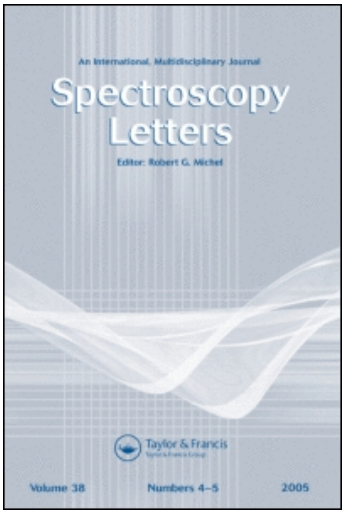

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Raman Spectroscopy for the Undergraduate Teaching Laboratory: Quantification of Ethanol Concentration in Consumer Alcoholic Beverages and Qualitative Identification of Marine Diesels Using a Miniature Raman Spectrometer

Danielle Cleveland[a]; Matthew Carlson[ab]; Evan D. Hudspeth[ac]; Lauren E. Quattrochi[ad]; Kathleen L. Batchler[ae]; Shrimati A. Balram[a]; Seongun Hong[a]; Robert G. Michel[a]
[a] Department of Chemistry, University of Connecticut, Storrs, Connecticut, USA [b] Department of Chemistry, University of California-Irvine, Irvine, CA [c] Science Department, United States Coast Guard Academy, New London, CT [d] Department of Chemistry & Biochemistry, Providence College, Providence, RI [e] Pfizer Inc., Groton, CT

To cite this Article Cleveland, Danielle , Carlson, Matthew , Hudspeth, Evan D. , Quattrochi, Lauren E. , Batchler, Kathleen L. , Balram, Shrimati A. , Hong, Seongun and Michel, Robert G.(2007) 'Raman Spectroscopy for the Undergraduate Teaching Laboratory: Quantification of Ethanol Concentration in Consumer Alcoholic Beverages and Qualitative Identification of Marine Diesels Using a Miniature Raman Spectrometer', Spectroscopy Letters, 40: 6, 903 — 924
To link to this Article: DOI: 10.1080/00387010701525638
URL: http://dx.doi.org/10.1080/00387010701525638

Spectroscopy Letters, 40: 903–924, 2007

ISSN 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010701525638

Raman Spectroscopy for the Undergraduate Teaching Laboratory: Quantification of Ethanol Concentration in Consumer Alcoholic Beverages and Qualitative Identification of Marine Diesels Using a Miniature Raman Spectrometer

Danielle Cleveland, Matthew Carlson[†], Evan D. Hudspeth[§], Lauren E. Quattrochi[#], Kathleen L. Batchler[‡], Shrimati A. Balram, Seongun Hong, and Robert G. Michel
Department of Chemistry, University of Connecticut, Storrs, Connecticut, USA

Abstract: Raman spectroscopy has steadily gained popularity as a powerful tool in both the analytical lab and the undergraduate classroom. The technique is attractive because it allows for rapid, nondestructive qualitative or quantitative analyses of many analytes with little or no sample preparation requirements. The introduction of less expensive, smaller, and more powerful diode laser excitation sources and the recent availability of rugged, red-sensitive, charge-coupled device–based miniature modular spectrometers has prompted the integration of Raman spectroscopy into the

Received 17 February 2007, Accepted 11 April 2007
This contribution is part of a special section of *Spectroscopy Letters* on "Spectroscopy in Undergraduate Education".

[†]Present address: Department of Chemistry, B-35 Rowland Hall, University of California-Irvine, Irvine, CA 92697.
[§]Present address: United States Coast Guard Academy, Science Department, 316 Smith Hall, 27 Mohegan Ave., New London, CT 06320.
[‡]Present address: Department of Chemistry & Biochemistry, Providence College, Providence, RI 02918.
[#]Present address: Pfizer Inc., Eastern Point Road, MS 4135, Groton, CT 06340.
Address correspondence to Robert G. Michel, Department of Chemistry, University of Connecticut, 55 North Eagleville Road, Storrs, CT 06269, USA. E-mail: robert.g.michel@uconn.edu

undergraduate curriculum. We have evaluated the analytical utility of a small, portable Raman instrument for the qualitative and quantitative analyses of two "real" samples. The experiments in this paper were designed to be used as a laboratory component for undergraduate education and include the quantification of ethanol in consumer alcoholic beverages and the qualitative identification of marine diesel fuels that had been spilled on surface waters. In the case of the liquor samples, the ethanol concentration in colorless, odorless alcoholic beverages could be determined very rapidly, but colored and heavily scented liquors proved more difficult and required pretreatment with activated carbon to remove fluorescence that masked the Raman signal. Similarly, a high-intensity fluorescence background was observed to mask characteristic Raman bands of the diesel fuels. Some reduction in the intensity of the fluorescence was observed after carbon pretreatment of the fuels. The set of undergraduate experiments described in this paper treat the concepts of quantitative and qualitative analysis using portable instrumentation, instrumental calibration by the standard addition and external curve methods, and method development for the analysis of real consumer and environmental samples.

Keywords: Calibration curve, consumer products, environmental samples, ethanol in alcoholic beverages, fuel oil spills, method development, petroleum products, portable Raman instrumentation, qualitative analysis, quantitative analysis, Raman spectroscopy, standard addition, surface waters, undergraduate experiments

INTRODUCTION

A recent effort toward the integration of CCD-based modular Raman spectroscopy into an undergraduate setting is evidenced by an increasing number of publications dedicated to the subject.[1–4] Traditionally, Raman spectroscopy has been precluded from the undergraduate curriculum because of the high cost of reliable, high-resolution, benchtop Raman spectrometers. However, new technology including sensitive low-noise CCD detectors, compact solid-state diode lasers, efficient holographic notch filters, and fiber-coupled filtered probes[5–8] has allowed the manufacture of cost-effective, compact, portable, and rugged Raman instruments. These miniaturized spectrometers can be used for on-site, *in situ*, real-time monitoring and offer rapid and nondestructive qualitative and quantitative analyses of a variety of analytes, often with little or no sample preparation requirements. One approach for the pedagogical use of Raman spectroscopy has been described in depth elsewhere.[9] In the current paper, a portable Raman spectrometer was evaluated for possible integration into the undergraduate curriculum for the analysis of two real samples, namely ethanol in alcoholic beverages and diesel oils that had been spilled on surface waters.

Ethanol in Alcoholic Beverages

Various methods have been explored for the determination of ethanol content in commercial as well as illegally produced alcoholic beverages.

The quantification of ethanol is important for quality control and product labeling in the food industry and for taxation purposes under regulation by various government agencies. For example, wine is defined in the Federal Alcohol Administration Act as, among other things, containing not less than 7% and not more than 24% alcohol by volume [27 U.S.C. 211(a)(6)].[10]

Traditionally, the coupling of direct injection or headspace gas chromatography (GC) with flame ionization or mass spectrometric (MS) detection has been the laboratory standard for determination of ethanol and other components in alcoholic beverages.[11–15] However, this process is often time-consuming, requiring sample preparation and extensive method development. Additionally, Mendes et al.[16] observed that the sugar content in various alcoholic beverages affected the quantification of ethanol by gas chromatography. The authors compared the intensities of the ethanol signal for ethanol-water mixtures with those of ethanol-water-sugar mixtures and observed that the intensities of the ethanol signals of the mixtures that contained sugar were lower than the signals from the mixtures without sugar.

