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HYDROGEN-DEUTERIUM EXCHANGE AND ULTRAVIOLET RESONANCE RAMAN SPECTROSCOPY OF BACTERIA IN A COMPLEX FOOD MATRIX¹

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ABSTRACT

The rapid identification and quantification of pathogenic foodborne bacteria is a national research priority. Data collected with a new spectrochemical method suggests that resonance Raman spectroscopy can rapidly and selectively identify and quantify bacteria in foods and other complex biomatrices. This method utilizes hydrogen-deuterium exchange (HDE) to resolve the spectral fingerprints of individual components in the overlapped regions of the ultraviolet resonance Raman spectra of heterogeneous samples. We illustrate this concept with HDE-induced changes in the spectra of bacteria and beef carcass wash samples. The data presented here suggest that the combination of ultraviolet resonance Raman spectroscopy and HDE can potentially establish the identities and quantities of bacteria in heterogeneous samples within seconds to minutes.

¹Mention of a trade name, proprietary product or specific equipment is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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INTRODUCTION

Outbreaks of foodborne bacterial illness from foods of animal origin are the cause of great concern throughout the world. In the United States, one reaction to this concern has been the institution of new meat and poultry inspection policies (Anon. 1996). Reactive probes are detected using PCR formats, standard nucleic acid hybridizations, ELISA, immunomagnetic particle concentration and others (Swaminathan and Feng 1994). These methods offer many advantages over traditional culture based bacterial identification and quantification techniques, and have improved in accuracy, sensitivity and speed over the last few years. However, the aforementioned techniques are generally not quantitative, require several handling steps, and require a new or updated reactive probe for each new pathogen or variant. In addition, these methods usually are not rapid enough to provide presence/absence information for specific pathogens in a time frame suitable for a modern processing facility.

In their groundbreaking work, Nelson *et al.* (1989; 1991a,b) have shown that ultraviolet resonance Raman (UVRR) spectroscopy scattering has the potential to identify bacteria. Manoharan *et al.* (1993) showed that the 242.54 nm excitation UVRR spectral fingerprints of bacteria are sensitive to the bacteria's growth phase and enrichment medium, while the 222.65 nm excitation spectral fingerprints are nearly growth-rate and media independent. These studies, however, were qualitative and restricted to suspensions of pure cultures. UVRR identification and quantification (IdQ) of specific constituents in complex biomolecular mixtures is difficult, if not stymied altogether, when the vibrational bands of the constituents overlap. The identification of individual components from vibrational spectra has been accomplished in simple mixtures of a few components using partial least squares analysis (Jacobsson and Hagman 1993) and neural networks (Tanabe *et al.* 1992, 1994; Jacobsson and Hagman 1993; Jasper and Kovacs 1994; Mittermayr *et al.* 1994; Wienke and Kateman 1994). However, complex mixtures of bacteria found in a food processing environment commonly contain hundreds of distinct molecular constituents in addition to the target bacteria. Further confounding the analysis, the concentrations of the target bacteria may be much lower than the concentrations of the nontarget organisms or other constituents. The problem reduces to extracting weak bacterial spectral fingerprints from a stronger extra-bacterial background in the Raman spectrum of a sample.

A solution to this problem of spectral fingerprint extraction of weak signal from a stronger background is to perturb the system such that the Raman fingerprints of sample constituents time-evolve in a predictable way, causing the spectral fingerprints to become less overlapped. When the response of particular constituents (e.g., bacteria-specific components) to the perturbation has been predetermined, the weak signal associated with the target may be distinguished from the background. The "static" bacteria identification and quantification

problem is attacked with a dynamic method. In addition, quantitative information can be obtained by referencing the areas or heights of the Raman bands of the constituent of interest to the area or height of a Raman band of an absolute differential Raman cross-section standard. We employ hydrogen-deuterium exchange (HDE) to perturb the sample and induce time-dependent changes in the vibrational spectra of the constituents of food samples.

HDE has been used to study biomolecular dynamics (Englander and Kallenbach 1984; Englander and Mayne 1992). The HDE rates of biomolecules are complicated functions of the chemical structure of the moieties undergoing exchange, solvent exposure, pH, salt, and other environmental parameters. Raman spectroscopy can monitor this exchange process; for instance, the exchange of the peptide H for D in N-methylacetamide dramatically changes the frequencies and intensities of the vibrational bands in N-methylacetamide's 218 nm excitation Raman spectrum (Mayne *et al.* 1985). Similarly, the HDE effect was used in conjunction with UVR spectroscopy to observe changes in the time- and temperature-dependent UVR spectra of nucleic acids and correlated these changes with the dynamics of nucleic acid denaturation (Benevides and Thomas, Jr. 1985). Raman spectroscopy has been used to observe the differences between protein and nucleic acid HDE dynamics in bean pod mottle virus (Li *et al.* 1993). These results suggest HDE-induced changes in the frequencies and intensities of the Raman bands of biomolecules are suited for spectral fingerprint extraction of weak signal from a stronger background.

