Environ. Sci. Technol. **2010,** *44,* 7749–7755

Environmental Science & Technology
Feature

Surface-Enhanced Raman Spectroscopy (SERS) for Environmental Analyses

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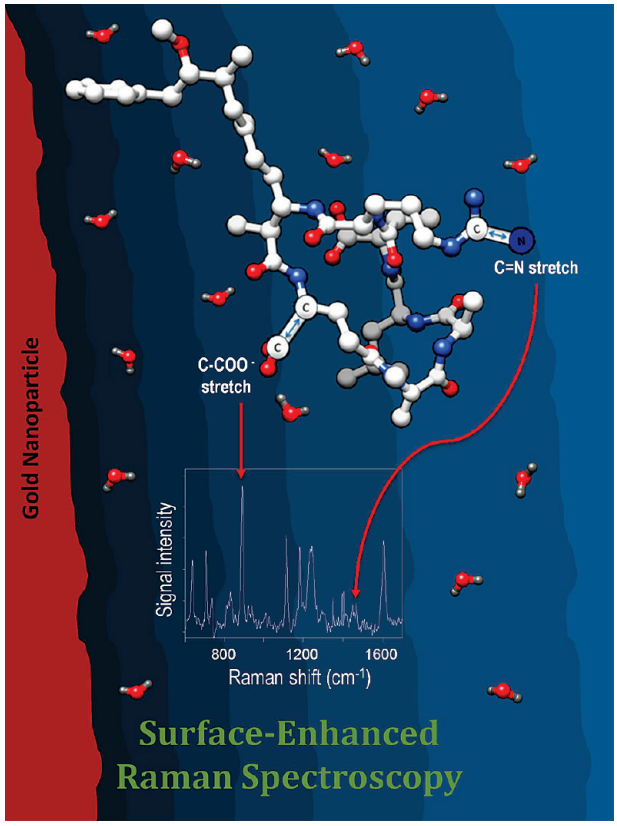
Received April 17, 2010



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Despite extensive efforts to protect public health, disease outbreaks still occur when toxic chemicals and microbial threats evade detection. Peanut butter and produce tainted with *Escherichia coli* 0157:H7, drinking water in Milwaukee, WI and Walkerton, ON (Canada) polluted by *Cryptosporidium*, surface waters contaminated by cyanotoxins, and mail laced with anthrax spores each represent an outbreak that may have been prevented with faster and more readily available pathogen or chemical detection methods. Although numerous techniques for contaminant detection in a variety of matrices exist, monitoring each analyte class generally requires specific instrumentation. Today, however, there is growing excitement about the potential use of surface-enhanced Raman spectroscopy (SERS) for simultaneous multiplex detection of infectious and noninfectious contaminants in a range of environmental milieu. As currently envisioned, SERS is a platform for simple, fast, inexpensive, reliable, and robust methods to screen single or multiple contaminant classes simultaneously (*1*−*4*). Thousands of SERS publications document significant progress in achieving such a vision; however, as outlined herein several surmountable obstacles must be overcome to achieve these goals.

Raman Scattering and SERS

As Raman and Krishnan first observed in 1928, natural molecular vibrations inelastically scatter light in a unique manner for every given molecular structure (*5*). This light is collected to create a Raman spectrum (Figure 1a). Raman measurements are simple to acquire, require little sample preparation, do not generally destroy samples or produce waste; spectra can be acquired through transparent glass, plastic, water, or solvent (*6*). However, because of the low strength of Raman signals, the technique has not gained significant traction as a tool for everyday environmental monitoring. The recent development of reproducible SERS substrates and methodologies has the potential to bring Raman-based methods into more widespread use (*2*).

SERS, first observed in 1974, is an extension of normal Raman spectroscopy that relies on electronic and chemical interactions between the excitation laser, analyte of interest, and SERS substrate (*9*). Raman signal enhancements as high as 10^{14} can occur for resonant molecules found in "hot spots" on a SERS substrate because of combined electromagnetic (EM), charge transfer (CT), and resonance signal enhancement mechanisms (*10, 11*). EM enhancement occurs when the incident laser excites surface plasmons (electrons at the metal surface that collectively oscillate upon excitation), thereby creating an electromagnetic field extending up to 20 nm from the metal and enhancing Raman signals of exposed molecules by an average of 10^4 (Figure 1b; refs *12* and *13*). However, single molecule enhancements of 10^{11} can occur at nanoparticle junctions where EM fields overlap (*10*). CT, the transfer of electrons between the analyte and metal surface, contributes an additional 10−100× enhancement when the analyte directly contacts the metal (*1, 13, 14*). Additional resonance enhancement is possible if the laser wavelength falls near an absorption wavelength of the sample (*13, 14*). To achieve the lowest possible detection limits, all potential signal enhancements (EM, CT, and resonance) must be considered and maximized (Figure 2 and Table 1).

