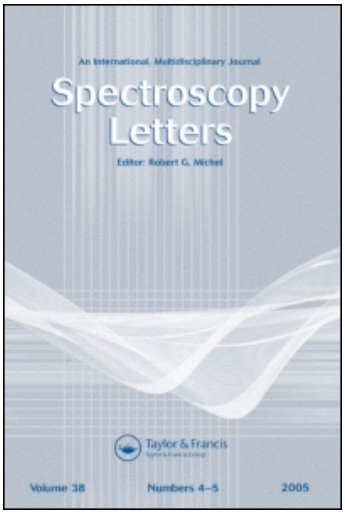

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FT-Surface-Enhanced Raman Scattering of Phenylalanine Using Silver-Coated Glass Fiber Filters

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ABSTRACT A simple and facile method has been developed to produce surface-enhanced Raman scattering (SERS) active surfaces. Tollen's reagent was used to coat silver onto the surface of glass fiber filters. Using a Fourier transform (FT) Raman instrument, strong surface-enhanced Raman signals have been observed for L-phenylalanine on these surfaces. The use of an FT-Raman instrument combined with this new SERS method allows for a method with both high sensitivity and selectivity. A linear dependence of the Raman signal on L-phenylalanine concentration (0.01–1.0 mM) has been demonstrated. The SERS technique presented here is both novel and promising for biochemical analysis.

KEYWORDS FTIR, L-phenylalanine, Raman spectroscopy, SERS

INTRODUCTION

The Raman effect, although a powerful tool for chemical analysis, is limited by its extremely small cross section compared with fluorescence spectroscopy (12–14 orders of magnitude lower).[1] It was not until the advent of surface-enhanced Raman scattering (SERS) that this limitation was overcome. The SERS phenomenon was first reported by a research group in 1974 when they observed an abnormally large Raman signal from pyridine that was adsorbed onto the surface of a roughened silver electrode.[2] The increase in Raman signal has been attributed to the attachment of molecules to the surface of "atomically rough" metal surfaces. This effect has led to the enhancement of Raman signals by as much as 14 orders of magnitude and has enabled Raman spectroscopy to combine structural information with high sensitivity.[3,4]

Advances in the SERS technique have led to many exciting new applications for chemical and biological analysis. The high sensitivity and selectivity it provides along with the ability to gain information on molecular structure, surface processes, and interface reactions has heightened its popularity. SERS signals have been reported using a variety of substrates including metal electrodes, metal colloids, metal-coated nanospheres/nanoparticles, metal-coated silica substrates, and silver membranes.[5–7] Gold and silver

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are the most common SERS active metals but SERS enhancements have also been reported using several transition metals including platinum, cobalt, iron, nickel, and palladium.[1] The SERS technique has become an invaluable tool for studying biological systems.[8–14] The use of near-infrared (NIR) or visible lasers allows for noninvasive application of SERS under ambient conditions in an aqueous environment. Additionally, SERS measurements can be made with exceptionally low spatial and temporal resolutions. Spatial resolutions of about $1\,\mu m^3$ have been reported along with temporal resolutions on the femtosecond scale.[3,15–17] This allows for the monitoring of biochemical processes in real time. SERS spectra have been acquired from amino acids, proteins, neurotransmitters, and nucleic acids.[3,8–14,18] SERS has been used to probe lipid chemistry, study membrane transport processes and immunoassay enzymes, and probe for DNA.[3,18–24] Several studies have even demonstrated the use of SERS to detect single molecules.[3,25–27]

