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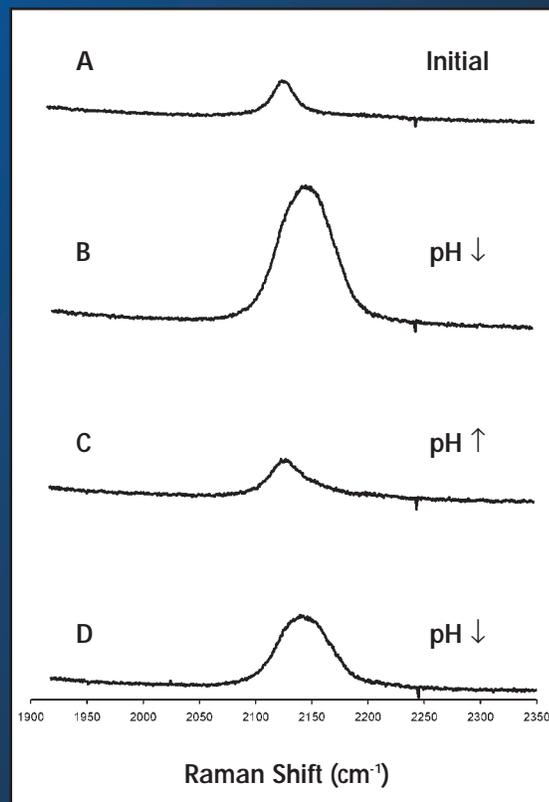
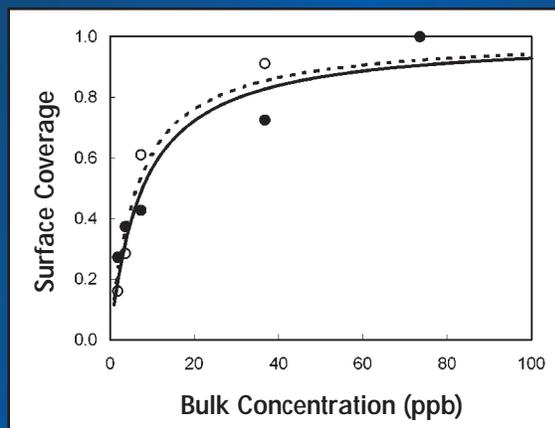
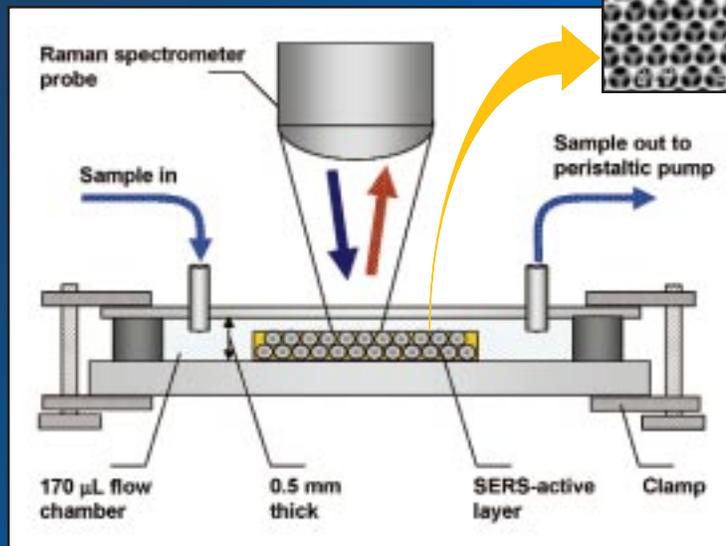


Rachael Barbour
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Nanostructured gold film (SEM)



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CONTINUOUS SERS MONITORING OF CYANIDE ON NANOSTRUCTURED GOLD

On-Line Spectroscopic Characterization of Sodium Cyanide with Nanostructured Gold Surface-Enhanced Raman Spectroscopy Substrates

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To implement surface-enhanced Raman spectroscopy as a practical detection method, highly enhancing, stable, and reproducible substrates need to be fabricated in an efficient manner, and their performance in different solution environments should be well characterized. In this work structured porous gold films have been fabricated using colloidal crystals to template gold nanoparticles. These films were integrated into an on-line flow chamber and used to study the effects of pH and other additives on the detection of sodium cyanide. The gold films proved to be highly enhancing and were used to detect cyanide over a wide range of pH values in the concentration range of ~2 to 200 ppb. The Raman signal intensity could be increased by lowering the pH after the adsorption of cyanide, which was likely due to both a change in the ionization state and a conformational change of the bound molecules. The peak intensity could also be enhanced multifold by treating the substrate with silver nitrate. Cyanide could be removed from the substrates using hydrochloric acid, although this also passivated the structures, and the activity could only be restored partially with tannic acid. These results provide a rational method to optimize the on-line detection of cyanide in water.