Raman and SERS Analytes. All polarizable molecules (molecules whose electron clouds are distorted by the electric field produced by a laser) generate Raman spectra. In general, nonpolar groups and symmetric vibrations generate the strongest Raman bands, and isomerization or the proximity of electron withdrawing or donating groups shift the positions of Raman peaks, allowing similar analytes to be distinguished from one another. Concentration data is also embedded

10.1021/es101228z
Published on Web 09/13/2010

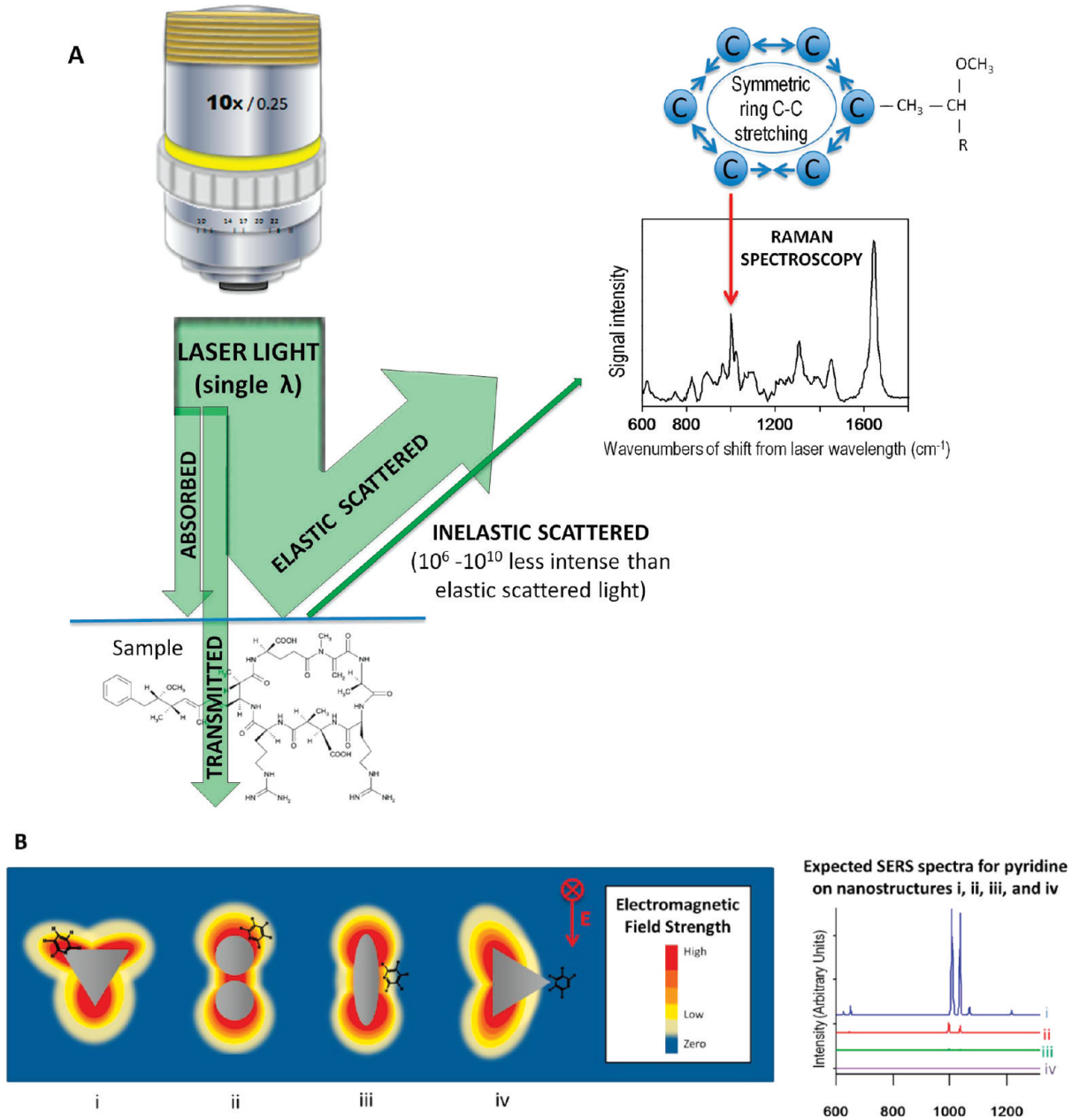

FIGURE 1. Raman Spectroscopy and SERS. (A) Interaction between laser light and a molecule produces inelastic scattered light (light with a different wavelength than the incident light). Each molecular vibration uniquely shifts the wavelength of a portion of scattered light and is visible as a specific band in the Raman spectrum (*6*). (B) Particle shape, size, and proximity to other particles affect the electromagnetic field (EM) that forms around a metal nanoparticle (adapted from ref *7*). Expected SERS spectra for pyridine illustrate that signal enhancements are greatest for molecules subject to both EM and charge transfer (CT) (shape i), while no enhancement is expected if the analyte is positioned outside the EM field (adapted from ref *8*).

within relative peak heights or peak areas, although it is not always straightforward to extract (*6*). Importantly, the low polarizability index of water means that it produces negligible signal relative to more polarizable analytes dissolved or suspended within it, thus