The method we are presenting employs the extremely rapid spectroscopic technique, ultraviolet resonance Raman (UVR) spectroscopy. Laser-generated monochromatic light is used to irradiate a sample, generating inelastically scattered light that is collected and analyzed. The energy of the inelastically scattered light corresponds to characteristic molecular vibrational modes of the constituents of the sample. An UVR spectrum is produced when the energy of the monochromatic ultraviolet light is coincident with the energy of an ultraviolet electronic transition of a constituent in the sample.

UVR provides quantitative information on sample constituents simply by including absolute differential Raman cross-section standards in the sample (Dudik *et al.* 1985).

The goal of our experiments was to test the feasibility of using the combination of HDE and UVR spectroscopy to dynamically detect specific bacteria against a background of other microorganisms and other materials (e.g., blood, feces, bone fragments, etc.) present in beef menstua. This goal requires that the following conditions be met: (1) bacteria produce distinct spectral fingerprints when placed in D₂O buffer, (2) that an UVR spectrum can be collected from highly turbid beef menstua, (3) that the Raman bands in the UVR spectra of beef menstua shift in response to HDE, and (4) that predetermined responses of the spectral fingerprints of the target bacteria to HDE can be observed in beef menstua. We present data

that directly support our contention that the first three conditions have been met, and the fourth can be met, suggesting that this technique can identify and quantify bacteria in unenriched samples with sufficient speed to be useful in automated food production facilities (Harhay, approved 1997).

MATERIALS AND METHODS

Organisms

Brochothrix thermosphacta (*Bt*, ATCC 11509) and *Pseudomonas fluorescens* (*Pf*, ATCC 13525) were cultivated in tryptic soy broth at 26C and 30C, respectively. Beef carcass sponge samples were obtained as previously described (Siragusa *et al.* 1995).

Sample Preparation

Stock suspensions of *Bt* and *Pf* were prepared by swabbing cultures from agar slants into H₂O buffer solution of 0.15 M Na₂SO₄ and 0.2 M Na₂H₂PO₄ in H₂O, adjusted to pH 7.25 with concentrated HCl. The SO₄⁻² ion served as the absolute differential Raman cross-section standard. The optical density at 600 nm was used to roughly estimate the concentration of bacteria. D₂O buffer solution was prepared by mixing a solution of 0.15 M Na₂SO₄ and 0.2 M Na₂H₂PO₄ in D₂O, adjusted to pD 7.25 with concentrated HCl. The pH/D measurements were made with a pH electrode without corrections for the deuterium isotope effect.

Bt or *Pf* sample suspensions were prepared by adding 2.0 mL of stock suspension to 10 mL of H₂O buffer or 10 mL of D₂O buffer. The HDE times are specified beginning with the mixing of the bacterial suspensions with D₂O buffer to the conclusion of spectral data acquisition. The concentration of bacteria in both of these suspensions was estimated to be in the range from 2 to 50 × 10⁶ bacteria/mL from the optical density measurement at 600 nm. Using this concentration range the absolute differential Raman cross-sections were calculated using excited state parameters for SO₄⁻² given by Dudik *et al.* (1985) and methods given by Dudik *et al.* (1985) and by Myers and Mathies (1987). Beef menstrua samples were prepared by diluting 3.3 mL of beef menstrua-bacteria suspension to 10.0 mL with either H₂O buffer or D₂O buffer.

UV Resonance Raman Spectrometer System and Calculations

The design of the ultraviolet resonance Raman spectrometer is similar to the design described previously (Harhay and Hudson 1993) with the following exceptions. A Lambda-Physik excimer laser producing 308 nm laser light pumping a Lambda-Physik dye laser producing 444 nm light that was doubled with BBO

crystal to produce the 222 nm excitation beam that impinged upon a “windowless” flowing planar sheet of liquid (Harhay and Hudson 1993) with the specular component (Rayleigh light) directed away from the entrance slit of the monochromator. The excimer was operated at 150 Hz. The Raman light was collected in a 180 degree backscattering geometry with a 2” f/1 lens, focused with a 2” diameter f/6.5 quartz lens through a depolarizer onto the entrance slit of a Spex 1401 double monochromator. The Spex 1401 was modified to function in the ultraviolet between 200-300 nm and was fitted with 2400 groove/mm holographic gratings. The resonance Raman spectra of *Bt* and *Pf* were collected with a Princeton Instruments 1454 intensified photodiode array detector and operating in gated mode with a 125 nanoseconds wide gate. Typically, 25 sixty second integration time scans were averaged together. The spectra were corrected for gain variation across the intensified photodiode array (Howard and Maynard 1986). The resonance Raman spectra of bacteria in beef menstrea were collected using an Oriol intensified charge coupled device operating in ungated mode. Typically, 50 twenty second scans were averaged together.