Flow injection and sequential injection analysis systems have been explored for use in ethanol determination in an attempt to automate the process and make it suitable for on-line process control. Refractive index detection,[17] enzymatic spectrophotometric detection,[18–20] and gas-permeable membrane flame ionization detection[21] have been successfully utilized as flow injection methods. Ion-selective electrodes[22] and titrimetric methods,[23] as well as microbe-based biosensors,[24] have also been introduced. The use of proton nuclear magnetic resonance (^{1}H-NMR) has also been explored for the determination of ethanol content in liquors,[25] but the utility of the method was thought to be limited due to the presence of interfering peaks at the same positions as the methyl peaks of ethanol. Further, the authors observed no significant difference between the values obtained from ^{1}H-NMR and GC at a confidence levels of $>95\%$. However, it was noted that the ^{1}H-NMR method was advantageous because it required no sample pretreatment, even for highly colored samples, which is often not the case for GC methods.

In general, spectroscopic techniques offer a simpler and more rapid approach to the determination of ethanol content compared with more traditional analytical methods listed above. Frausto-Reyes et al.[26] used Raman spectroscopy combined with principal component analysis to qualitatively distinguish silver tequila from aged tequilas by observation of ethanol content. Mendes et al.[16] evaluated the use of Fourier transform Raman spectroscopy (FT-Raman) combined with partial least-squares calibration models for the quantification of ethanol in beverages. Spectra were preprocessed with 17-point smoothed second derivatives, and partial least-squares calibration models were used to quantify ethanol concentration. The second-derivative spectra successfully reduced the fluorescence background from the beverage samples to allow for quantification of ethanol.

Nordon et al.[27] compared the use of noninvasive near-infrared (NIR) and Raman spectroscopies for the determination of ethanol concentration in whiskey, vodka, and sugary alcoholic beverages. Although the NIR spectra were dominated by water and ethanol concentrations had to be derived by multivariate methods, the Raman spectra offered direct correlation of signal strength with ethanol concentration. Unfortunately, high-intensity fluorescence backgrounds were observed for the whiskey and the alcoholic beverages with high sugar content. The fluorescence was so high for the sugary beverages that quantification by direct Raman spectroscopy was impossible. However, the authors were able to successfully quantify the ethanol concentration in all three types of alcoholic beverages using univariate analysis of the signal intensity at 873 cm^{-1} in the first-derivative spectra.

A first approach to integrating Raman spectroscopy into the undergraduate laboratory for the quantification of ethanol in alcoholic beverages was done by Sanford et al.[3] using a laboratory-constructed CCD-based modular Raman instrument with a 488-nm argon-ion laser excitation source. In their work, the observed Raman shift at 2941 cm^{-1}, which was attributed to the C-H stretch in ethanol, was used to create a calibration curve for ethanol content by plotting peak area as a function of percent ethanol in solution. The authors then examined the ethanol content in whiskey, vodka, clear rum, gin, and neutral grain spirits without any sample preparation requirements. Broadband fluorescence was observed for the gin and whiskey samples, which was attributed to the presence of other organic hydrocarbon compounds in the liquor samples. The limit of detection for ethanol was determined to be 1%.

In the current paper, the ethanol content of various commercial alcoholic beverages was determined using portable, low-cost Raman instrumentation. A determination of ethanol content in various commercially available alcoholic beverages was performed using 532-nm and 785-nm excitation Raman spectroscopy. The quantitative results were considered for use in an undergraduate teaching setting. Further, a method to remove fluorescent compounds from the samples was presented, and a comparison of external aqueous calibration with the standard addition method was done.

Fingerprinting of Fuels Spilled in Water

The qualitative determination of oils and fuels spilled on surface waters is a second example of real sample analysis that might be readily inserted into the undergraduate curriculum. During oil spill situations in open waters, the United States Coast Guard seeks to rapidly and irrefutably identify the source of the oil spill. Gas chromatography (GC), infrared spectroscopy (IR), fluorescence spectroscopy, and gas chromatography with mass spectrometric detection (GC-MS) are routinely used offsite in the U.S. Coast Guard Marine Safety Laboratory as part of a multiple-detection method approach to fingerprint petroleum products and to subsequently identify offending ships. There have

been many recent Raman studies on fuel additives and adulterants for quality control,[28–30] examinations of the structure and composition of hydrocarbon fractions,[31–33] and efforts to determine the concentration of petroleum that had been spilled on water.[34] However, only limited studies have been completed for the use of Raman spectroscopy for *identification of* and *discrimination* among petroleum products that have been spilled on water.[35,36]

Raman spectroscopy of oils is problematic under even ideal conditions, as oils are known to be weak Raman scatterers that often give high-intensity fluorescent backgrounds. Also, the relatively high volatility of oils and petroleum products causes problems as the sample can be vaporized in the laser beam. Ahmadjian and Brown[36] attempted to use Raman spectroscopy to fingerprint and differentiate among kerosene, no. 2 fuel oil, and lubricating oil. The authors found that the addition of powdered charcoal to the samples reduced the intensity of the fluorescence of the sample during measurement of Raman signals. Using gas chromatography and infrared spectroscopy, the authors determined that the charcoal treatment did not significantly change the composition of the fuels. Also, the authors examined charcoal-treated samples taken from an oil spill and compared the Raman signatures with oil from two suspected sources. The authors were able to correctly match the spilled oil to the source of the spill by careful examination of the Raman bands of the samples.

In other work,[35] Ahmadjian and Brown examined the feasibility of remote detection of oils slicks by Raman spectroscopy. Raman bands corresponding with the C-H stretch in the 2830–2870 cm^{-1} region were readily observed for an oil sample, while the oil was on the surface of water, using both conventional and long-path remote Raman experimental arrangements. Spectra exhibited the same Raman shifts whether the oil was sampled neat or observed on the surface of water. Indeed, remote Raman analysis has been applied successfully to process monitoring applications in the petroleum industry.[37,38] However, those studies focused on the differentiation of pure analytes inside a process pipeline rather than on materials that have been spilled in the environment. In the current work, the use of Raman spectroscopy for the *in situ* identification of spilled fuel oils was investigated both as an undergraduate teaching exercise and as an experimental technique that might be used by the U.S. Coast Guard in a complementary way to the traditional laboratory-based GC, GC-MS, IR, and fluorescence spectroscopic methods.

MATERIALS AND METHODS

Laser Raman Spectroscopy

The experimental arrangement used in this paper has been described in detail elsewhere.[9] Briefly, a BWT-40-OEM diode pumped solid-state green laser

(B&W Tek, Inc., Newark, DE, USA), provided 40-mW output power and 532 nm to the sample through a 90-μm-diameter, 1.5-m-long excitation fiberoptic cable connected to an RPB-532 Raman Probe (InPhotonics, Inc., Norwood, MA, USA). The probe and standard sample cells were held in place by a lab-constructed probe and sample cell holder designed to provide reproducibility of sample cell placement and light source positioning. The Raman scattered light was then transmitted through a 200-μm-diameter, 1.5-m-long collection fiberoptic cable to the BTC110E miniature TE-cooled, fiber-coupled 2048-element linear silicon array CCD spectrometer (B&W Tek, Inc.). The spectrometer was configured with a 10-μm slit and a fixed 1800 lines mm^{-1} diffraction grating blazed at 500 nm. Spectrometer to computer communication was provided by an RS-232 cable, via the BW-Spec software, which was included on a CD for use with a Microsoft Windows–based computer. An integration time of 7 s was used to collect the Raman signals unless otherwise noted.