Index Headings: Surface-enhanced Raman spectroscopy; Structured gold films; Cyanide detection; Colloidal crystal templating; Nanoparticles.

INTRODUCTION

The high sensitivity of surface-enhanced Raman spectroscopy (SERS) derives from the strong field intensities generated by the surface plasmons at roughened metallic surfaces.¹ There has been significant interest in developing SERS into a routine detection method for a wide range of molecules,^{2,3} including chemical agents,⁴⁻⁶ biomolecules,^{7,8} and DNA.⁹⁻¹¹ To be of practical use, however, SERS must be selective, reproducible, and robust, in addition to being highly sensitive. The most significant barrier to the practical use of SERS is the lack of inexpensive and simple methods for fabricating stable metallic substrates with well defined performance properties over a wide range of solution environments.³

Many of the original ideas for making substrates for SERS detection systems have revolved around electrochemical roughening of a metallic surface,⁵ vapor deposition of metal onto a roughened surface¹² or through a

natural nanosphere mask,^{13,14} and microfabrication of patterned metallic structures.^{15,16} Although the sensitivity of the substrates made by these techniques is adequate for many applications, they have the drawback of being relatively complex and expensive. Further, electrochemical roughening has the disadvantage of poor reproducibility, and microfabrication is time consuming.

One of the most promising and simple methods for forming SERS substrates is the deposition of metallic nanoparticles on a substrate.^{2,17} Metallic nanoparticles adsorbed on a glass surface using either biospecific or metal-affinity interactions⁸ have been used as SERS substrates. However, these structures lack advanced features such as periodicity that are present in microfabricated substrates.^{13,18} Also, it can be expected that the activity of the substrates would be increased if the roughened metallic surface could be extended in the third dimension, yet simple adsorption or surface aggregation of nanoparticles would generate structures that are too dense to possess SERS activity throughout the bulk of the material.

We have recently introduced a method of forming three-dimensional structured metallic films on microscope slides by templated self-assembly.^{19,20} The porous films are formed using colloid crystals to template the assembly of gold nanoparticles. Gold nanoparticles and polystyrene microspheres are mixed and slowly coated onto the surface of a glass plate. As the solvent evaporates from the film, the latex microspheres assemble into colloidal crystals, and, as the liquid recedes into the interstitial voids of the colloidal crystals, the gold nanoparticles are packed together around the latex crystal template (Fig. 1A). The latex spheres can then be removed by dissolution in an organic solvent, leaving a three-dimensional array of gold nanoparticles arranged into an ordered structure (Fig. 1B). The advantages of this method are the rapid, simple, and inexpensive fabrication of SERS substrates with uniform controllable pore size and periodicity. We have shown that these structures are highly enhancing SERS substrates using trans-1,2-bis(4-pyridyl)ethylene as a model compound.^{19,20}

In this paper we describe how novel gold SERS substrates can be integrated into an on-line flow chamber and how this system can be used to study the effects of solution conditions and additives on the detection of sodium cyanide. Cyanide has been almost exclusively studied on silver substrates,^{4,21-25} which are known to be highly enhancing. However, gold substrates have advantages over