The absolute differential Raman cross-sections were calculated with the following equation.

$$\left(\frac{d\sigma}{d\Omega} \right)_{bac} = \left(\frac{d\sigma}{d\Omega} \right)_{std} \frac{I_{bac}}{I_{std}} \frac{C_{std}}{C_{bac}}$$

where:

$$\left(\frac{d\sigma}{d\Omega} \right)_{bac} \quad \text{is the absolute differential Raman cross section of the bacteria in cm}^2/\text{bacteria-steradian}$$

$$\left(\frac{d\sigma}{d\Omega} \right)_{std} \quad \text{is the absolute differential Raman cross section of the internal standard, } \text{SO}_4^{-2} = 5.8 \times 10^{-28} \text{ cm}^2/\text{molecule - steradian at 222 nm excitation}$$

I_{bac} , I_{std} are the intensities, heights or areas, of the bacterial or standard Raman vibrational band

C_{std} , C_{bac} is the molar concentration of the standard and bacteria

The instrument efficiency was measured not to vary more than 3-5% in the spectral region; no correction for instrument efficiency was used. Corrections for local-field, solid angle, and self-absorption were not performed.

RESULTS AND DISCUSSION

Figure 1a shows that the 222 nm excitation spectra of *Brochothrix thermosphacta* (*Bt*, ATCC 11509) in H₂O and D₂O buffer are dominated by the 981 cm⁻¹ SO₄²⁻ symmetric stretching vibration of the absolute differential Raman cross-section standard. The 1048 cm⁻¹ band is the only band in this spectrum of *Bt* in H₂O buffer that is unambiguously assignable to *Bt*. Immediately after mixing the stock *Bt* suspension with D₂O buffer, the 0.4 hour spectrum was collected. The 1048 cm⁻¹ *Bt* band lost all of its intensity and was eliminated in this spectrum. The HDE process in the sample suspension was allowed to proceed at room temperature for 9.0 h without laser irradiation, then another Raman spectrum was collected. After 9.5 h of HDE, a 1044 cm⁻¹ band of reduced intensity, relative to the 1048 cm⁻¹ in H₂O buffer, appeared in the spectrum.

Figure 1b shows the 222 nm excitation spectra of *Pseudomonas fluorescens* (*Pf*, ATCC 13525) in H₂O and D₂O buffer are dominated by the 981 cm⁻¹ SO₄²⁻ symmetric stretching vibration of the absolute differential Raman cross-section standard. The 1048 cm⁻¹ band is the only band in this spectrum of *Pf* in H₂O buffer that is unambiguously assignable to *Pf*. This 1048 cm⁻¹ appears on a very broad spectral artifact centered at 1078 cm⁻¹. Immediately after mixing the stock *Pf* suspension with D₂O buffer, the 0.4 h spectrum was collected. The 1048 cm⁻¹ *Pf* band lost all of its intensity and was eliminated in this spectrum. The HDE process in the sample suspension was allowed to proceed at room temperature for 9.0 h without laser irradiation, then another Raman spectrum was collected. After 9.5 h of HDE, a 1050 cm⁻¹ band of reduced intensity, relative to the 1048 cm⁻¹ band in H₂O buffer, appeared in the spectrum. The 1050 cm⁻¹ band isn't significantly shifted from the 1048 cm⁻¹ band; however, the shape of the 1050 cm⁻¹ band has broadened relative to the 1048 cm⁻¹ band in H₂O.

