2007

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Goeller, Lindsay J. and Riley, Mark R., "Discrimination of Bacteria and Bacteriophages by Raman Spectroscopy and Surface-Enhanced Raman Spectroscopy" (2007). *Biological Systems Engineering: Papers and Publications*. 352.
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Discrimination of Bacteria and Bacteriophages by Raman Spectroscopy and Surface-Enhanced Raman Spectroscopy

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Detection of pathogenic organisms in the environment presents several challenges due to the high cost and long times typically required for identification and quantification. Polymerase chain reaction (PCR) based methods are often hindered by the presence of polymerase inhibiting compounds and so direct methods of quantification that do not require enrichment or amplification are being sought. This work presents an analysis of pathogen detection using Raman spectroscopy to identify and quantify microorganisms without drying. Confocal Raman measurements of the bacterium *Escherichia coli* and of two bacteriophages, MS2 and PRD1, were analyzed for characteristic peaks and to estimate detection limits using traditional Raman and surface-enhanced Raman spectroscopy (SERS). MS2, PRD1, and *E. coli* produced differentiable Raman spectra with approximate detection limits for PRD1 and *E. coli* of 10^9 pfu/mL and 10^6 cells/mL, respectively. These high detection concentration limits are partly due to the small sampling volume of the confocal system but translate to quantification of as little as 100 bacteriophages to generate a reliable spectral signal. SERS increased signal intensity 10^3 fold and presented peaks that were visible using 2-second acquisitions; however, peak locations and intensities were variable, as typical with SERS. These results demonstrate that Raman spectroscopy and SERS have potential as a pathogen monitoring platform.

Index Headings: Raman spectroscopy; Pathogen detection; Bacteriophage; Surface-enhanced Raman spectroscopy.

INTRODUCTION

The presence of pathogenic organisms in drinking water represents a substantial risk to widespread transfer of disease. According to the U.S. Center for Disease Control and Prevention (CDC), in 1999, contaminated drinking water caused 39 outbreaks; sickening 2068 people; hospitalizing 122 people; and killing two people.¹ These unnecessary infections could be decreased with continuous pathogen monitors placed in community water systems that could warn citizens of contaminations. Similarly, concerns have been raised for homeland security and the spread of pandemics such as avian flu, all of which pose significant challenges for detection and screening of large numbers of samples.

Current water-borne pathogen detection methods are slow and expensive and often reveal only limited information about the contaminant. Standard pathogen detection methods are performed primarily on a diagnostic basis using standardized methods that typically require manual sampling, large sample sizes, 2–14 days of laboratory time, expensive manual labor, long purification processes, and costly equipment.

The complexity and diversity of pathogens (bacteria, viruses, and protozoa) presents many challenges to the design of detection schemes. There has been recent success using polymerase chain reaction (PCR), antibody-based detection,

and mass spectrometry to detect many specific pathogens based on biological structure.² Such approaches have potential for use as pathogen monitors; however, there are fundamental limitations that restrict their use outside of a controlled laboratory. For example, PCR suffers from the frequent presence of compounds, such as humic acids, that inhibit the use of reverse transcriptase enzymes.³ Antibodies are not robust for continual field use. Mass spectrometry is expensive and not readily portable.⁴

An alternative approach that offers many potential advantages in water monitoring is provided by spectroscopic techniques that are specific, noninvasive, nondestructive, and can be performed very rapidly. In contrast to infrared (IR) spectroscopy, Raman spectroscopy offers other advantages for water monitoring because water itself does not significantly affect Raman spectral data. Although Raman sensitivity is low in comparison to IR spectroscopy, this can be increased by applying signal-enhancement methods (surface-enhanced Raman spectroscopy, or SERS), in which metal particles are placed in direct contact with the sample. When the incident laser wavelength matches the resonant frequency of the metal, Raman scattering is enhanced. This effect is known to increase signal intensities by up to 10^{14} .⁵

There are many studies that use Raman spectroscopy to study bacteria for clinical diagnosis or food safety.^{6–12} Raman can provide species discrimination amongst food-borne pathogens^{9,13,14} using either dried or viable cells, but not using diluted samples, probably due to low signal intensity.