allowing aqueous contaminants to be observed by Raman spectroscopy (*13, 15*). However, even species with weak Raman signals can be probed if the experiment is designed to accommodate lower intensity signals. For example, the extent of hydrogen bonding in water as determined by Raman spectroscopy can be used to infer temperature in a sample with an accuracy of ±0.1 °C, but water also produces negligible background signal compared to 3 nM saxitoxin (*16, 17*).

In theory, any analyte can be detected by SERS if it is polarizable when it interacts with a SERS substrate. Even Raman-inactive molecules can become SERS-active if the EM field disrupts the normal symmetry of the analyte, the analyte is in resonance with the excitation laser, or CT occurs (*9*). SERS spectra are not always enhanced versions of Raman spectra; band positions migrate when metal-analyte bonds form, and relative band intensities shift when the analyte orients in a specific direction relative to the SERS substrate, thus allowing one portion of the molecule to be more highly affected by the EM field (*12*−*14*). Despite potential differences between Raman and SERS, the polarizable bonds within a molecule or complex are observed in both types of spectra, water exhibits a weak SERS signal, and although normal Raman can be hindered by background fluorescence signals 10^{14} times more intense than Raman signals (produced when impurities or the sample itself absorb the laser line and re-emit it as the excited electrons return to the ground state), SERS substrates often provide nonradiative decay channels