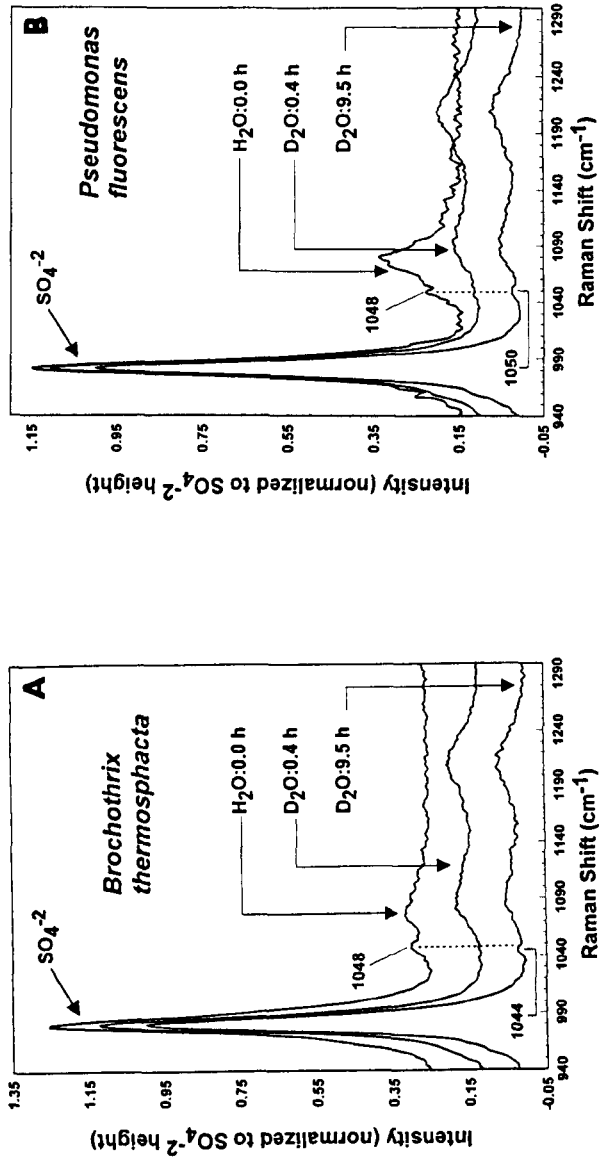


FIG. 1. THE 222 nm EXCITATION RESONANCE RAMAN SPECTRA OF (A) *BROCHOTHRIX THERMOSPRACTA* (ATCC 11509) AND (B) *PSEUDOMONAS FLUORESCENS* (ATCC 13525) IN H₂O OR D₂O BUFFER AT pH(D) 7.25 CONSISTING OF 0.15 M Na₂SO₄ AND 0.2 M Na₂H₂PO₄. The HDE times are specified with T₀ at the time of mixing the bacterial suspensions with D₂O buffer. The height of each spectrum was scaled to bring the height of the 981 cm⁻¹ SO₄⁻² band to 1.00.

These results for pure cultures of *Bt* and *Pf* are summarized in Table 1. To our knowledge, this is the first time that absolute differential Raman cross-sections have been reported for any bacteria. The large absolute differential Raman cross-sections result from the millions of potential chromophores comprising each bacterium. Two other important features are evident in Table 1. This establishes a new basis for the specific IdQ of *Bt* and *Pf*. The first is that the height of the observed vibrational bands of the bacteria, relative to the absolute differential Raman cross section standard, change as a function of HDE time. The second is that while the HDE changes the position of the observed *Bt* vibrational band, this does not occur for *Pf*. We expect to observe similar differences in the behavior of HDE induced changes in the resonance Raman spectra of other bacteria species because of the differences in their biomolecular composition.

TABLE 1.
ABSOLUTE DIFFERENTIAL RAMAN CROSS SECTIONS OF BACTERIA *BROCHOTHRIX THERMOSPACTA* AND *PSEUDOMONAS FLUORESCENS*, 222nm LASER EXCITATION

Height of the SO_4^{2-} 981 cm^{-1} band = $1.00 \text{ } 2\text{-}50 \times 10^6$ bacteria/mL

Bacterium	H-D Exchange time (Hours)	Frequency cm^{-1}	Height	Cross Section, $\text{cm}^2/(\text{bacteria-steradian})$
<i>BT</i> (H_2O)	-	1048	0.027	$1.2\text{-}30 \times 10^{-17}$
<i>BT</i> (D_2O)	.3	1044	0	0
<i>BT</i> (D_2O)	9.5	1044	0.014	$0.60\text{-}15 \times 10^{-17}$
<i>PF</i> (H_2O)	-	1048	0.030	$1.3\text{-}33 \times 10^{-17}$
<i>PF</i> (D_2O)	.3	1050	0	0
<i>PF</i> (D_2O)	9.5	1050	0.013	$0.56\text{-}14 \times 10^{-17}$

The HDE effects on the spectra of beef menstria samples were next examined to determine whether or not UVRR spectra could be collected from these highly turbid suspensions and to measure the extent of the shifting in the frequencies and intensities of Raman bands in the spectra. Figure 2 shows the effects of HDE on the 222 nm excitation UVRR spectrum of a beef menstria/bacteria suspension. This suspension is typical of the samples obtained from the surface of a carcass acquired according to the method of the USDA Food Safety and Inspection Service (FSIS).