Much less effort has been applied to evaluate viruses using Raman spectroscopy. Viruses range in size (5 to 300 nm), composition (protein, DNA and/or RNA, some contain lipids), and shape (helical, polyhedral, or irregular). Raman spectroscopy has been applied to plant and bacterial viruses to evaluate the interaction between proteins and nucleic acids.^{15,16} There are few reports of limit of detection evaluations for quantification of viruses, the smallest type of pathogen and therefore the most difficult to detect. Since exposure to less than 10 viruses may cause illness,¹⁷ the viral detection limit requires careful investigation.

Low signal intensities can be improved using SERS as has been used to detect bacteria after they absorb silver colloids¹⁸ or intestinal epithelial cells after absorption of gold colloids.¹⁹ The SERS fingerprint of a cell has been found to be sensitive to very subtle chemical changes inside the cell²⁰ and can be used to study the movement of a chemical within the cell.²¹ Grow and co-workers²² combined SERS with an antibody microarray to create a system that has the potential to identify an array of possibly 64 pathogens and pathogen-produced toxins.

The overall objective of this study was to evaluate the ability of Raman spectroscopy and SERS to discriminate between specific species of bacteria and bacteriophages and to estimate

Received 28 September 2006; accepted 20 April 2007.

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FIG. 1. Transmission electron microscopy (TEM) images of bacteriophages (a) PRD1 and (b) MS2. Solid arrows point to intact (solid white) bacteriophages; dotted arrows point to damaged (hollow) bacteriophages. Estimated concentrations from these images, within two orders of magnitude, are 10^6 pfu/mL PRD1 and 10^9 pfu/mL MS2. The dark and light spots are due to uneven application of negative dye. These images confirmed the presence of the bacteriophage; plaque assays were used for more accurate concentration estimates. Image size: (a) 1240×1240 ; (b) 400×400 nm.

these pathogens' detection limits, particularly to identify and differentiate pathogens in water systems.

Bacteriophage MS2 and PRD1 were selected as the focus of study because they have diverse physical and biochemical characteristics. MS2 is a 25 nm RNA virus that infects *E. coli* bacteria, and has structural similarities to enteroviruses. PRD1 is a 75 nm DNA lipid-containing virus that infects *Salmonella* and *E. coli* and has structural similarities to adenoviruses. PRD1 has a mass 33 times greater than that of MS2. A non-infective *E. coli* strain was also studied. These microbes were analyzed to estimate the detection capabilities in specificity and sensitivity using Raman spectroscopy and SERS.

MATERIALS AND METHODS

The bacterium *E. coli* and two bacteriophages (PRD1 and MS2) were cultured using standard methods.²³ Each species was then placed on the microscope of a confocal Raman microspectrometer stage at known concentrations. Spectra were collected and analyzed to identify unique spectral features and to provide an estimate of the potential detection limit for each microorganism. Methods to perform these experiments are described in detail below.

The rationale for utilizing bacteriophages was that these microorganisms are safe surrogates for viral release studies in the environment. The moderate term goal of the work is to perform viral surrogate release studies into water systems to assess transport and distribution so as to simulate the spread of viruses in an intentional (or accidental) release. To make such studies practical requires a continual monitoring process to detect the viral simulants. The two bacteriophages studied here (MS2 and PRD1) are the most common safe viral surrogates utilized in field release studies.

To produce MS2 and PRD1 bacteriophages, one host bacterial colony was added to 3 mL of TSB and incubated at 37 °C for 24 hours. *E. coli* was used as the MS2 host and *Salmonella* was used as the PRD1 host. One milliliter (1 mL)

of stock bacteriophage solution was added to the host and incubated in a shaker for 8–12 hours at 37 °C. Then, 0.35 g EDTA and 0.01 g crystallized lysozyme were added to the infected host solution and incubated in a shaker for 30 minutes at 37 °C. The solution was centrifuged at 10 000 rpm for 20 minutes. The supernatant was vacuum-filtered through a 0.45 μ m filter and stored at 4 °C for up to one month before use.