A second portable laser–spectrometer system delivered red radiation to the sample cell. Here, a BWT-250-OEM diode pumped solid-state red laser (B&W Tek, Inc.), provided 250-mW output power at 1.8 A and 785 nm to the sample through a 90-μm-diameter, 1.5-m-long excitation fiberoptic cable connected to an RPB-785 Raman Probe (InPhotonics, Inc.). As before, the probe and standard sample cells were held in place by a lab-constructed probe and sample cell holder designed to provide reproducibility of sample cell placement and light source positioning. The Raman scattered light was then transmitted through a 200-μm-diameter, 1.5-m-long collection fiberoptic cable to a BTR111 P-785 miniature TE-cooled, fiber-coupled, 2048-element linear silicon array CCD spectrometer (B&W Tek, Inc.). This spectrometer had a 5 V DC power input at less than 1.2 A and an operating temperature range of 15–35°C. The spectrometer had a slit width of 25 μm, and a diffraction grating with 1200 lines mm^{-1} blazed at 750-nm. Spectrometer to computer communication was provided by a high-speed USB cable, via the BW-Spec software. An integration time of 30 s was used to collect the Raman signals unless otherwise noted. For all experiments, a sample blank was recorded and automatically subtracted from the Raman signal of the analyte using the BW-Spec software. The details of both Raman systems are summarized in Table 1. Three trials were performed for each sample.

Samples

Various types of alcoholic beverages, including vodka, rum, gin, and other liquors, were obtained from local commercial vendors and analyzed for ethanol content. Aqueous calibration standards, ranging in concentration from 0 to 100% ethanol, were prepared using standard ACS/USP grade 200-proof ethanol (Pharmco Products, Inc. Brookfield, CT, USA). In

Table 1. Specifications of the portable Raman instrumentation

Parameter	Raman system 1 (green)	Raman system 2 (red)
Excitation wavelength (nm)	532	785
Laser output power (mW)	40	250
Laser linewidth (nm)	0.1	0.3
Diameter, Raman excitation fiber (μm)	90	90
Length, Raman excitation fiber (m)	1.5	2
Diameter, Raman collection fiber (μm)	200	200
Length, Raman collection fiber (m)	1.5	2
Model, fiber-coupled CCD spectrometer	TE-cooled, BTC110E	TE-cooled, BTC111E
CCD type	2048-element linear silicon array	2048-element linear silicon array
CCD spectral resolution	0.66 at 546.1 nm	0.75 at 772.5 nm
CCD entrance slit width (μm)	10	25
CCD grating (lines/mm)	1800	1200
CCD wavelength range (nm)	500–650	780–1050
CCD high pass filter (nm)	495	570

between scans, the cuvette was rinsed twice using deionized water and then once with acetone. The cuvette was then dried in a low-flow nitrogen stream. Three marine diesel fuels, "SH" Diesel, "SK" Diesel, and "Helcat" Diesel, were on loan from the U.S. Coast Guard Marine Safety Laboratory (Groton, CT, USA). Automotive diesels were obtained commercially from gas stations around Connecticut.

Sample Pretreatment

Some liquor and oil samples were treated with a color-removal procedure prior to analysis. This procedure is similar to a method devised by Ahmadjian and Brown for use with petroleum and oil samples.[36] For liquor samples, 0.5 g of NORIT 211 decolorizing active carbon (Acros Organics, Morris Plains, NJ, USA) was thoroughly mixed with 5 mL of calibration standard or unknown sample solution for about 2 min in a covered 13 × 100 mm test tube. It should be noted that the liquor samples were kept tightly covered as much as possible during the entire pretreatment procedure in an effort to prevent loss of ethanol by evaporation into the air. The covered solutions were then centrifuged at 4000 rpm for 25–35 min, until the supernatants were relatively transparent. The supernatants were drawn from the test tube and placed in a cuvette for immediate analysis. In the case of the oil samples, the pretreatment procedure was modified to maximize the loss of

volatile compounds from the oils. The removal of lower boiling hydrocarbons from the oils, by evaporation, served to minimize difference in the degrees of weathering between oil samples.[36] In this way, the laboratory standards were made to mimic spill and suspect spill source samples that had been exposed to the environment. A 1-mL aliquot of the unknown diesel sample was thoroughly mixed with pentane in a 1 to 2 ratio to decrease the viscosity of the oil. Two grams of powdered charcoal were added, and the diesel-pentane solution was stirred for approximately 2 min. A standard Buchner funnel/vacuum flask assembly was used to remove the solid charcoal from the diesel solution. The resulting solution was allowed to sit, uncovered, in a fume hood overnight to allow the pentane to evaporate. This procedure yielded pale-yellow colored oil samples.

RESULTS AND DISCUSSION

The experiments described here were designed for use in an undergraduate teaching setting as examples of practical applications of Raman spectroscopy. The use of miniature, portable Raman instrumentation allows for lecture-style demonstrations of Raman principles, traditional benchtop laboratory work, and *in situ* field work. The experiments are suitable for classroom discussions of qualitative and quantitative analysis, calibration curves and limits of detection, assignment of Raman bands, the fundamental principles of Raman spectroscopy, and advances in modern instrumentation.

Quantification of Ethanol in Alcoholic Beverages

The use of Raman spectroscopy for the determination of ethanol content in alcoholic liquors was evaluated here as one example of real sample analysis that might be readily integrated into an undergraduate curriculum. One pedagogical approach to teaching the principles of Raman spectroscopy and a list of suitable analytes were presented by Hudspeth et al.[9] Initially, untreated samples of rum, vodka, gin, and Southern Comfort were evaluated for ethanol content using Raman spectroscopy with 532-nm excitation. Ethanol was quantified in the samples using both external aqueous calibration and the standard addition method. The principles of the external and standard addition methods of calibration have been covered at length in undergraduate textbooks on analytical 'chemistry,[39–41] and a discussion of these methods might usefully be included in the instruction at this point.

Briefly, external calibration is achieved by the careful preparation of a series of known concentrations, or standards, of the analyte of interest. It should be noted that although the calibration standards are prepared separately from the unknown sample, both the standards and the unknown sample should be processed in identical ways. The instrument response is measured for each

calibration standard, and a calibration curve that plots instrumental response as a function of analyte concentration is prepared. For example, the intensity of a particular Raman band that is unique to the analyte of interest can be recorded and plotted as a function of the concentration of the analyte in the standard. The curve can then be used to determine the concentration of the analyte in the unknown sample. In contrast, standard addition calibration is achieved by the addition of a known amount of standard to a known amount of sample containing the analyte of interest. The overall signal of the combined solutions is used to extrapolate the concentration of unknown that was originally present in the sample.

In the current work, external aqueous calibration was performed using both single and multiple Raman band approaches. Multiple-band external calibration is similar to the traditional, single-band calibration described above, except that the intensities of several Raman bands are used to normalize the intensity of the most intense Raman peak.[42] The normalized intensity is then plotted as a function of analyte concentration to create a calibration curve for quantification of the analyte of interest. It should be noted that for single-band calibration, the intensity of the Raman band at 888 cm^{-1} was used to generate the calibration curves and to quantify the ethanol in each sample in the current work. The peak at 888 cm^{-1} corresponded with a symmetric C-C-O stretch[43] and had a very strong relative intensity (Fig. 1). A prominent Raman band at 2854 cm^{-1} was rejected for

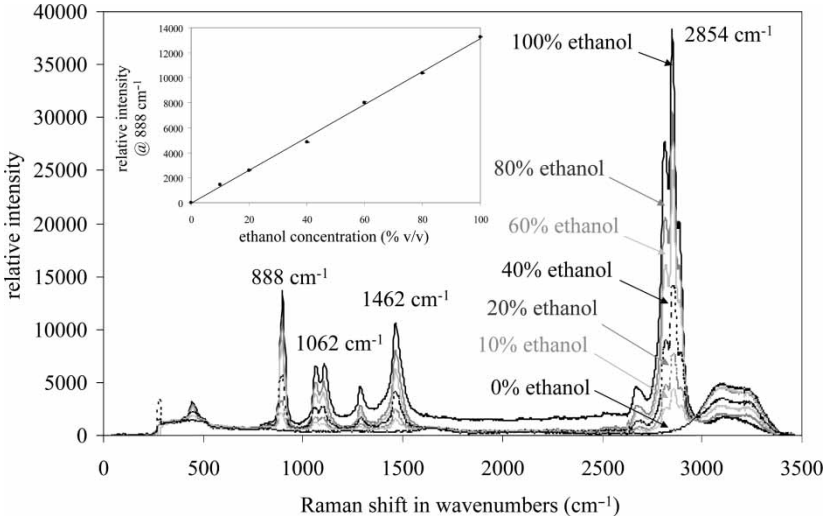

Figure 1. Typical Raman spectra of ethanol calibration standards obtained using excitation at 532 nm for untreated samples. The Raman band at 888 cm^{-1} was used for quantification. The inset shows a typical calibration curve of aqueous solutions of ethanol ranging in concentration from 0 to 100% v/v.