The topmost spectrum is that of a beef menstrua-bacteria suspension in H₂O buffer. Besides the 981 cm⁻¹ SO₄⁻² absolute differential Raman cross-section band, there are many menstrua-bacteria Raman bands present. There is a broad shoulder on the side of the 981 cm⁻¹ band at 1003 cm⁻¹, a broad band at 1173 cm⁻¹, and a band at 1556 cm⁻¹ due mainly to the scattering of O₂ in the air. A broad band centered at 1640 cm⁻¹ is in part attributable to the H₂O bending mode and the peptide amide I mode of proteins. The height of this 1640 cm⁻¹ band is about equal to the 981 cm⁻¹ SO₄⁻² standard. Throughout the entire observed range, structure of low (<0.05) height relative to the 981 cm⁻¹ stand is observed. The spectrum of beef menstrua-bacteria subjected to 15 min of HDE shows a reduction in the height and/or broadening of the 1173 cm⁻¹ band and a reduction in the height of the 1640 cm⁻¹ band relative to the 981 cm⁻¹ standard. This reduction in the 1640 cm⁻¹ band is due in part to the replacement of the H₂O solvent for D₂O, diminishing the contribution of the H₂O bending mode to this Raman band and is not considered significant in bacterial IdQ. After 15 min of HDE, a weak band at 1151 cm⁻¹ appears. As HDE progresses, we observe a general increase in this 1151 cm⁻¹ band, the appearance of a 1054 cm⁻¹ band, and continuous diminishment in the 1173 cm⁻¹ band. The 1003 cm⁻¹ band that was approximately one-half the height of the 981 cm⁻¹ standard before HDE, is considerably reduced in height and shifted to 1007 cm⁻¹ after 115 min of HDE. The increase in structure in the 92, 102, and 115 min spectra is due in part to the reduction in the slit width from 200 microns to 75 microns. This reduction in the slit width was performed because of the appearance of the 1054 and 1151 cm⁻¹ bands at 81 min, to take advantage of the increase in the resolution in the Raman spectrometer.

The observed response of three selected Raman bands of beef menstrua to HDE from Fig. 2 are summarized in Fig. 3. We observe three distinct behaviors of the heights or normalized heights of these Raman bands as a function of HDE time. While the height of the 1003-7 cm⁻¹ band normalized to its value in H₂O buffer tends to increase its magnitude with HDE time, the height of the 1151 cm⁻¹ band normalized to its value in H₂O buffer tends to decrease its magnitude with HDE time. While the height of the 1054 cm⁻¹ band tends to increase with HDE, its time-dependent behavior is different from the 1151 cm⁻¹ band. The different behaviors of these three Raman bands exhibit distinctive patterns expected for an unambiguous identification.

Figures 2 and 3 show HDE has a dramatic impact on the UVRR spectra of the beef menstrua-bacteria suspension. With these preliminary data, we cannot attribute any Raman bands observed solely to bacteria or bacteria-free beef menstrua. However, these data suggest a novel way to separate the Raman signal of one biomolecular component with exchangeable protons, such as "target" bacteria, from the Raman spectrum of a complex mixture where the Raman spectra of the individual components overlap. HDE changes the intensity and frequency of some of the Raman bands in the UVRR spectra of all of the samples that we have tested.