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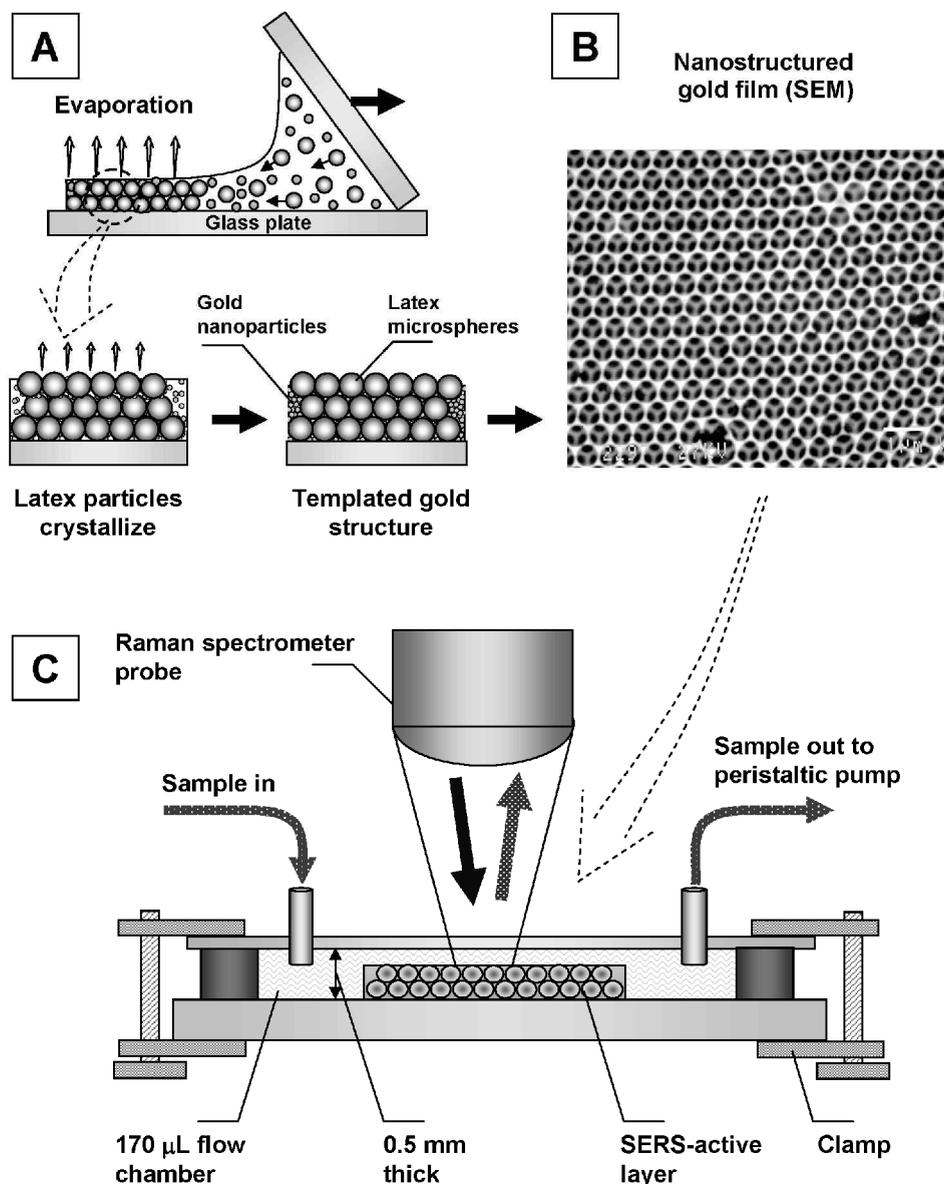


FIG. 1. (A) Schematic diagram of the colloidal crystal templating method used to assemble three-dimensional arrays of gold nanoparticles on glass substrates. (B) SEM micrograph of a gold nanostructured film after the removal of the latex microspheres. (C) Schematic diagram of the flow chamber integrated with a gold substrate, which was used for the on-line detection of cyanide in a flowing stream of liquid.

silver substrates in terms of long-term stability and storage life.

In addition to testing the performance of the new structured porous gold films, we have characterized the cyanide spectra in a continuous flux of liquid, simulating long-term practical monitoring of water sources for defense, industrial, or environmental purposes. A shortcoming of many previous studies was that the analyte was applied to the substrate by encapsulation of a stagnant solution or by evaporation of a small amount of liquid.^{12,17,18} These methods assume that the substrates are uniform in structure and that the analyte is evenly dried on the surface. These assumptions may often be invalid, which complicates the evaluation of the SERS performance as a function of solution conditions and analyte concentration. We present data obtained by sampling the same position on the metal film in a flow chamber repeatedly, which enables the evaluation of the adsorption

equilibria and surface configuration of cyanide bound to gold in real time.

EXPERIMENTAL

Materials and Substrate Preparation. The gold nanoparticles were prepared by standard procedures reported previously.^{26,27} The diameter of the gold nanoparticles was approximately 20 to 25 nm. The gold particles were concentrated approximately 100 fold using 5 kD MWCO centrifuge filters and then further concentrated another order of magnitude by sedimentation at 1600 g for 2 h. The final gold concentration was typically 1–5 wt %.

Negatively charged polystyrene microspheres (650 nm, IDC, Portland, OR) were diluted 10 fold in deionized water. Nonionic surfactant (Tween 20, Sigma, St. Louis, MO) was added to stabilize the latex particles further

(final conc. of 0.0002 wt %), and then the microspheres were settled by centrifugation. The final concentration of the microspheres in suspension was ~20 wt %. Approximately 8 μL of concentrated gold and 4 μL of concentrated latex suspensions were mixed and spread onto a glass slide (Fig. 1A). The top plate was attached to a linear motor and translated horizontally at 2 $\mu\text{m/s}$. The latex/gold films were deposited and dried typically within 45 min. The substrates were then washed in toluene for approximately 10 min to remove the latex templates. No subsequent cleaning procedures were required, even after extended periods of storage.