Fourier transform (FT) Raman instrumentation, although not commonly used in SERS methods, represents a promising alternative to traditional Raman instrumentation. The primary disadvantage of FT-Raman relative to dispersive Raman is its low sensitivity caused by the necessity of using high-noise solid-state detectors. However, this lower sensitivity relative to dispersive instruments is not limiting when utilizing SERS techniques. There are several advantages to using an FT-Raman instrument. The FT instruments are commercially available with sample interfaces that provide turnkey operation. The use of a NIR excitation source (1064 nm) significantly reduces the background fluorescence and often helps minimize photodegradation of the sample. Typically visible-light sources are used to generate SERS enhancements on silver surfaces due to the surface plasmons being in resonance at this wavelength. However, SERS enhancements from silver colloids have been observed using off-resonance NIR excitation sources.[28–31] The observed enhancements are thought to be due to aggregation of the colloids. Aggregation of the colloids leads to broadening of the surface plasmon frequencies, allowing them to be excited with NIR light. The FT instrument also uses an interferometer, which allows the resolution of the spectrometer to be adjusted for experimental conditions; for example, high-resolution scans at $1\,cm^{-1}$ resolution in order to detect subtle peak shifts or low-resolution scans when signal to noise is the limiting factor. Perhaps most importantly, the use of an interferometer that is referenced to a helium–neon (HeNe) laser ensures that the wavelength axis of the spectrum is self-calibrated and accurate. This is crucial for experiments involving comparative studies of subtle spectral shifts.

In the following report, the preparation of a novel and practical SERS-active Ag substrate is described. The analytical utility of this substrate coupled with an FT-Raman instrument is demonstrated using the amino acid L-phenylalanine (Phe). The results illustrate the potential for developing a SERS substrate for the routine qualitative and quantitative analysis of biological molecules.

MATERIALS AND METHODS

Instrumentation

A RAMAN 960 (Thermo Nicolet, Waltham, MA) FT-Raman spectrometer was used for the acquisition of all FT-SERS spectra. The RAMAN 960 utilizes a Spectra Physics Nd:YVO4 laser (1064 nm) as an excitation source (Santa Clara, CA). Output laser power could be adjusted from 0.015 to 5.0 W. An He-Ne laser (632.8 nm, Melles Griot, Albuquerque, NM) was used as the reference source. Nominal He-Ne laser power was 2.0 mW.

Substrate Preparation

Silver can be deposited on glass silica surface by a reaction known as the *mirror* or Tollen's reaction. A thin silver layer was formed on the surface of glass fiber filters following the procedure of Saito et al.[32] All solutions used in this procedure were prepared using 18 MΩ MilliQ water. Six milliliters of a 2% (w/w) silver nitrate solution was placed in a glass Petri dish. A 3.2% KOH solution was added drop-wise until a brown precipitate (Ag_2O) was formed. The solution was swirled to mix. To this solution a 30% (w/w) ammonia solution was added drop-wise until the precipitate was completely dissolved ($[Ag(NH_3)_2]^+$ was formed). Next, a 6% (w/w) silver nitrate solution was added drop-wise until the solution became yellowish brown. Three drops of 6% ammonia were added and the solution became transparent. The Petri dish was swirled to ensure mixing.

Six glass fiber filters were placed in the Petri dish and were completely immersed in the solution. The Petri dish was placed in a water bath set at 35°C for 15 min to allow the solution to warm. In a separate glass vial, 2 mL of a 35% (w/w) glucose solution was mixed together with 1 mL of methanol. The glucose–methanol mixture was added to the Petri dish and mixed by swirling. The solution immediately became gray in color and turned completely black after a few minutes. The filters remained in the Petri dish for one hour, after which they were removed. Immediately after removal the filters were flushed with 1 L of 18 MΩ MilliQ water. The filters were left overnight to dry at room temperature. Scanning electron microscope (SEM) images of the coated filters were obtained using a LEO 435 VP (Oberkochen, Germany) scanning electron microscope. No sample pretreatment was required prior to obtaining SEM images.

Measurements

Amino acid solutions (10 mM) were prepared from one molar stock solutions and 18 MΩ MilliQ water. One hundred microliters of the amino acid solution was pipetted directly onto the center of the coated side of a silver-coated glass fiber filter. The filter was then placed onto a sample holder, which was inserted into the sample compartment of the Raman 960 FT-Raman spectrometer. Using the He-Ne laser the sample position was adjusted manually so that the laser was focused directly onto the surface of the filter. The sample compartment was then closed. Using the computer interface software Omnic Version 6.0a (Waltham, MA) the sample position was adjusted further to obtain the maximum Raman signal using the Nd:YVO4 laser set at 0.2 W. A Raman spectrum was then obtained from the sample using the Omnic software interface. The instrument was set to acquire spectra after 64 scans with a resolution of 8 nm and laser power set at 0.2 W.