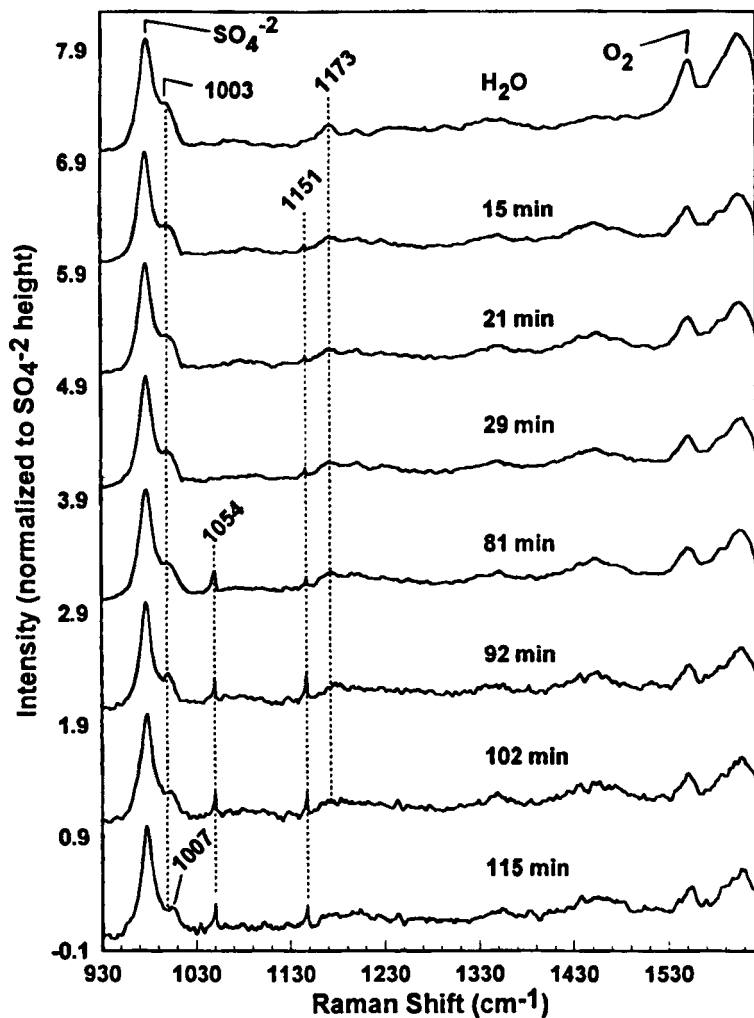


FIG. 2. THE 222 nm EXCITATION RESONANCE RAMAN SPECTRA OF BEEF CARCASS SPONGE SAMPLES (BEEF MENSTRUUA) IN AN H₂O OR D₂O BUFFER AT pH(D) 7.25 CONSISTING OF 0.15 M Na₂SO₄ AND 0.2 M Na₂H₂PO₄. The HDE times are specified with T₀ at the time of mixing the menstrua suspensions with D₂O buffer. The height of each spectrum was scaled to bring the height of the 981 cm⁻¹ SO₄²⁻ band to 1.00.

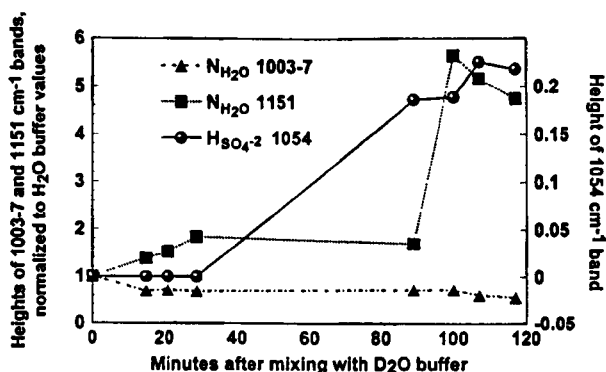


FIG. 3. RESPONSE OF SELECTED BEEF MENSTRUA RAMAN BANDS FROM FIG. 2 TO HDE. The heights of the 1003-7 cm^{-1} and 1151 cm^{-1} bands are normalized to their values in H_2O buffer, while the height of the 1054 cm^{-1} band is normalized to the height Raman cross section standard SO_4^{2-} at 981 cm^{-1} .

A neural net may be trained to identify these components in complex mixtures by acquiring the HDE time-dependent spectra of the components separately, and in combination at different concentrations.

The implications of combining the selectivity afforded by HDE and UVRR are clear. This dynamic method increases the probability of finding a unique UVRR fingerprint, or series of fingerprints, of bacteria that can be extracted from the Raman spectra of a complex mixture. The HDE rate in biomolecules has been shown to depend upon many chemical and physical factors, and therefore, the HDE time-dependent behavior of the UVRR spectra will depend parametrically on these factors. When intact bacteria are initially subjected to HDE, some of the bacterial components on the exterior surface will be the first to undergo HDE. It is reasonable to surmise that some of the more protected bacterial components in the cytoplasm of the bacteria will exchange more slowly than the exterior components depending on the permeability of the cell. Chemical and physical factors provide a high degree of variability in the HDE process. This variability compliments the selectivity afforded by UVRR. Changing the laser excitation frequency selectively enhances the scattering from nucleotides (Ziegler *et al.* 1984), nucleic acids (Benevides and Thomas, Jr. 1985; Kubasek *et al.* 1985; Hudson and Mayne 1986; Manoharan *et al.* 1990), amino acids (Asher *et al.* 1986; Hildebrandt *et al.* 1989); and peptides (Rava and Spiro 1985; Mayne and Hudson 1987) are selectively enhanced. As a consequence, the combination of HDE and UVRR spectroscopy for bacterial IdQ provides more "new" dimensions than the single dimension of HDE time.