quantification purposes, as that spectral region corresponds with a C-H stretch, and therefore, the presence of other organic species might interfere with the ethanol signal in that region.[3,43] The intensities of the Raman peaks at 888, 1062, and 1462 cm^{-1} were used for the multiple-band normalization method. The band at 1062 cm^{-1} corresponded with an asymmetric C-C-O stretch, and the peak at 1462 cm^{-1} probably corresponded with a asymmetric CH_3 bend or a CH_2 vibration.[43] Both bands showed moderately strong relative intensities in the Raman spectrum of ethanol.

A typical set of Raman spectra for 532-nm excitation of untreated ethanol standards is shown in Fig. 1. The intensities of the Raman band at 888 cm^{-1} was plotted as a function of ethanol concentration in the standards to produce the inset calibration curve. As shown in Fig. 2, the use of 532-nm excitation was insufficient for quantification of the ethanol in untreated Southern Comfort and gin, as fluorescence from these samples obscured the true intensities of the Raman bands across the whole region of interest. Therefore, it was determined that, prior to quantification, the carbon treatment procedure described above should be attempted to remove fluorescence from the spectra in order to obtain more reliable quantification results. A summary of the results of the effect of carbon treatment on the quantification of ethanol in liquors by 532-nm excitation Raman is presented in Table 2. As shown in Fig. 2, for 532-nm excitation Raman, the carbon treatment process was able to remove some of the fluorescence from the Southern Comfort sample, such that some of the characteristic Raman bands for ethanol could

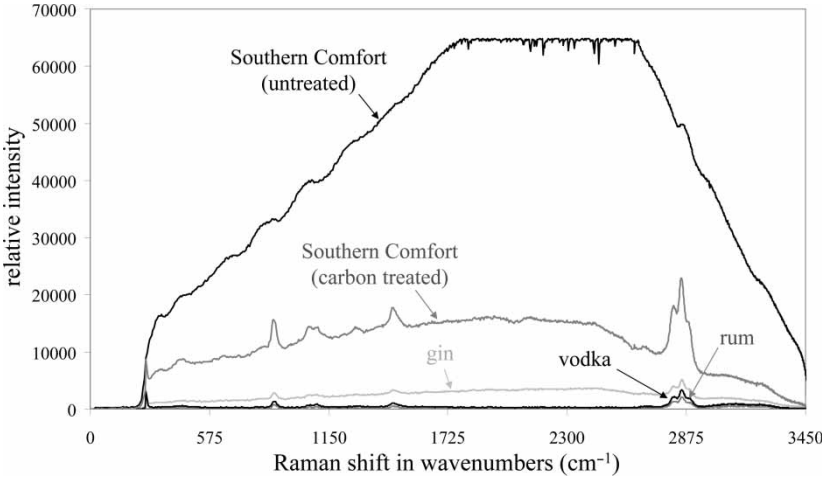

Figure 2. Raman spectra of untreated liquor samples obtained using 532-nm excitation. The fluorescence from the untreated Southern Comfort sample obscured the Raman band at 888 cm^{-1} and prevented quantification of ethanol in the liquor. The carbon treatment procedure removed some of the fluorescence from the Raman signal of the Southern Comfort.

Table 2. Quantification of ethanol in carbon-treated alcoholic beverages by 532-nm excitation Raman spectroscopy

Sample	% Ethanol from manufacturer	% Ethanol, single-peak aqueous calibration	% Ethanol, multipeak aqueous calibration	% Ethanol, standard addition
Rum	40	45 ± 3	36 ± 3	37 ± 1
Gin	40	34 ± 3	31 ± 4	34 ± 2
Vodka	40	46 ± 2	34 ± 5	31 ± 1
Southern comfort	50	80	NP	NP

NP, quantification not performed because of broadband fluorescence.

be observed. However, the remaining fluorescence signal masked the true height of the Raman band, and the amount of ethanol in Southern Comfort could not be quantified accurately, as shown in Table 2. Therefore, multiple trials for carbon-treated Southern Comfort were not performed with 532-nm excitation. In general, the single-peak aqueous external calibration procedure overestimated the amount of ethanol in the sample, and the multiple-peak and standard addition procedures underestimated the ethanol content of the alcoholic beverages. A Student's *t*-test was performed to determine whether or not the results agreed, within experimental error, with the manufacturer's label. For rum and gin, the results obtained by the single-peak and multiple-peak calibration methods were statistically the same as the amount of ethanol given by the manufacturer at a 95% confidence level. For vodka, the results obtained by multiple-peak normalized calibration method agreed with the manufacturer's value, but the results obtained by single-peak calibration were statistically different from the value on the label.

In an effort to further reduce fluorescence, the same experiments were performed using 785-nm excitation radiation. The use of longer-wavelength radiation for Raman excitation has been shown to reduce sample fluorescence because the lower energy radiation reduces the amount of electronic absorption by the molecule of interest.[44] The results of this study are shown in Table 3. A Student's *t*-test was performed to compare the experimental results with the manufacturer's label. For rum, gin, and vodka, the single-peak, multiple-peak, and standard addition calibration methods gave results that are statistically the same as the amount of alcohol listed on the manufacturer's label. It should be noted that the results shown in Table 3 for untreated Southern Comfort with 785-nm were possible only when an average of the intensity of the fluorescence baseline was subtracted from the total Raman signal at 888 cm^{-1}. Because the sample was highly fluorescent, the calculated amount of ethanol in the Southern Comfort was found to be much higher, similar to the results given in Table 2, when only the sample blank was

Table 3. Quantification of ethanol in untreated alcoholic beverages by 785-nm excitation Raman spectroscopy

Sample	% Ethanol from manufacturer	% Ethanol, single-peak aqueous calibration	% Ethanol, multipeak aqueous calibration	% Ethanol, standard addition
Rum	40	41 ± 2	35 ± 7	40 ± 2
Gin	40	39 ± 2	38 ± 5	40 ± 2
Vodka	40	39 ± 1	36 ± 4	41 ± 2
Southern comfort	50	37 ± 2[a]	55 ± 4[a]	27 ± 2[a]

[a]Subtraction of fluorescence baseline; see text for details.

subtracted from the total Raman signal at 888 cm^{-1}. Unfortunately, for 532-nm excitation Raman spectroscopy of the untreated and treated Southern Comfort samples, the broadband fluorescence background obscured the Raman peaks, and baseline fluorescence subtraction was not sufficient for accurate quantification of the amount of ethanol in the samples. For 785-nm excitation spectra of Southern Comfort where fluorescence baseline subtraction was attempted, both single-peak and standard addition calibration gave results that were statistically different from the amount of ethanol provided by the manufacturer. However, for Southern Comfort samples where calibration with multiple-peak normalization was combined with subtraction of the fluorescence baseline, the experimental amount of ethanol was statistically the same as the amount provided by the manufacturer's label.