RESULTS AND DISCUSSION

Silver was successfully deposited on the surface of the glass fiber filters. A representative SEM micrograph of a silver-coated glass fiber filter is shown in Fig. 1. As can be seen from the image, the entire surface of each fiber was covered with silver. There was some noticeable aggregation or clumping of particles but the overall coating of the fibers appears fairly regular. The utility of the resulting filter as a SERS-active substrate is illustrated in Fig. 2. The resulting spectrum is representative of previously reported SERS spectra of L-phenylalanine generated from silver colloids.[33] The bands that appear at 622, 1001, 1031, 1205, and 1600 cm^{-1} correspond to the characteristic aromatic ring bands. The strong enhancements of these bands, specifically the band at 1001 cm^{-1}, is believed to be due to the participation of the π-system of the phenyl ring in the complex formation. The bands at 931 and 1386 cm^{-1} correspond to C$-$COO$^-$ stretching and COO$^-$ symmetric stretching, respectively. The enhancement of the band at 1386 cm^{-1} suggests that the L-phenylalanine is adsorbed to the silver surface via the carboxylate group.[33] The peak assignments for SERS spectrum of L-phenylalanine are given in Table 1.

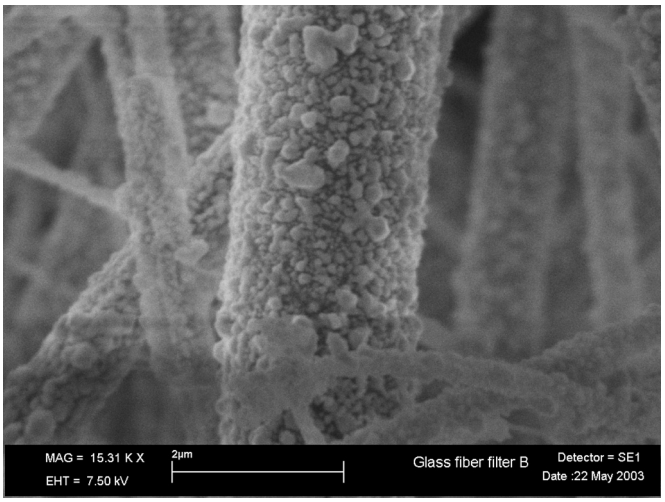

FIGURE 1 **Photomicrograph taken by scanning electron microscopy (SEM) of the silver surface prepared from the Tollen's reagent.**

The resulting SERS enhancements demonstrated in Fig. 2 are surprising when considering the use of an NIR excitation source. The surface plasmon frequency of the silver surface on the fibers would not be expected to be excited in the near-infrared and therefore would not contribute the electromagnetic enhancement of the Raman signal. However, it has been demonstrated that through aggregation of silver colloids that the plasmon frequency of the silver surface can broaden enough to be exited by an NIR source.[28–31] It is believed that at points on the filter surface where fibers intersect at close enough proximity, the surface plasmon frequency will broaden