On-Line Measurements and Instrumentation. Figures 1B and 1C illustrate the integration of the structured metallic films into a flow chamber for on-line detection. A 170 μL microscope chamber (PC1R-0.5, Grace Bio-labs, Bend, OR) was clamped to the substrate using two steel plates with circular openings to access the gold film optically. Teflon tubing (0.08 cm inner diameter at the inlet, 0.16 cm inner diameter at the outlet) was then connected to the two microscope chamber ports and a peristaltic pump was used to pass the sample through the flow chamber at 0.4 mL/min. Teflon grease was used to seal the microscope chamber ports.

The gold substrate and microscope chamber were placed inside a Raman detection system. The Raman instrument consisted of an Echelle spectrograph (Model NIR700, EIC), a RamanProbe[™] fiber-optic Raman probe from EIC Laboratories, and a diode laser operating at 785 nm from SDL. Spectra were collected and processed using EIC2000 software. The laser power was limited to approximately 30 mW to avoid damaging the substrate, and the data collection time was 60 s. To avoid background noise due to the room lighting, the flow cell was placed on an X-Y translation stage in a light-tight box containing the fiber-optic probe. The translation stage was used to align the substrate at the focus of the Raman probe, and the position of the scanning beam on the substrate was monitored with a charge-coupled device (CCD) camera.

Substrate Treatment and Experimental Methodology. Since gold is generally hydrophobic and glass is generally hydrophilic, the gold structured films did not adhere well to the microscope slides initially and could be peeled away by flowing liquid. This problem was eliminated by treating the porous gold substrates with dimethylchlorosilane vapors (Sigma, St. Louis, MO) for approximately 15 min, which increased the hydrophobic nature of the glass surface and enhanced the adhesion of the gold film. Afterward, the substrates were stable mechanically in a flowing water stream and could be used for extended periods of time without the metal releasing from the glass substrate. Based on the substrate stability, repeated experiments could be performed on the same position at various solution conditions, allowing the effects of the substrate structure and the solution environment to be separated.

In a typical experiment, distilled water was passed through the microscope chamber for 15 min, and the background spectrum was collected. The cyanide sample was then passed through the microscope chamber for 15 min, and the spectrum was collected at regular intervals. The cyanide signal typically reached a steady value in 5

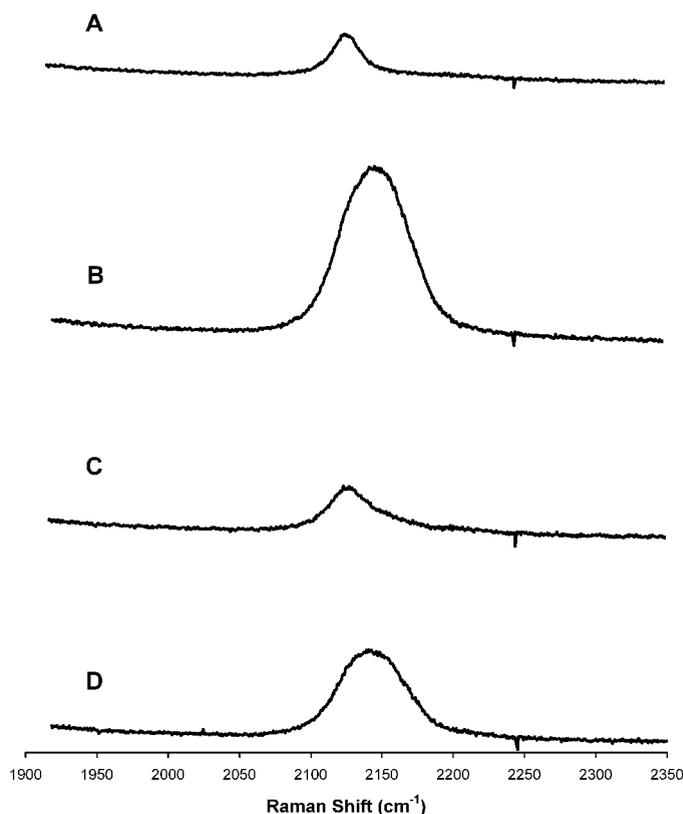


FIG. 2. Raman spectra of sodium cyanide after 15 min of: (A) 40 ppb sodium cyanide in 0.1 M NaOH, (B) 0.01 M HNO_3 without cyanide, (C) 0.1 M NaOH without cyanide, and (D) 0.01 M HNO_3 without cyanide.

to 10 min. The background spectrum obtained for water was subtracted from the spectra for cyanide. The cyanide peak was then fit to a Gaussian form using Grams 32/AI software from Thermo Galactic. The peak area and peak height were determined using the fitted cyanide peak.