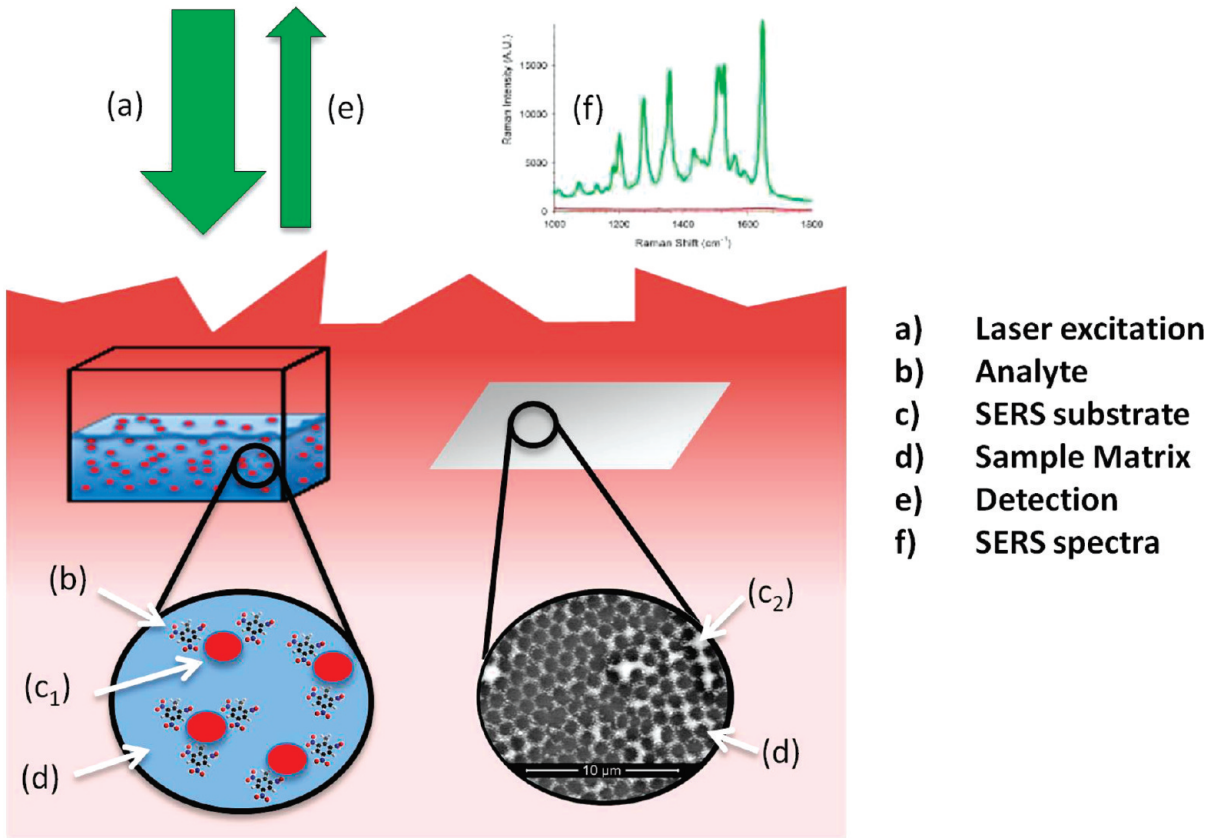

FIGURE 2. Components of a SERS measurement. Laser (a) illumination of an analyte (b) results in Raman signal emission and creates an EM field about the SERS substrate, commonly gold (Au) or silver (Ag) nanoparticles (c_1) or nanostructured surfaces (c_2; SEM courtesy of Weinan Leng, Virginia Tech) that are influenced by the sample matrix (d). The detector (e) collects inelastic scattered light from the sample. SERS occurs for RBITC using a 633 nm laser and 40 nm Au nanoparticles (f, green) but not 13 nm nanoparticles (f, red) because of the difference in the surface plasmon absorption band of the two particle sizes (*4*).

that quench fluorescence (*13*). These facts are critical for environmental applications of SERS as they allow acquisition of spectra of many classes of contaminants in aqueous matrices.

Environmental Applications

Although the complexity of SERS elicits some skepticism about the utility of the technique, its growing list of remarkable analytical achievements cannot be ignored (*2, 4, 11, 21, 22, 24*). For example, in highly controlled environments, SERS can be used to detect contaminants at femtomolar concentrations over practical time scales (*11*). SERS is also capable of simultaneously detecting multiple contaminants of varying polarities and molecular weights, a feat that most existing detection strategies cannot achieve (*1, 2, 13*). Furthermore, SERS is exquisitely sensitive to subtle differences in material structure, allowing differentiation of similarly structured organic molecules and bacterial strains (*4, 22, 25*). Over the past 30 years, SERS has been used for quantitation and identification of organic and inorganic contaminants, pathogens, and nanomaterials in environmental samples (*1−3, 23−26*). Detailed descriptions documenting the history of environmental applications of SERS, capabilities of SERS for single molecule detection, biowarfare agent detection, and medical applications are available elsewhere (*1, 2, 10, 24*). Herein, we focus on recent applications of SERS for qualitative identification and quantitative analysis of aqueous and airborne organic and inorganic contaminants and pathogens.

Aqueous and Airborne Organic and Inorganic Contaminants. SERS of aqueous species is relatively straightforward: the sample is dried onto a solid SERS substrate or dissolved in an aqueous phase containing nanoparticles. SERS of airborne samples requires an extra step to bring the analyte into contact with the SERS substrate. Airborne analytes may be deposited on solid SERS substrates, pumped through impactors that direct samples into cuvettes containing aqueous nanoparticles, bubbled into nanoparticle solutions, or partitioned into water using free-surfaces on open fluidic devices (*27, 28*).