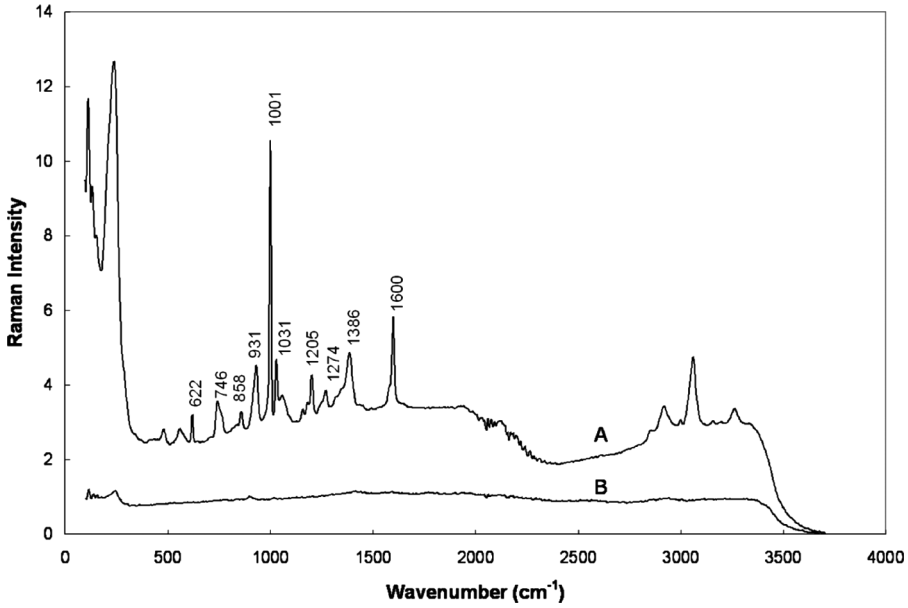

FIGURE 2 SERS spectrum of phenylalanine on the silver surface prepared from the Tollen's reagent (A) and a Raman spectrum of phenylalanine in aqueous solution (B).

similar to that observed by colloid aggregation. It is at these points or "hot spots" where strong electromagnetic Raman enhancement can occur.

The effect of solution concentration on relative Raman intensity is illustrated in Fig. 3. Each spectrum was the mean of four spectra obtained at four random locations on the filter surface. The spectra were acquired using an FT-Raman instrument as described in the Experimental section. The spectra show the increase in relative Raman intensity over a range of L-phenylalanine concentrations (0.01–1.0 mM). The detection limit for L-phenylalanine was 0.01 mM under the stated experimental conditions. No L-phenylalanine peaks were identifiable at lower concentrations.

TABLE 1 Wavenumbers and Assignments for Phenylalanine

Raman shift (cm^{-1})	Assignments[a]
622	ν_{6b} Inplane ring deformation
746	ν_1 Symmetric ring breathing
858	
931	C–COO^- Stretching
1001	ν_{12} Symmetric ring stretch
1031	ν_{18a} Inplane CH bending
1205	ν_{7a} Phenyl-C stretch
1274	
1386	COO^- Symmetric stretching
1600	ν_{8a} Inplane ring stretching

[a]Spectra assignments based on Podstawka et al.[33].

A plot of relative intensity versus concentration for the band at 1001 cm^{-1} is given in Fig. 4. This band was selected because it was the most intense. The plot reveals an initial sharp increase in the relative Raman intensity as the L-phenylalanine concentration increases. The relative intensity eventually reaches a maximum and then plateaus and in some cases decreases for increasing L-phenylalanine concentrations. These three phenomena, sharp increase to maximum, plateau, and slight decrease, can best be explained by the film morphology on the filter surface. As described above, the Raman enhancement is believed to occur at certain hot spots on the film surface created at points on the filter where fibers intersect in close proximity. The number of these hot spots is limited throughout the surface of the fiber filter. At low concentrations the L-phenylalanine adsorbs on the surface of the hot spots and causes the relative intensity to increase. As the concentration of L-phenylalanine increases, the hot spot will eventually become saturated, resulting in a multilayer of adsorbed molecules. This would explain the plateau and subsequent decrease in relative intensity. However, it appears that at low concentrations the increase in relative intensity is linear. A plot of the relative intensity versus concentration over the linear range is given in the inset of Fig. 4.

This plot exhibits a good linear relationship between 0.01 and 0.15 mM L-phenylalanine. This