RESULTS AND DISCUSSION

Effect of pH on Cyanide Adsorption and Signal Intensity. A major finding of this work was that the Raman signal for cyanide was dependent on both the pH of the solution used to adsorb cyanide initially and the pH of solutions without cyanide that were passed through the flow chamber after the initial cyanide adsorption. We found that cyanide solutions at neutral pH resulted in a weak Raman signal, suggesting that cyanide does not adsorb on the gold in the non-ionized state (sodium cyanide pK_a is approximately 9.5).²⁸ However, a strong Raman peak at 2125 cm^{-1} confirmed that cyanide adsorbed on the gold readily at high pH (~13) (Fig. 2A).

A surprising SERS effect was observed after the removal of the cyanide solution from the flow chamber with water, which reduced the solution pH. The Raman signal after the removal of the cyanide in solution originated from the layer of cyanide adsorbed on the metallic surface. The height and area of the cyanide peak increased multifold when distilled water was passed through the chamber. The increase was even larger with dilute nitric acid (0.01 M), as shown in Fig. 2B. This was accompanied by a small shift of the cyanide peak from

2125 cm^{-1} at basic pH to higher frequencies at neutral and acidic pH. The degree to which the cyanide peak shifted was dependent on the solution pH; the peak shifted to 2130 cm^{-1} in the presence of water, while the peak shifted to 2140 cm^{-1} in the presence of nitric acid. This effect was largely reversible: when the pH was increased again with 0.1 M sodium hydroxide without further addition of cyanide, the substrates gave a similar spectrum as before (Fig. 2C). This shift in peak intensity and peak position with pH could be repeated for many cycles. However, the use of nitric acid caused a small reduction of the peak intensity for each subsequent cycle of detection at low pH (Fig. 2D), which was not observed when water was used to reduce the solution pH (data not shown).

The dependence of the Raman spectra of cyanide as a function of pH for silver substrates has been reported previously,⁴ but it cannot be directly compared with our results. Shelton et al.⁴ reported a decrease in the peak intensity for cyanide at low pH. However, these results were collected for the initial cyanide adsorption on a clean electrode surface and are in good agreement with our observation that a very weak Raman signal is obtained when attempting to absorb cyanide at neutral or acidic pH. The effect of lowering the pH *after* the irreversible adsorption of cyanide at high pH has not been studied previously.

The reversible shift of the peak position and peak intensity for cyanide adsorbed on a gold surface can be attributed to a conformational or ionization transition as pH is varied. It has been shown previously that the mechanism of cyanide binding to gold in an aqueous environment is largely ionic,²⁹ in contrast to carbon monoxide, which binds to gold largely in a covalent manner. Otto et al.^{25,30,31} present a model where the increased positive charge on a silver surface at low pH in the presence of cyanide can lead to the transition $\text{AgCN} \rightarrow \text{Ag}(\text{CN})_3^{2-}$. The cyanide ions in the latter complex are oriented parallel to the surface, and are much more SERS active. It has also been shown that the adsorption energy of cyanide on nickel in different orientations varies by only a few percent, indicating that a change in orientation is possible due to a change in the solution pH.^{32,33} Kellogg and Pemberton²⁴ have observed that for cyanide adsorbed on a silver surface in an acidic environment, the cyanide peak shifts to higher frequencies by $\sim 30 \text{ cm}^{-1}$, which is consistent with our results. They discuss the possibility of a change in the ionization state and a transition from an end-bound to a side-bound conformation for cyanide. Neither of these effects can be ruled out, and it is possible that both phenomena may co-exist on the gold surface. Further theoretical analysis is necessary to clarify the origin of the SERS effects that we have observed.

Effect of Cyanide Concentration and Lower Limit of Detection (LOD). Two major goals of this study were to estimate the limits of the detection for cyanide and to understand the relationship between the Raman peak intensity and the cyanide solution concentration. To characterize the relationship between the peak intensity and the cyanide concentration, we used the following methodology: for each cyanide concentration, a spectrum was recorded for the original cyanide solution at high pH, and again after washing the chamber with a 0.01 M nitric acid