Qualitative identification of TNT (2,4,6-trinitrotoluene), fullerenes, and numerous other compounds often rely on detection of a specific SERS spectrum to infer the presence of a contaminant (Figure 3a; refs *11, 25, 29*). Impressively, the in situ identification of benzenethiol, 1-naphthylamine, and pyridine sorbed to humic acid was achieved simply by adding Au salt to humic acid. Humic acid acts as a reductant and produces Au nanoparticles in situ. The background SERS spectrum for these nanoparticles provides an open spectral window through which SERS spectra of pollutants at 10^{-5} M concentrations are clearly visible (*25*).

Quantitative SERS is more challenging than qualitative but can be achieved using internal standards that allow signal normalization or microfluidic cells that produce turbulent flow to mix samples and facilitate time-based signal averaging (Figure 3b). These methods reduce variability between measurements and create robust calibration curves. Such approaches have been used for quantitation of pesticides, nicotine, cyanide, methyl parathion, saxitoxin, dipicolinic acid (anthrax biomarker), and uranyl ions in aqueous samples (*17, 21, 26, 30*).

Pathogen Detection. SERS for pathogen detection is achieved by coating cells with a SERS-active material, reducing a noble metal at the cell wall to produce nano-

TABLE 1. Components of a SERS Measurement

	SERS step	purpose	design considerations	choices	refs
(1)	Laser	Excites the analyte to produce Raman signals, generates EM field, enables resonance in the analyte.	Wavelength (consider plasmon absorption band of the SERS substrate, electronic transitions of analyte, and potential sample fluorescence) and power (maximize SERS signal while avoiding sample degradation).	Visible to IR lasers (488−1064 nm), 0−1000+ mW of power.	*6, 14, 18*
(2)	Analyte	Raman active analytes have polarizable bonds that vibrate and emit inelastic scattered light. SERS active molecules are polarizable upon interaction with a SERS substrate.	Polarizability in the presence of the SERS substrate, affinity for the SERS substrate, resonance at the laser wavelength, concentration, strength of polarizability with respect to background.	Endless possibilities. Any molecule that is polarizable upon interaction with a SERS substrate.	*1, 6, 9, 12*
(3)	SERS Substrate	Provides EM field to enhance the Raman signal. Upon analyte contact, the substrate can also transfer charge to facilitate additional enhancement.	Size and sharpness of surface features, metal choice, surface functionalization, s tability, synthesis complexity, reproducibility, biocompatibility, cost, wavelength of plasmon absorption band.	Gold, silver, copper, other metals; nanoparticles, ordered thin films, functionalized particles or surfaces.	*1, 9, 12, 19, 20*
(4)	Sample Matrix and Analysis Condition	Can encourage or discourage interactions between analyte and SERS substrate, certain matrices quench SERS, solvent or fluorescent backgrounds can overshadow analyte spectra, experimental conditions may slig htly shift peak locations and relative band heights.	Surface chemistry at SERS substrate, charge of analyte, influences of ions, peak locations of analyte in relation to solvent or background, laser wavelength corresponding to fluorescence, consisten cy between samples and experiments, homogeneity of samples.	Sample dissolved in water or organic solvent, dried sample, solid sample, ion addition, sample vessel material, sample flow through cell.	*6, 10, 13, 14, 21*
(5)	Detection	Records Raman signals.	Wavelength range of detector, sensitivity, signal-to-noise ratio, response time, robustness, vulnerability to light.	CCD, multichanneled photomultipliers, AlGaAs photocathodes, intensified CCDs, NIR detectors.	*6, 18*
(6)	Spectral Analyses	Peak area and band locations can be used for qualitative identification when compared to existing spectra. Peak areas and heights can be correlated to concentration for quantitative analyses.	Raman peak locations sensitive to bond's local environment, major peak shifts result from influence of SERS substrate on polarizability of analyte and formation of analyte-metal bonds, small position and intensity shifts occur when analyte favors a specific orientation to the metal surface, software packages available for analysis of large data sets, peak identification possible by comparing to existing spectra.	Peak identifications, principal component analysis, partial least-squares (PLS), soft independent modeling of class analogies, and PLS-discriminant analysis are available when spatial and sample variability cause two spectra of the same sample to differ.	*6, 12, 21−23*

structures, using a solid substrate, or marking pathogens with SERS-active immunotags; however, overcoming sample variablility can be challenging (*1*, *4*). The surface of a pathogen, which can vary in size from 10 nm for a small prion to many micrometers for protozoa, consists of a diverse array of biomolecules each with a characteristic Raman