By comparison of the relative intensities of the fluorescence baselines of the spectra for untreated Southern Comfort in Fig. 2 and Fig. 3, it was observed that the fluorescence of the Southern Comfort sample was somewhat reduced under 785-nm excitation relative to 532-nm excitation. Indeed, as shown in Fig. 3 for Southern Comfort, some Raman bands that had been completely obscured for 532-nm excitation began to appear by use of 785-nm excitation radiation. In addition to the use of longer wavelengths for Raman excitation, it has been shown that sample fluorescence may be reduced by pretreatment of the sample with charcoal.[36] Therefore, in the current work, 785-nm Raman excitation was paired with carbon pretreatment of the sample in order to minimize the intensity of the fluorescence of the sample and to optimize the determination of ethanol concentration in liquor samples. As shown in Table 4, for rum, gin, and vodka, the single-peak, multiple-peak, and standard addition calibration methods gave results that were statistically the same as the amount of ethanol provided by the manufacturer. Quantification of the amount of ethanol in Southern Comfort could not be performed successfully, as a large fluorescence baseline remained, despite carbon treatment and the use of 785-nm for Raman excitation.

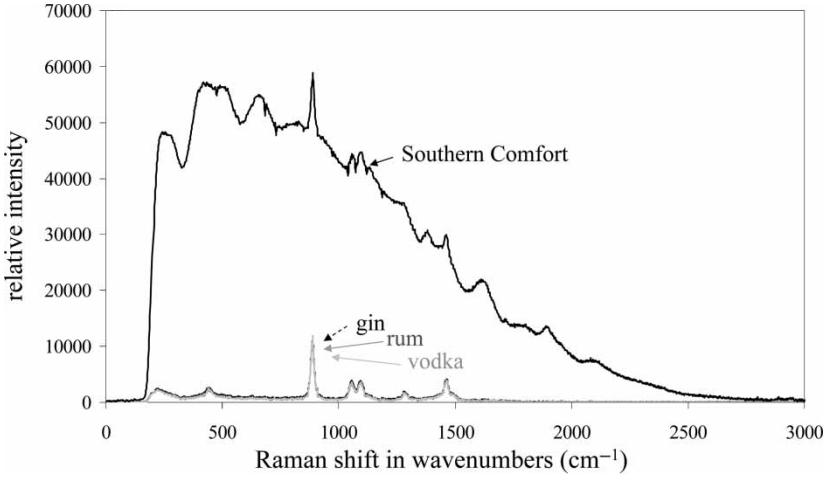

Figure 3. Raman spectra of untreated liquor samples obtained using 785-nm excitation. The intensity of the fluorescence of the Southern Comfort sample was visibly reduced compared with the fluorescence observed for 532-nm excitation. However, accurate quantification of ethanol in Southern Comfort was prevented because the Raman signal was still partially obscured by the intense fluorescence.

Probably, organic hydrocarbons or sugars[27] in the matrix contributed to the broadband fluorescence of the sample.

Standard addition is well established as a method that can be used to compensate for matrix effects,[39] such as the effect of the sugar content of the liquor on the overall Raman signal.[27] Therefore, standard addition was used in an attempt to quantify the amount of ethanol in a wide range of other types of liquors. Unfortunately, as shown in Table 5, using the optimized procedure (785-nm excitation, carbon-treated samples), the

Table 4. Quantification of ethanol in carbon-treated alcoholic beverages by 785-nm excitation Raman spectroscopy

Sample	% Ethanol from manufacturer	% Ethanol, single-peak aqueous calibration	% Ethanol, multipeak aqueous calibration	% Ethanol, standard addition
Rum	40	37 ± 6	37 ± 6	40 ± 2
Gin	40	33 ± 6	33 ± 8	40 ± 1
Vodka	40	36 ± 6	36 ± 7	40 ± 1
Southern comfort	50	NP	NP	17 ± 1

NP, quantification not performed because of broadband fluorescence.

Table 5. Quantification of ethanol in carbon-treated alcoholic beverages by 785-nm excitation Raman spectroscopy using standard addition calibration

Sample	% Ethanol from manufacturer	% Ethanol, experimental
Agwa herbal liqueur	30	31 ± 0.1
Jose Cuervo	40	36 ± 0.1
Sambuca Light	42	39 ± 1
Sambuca Dark	40	31 ± 1
Dewar's Scotch Whiskey	40	31 ± 1
Blackberry brandy	35	33 ± 1
Fireball Liqueur	33	30 ± 1
Christian Brother's Brandy	40	24 ± 1
Hennessy cognac	40	25 ± 1
Grand Marnier	40	1 ± 0.01
Jagermiester	35	1 ± 0.01
Bailey's Irish Cream	17	70 ± 11
Kahlua coffee liqueur	20	1 ± 1

experimental values of ethanol did not closely match the known values for many of the liquor samples. A student's *t*-test was performed, and for the liquors shown in Table 5, only the blackberry brandy beverage gave results that were statistically the same as the amount of ethanol on the manufacturer's label. A majority of the liquors were found to fluoresce, despite carbon treatment and the use of a 785-nm excitation wavelength. In particular, the largest margin of error was observed for liquors with deep colors, heavy fragrances, or higher viscosities, such as Jagermeister, Bailey's Irish Cream, and Kahlua. Also, as shown in Table 5, the actual amount of ethanol that was quantified was less than the expected value for samples such as Christian Brother's Brandy, Grand Marnier, and Hennessy cognac. Possibly, evaporation of a fraction of the ethanol content in these samples occurred during the carbon treatment procedure or by laser-heating of the sample during analysis. Also, some of the ethanol might have been lost to adsorption onto the carbon surface. In addition, some discrepancy between the experimental results and the expected amount of ethanol in the liquor samples may have been a result of inaccurate labeling by the manufacturer.

These results suggest that there remains a need to develop an improved method for removal of the fluorescent compounds from the alcoholic beverage samples. More rigorous methods, such as first-derivative spectroscopy or Fourier transform,[16,27] may be required for the reliable quantification of ethanol in alcoholic beverages by Raman spectroscopy. Also, although standard addition was universally applied for all liquors in the current paper, there may be no single most appropriate method for the quantification of ethanol across all types of liquors.

Identification of Diesel Samples Using Raman Spectroscopy

Raman spectroscopy was also evaluated as a method for fingerprinting oils that had been spilled in open waters. Samples of various fuel oils including home heating oil diesel, and crude oil were placed in a water matrix and analyzed using 532-nm excitation Raman spectroscopy. An empty sample cell was used as a blank for dark subtraction by the BWSpec Software, and an integration time of 0.2 s was used to acquire spectra. As shown in Fig. 4, some Raman bands are visible in the C-H stretch region, but much of the Raman spectrum of the diesel fuel was obscured by an intense fluorescence background. At longer integration times, the fluorescence background completely masked the Raman signals. Similar spectra (not shown) were obtained for the other oil samples. In an effort to reduce the fluorescence background, the oil samples were pretreated with the charcoal procedure that was previously described. A comparison of treated and untreated diesel fuel performed using the same 0.2-s integration time is presented in Fig. 5. It was observed that the charcoal procedure significantly decreased the intensity of the fluorescence across the entire wavelength range of the spectrometer. Also, it appeared that the fluorescence had obscured many of the characteristic Raman peaks for the diesel fuel in the 750–1750 cm^{-1} fingerprint region. The bands observed in that region for carbon-treated samples were similar to those found in the literature for diesel fuel.[33,36]

In order to determine the ability of Raman spectroscopy to distinguish among the various fuel oils, commercial automotive diesel fuels were obtained from three different filling stations in cities around Connecticut.