A critical consideration for application of any bacterial detection system as a HACCP monitor in food production is the length of time required for initial detection. The data presented here allow an estimate of the time required to collect an UVRR spectrum. Assuming a concentration of 100 bacteria/mL, an estimated Raman cross-section of 6×10^{-17} cm²/steradian-bacteria for Raman band X, a 25 milliWatt 210 nm excitation beam, 27% detector quantum efficiency for a CCD detector, 7% monochromator efficiency, a 0.6π steradian solid angle of collection of the Raman light from the sample, and a $3 \times 3 \times 2$ mm Raman scattering volume, we calculate using the formulas discussed in Bilhorn *et al.* (1987) and Schwab *et al.* (1986) it will take 115 s to acquire an UVRR spectrum with signal to noise ratio of 10 for Raman band X. Spectral acquisition times on this timescale may make it possible to analyze food samples on the food processing lines, as opposed to in the laboratory. In addition, 100 bacteria/mL is substantially below that required for more commonly applied techniques of bacterial identification, most or all of which require an incubation period to allow growth of the bacteria to a detectable level.

This dynamic method suggests a potential means to identify bacteria in complex menstua without time-consuming culture steps. Further research will determine the levels of sensitivity in both numerical (i.e., lowest cell density needed for an identifiable signal) and taxonomic terms (i.e., phylogenetic level, e.g., genus, species, subspecies). In addition, the distinction between viable, viable but not culturable, and dead cells will be explored. However, recognition of time-evolving, HDE-induced changes in the UVRR spectral fingerprints of bacteria from complex menstua represents the first step in the rapid spectral analysis of biological samples for bacterial analytes.

REFERENCES

- ANON. 1996. Part II, United States Department of Agriculture. 9 CFR Part 304, *et al.* Pathogen reduction; hazard analysis and critical control point (HACCP) systems; final rule. Federal Register *61*, 38806-38989.
- ASHER S.A., LUDWIG, M. and JOHNSON, C.R. 1986. UV Resonance Raman excitation profiles of the aromatic amino acids. *J. Am. Chem. Soc.* *108*, 3186-3197.
- BENEVIDES, J.M. and THOMAS, JR., G.J. 1985. Dependence of Purine 8C-H exchange on nucleic acid conformation and base-pairing geometry: A dynamic probe of DNA and RNA secondary structures. *Biopolymers* *24*, 667-682.
- BILHORN, R.B., SWEEDLER, J.V., EPPERSON, P.M. and DENTON, M.B. 1987. Charge transfer device detectors for analytical optical spectroscopy--Operation and characteristics. *Appl. Spectrosc.* *41*, 1114-1125.

- DUDIK, J.M., JOHNSON, C.R. and ASHER S.A. 1985. Wavelength dependence of the preresonance Raman cross sections of acetonitrile, sulfate ion, perchlorate ion and nitrate ion. *J. Chem. Phys.* **82**, 1732-1740.
- ENGLANDER, S.W. and KALLENBACH, N.R. 1984. Hydrogen exchange and structural dynamics of proteins and nucleic acids. *Q. Rev. Biophys.* **16**, 521-655.
- ENGLANDER, S.W. and MAYNE, L. 1992. Protein folding studied using hydrogen-exchange labeling and two-dimensional NMR. *Annu. Rev. Biophys. Biomol. Struct.* **21**, 243-265.
- HARHAY, G.P. Resonance Raman spectroscopy for identifying and quantitating biomatter, organic, and inorganic analytes. U.S. Patent and Trademark Office: Harhay, Gregory P., submitted-1996, approved, 1997.
- HARHAY, G.P. and HUDSON, B.S. 1993. Quantitative resonance Raman spectroscopy of N-acetylpyrrolidine in aqueous solution. *J. Phys. Chem.* **97**, 8158.
- HILDEBRANDT, P., CZERNUSZEWICZ, R.S., GRYGON, C.A. and SPIRO, T.G. 1989. Ultraviolet resonance Raman enhancement of carboxylate and guanidinium groups in amino acids. *J. Raman. Spectrosc.* **20**, 645.
- HOWARD, R. and MAYNARD, M. 1986. Correction for gain variation in microchannel plate intensified detectors. *Appl. Spectrosc.* **40**, 1245-1246.
- HUDSON, B. and MAYNE, L. 1986. Ultraviolet resonance Raman spectroscopy of biopolymers. *Meth. Enzymol.* 331-350.
- JACOBSSON, S.P. and HAGMAN, A. 1993. Chemical-composition analysis of carrageenans by infrared-spectroscopy using partial least-squares and neural networks. *Anal. Chim. Acta* **284**, 137-147.
- JASPER, W.J. and KOVACS, E.T. 1994. Using neural networks and Nir spectrophotometry to identify fibers. *Text. Res. J.* **64**, 444-448.
- KUBASEK, W.L., HUDSON, B. and PETICOLAS, W.L. 1985. Ultraviolet resonance Raman excitation profiles of nucleic acid bases with excitation from 200 to 300 nanometers. *Proc. Natl. Acad. Sci. USA* **82**, 2369-2373.
- LI, T., JOHNSON, J.E. and THOMAS, G.J. 1993. Raman dynamic probe of hydrogen exchange in bean pod mottle virus: Base-specific retardation of exchange in packaged ssRNA. *Biophys. J.* **65**, 1963-1972.
- MANOHARAN, R., GHAMATI, E., CHADHA, S., NELSON, W.H. and SPERRY, J.F. 1993. Effect of cultural conditions of deep UV resonance Raman spectra of bacteria. *Appl. Spectrosc.* **47**, 2145-2150.
- MANOHARAN, R., GHAMATI, E., DALTERIO, R.A., BRITTON, K.A., NELSON, W.H. and SPERRY, J.F. 1990. UV resonance Raman spectra of bacteria, bacterial spores, protoplasts and calcium dipicolinate. *J. Microbiol. Meth.* **11**, 1-15.
- MAYNE, L. and HUDSON, B. 1987. Applications of ultraviolet resonance Raman spectroscopy to protein structure. *AIP Conf. Proc.-Adv. Laser Sci.* **2**, 583-585.