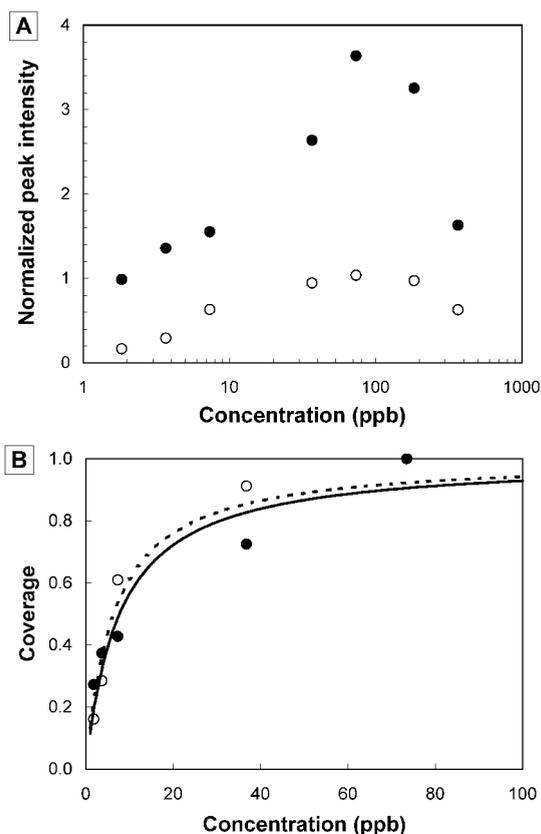


FIG. 3. (A) Sodium cyanide peak intensities normalized to a reference peak at 888 cm^{-1} in the presence of 0.1 M NaOH (○), and after washing with 0.01 M HNO₃ (●). (B) Calculated surface coverage values for cyanide in the same solution environments and the corresponding fitted results using the Langmuir isotherm for 0.1 M NaOH (-----) and 0.01 M HNO₃ (—).

solution for 15 min to enhance the Raman intensity as mentioned above. The cyanide peak areas were normalized to a background peak at 888 cm^{-1} that did not change as a function of the concentration of cyanide in solution. The data are shown in Fig. 3A. The cyanide peak intensity increases approximately linearly with the logarithm of the concentration in the range of 2 to 70 ppb (molar basis). This is in good agreement with previous work for cyanide adsorption on silver that reported a linear dependence between peak area and concentration on a log–log plot.⁴ The Raman peak intensities saturated at ~ 70 ppb, and then began to decrease at higher cyanide concentrations. This correlates with the cyanide concentration of approximately 100 to 200 ppb that corresponds to a monolayer of cyanide on gold,³⁴ which may cause dissolution of the gold surface and lead to substrate degradation.

The major findings obtained from our data are summarized in Table I. The average cyanide peak intensity increased by a factor of four after washing the substrate with a low pH solution. We averaged the noise level in the region of 2300–2400 cm^{-1} and used this as a basis for estimating the signal-to-noise ratios (S/N) for the different solution environments. The S/N ratio is approximately 3.7 for the lowest cyanide concentrations studied in this work (2 ppb) at high pH, and this ratio improves by a factor of ~ 3 after reducing the pH. There is no improvement in the S/N ratio at high cyanide concentra-

TABLE I. Effect of the solution pH after initial adsorption of cyanide on the signal-to-noise (S/N) ratio and the lower limit of detection (LOD) for sodium cyanide.

Sample	Peak maximum, cm^{-1}	Average increase of the peak area on "washing"	S/N ratio at 2 ppb	S/N ratio at 180 ppb	Extrapolated LOD at S/N = 3, ppb
Cyanide in 0.1 M NaOH	2125	1.0 (basis)	3.7	26	1.72
0.01 M nitric acid (no cyanide in solution)	2140	4.0	10	25	0.73

tions (>100 ppb) after washing the substrate with a low pH solution, but the noise at high cyanide concentrations is also less significant.

In order to make quantitative estimates of the lower limit of detection for our substrates, a model is needed for the relationship between the Raman signal and the solution concentration of cyanide. We assume that the cyanide signal is proportional to the surface concentration of bound cyanide. In order to correlate the surface concentration to the cyanide concentration in solution, we used the Langmuir adsorption isotherm:

$$\theta = \frac{Kc}{Kc + 1} \quad (1)$$

where θ is the fractional surface coverage, K is the adsorption equilibrium constant, and c is the solution concentration. The Langmuir isotherm is known to describe both physical and chemical adsorption relatively well,³⁵ the latter of which is the relevant case for this work. We

assume that in the concentration region of 100 to 200 ppb, where the Raman signal levels off, the surface coverage approaches saturation, i.e., $\theta \rightarrow 1$, which has been reported previously.³⁴

The normalized peak areas for cyanide at high pH show a good fit with the Langmuir isotherm (Fig. 3B) using a single fitting parameter of $K = 0.16 \text{ ppb}^{-1}$. At low pH the value of the adsorption equilibrium constant is approximately the same (0.13 ppb^{-1}) as the value found at high pH. This is consistent with the idea that the variation of solution pH does not change the binding affinity of cyanide for gold, but rather enhances the Raman signal of the cyanide that is already bound. Finally, the Langmuir isotherm was used to estimate the theoretical lower limit of detection (LOD) by extrapolating to a S/N ratio of 3, and the results are reported in Table I.