PRD1 was further purified using the following approach. Eighty milliliters (80 mL) of PRD1 stock was pelleted at 25 000 rpm for 3 hours at 4 °C. The supernatant was poured off and the pellet was re-suspended in 1–2 mL sterile saline solution. A sucrose gradient maker dispensed a 10 mL continuous gradient of 40% to 5%, on top of the 50% cushion. The tube was then topped off with the re-suspended PRD1 pellet. The gradients were centrifuged at 21 000 rpm for 70 minutes at 4 °C and then continuously removed from the tube. Absorbance was monitored continuously and recorded. The fifth and sixth sequentially filled tubes, which contained the bacteriophage band, were combined and washed by centrifuging at 36 000 rpm for 2 hours at 4 °C. Bacteriophage purification was verified using standard plaque assays.

The purification processes for MS2 were the same as those used for PRD1 purification with the following exceptions. The sucrose gradient was made with 1 mL of 50% cushion and 10 mL of 15% to 30% gradient. No visible band formed, but tubes seven and eight had the maximum absorbance peak and were subsequently washed by centrifugation at 36 000 rpm for 2 hours at 4 °C.

Transmission electron microscopy was used to evaluate phage purification. Copper grids, plated with palladium, were exposed to a 10 μ L drop of purified bacteriophage for 3 min. The grid was then exposed to a 10 μ L drop of negative stain (2% phosphotungstic acid, pH 7.0) for 3 min, twice. MS2 and PRD1 scanning electron micrograph (SEM) images are shown in Fig. 1.

Confocal Raman measurements were performed on an HR800 system (HORIBA Jobin Yvon Inc.), consisting of a

FIG. 2. Raman spectra of media components. (Top) Sucrose (15% w/v) and (bottom) TSB. Many of the sucrose features are also in TSB. Peaks with carbohydrate assignments are identified with arrows.

784.85 nm laser diode, notch filter, inverted confocal microscope, 60× water immersion IR objective, spectrograph, charge-coupled device (CCD) detector, and LabSpec software. The Raman system was calibrated before each use with a silicon wafer, which produces a strong Raman peak at 520.7 cm^{-1} . Mirrors were adjusted daily to center the laser on the CCD. The confocal hole and slit were set at 400 and 140 μm , respectively.

The Raman system is equipped with a water-immersion objective with a coating optimized for infrared measurements. This objective has a very small working distance, so thin coverslips were used as slides. Sapphire windows, an ideal material for Raman IR measurements, are not available in the necessary thickness (0.13 to 0.21 mm), so 0.2 mm quartz slides were utilized. Quartz slides produce significant Raman scatter compared to sapphire, but produced adequate differentiation within spectra.

According to the manufacturer, when using the default 100× objective, a slit of 145 μm projects the image onto 8.6 pixels, each with an area of 225 μm^2 . These experiments used a 60× objective and a 140 μm slit. The cross-sectional area sampled by each measurement is then 3225 μm^2 . Using an average hole size of 400 μm yields a sample thickness of 2 μm . Therefore, the volume sampled was approximately 6.4×10^{-9} mL. This small sample size greatly impacts the detection limit of bacteria and bacteriophages and is described in more detail below.

Before use, slides were cleaned in deionized water and wiped with acetone-soaked-lens paper. Forty microliters (40 μL) of DI water was placed on the water-immersion objective. One hundred and twenty microliters (120 μL) of sample solution was placed on the slide. Acquisition times up to 60 minutes were employed. When colloids were applied, acquisition times were reduced to 200 seconds per 4000 cm^{-1} . GRAMS software was used to combine and analyze the data. Excitation of the gold colloids using 785 nm laser light represents a compromise based on adsorption of the gold and minimization of light-induced damage to the biological samples. Gold does absorb 785 nm photons.²⁴ Selection of the laser wavelength has been addressed previously.^{12,24}

Colloid slides were prepared using new coverslips to ensure a clean surface. The slides were prepared with deionized water and an acetone wipe. Sixty to one hundred-twenty microliters

FIG. 3. MS2 (10^{12} pfu/mL), PRD1 (10^{12} pfu/mL), and *E. coli* (10^8 cells/mL) can be differentiated by unique Raman spectral features. Spectra include saline and silica background signals.

