M HNO_3) on a 9-month-old alumina-modified AgFON substrate. The inset shows the intensity ratio (I_{1020}/I_{1050}) variation with time. Laser excitation, 785 nm; laser power, 50 mW; acquisition time, 10 s; $D =$ 590 nm; $d_m = 200$ nm; and two ALD cycles of alumina.

measured to quantitatively compare the substrates of different ages (shown in Figure 7 inset). The temporal stability of alumina-modified AgFON substrates is evident in the fact that the SERS intensities of extracted CaDPA remained constant over the course of 9 months. The excellent long-term stability, coupled with precision and low cost, makes alumina-modified AgFON substrates ideally suited for potential field sensing applications.

Conclusions

Alumina-modified AgFON substrates were fabricated using atomic layer deposition. The surface chemistry of this new type of SERS substrates is predictable on the basis of the polar interaction between the analyte molecules and the stationary phase, alumina. This hypothesis was verified by both the TLC and SERS analyses of the competitive adsorptions between dipicolinic acid, diacetylpyridine, and dimethoxypyridine. As expected, dipicolinic acid has the highest binding affinity to alumina-modified AgFON substrates. The high adsorption affinity of carboxylic acid to alumina-modified AgFON surfaces was exploited to improve the detection of CaDPA, a bacillus spore biomarker. The SERS intensity of CaDPA was measured as a function of alumina overlayer thickness. The AgFON substrates modified with two ALD cycles of alumina optimize for the detection of CaDPA. This case demonstrates the importance of understanding the SERS signal transition mechanism. Specifically, the tradeoff between SERS intensity increase due to the greater binding affinity of CaDPA toward alumina compared to silver and the intensity decrease due to the decay of the electromagnetic field around silver nanostructures is demonstrated.

This work also further details our improvements in detecting bacillus spores using SERS. Most notably, the sensor system is now more sensitive and maintains its performance over a long period of 9 months. The SERS signal of extracted CaDPA was measured over the concentration range 10^{-14}–10^{-12} M to determine the saturation binding capacity of the alumina-modified AgFON surface and calculate the adsorption constant ($K_{CaDPA} = 4.9 \times 10^4$ M^{-1}). At present, a 10-s data collection time is capable of achieving a LOD of $\sim 1.9 \times 10^{-6}$ M using 785 nm laser excitation. For bacillus spores on alumina-modified AgFON, the adsorption constant of extracted CaDPA was determined to be 9.0×10^{13} M^{-1} and the LOD 1.4×10^3 spores.

This experiment demonstrates two very important future trends in SERS. First, the addition of the alumina layer imparts a new chemical functionality to the SERS active surface. We will expand our palette of available ALD layer materials, which will enable greater chemical control over the nature of the selectively adsorbed analytes. Second, the novel SERS substrates reported herein hold enormous promise for combining both TLC and SERS without extra deposition steps of silver colloids or positively charged polymer.[30,31] This combination, with the development of miniaturized Raman spectrometers and nanofabrication methods, will allow for effective separation and sensitive identification of components in a mixture analyte, which is an important task for the development of chip-based chemical and biological detection systems.

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