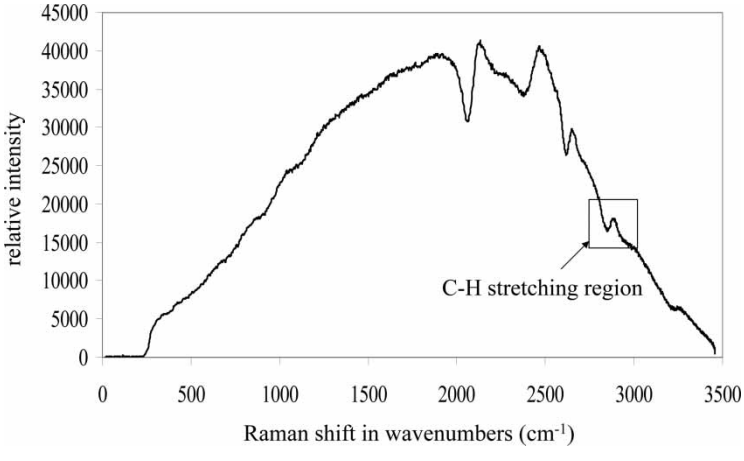

Figure 4. Observed Raman spectrum of diesel fuel in a water matrix. The Raman band observed in the C-H stretching region of the spectrum was similar to results previously published in the literature.

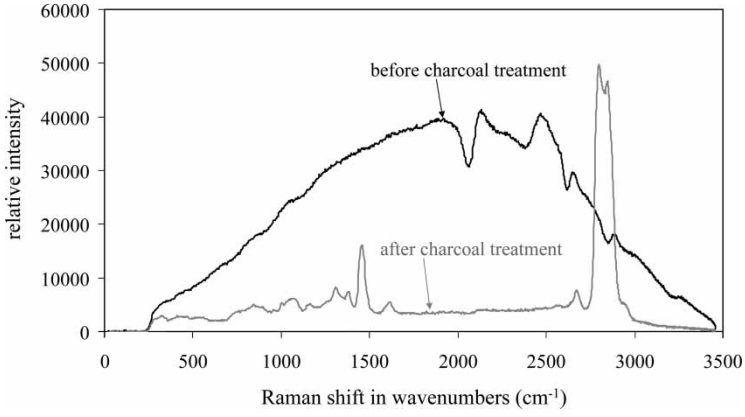

Figure 5. Comparison of Raman spectra of diesel fuel before and after the carbon treatment procedure. It was observed that the carbon procedure removed a significant amount of fluorescence from the diesel spectrum. This process may allow for the use of Raman spectroscopy to distinguish among various fuel oils.

One sample was collected from a Getty station in Avon, CT, while a second sample was collected from a Mobil station in Oakdale, CT. A third sample was taken from CITGO in Storrs, CT. Each sample was pretreated using the charcoal procedure described above. Raman spectra were collected using a 20-s integration time, and the result is shown in Fig. 6. No significant differences were observed in the Raman signals of the three diesels. The fuels were further analyzed by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), and infrared spectroscopy (FTIR) at the U.S. Coast Guard Marine Safety Laboratory in Groton, CT. Only

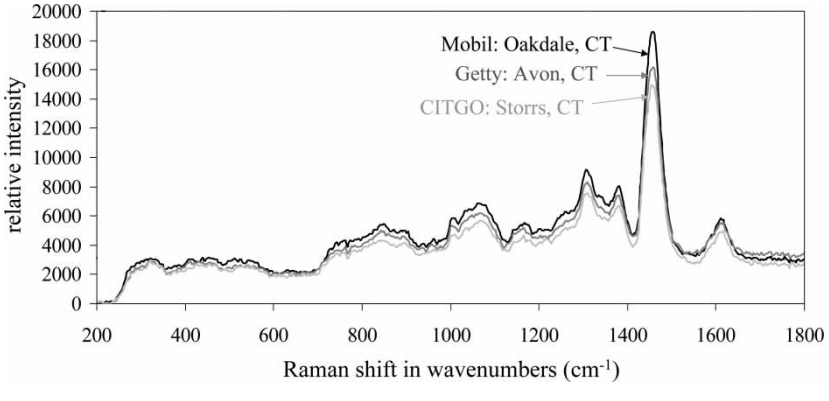

Figure 6. Comparison of Raman spectra of three carbon-treated commercial diesel fuels. Raman spectra of diesels sampled from three different service stations around Connecticut appeared similar in the fingerprint region.

slight differences in the lighter, more volatile components of the fuels were observed, and it was concluded that probably, all three fuel samples were identical and had been delivered from the same fuel distribution center.

Marine diesel fuel standards, previously identified by the U.S. Coast Guard Marine Safety Laboratory using GC, GC-MS, and FTIR, were obtained and analyzed by portable Raman spectroscopy. The fuels, namely "SH," "SK," and "Helcat" diesels, are known to give statistically different spectra by GC, GC-MS, and FTIR. For taxation purposes, marine diesels are required to be colored a deep red with synthetic Solvent Red 164 diazo dye. In general, the red coloration causes significant sample fluorescence. Therefore, the charcoal pretreatment procedure described above was used to remove the red coloration from the fuels prior to analysis. As shown in Fig. 7, Raman spectroscopy was able to differentiate among the three diesel standards. The unknown spectrum appears to have features most similar to the "SK" standard spectrum.

Ultimately, it appeared that the *in situ* analytical utility of 532-nm excitation for identification of marine diesel samples is limited by an excessive amount of sample fluorescence. Extensive sample pretreatment was required to reduce sample fluorescence so that Raman spectral bands could be observed. Therefore, 532-nm excitation Raman spectroscopy is probably not suitable for use as a field instrument by the U.S. Coast Guard for rapid identification of oil spills and offending tanker ships. However, as previously mentioned, it has been shown that the amount of fluorescence exhibited by a sample can be reduced by using a longer wavelength for Raman excitation.[44] Here, a 785-nm laser was used to provide lower-frequency Raman excitation compared with 532-nm excitation. In order to better evaluate the utility of 785-nm for rapid, *in situ* evaluation of oil spills, or for other situations where

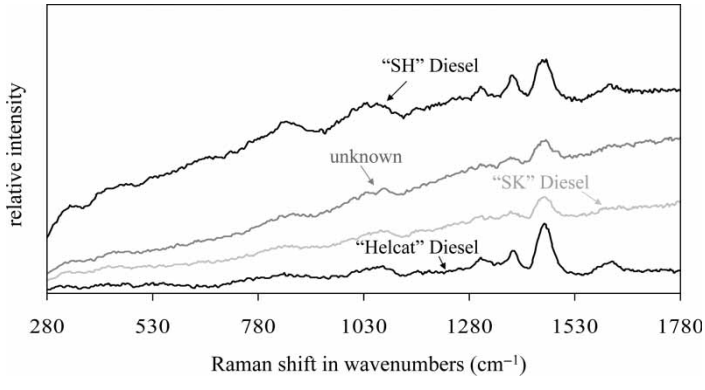

Figure 7. Identification of unknown marine diesel by 532-nm excitation Raman spectroscopy. Raman spectra of carbon-treated marine diesel standards were compared with the spectra of an unknown carbon-treated marine diesel fuel. The spectrum of the unknown is most similar to that of the "SK" standard. The spectra have been offset for clarity.