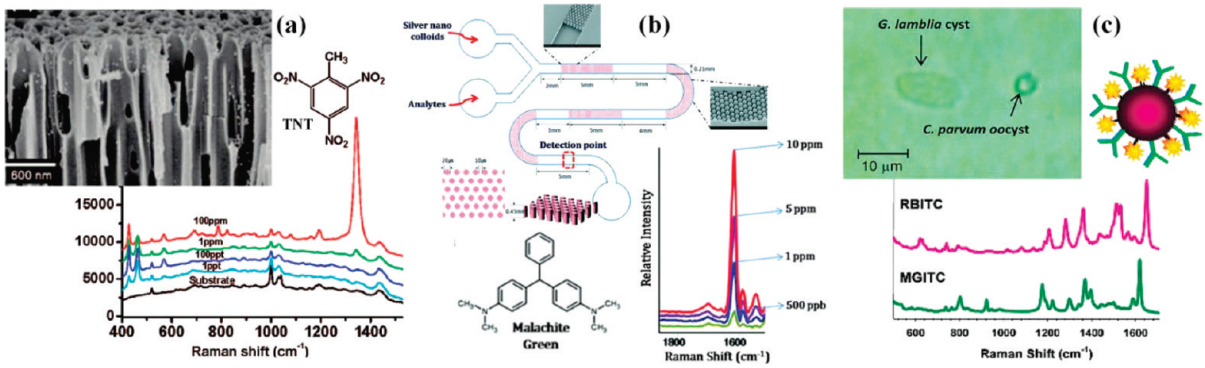

FIGURE 3. SERS applications: (A) detection of TNT at zeptogram levels using alumina membrane pores coated with polyelectrolyte and Au nanoparticles, (B) quantitation of malachite green fungicide solutions using a PDMS microfluidic channel and Ag nanoparticles, and (C) identification of *Cryptosporidium* and *Giardia* using immunogold labels tagged with SERS-active dyes (*3, 4, 11*).

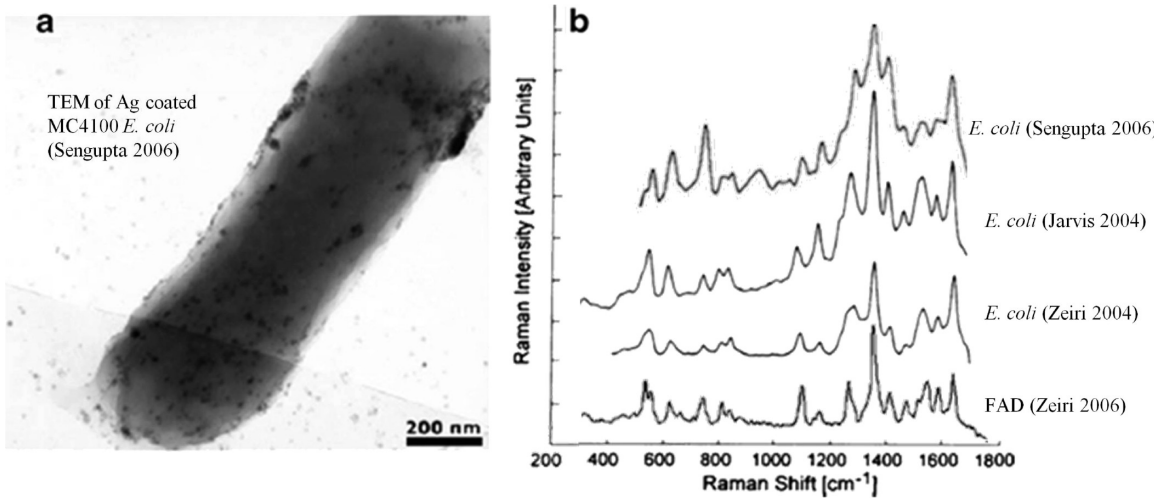

FIGURE 4. SERS spectra of *E. coli* from separate laboratories produced similar spectra with varied signal intensities (adapted from refs *1, 31, 32*). An external Ag coating on the cell membrane (*33*) and Ag nanoparticle coatings (*32, 34*) facilitated the enhancements. FAD spectra compared to *E. coli* spectra demonstrate the preferential association of Ag nanostructures with FAD on the cell surface (*31*).

spectrum (*23, 31*). SERS spectra reflect the region of the sample illuminated by the laser (the size of which is dependent upon the aperture of the microscope objective used to take the spectrum) and the surface features that associate with the SERS substrate. Variations in these parameters cause a pathogen to exhibit multiple SERS spectra. The effects of such variability can be minimized when SERS-active nanostructures associate with a specific biomolecule on the cell surface, microfluidic cells fully mix samples during spectral acquisition, or cells are deposited on solid substrates and SERS signals acquired over a large area are averaged (*31, 32*). For example, SERS spectra for *E. coli* obtained using colloidal Ag exhibit band positions for flavin adenine dinucleotide (FAD) molecules because the nanosilver associates closely with flavins and thiol-containing flavoprotein pockets on the cell surface (Figure 4, refs *1*, *27*, and *31*).