(60 to 120 μL) of 60 nm gold colloid (British Biocell International) was added to the slide and dried overnight, forming a rough gold surface that would be in close contact with the biological samples. Just prior to acquisition, 120 μL samples were added directly on top of the dried colloid. Colloid-prepared slides were not reused because the dried colloid could not be completely removed. The gold itself did not produce enhanced scattering of silica or water signals. Aqueous colloids resulted in scattering that is almost identical to water, in both intensity and features.

RESULTS

The goal of this research was to evaluate the feasibility of using Raman spectroscopy to detect pathogenic organisms, discriminate between classes and species, and provide concentration information.

The first step was to evaluate methods for collecting reliable and reproducible spectra. Background spectra of potentially interfering components (silica, saline, sucrose, TSB culture broth, and water) were collected. Within spectra of the culture media are signals from silica (from the microslides), water, and a sugar feature (Fig. 2). These carbohydrate features (1460, 1340, and 1128 cm^{-1}) are significantly more intense in sucrose than in TSB. Water and saline show similar spectra as these salts did not produce Raman scattering at the probed frequencies.

Due to the overlapping nature of the spectral features of the culture medium and the features of the microorganisms, it was necessary to purify the microorganisms prior to spectral collection. Without such clean up, bacteriophage spectra did not display significant differences from the culture medium spectra of Fig. 2. Due to the large size of *E. coli*, purification required only rinsing with an osmotically balanced salt solution followed by centrifugation. The bacteriophages cannot be collected by centrifugation and thus required a more elaborate purification process.

After samples were purified, significant differences in spectral features were readily apparent (Fig. 3). Many peak locations are similar in all three spectra, notably at 1449, 1320, 1301, and 1128 cm^{-1} . These peaks are assigned, respectively, to carbohydrates, proteins, lipids, and proteins (Table I). The

TABLE I. Common biological peak locations and assignments for *E. coli* spectra.

Frequency center of major peaks (cm ⁻¹) for <i>E. coli</i>	Peak of reference and source	Peak assignments			
		DNA/RNA	Protein	Lipids	Carbohydrates
1607	1607 ^a		C=C Phe, Tyr		
1578	1578 ^a	G, A			
1460		G, A, CH def	CH def	CH def	CH def
1449	1460 ^b and 1420–1480 ^a	G, A, CH def	CH def	CH def	CH def
1340	1449 ^b and 1420–1480 ^a	G, A	CH def		CH def
1320	1340 ^b and 1342 ^a				
1301	1320 ^a	G	CH def		
1249	1301 ^b			CH ₂ twist	
1209	1220–1284 ^a	T, A	Amide III	CH bend	
1176	1209 ^a		C–C ₆ H ₅ str., Phe, Trp		
1158	1176 ^a		CH bend Tyr		
1128	1158 ^a		C–C/C–D str.		
1088	1128 ^a		C–N str.		
1066	1060–1095 ^a	PO ₂ ⁻ str.		Chain C–C str.	C–O str.
1033	1066 ^b		C–N str.	Chain C–C str.	C–O, C–C str.
1002	1033 ^a		C–H in-plane, Phe	Chain C–C str.	
973	1005 ^a		Sym ring br., Phe		
959	–				
937	937 ^a		C–C BK str., α-helix		C–O–C glycos bond
919	–				

^a Notingher and co-workers.^b Simmons.

prevalence of protein features can be explained by the dry-weight composition of *E. coli*, which is about 55% protein, 25% nucleic acids, 10% lipids, and 10% carbohydrates. All have the strong feature at 1650 cm⁻¹ and also a major feature at 1450 cm⁻¹ representative of a number of biological components including nucleic acids, protein, lipids, and carbohydrates (Table I). The sharp peak at 1002 cm⁻¹ is likely due to protein vibrations.¹⁴

The major common peaks in pure MS2 and PRD1 (1607, 1449, 1381, 1301, and 1005 cm⁻¹) and their assignments are listed in Table I. The 1607 and 1005 cm⁻¹ peaks are assigned to protein, the 1381 cm⁻¹ peak was not present in published peak assignments, and the 1301 cm⁻¹ peak has an assignment for lipid vibrations.