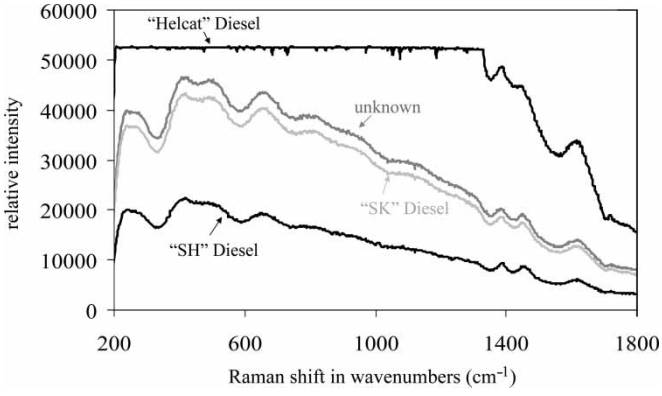

Figure 8. Identification of unknown marine diesel fuel using 785-nm excitation without carbon pretreatment. The Raman spectrum of the unknown diesel most closely resembled the spectrum for the "SK" diesel standard.

sample pretreatment is not feasible, untreated samples of the marine diesel standards were examined. For these experiments, the spectrometer integration time was 5 s. As shown in Fig. 8, the untreated diesel fuels still exhibited strong fluorescence under 785-nm excitation. Further, the broadband fluorescence of the "Helcat" fuel standard obscured much of the spectrum. Visually, the Raman spectra of the "SK" and "SH" diesels are very similar. A tentative assignment of the unknown as a match to the "SK" diesel could be made solely on the similar relative intensities of the unknown and SK spectra. Unfortunately, an intensity match is probably not definitive enough for identification of a spilled fuel, as relative intensity could be affected by the concentration of the analyte in Raman spectroscopy.[34] Further evaluation is required. For example, a statistical method such as Mahalanobis distance[45,46] might be used to improve the classification of the unknown sample over visual inspection. Although the data presented here for diesel fuels is inconclusive, probably, the reduced fluorescence lent by 785-nm excitation could be useful for the Raman identification of other samples of interest to the homeland security sector.[47,48] Also, portable Raman spectroscopic detection might usefully be paired with Fourier transform, surface-enhanced Raman spectroscopy,[49] or chemometrics[50,51] for improved on-site rapid discrimination of oils spilled in open waters or in other situations where extensive sample preparation is not practical.

CONCLUSIONS

Raman spectroscopic detection has traditionally been avoided in the undergraduate laboratory curriculum because of the high costs associated with

instrumentation. However, the recent introduction of miniature, portable Raman instrumentation has made Raman spectroscopy more accessible for undergraduate applications. In the current paper, two experiments were evaluated for use in an undergraduate teaching setting. The analysis of ethanol in alcoholic beverages offered the opportunity to introduce students to the concepts of calibration curves, standard addition, and quantitative analysis. The determination of oils in water matrices might usefully be included in the curriculum for studies of qualitative analysis, environmental science, and chemometrics. Although the results presented here suggest that, probably, further optimization is required for both sample types, these experiments offer the student the opportunity to learn about method development for the analysis of "real" analytical samples. Students might be encouraged to expand the investigation to validate the results obtained using Raman spectroscopy, or to verify the percent liquor supplied on the beverage label by the manufacturer, using complementary techniques such as gas or liquid chromatography. Finally, the principles of Raman spectroscopy and modern instrumentation, the assignment of Raman bands, and methods of statistical classification might usefully be incorporated into the experiments.

ACKNOWLEDGMENTS

Thanks are due to the National Science Foundation's program for Research Experience for Undergraduates, at the University of Connecticut, which supported two of us (M.C. and K.L.B.). In addition, thanks are due to B&W Tek, Inc., for the loan of the spectrometers and diode lasers and to InPhotonics, Inc., for the loan of the Raman probe heads. We thank the United States Coast Guard Marine Safety Laboratory for the generous loan of marine diesel samples and Dr. Pedro Cid-Aguero at the University of Connecticut for helpful discussions. We gratefully acknowledge the craftsmanship of machinist Dave Osier at the University of Connecticut for his expertise in crafting the probe and sample cell holder.

REFERENCES

1. DeGraff, B. A.; Hennip, M.; Jones, J. M.; Salter, C.; Schaertel, S. A. An inexpensive laser Raman spectrometer based on CCD detection. *Chem. Educator*, **2002**, *7*, 15–18.
2. Donohoue, D. L.; Earl, G. W.; Viste, A. Using the Ocean Optics R-2000 Raman spectrometer in the undergraduate laboratory. *Proc. South Dakota Acad. Sci.* **2000**, *79*, 63–70.
3. Sanford, C. L.; Mantooth, B.; Jones, B. T. Determination of ethanol in alcohol samples using a modular Raman spectrometer. *J. Chem. Educ.* **2001**, *78*, 1221–1225.

4. Lorigan, G.; Patterson, B. M.; Sommer, A. J.; Danielson, N. D. Cost-effective spectroscopic instrumentation for the physical chemistry laboratory. *J. Chem. Educ.* **2002**, *79*, 1264–1266.
5. Chase, B. A new generation of Raman instrumentation. *Appl. Spectrosc.* **1994**, *48* (7), 14A–19A.
6. Lewis, I. R.; Griffiths, P. R. Raman spectrometry with fiber-optic sampling. *Appl. Spectrosc.* **1996**, *50* (10), 12A–30A.
7. Pelletier, M. J. Quantitative analysis using Raman spectrometry. *Appl. Spectrosc.* **2003**, *57* (1), 20A–42A.
8. Pemberton, J. E.; Sobocinski, R. L.; Bryant, M. A.; Carter, D. A. Raman spectroscopy using charge-coupled device detection. *Spectroscopy* **1990**, *5* (2), 26–36.
9. Hudspeth, E. D.; Cleveland, D.; Batchler, K. L.; Nguyen, P. A.; Feaser, T. L.; Quattrochi, L. E.; Morenz, J.; Balram, S. A.; Zhou, J. X.; Lombardi, D.; Michel, R. G. Teaching Raman spectroscopy in the both the undergraduate classroom and the laboratory with a portable Raman instrument. *Spectrosc. Lett.* **2006**, *39*, 99–115.
10. Office of Regulatory Affairs, United States Food and Drug Administration. Sec. 510.400 Dealcoholized Wine and Malt Beverages—Labeling (CPG 7101.04). November 29, 2005. Available at http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg510–400.html.
11. Leary, J. J. A quantitative gas chromatographic ethanol determination. *J. Chem. Educ.* **1983**, *60*, 675.
12. Wang, M.-L.; Wang, J.-T.; Choong, Y.-M. Simultaneous quantification of methanol and ethanol in alcoholic beverage using a rapid gas chromatographic method coupling with dual internal standards. *Food Chem.* **2004**, *86*, 609–615.
13. Panosyan, A. G.; Mamikonyan, G. V.; Torosyan, M.; Gabrielyan, E. S.; Mkhitaryan, S. A.; Tirekyan, M. R.; Ovanesyan, A. Determination of the composition of volatiles in Cognac (Brandy) by headspace gas chromatography-mass spectrometry. *J. Anal. Chem.-USSR (Trans. Zhurnal Analiticheskoi Khimii)*, **2001**, *56* (10), 945–952.
14. Looney, M. M.; Ford, A. C. Differentiation of bourbon whiskies using gas chromatography and cluster analysis. *Texas J. Sci.* **2000**, *52*, 345–352.
15. Lanças, F. M.; Galhiane, M. S. Fast routine analysis of light components of alcoholic beverage using a large-bore open tubular fused silica column. *Bol. Soc. Chil. Quim.* **1993**, *38*, 177–182.
16. Mendes, L. S.; Oliveira, F. C.C.; Suarez, P. A.Z.; Rubim, J. C. Determination of ethanol in fuel ethanol and beverages by Fourier transform (FT)-near infrared and FT-Raman spectrometries. *Anal. Chim. Acta* **2003**, *493*, 219–231.
17. Bezerra dos Santos, S. R.; Ugulino de Araújo, M. C.; Aquino Barbosa, R. An automated FIA system to determine alcoholic grade in beverages based on Schlieren effect measurements using an LED-photocolorimeter. *Analyst* **2002**, *127*, 324–327.
18. Segunda, M. A.; Rangel, A. O.S.S. Sequential injection flow system with improved sample throughput: determination of glycerol and ethanol in wines. *Anal. Chim. Acta* **2002**, *458*, 131–138.
19. Mohns, J.; Künnecke, W. Flow analysis with membrane separation and time based sampling for ethanol determination in beer and wine. *Anal. Chim. Acta* **1995**, *305*, 241–247.
20. Rangel, A. O.S.S.; Tóth, I. V. Determination of ethanol in wines by flow injection spectrophotometry using gas-diffusion and immobilized enzyme reactor. *Am. J. Enol. Vitic.* **1999**, *50*, 259–263.