The LOD for cyanide at high pH is approximately 5 times lower than that previously reported for silver electrodes,⁴ which shows that our gold films perform at least as well as those fabricated previously from silver, even though silver is generally believed to produce higher enhancement factors.^{36,37} The low pH environment decreases the LOD further by a factor of 2.4. This is physically reasonable since the lower limit of detection as a function of solution pH is governed by the efficiency of the Raman scattering, which is greater at low pH. The estimated surface coverage at the LOD concentration is 0.21 for the high pH sample and 0.087 at low pH. Since the adsorption isotherm drops sharply in the region of low cyanide concentration, we would not expect a significant improvement in the LOD by improving the scattering efficiency of the substrates. However, chemical modification of the surface in a manner that promotes stronger cyanide absorption may reduce the LOD.

Substrate Fouling and Regeneration. The use of SERS substrates for long-term monitoring requires an understanding of how the substrates can be regenerated after the adsorption of cyanide, as well as an understanding of solution conditions that may inactivate the substrates. We studied the effect of hydrochloric acid (0.1 M) as a potential agent to remove bound cyanide from the gold surface. The introduction of hydrochloric acid completely removed the signal of the cyanide bound at high pH, but it also passivated the substrates, which could not be used for further cyanide detection (Fig. 4). To determine whether the presence of chloride ions could independently cause the passivation of the substrates, we tested the effect of 0.1 M sodium chloride at neutral pH, which had no discernible effect on the substrate performance. The low pH environment in the absence of chloride ions is also not the source of fouling since 0.1 M nitric acid did not remove bound cyanide or passivate the substrates. We conclude that the combination of low pH and the

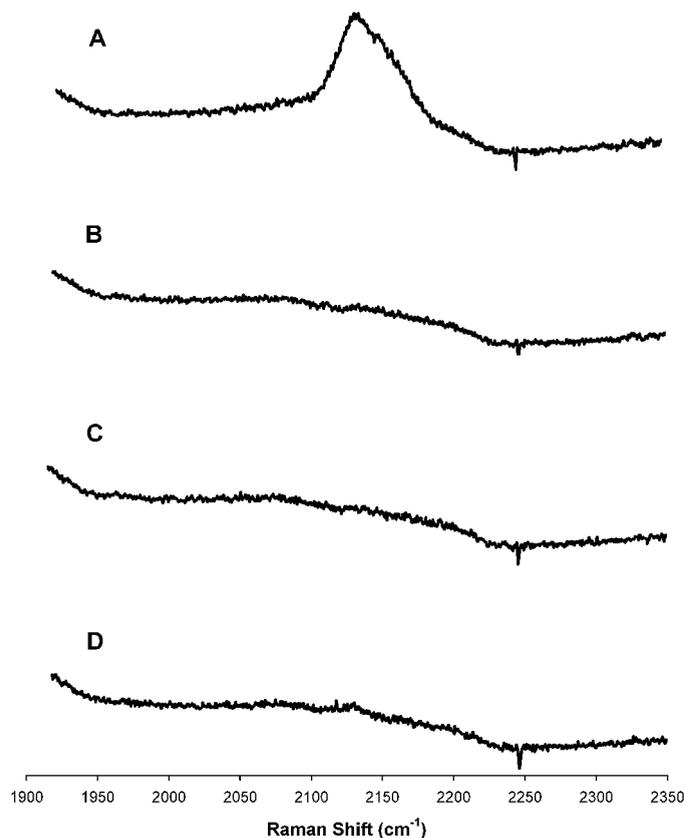


FIG. 4. Raman spectra of sodium cyanide after: (A) 15 min of 40 ppb cyanide in 0.1 M NaOH and 15 min of water, (B) 15 min of 0.1 M HCl, (C) 5 min of water, and (D) 15 min of 40 ppb cyanide in 0.1 M NaOH.