MS2 has strong features not present in spectra from TSB and PRD1, which have peak assignments for protein (1667 and 1320 cm⁻¹) and nucleic acids (1088 cm⁻¹). PRD1 has considerably stronger peaks at 1607, 1380, and 1249 cm⁻¹ (protein, undefined, and protein, respectively) compared to TSB and MS2 stock. The feature at 1301 cm⁻¹ (indicative of CH₂ twist of lipids) is broader and more intense in PRD1, which contains an internal lipid membrane not present in MS2. The carbohydrate signals appear to be minimal, indicating that little if any sucrose from the culture media remains.

In comparing PRD1 and *E. coli*, peaks that are centered around 1000 cm⁻¹ have different locations: 1005 cm⁻¹ for PRD1, and 1002 cm⁻¹ for *E. coli*. A number of spectral features are apparent in the bacteriophages that are not in *E. coli* (1620, 1380, and 1301 cm⁻¹), which may be used to differentiate phage from bacteria.

Experiments were performed to evaluate the limit of detection of *E. coli* and PRD1. *E. coli* was diluted from a stock of 10⁸ CFU/mL by decade increments with deionized water and then Raman spectra were collected. The *E. coli*

dilution results are shown in Fig. 4 along with a spectrum of pure saline. The strong *E. coli* specific features diminish in intensity with bacterial concentration. Using the peak at 1449 cm⁻¹ as a presence indicator, the limit of detection appears to be approximately 10⁶ CFU/mL for hydrated bacteria.

Raman spectra were collected from dilutions of PRD1 at decade increments in deionized water (Fig. 5). The features at 1449 and 1330 cm⁻¹ were used to evaluate the potential limit of detection of PRD1 because these are the largest and most prevalent features in the concentrated PRD1 trials (Fig. 3). The dilution experiments show that this peak is identifiable at concentrations as low as 10⁹ PFU/mL, but it is not apparent at

FIG. 4. Raman spectra of varying concentrations of *E. coli* in saline. Spectra labels from top to bottom: 10⁷ CFU/mL, 10⁶ CFU/mL, 10⁵ CFU/mL, 10³ CFU/mL, and saline.

Fig. 5. Raman spectra of varying concentrations of PRD1 in saline. Spectra labels from top to bottom: 10^{12} PFU/mL, 10^9 PFU/mL, 10^8 PFU/mL, 10^6 PFU/mL, 10^4 PFU/mL, and saline.

10^8 PFU/mL. The 10^8 PFU/mL measurement was repeated seven times to confirm this result.

Raman spectra were collected for microbial samples on glass slides coated with gold colloids so as to promote the SERS effect (Fig. 6). A substantial enhancement of signal intensity was observed for all measurements such that the integration time could be decreased 18-fold with no decline in spectral intensity compared to measurements without colloids.

E. coli measurements (Fig. 6a) show a substantial increase in intensity upon addition of gold colloids. Locations of features have shifted and so it is not feasible to perform a direct one-to-one comparison with colloid-free spectra. Spectra of hydrated PRD1 and dried PRD1, both with addition of colloids, show substantial differences (Fig. 6b). Dry PRD1 with colloids appears very similar to dry colloids without PRD1. Hydrated PRD1 with colloids has many of the features present in the saline solution with colloids, but has fewer peaks from the silica substrate. It appears that the wet colloid PRD1 spectra have collected more information from the surrounding solvent than from the underlying substrate.

The largest increase in signal intensity upon addition of gold colloids was observed with MS2 (Fig. 6c). Samples with as little as 10^3 PFU/mL with a 200 second spectral integration time provided strong spectral features. There was, however, sizeable variability in replicate spectra.

DISCUSSION

This study presents a comparison of spectral features of a bacterial species and two bacteriophages evaluated by Raman spectroscopy and Raman spectroscopy in the presence of gold colloids to stimulate the SERS effect.