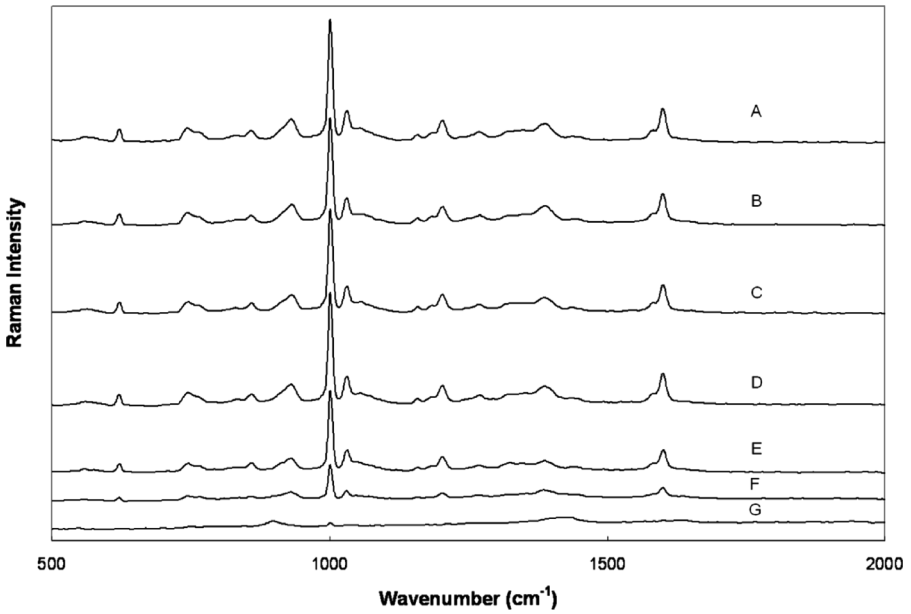

FIGURE 3 SERS spectra of phenylalanine as a function of solution concentration: (A) 1.0 mM, (B) 0.5 mM, (C) 0.25 mM, (D) 0.15 mM, (E) 0.1 mM, (F) 0.05 mM, and (G) 0.01 mM.

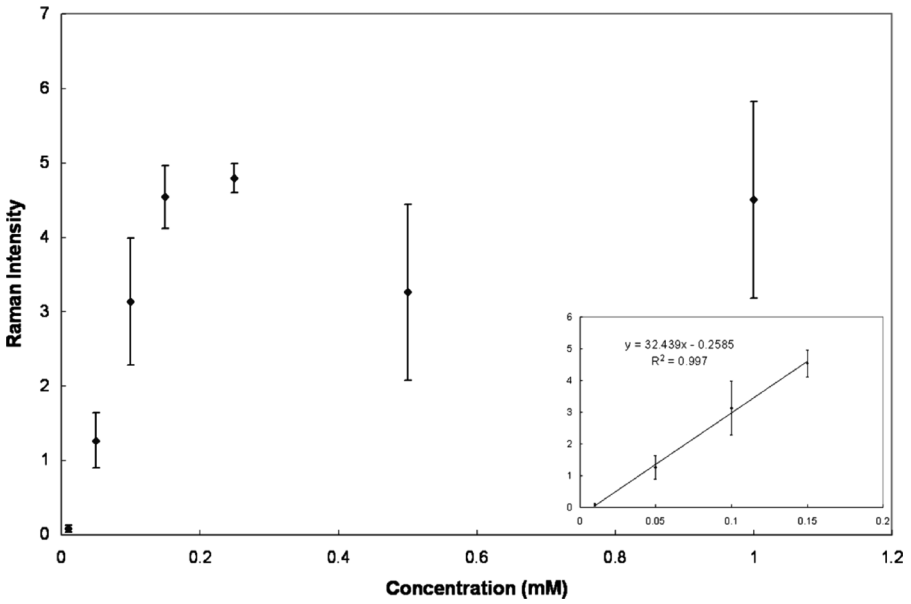

FIGURE 4 SERS intensity dependence on L-phenylalanine solution concentration. The linear range is highlighted in the inset.

result demonstrates the ability to predict L-phenylalanine concentrations over this range. The slope of the plot is such that there is a large increase in relative intensity (~36×) from the low to high end of the range, allowing for the ability to easily differentiate L-phenylalanine concentrations.

CONCLUSIONS

The silver-coated filter SERS method presented here is a novel and promising method for biochemical analysis. The demonstrated ability to detect amino acids qualitatively and quantitatively can be further extended to the detection of other biological molecules such as proteins, tumor markers, and glucose. The use of an FT-Raman instrument combined with this new SERS method allows for a method with both high sensitivity and selectivity. The increased sensitivity is a result of the high relative intensity signals generated from the SERS substrate. The increased selectivity is a result of the high spectral resolution of an FT instrument. This method is

relatively simple and requires no sample preparation prior to analysis.

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