21. Liu, S.; Augusto, F.; Pires Valene, A. L.; Tubino, M. Flow injection determination of ethanol in Brazilian brandies using a gas-permeable membrane and a gas flame ionization detector. *J. Flow Injection Anal.* **2001**, *18*, 144–149.
22. Kokovkin, V. V.; Smolyakov, B. S. Ethanol determination in aqueous solutions and wine stocks by ion-selective electrodes. *J. Anal. Chem.-USSR (Trans. of Zhurnal Analiticheskoi Khimii)*, **1995**, *50*, 519–523.
23. Comitre, A. L.D.; Reis, B. F. Automatic multicommutated flow system for ethanol determination in alcoholic beverages by spectrophotometry. *Lab Robotics Automat.* **2000**, *12*, 31–36.
24. Rotariu, L.; Bala, C.; Magearu, V. New potentiometric microbial biosensor for ethanol determination in alcohol beverages. *Anal. Chim. Acta* **2004**, *513*, 119–123.
25. Abayeh, O. J.; Ochekpe, N. A.; Kuteyi, F. T.; Chinoko, Y. D. The comparison of 1H-NMR spectroscopy and gas chromatographic techniques in the quantification of ethanol and methanol in alcoholic beverage mixtures. *J. Chem. Soc. Nigeria* **2004**, *29*, 49–53.
26. Frausto-Reyes, C.; Medina-Gutiérrez, C.; Sato-Berrú, R.; Sahagún, L. R. Qualitative study of ethanol content in tequilas by Raman spectroscopy and principal component analysis. *Spectrochim. Acta A* **2005**, *61*, 2657–2662.
27. Nordon, A.; Mills, A.; Burn, R. T.; Cusick, F. M.; Littlejohn, D. Comparison of non-invasive NIR and Raman spectrometries for determination of alcohol content of spirits. *Anal. Chim. Acta* **2005**, *548*, 148–158.
28. Jager, M. J.; McClintic, D. P.; Tilotta, D. C. Measurement of petroleum fuel contamination in water by solid-phase microextraction with direct Raman spectroscopic detection. *Appl. Spectrosc.* **2000**, *54*, 1617–1623.
29. de Bakker, C. J.; Fredericks, P. M. Determination of petroleum properties by fiber-optic Fourier transform Raman spectrometry and partial least-squares analysis. *Appl. Spectrosc.* **1995**, *49*, 1766–1771.
30. Choquette, S. J.; Chesler, S. N.; Duewer, D. L.; Wang, S.; O'Haver, T. C. Identification and quantitation of oxygenates in gasoline ampules using Fourier transform near-infrared and Fourier transfer Raman spectroscopy. *Anal. Chem.* **1996**, *68*, 3525–3533.
31. Tiwari, V. S.; Khijwania, S. K.; Yueh, F.-Y.; Singh, J. P. Optical fiber Raman sensor for monitoring hydrocarbon in fuel and industrial chemical processing. *Proc. Int. Soc. Opt. Eng.* **2004**, *5589*, 8–13.
32. Zhang, S. L.; Michaelian, K. H.; Bulmer, J. T.; Hall, R. H.; Hellman, J. L. Fourier transform Raman spectroscopy of fuels: curve-fitting of C-H stretching bands. *Spectrochim. Acta A* **1996**, *52*, 1529–1540.
33. Michaelian, K. H.; Zhang, S. L.; Hall, R. H.; Bulmer, J. T. Fourier transform Raman spectroscopy of Syncrude sweet blend distillation fractions derived from Athabasca bitumen. *Spectrochim. Acta A* **2001**, *57*, 73–81.
34. Matter, D.; Haffner, K. Y.; Kleiner, T. Method and apparatus for determining petroleum concentration in water. European Patent Application: EP 2002–405013, 2003.
35. Ahmadjian, M.; Brown, C. W. Feasibility of remote detection of water pollutants and oil slicks by laser-excited Raman spectroscopy. *Environ. Sci. Technol.* **1973**, *7*, 452–453.
36. Ahmadjian, M.; Brown, C. W. Petroleum identification by laser Raman spectroscopy. *Anal. Chem.* **1976**, *48*, 1257–1259.
37. Gamble, H. A.; Robbins, J. C.; Mackay, G. I.; Schiff, H. I. Development of a compact Raman spectrometer for detecting product interfaces in a gasoline pipeline, Proceedings of the Air and Waste Management Association's Annual

Conference and Exhibition, 95th, Baltimore, MD, June 23–27, 2002; Air & Waste Management Association: Pittsburgh, PA, pp. 3067–3075.
38. Purcell, F.; Grayzel, R.; Adar, F. Remote Raman analysis for process monitoring applications. *Proc. Int. Soc. Opt. Eng.* **1992**, *1681*, 149–158.
39. Harris, D. C. Calibration methods. In *Quantitative Chemical Analysis*, 5th edn.; W.H. Freeman and Company: New York, 1999; pp. 93–111.
40. Christian, G. D. Data handling. In *Analytical Chemistry*, 5th edn.; John Wiley & Sons: New York, 1994; pp. 14–64.
41. Skoog, D. A.; Holler, F. J.; Nieman, T. A. Introduction - Calibration of instrumental methods. In *Principles of Instrumental Analysis*, 5th edn.; Harcourt Brace & Co: Orlando, FL, 1998; pp. 15–18.
42. Sato-Berrú, R. Y.; Medina-Valtierra, J.; Medina-Gutiérrez, C.; Frausto-Reyes, C. Quantitative NIR Raman analysis in liquid mixtures. *Spectrochim. Acta A* **2004**, *60*, 2225–2229.
43. Lin-Vien, D.; Colthup, N. B.; Fateley, W. G.; Grasselli, J. G. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*; Academic Press: Boston, 1991.
44. Skoog, D. A.; Holler, F. J.; Nieman, T. A. Raman spectroscopy. In *Principles of Instrumental Analysis*, 5th edn.; Harcourt Brace & Co: Orlando, FL, 1998, pp. 429–444.
45. De Maesschalck, R.; Jouan-Rimbaud, D.; Massart, D. L. The Mahalanobis distance. *Chemom. Intell. Lab. Syst.* **2000**, *50*, 1–18.
46. Gnanadesikan, R. *Methods for Statistical Data Analysis of Multivariate Observations*; John Wiley & Sons: New York, 1977.
47. Harvey, S. D.; Vucelick, M. E.; Lee, R. N.; Wright, B. W. Blind field test evaluation of Raman spectroscopy as a forensic tool. *Forensic Sci. Int.* **2005**, *25*, 12–21.
48. Wright, C. W.; Harvey, S. D.; Wright, B. W. Detection of hazardous chemicals using field-portable Raman spectroscopy. *Proc. Int. Soc. Opt. Eng.* **2003**, *5048*, 107–118.
49. Brinkmann, U. SERS traces water pollution in real time. *Laser Focus World* **2004**, *40* (6), 12.
50. López-Díaz, E. C.; Bianchi, G.; Goodacre, R. Rapid quantitative assessment of the adulteration of virgin olive oils with hazelnut oils using Raman spectroscopy and chemometrics. *J. Agric. Food Chem.* **2003**, *51*, 6145–6150.
51. Baeten, V.; Aparicio, R. Edible oils and fats authentication by Fourier transform Raman spectrometry. *Biotechnol. Agron. Soc. Environ.* **2004**, *4*, 196–203.