It was anticipated that the two phage types would have similar characteristic peaks with some differences in peak intensities; although the phages have relatively similar compositions (mostly nucleic acids, protein, and some lipid), they have different proportions of these components. For example, PRD1 is larger than MS2 and contains internal lipid membranes, while MS2 has only minimal lipid content. Other structural variations in the phages could also produce

Fig. 6. (a) Influence of addition of gold colloids on Raman spectra. Spectra from top to bottom: dry colloid, saline on colloid, *E. coli* 10^6 CFU/mL (on colloid), and *E. coli* 10^6 CFU/mL (no colloid). Note that integration times are 200 seconds for samples with colloids and 1 hour for the spectrum without colloids. (b) Influence of sample hydration on Raman spectra of bacteriophages. Spectra from top to bottom: PRD1 10^8 pfu/mL (wet), PRD1 10^8 pfu/mL (dry), saline (wet), colloid (dry), and PRD1 10^{12} pfu/mL (no colloids). Note that integration times are 200 seconds for samples with colloids and 1 hour for the spectrum without colloids. (c) Influence of gold colloids in Raman spectra of MS2. Spectra from top to bottom: MS2 10^3 PFU/mL (wet), MS2 10^{11} PFU/mL (no colloids), and saline (wet). Note that integration times are 200 second for samples with colloids and 1 hour for the spectrum without colloids.

differentiable peaks. Differences between *E. coli* and phage were expected to be more apparent due to differences in composition.

Detection limits for *E. coli* and PRD1 were compared and it was found that the detection limit for PRD1 was higher than predicted based on the *E. coli* detection. An *E. coli* bacterium, at 2 μm in length, is about 27 times larger than a 75 nm diameter PRD1 virus. One would expect to have a detection limit for viruses that is 27 times larger than that for *E. coli*. If this is the true limit for the system, then PRD1 would be expected to have a limit of detection of 2.7×10^8 PFU/mL rather than the determined limit of 1×10^9 PFU/mL. It is probable that the detection limit depends on variables other than size; the *E. coli* scattering may be inherently stronger than phage scattering due to composition.

The detection limits reported here must take into consideration the biomass evaluated rather than the more traditional analysis based on concentration. The confocal system evaluates only a small volume element of the sample at any one time, which impacts translation of the measurements here to a concentration-based detection limit. Typically, sample thickness using a confocal system is on the order of a few micrometers.²⁵ Based on the dimensions described above, the estimated probed sample volume is 6.5×10^{-9} to 6.5×10^{-8} mL. Applied to the measurements of PRD1 at 10^9 PFU/mL, the Raman system is detecting less than 100 individual viruses. This small sample number is surprising especially because samples were not dried, but left with full hydration. While we do not have a direct count of PRD1 for these measurements, the transmission electron microscope (TEM) images (Fig. 1) and a geometric evaluation indicate that the largest number of phages that could be localized in the given probed volume is no greater than 10^5 PFU. Therefore, measurements of PRD1 using 10^9 PFU/mL in suspension translate to a range of 10^2 – 10^5 total PFU probed and yielding reproducible spectra.

The long integration times required here motivated the search for methods to improve signal intensity and decrease collection time, thus leading to the addition of gold colloids to the microbial samples. Colloids of metals can induce the surface-enhanced Raman effect, which can enhance a Raman signal as much as 10^{14} times.⁵ This enhancement occurs when metal particles interact with the incident light oscillating at a frequency similar to the surface plasmon frequency of the metal structure, resulting in an enhancement of the electric field in the vicinity around the metal.^{26,27}

There is substantial variation in replicate spectra of the bacterium and the bacteriophage with colloids. In the case of the aqueous SERS spectra, at least two factors could play a role in producing the dissimilar baseline pattern. The water may diffuse background signal that is due to the gold itself, causing a different baseline to form, and/or particles in the saline solution could be producing the peaks.