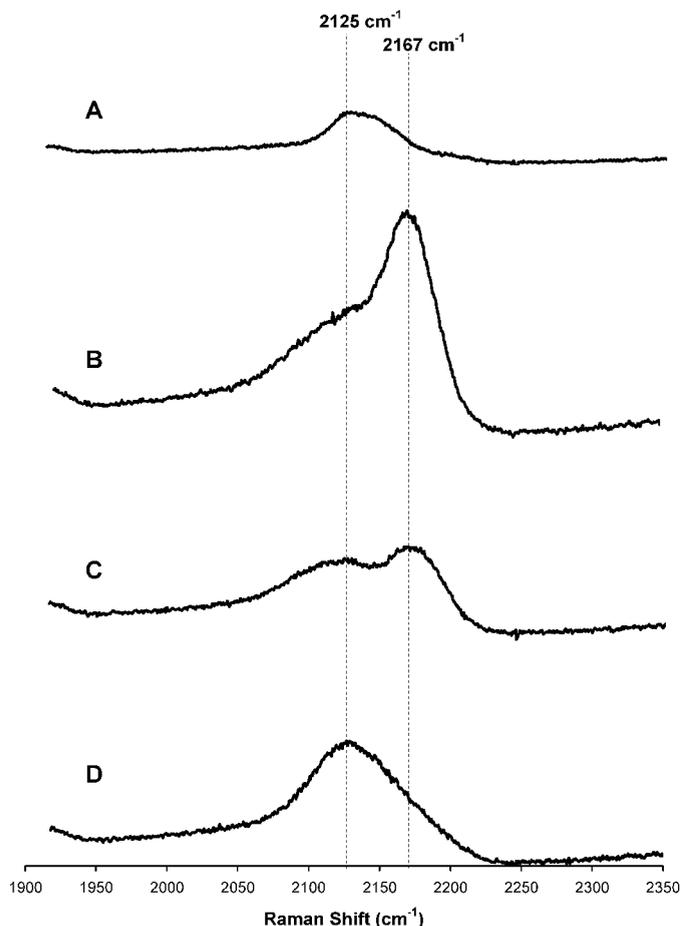


FIG. 5. Raman spectra of sodium cyanide after: (A) 10 min of 40 ppb cyanide in 0.1 M NaOH and 10 min of water, (B) 15 min of 0.01 M AgNO₃, (C) 5 min of water, and (D) an additional 10 min of water.

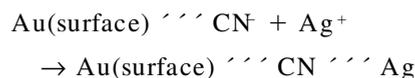
presence of chloride ions created a layer of gold chloride on the gold surface that prevents adsorption of cyanide. To confirm this hypothesis, we treated the substrates that were passivated with hydrochloric acid with the reducing agent that was used to make the gold nanoparticles from gold chloride (1% tannic acid). We found that the reducing agent was able to partially restore the SERS activity of the passivated gold films. A regeneration procedure involving the use of hydrochloric acid followed by tannic acid is not practical, and a simpler, single-step process would be more desirable.

Effects of Silver Ions as Spectra Enhancers. It has been demonstrated that the presence of single silver atoms protruding from silver substrates can be important for achieving high SERS efficiencies.^{16,25,30,38} We studied the effect of silver ions on the detection of cyanide using gold substrates that were treated previously with cyanide. The cyanide was bound to the gold at high pH, after which the substrates were washed with water and then treated with 0.01 M silver nitrate. This procedure resulted in a cyanide peak of ~5 times higher intensity than that obtained for the same substrate before addition of the silver nitrate (Figs. 5A and 5B). The increased SERS intensity is a result of a new peak at 2167 cm⁻¹, but the original peak at 2125 cm⁻¹ also increases in intensity. Further, when substrate that was treated with silver nitrate was washed with water, the peak at 2167 cm⁻¹ was re-

moved (Figs. 5C and 5D), although the original peak at 2125 cm⁻¹ remained at a slightly elevated intensity, which did not appear to be reversible.

The most plausible explanation for this remarkable effect is that the silver ions adsorb on the surface, forming complexes with cyanide that result in high SERS activity. The frequency that we have measured, 2167 cm⁻¹, coincides with the literature value for the SERS peak position of AgCN.²⁵ One alternative hypothesis is that the increase in the Raman intensity may be due to the formation of silver nanoparticles in the flow chamber by photoreduction. However, we passed a solution of silver nanoparticles formed by photoreduction through the flow chamber in the presence of a gold substrate and did not observe the type of strongly enhancing behavior that is observed in Fig. 5.

We therefore speculate that the observed SERS enhancement arises from the formation of complexes due to silver ion binding:



The effect causes a significant increase in the S/N ratio, and would likely further reduce the LOD, although this was not quantified. The combination of gold substrates with adsorbed silver or silver ion layers appears to be a promising system for the development of highly enhancing SERS substrates.

CONCLUSION

The results of this study provide further evidence that SERS is a highly sensitive and selective method that enables the determination of both the chemical nature and the concentration of analyte in solution. The integration of highly inert gold substrates with a flow chamber could make SERS applicable for on-line long-term monitoring of water for environmental or chemical pollutants and poisons. Our results show that the strength of the Raman signal could be increased significantly by modulating the solution pH or by the addition of silver ions. However, the detection of cyanide could occur only after adsorption at high pH, and the substrates could be passivated by hydrochloric acid. These effects warrant further experimental and theoretical research and may aid in the design and optimization of robust monitoring devices.

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