Once sample variability is overcome, SERS can distinguish between viable and nonviable pathogens, Gram positive (*Listeria, Staphylococcus aureus, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis*) and Gram negative (*Escherichia coli, Salmonella*) bacteria, and protozoa (*Cryptosporidium parvum, Giardia lamblia*) despite structural and biomolecular similarities (*2, 22, 23, 31*). Spectral interpretation that allows species to be distinguished from one another often involves principal component-discriminant function analysis or plots of ratios of SERS peak intensities (*22, 23*). When surface features between pathogen species are not sufficiently unique to allow species determination, immunotags can be created that provide SERS substrates linked to both SERS-active molecules and antibodies for the targeted pathogen; such a scheme was recently implemented by our laboratory to tag *Cryptosporidium parvum* and *Giardia lamblia* (Figure 3c, ref *4*).

Remaining Challenges and Future Outlook

Like any analytical technique, SERS has its own challenges, the greatest of which result from its strengths: SERS is not only sensitive to slight details of molecular structure but also to local environment and molecular orientation at SERS substrates (*6, 9*). Hence, it is not uncommon to acquire several completely distinct SERS spectra of a single sample within a fraction of a second, thus causing some SERS experiments to be considered highly variable. Slight changes in SERS substrate features, extent of aggregation of nanosilver or nanogold, substrate surface chemistry, sample matrix chemistry, presence of impurities, analyte orientation, or analysis conditions can influence SERS spectra (*3, 13, 20*). Surface and sample matrix chemistry can be manipulated by adding ions to the sample, but ions can function as either SERS activators or quenchers, and their effects, which are difficult to predict, must be analyzed on a case by case basis (*10, 26*). Unstable SERS spectra are also produced when signals from "SERS hotspots" dominate spectra or when laser exposure and thermal or photochemical mechanisms degrade analytes or SERS substrates over short time scales (*13, 21*). Encouragingly, efforts to improve reproducibility have been successful.

Precise quantitative SERS studies have been conducted using low laser power, internal standards that allow signal normalization throughout an experiment, microfluidic cells that create turbulent flow conditions to both mix the sample and allow an average over time to be measured, and reproducible SERS substrates that are currently being improved, tested, and commercialized (*1, 20, 21*).

SERS is also associated with its own specificity; although simultaneous detection of multiple contaminant classes is possible, it is not always straightforward to apply the SERS setup for one molecule to another with a similar structure, polarity, or molecular weight. Instead, the experimental parameters must often be carefully examined and modified for each SERS analyte. Analytes must be precisely matched to a compatible SERS substrate, excitation laser, and set of experimental parameters. Developing each specific SERS application requires trained laboratory personnel, research dollars, and patience in the laboratory, and only further study will allow SERS to achieve its full potential. Nevertheless, once developed, many SERS methods will tolerate simplification to allow practical application for routine monitoring.

Despite these stumbling blocks, SERS is rapidly evolving into a practical analytical tool that can be simultaneously applied to multiple analyte classes, and existing environmental applications are both plentiful and remarkable. Furthermore, SERS applications are not limited to the laboratory bench: microfluidic cell, lab-on-a-chip applications with portable Raman systems have facilitated quantitative field analyses for species such as dipicolinic acid and malachite green (a genotoxic antiseptic; ref *3*). At the current rate of development of reproducible SERS substrates, microfluidic devices that reduce spectral variability, and practical applications for environmentally relevant analytical problems, SERS has great potential to become a rapid, reliable, and widespread technique. The future of SERS is bright indeed.

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Acknowledgments

This work was supported by an EPA STAR graduate research fellowship to R.A.H. (F08B20329) and research grants from the National Science Foundation (BES-0606995) and ICTAS. The constructive comments of Dr. Weinan Leng are much appreciated.

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