In both baselines it is possible that neither the silica nor the water is affecting the baseline peaks. This is a key difference between the SERS and non-SERS data. The medium surrounding the colloids in dry SERS measurements is merely silica; in wet SERS measurements the medium includes silica and water. In non-SERS measurements, these media have distinct spectra, but in SERS measurements, these media may not have any effect on the signal intensity (with the exception of water reducing signal by diffusion). In other words, the silica slide material and surrounding water in aqueous measurements

may generate a smaller or essentially nonexistent enhancement by the SERS effect. This is an advantage for applying this technique to pathogen detection.

According to previous reports, SERS spectra for a sample can be similar to its Raman spectra;²⁷ however, SERS probes only the surface of the sample when evaluating a membrane-enclosed bacterium or viral particle. There also can be chemical shifts of the signal and a lack of signal enhancement for interior components that do not directly contact the gold colloids.

The results presented in Fig. 6 show major differences in peak locations in the two types of measurements. SERS variability was evident in many ways: Intensity varied from one second of acquisition to another during a single measurement (by up to hundreds of measurement units). This is typical in SERS measurements.⁵ It is possible that some of the Raman scatter from the phage is shifted in frequency by the enhancement field, and/or that certain frequencies of scattering are more sensitive to the enhancement than others. Additionally, different portions of PRD1 could have been subjected to the enhancement during each measurement. This could be caused by movement of the phage due to thermal motion (the phage is not anchored) or to the inherent instability in SERS. Due to the very small probe area and the lack of anchoring the bacteriophage onto the SERS support, the number of bacteriophage present within the sampling element could vary with time due to thermally induced movement. Thus, changes in the interactions between the phage and the colloids could be responsible for the variability. Anchoring the bacteriophage using antibodies could address this issue.

The spectra presented here show a lower quality than has been achieved in other systems. The confocal nature restricts the sample volume probed at any one time and thus biomass analyzed is small. Measurement is made in fully hydrated samples rather than the often-used dry biomass. Spectral quality can be increased by not performing confocal measurements (utilizing a larger sample volume), by anchoring the bacteriophages using antibodies or other targeting compounds, or by drying the samples. Most reported microbial spectra utilize dried material and thus direct comparisons to the spectra shown here are not appropriate.

The repeatability of the SERS measurements is a major difficulty of this approach, and one of the primary goals of presenting these SERS studies is to show the types and magnitude of variations obtained with SERS on viruses that have a size similar to the commonly employed metal colloids. Despite the large signal enhancement obtained (and the greatly decreased integration time required to achieve a good signal-to-noise ratio), the variability in the SERS measurements along with differences in the virus structural information presented demonstrate the challenges in utilizing a non-specific SERS approach for environmental monitoring without use of a targeting scheme such as antibody-based delivery.

CONCLUSION

The need for a rapid, sensitive, and specific pathogen monitor is emphasized by reports of disease outbreaks, EPA health advisories for waterborne pathogens, and warnings of bioterrorism from the Center for Disease Control and Prevention. The potential for Raman spectroscopy to provide a rapid measurement requires an ability to distinguish between related microbial species quantified in a rapid manner. Spectral

features distinct across three microorganisms (one bacterium and two bacteriophages) are apparent.

Possible detection limits using Raman spectroscopy are within the range appropriate for monitoring schemes. In the confocal micro-Raman measurements here, a volume of 6.5×10^{-9} mL was probed in each measurement resulting in detection limits of 10^6 CFU/mL for *E. coli* and 10^9 PFU/mL for PRD1. Other Raman sampling schemes that evaluate larger volumes would quite likely generate much lower detection limits.

Addition of gold colloids led to significant increases in signal intensity and decreased required measurement time 18-fold. These enhanced spectra displayed substantial variability in peak features. Importantly, silica and water signals did not appear to affect SERS spectra; presumably, signals were primarily due to the interaction between the particles and the enhancement field, in close proximity to the gold colloids. This interaction should be studied more closely to determine the cause of peak variations in SERS spectra.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Charles Gerba and his laboratory for assistance with bacteria and virus production, Dr. Joseph Simmons for use of his Raman spectrometer, and Dr. Donald Lightner for assistance in virus purification. This work was supported by the Arizona Board of Regents Technology and Research Initiative Fund and by the NIEHS sponsored Southwest Environmental Health Sciences Center # P30 ES06694.

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