- MAYNE, L.C., ZIEGLER, L.D. and HUDSON, B. 1985. Ultraviolet resonance Raman studies of N-methylacetamide. *J. Phys. Chem.* **89**, 3395-3398.
- MITTERMAYER, C.R., DROUEN, A., OTTO, M. and GRASSERBAUER, M. 1994. Neural networks for library search of ultraviolet-spectra. *Anal. Chim. Acta* **294**, 227-242.
- MYERS, A.B. and MATHIES, R.A. 1987. Resonance Raman intensities: A probe of excited-state structure and dynamics. In *Biological Applications of Resonance Raman Spectroscopy*, (T.G. Spiro, ed.) pp. 1-58, Wiley, New York.
- NELSON, W.H., DALTERIO, R.A. and PEACEDALE, S. 1989. Detection and identification of bacteria by means of ultra-violet excited resonance Raman spectra. U.S. Patent and Trademark Office, Patent 4,847,198: The Board of Governors for Higher Education, State of Rhode Island and Providence Plantations.
- NELSON, W.H., MANOHARAN, R. and SPERRY, J.F. 1991a. UV resonance Raman studies of bacteria. *Appl. Spectrosc. Rev.* **27**, 67-124.
- NELSON, W.H., SPERRY, J.F. and NELSON, W.H. 1991b. UV resonance Raman spectroscopic detection and identification of bacteria and other microorganisms. *Mod. Tech. Rapid Microbiol. Anal.*, P97 **143**, 97-143.
- RAVA, R.P. and SPIRO, T.G. 1985. Ultraviolet resonance Raman spectra of insulin and α -lactalbumin with 218 and 200-nm laser excitation. *Biochemistry* **24**, 1861-1865.
- SCHWAB, S.D., McCREERY, R.L. and GAMBLE, F.T. 1986. Normal and resonance Raman spectroelectrochemistry with fiber optic light collection. *Anal. Chem.* **58**, 2486-2492.
- SIRAGUSA, G.R., CUTTER, C.N., DORSA, W.J. and KOOHMARAIE, M. 1995. Use of a rapid microbial ATP bioluminescence assay to detect contamination on beef and pork carcasses. *J. Food Prot.* **58**, 770-775.
- SWAMINATHAN, B. and FENG, P. 1994. Rapid detection of food-borne pathogenic bacteria. *Annu. Rev. Microbiol.* **48**, 401-426.
- TANABE, K., TAMURA, T. and UESAKA, H. 1992. Neural network system for the identification of infrared spectra. *Appl. Spectrosc.* **46**, 807-810.
- TANABE, K., UESAKA, H., INOUE, T., TAKAHASHI, H. and TANAKA, S. 1994. Identification of mineral components from near-infrared by a neural-network. *Bunseki Kagaku* **43**, 765-769.
- WIENKE, D. and KATEMAN, G. 1994. Adaptive resonance theory-based artificial neural networks for treatment of open-category problems in chemical-pattern recognition--application to UV-Vis and IR spectroscopy. *Chemometrics Intelligent Lab. Syst.* **23**, 309-329.
- ZIEGLER, L.D., HUDSON, B., STROMMEN, D.P. and PETICOLAS, W.L. 1984. Resonance Raman spectra of mononucleotides obtained with 266 and 213 nm ultraviolet radiation. *Biopolymers* **23**, 2067-2081.