A Raman Waveguide Detector for Liquid Chromatography

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A novel real-time liquid core Raman waveguide detector designed for liquid chromatographic applications is described. The Raman waveguide detector provides enhanced selectivity over typical high-performance liquid chromatography (HPLC) detectors. The waveguide detector also greatly improves the sensitivity of a typical Raman measurement without resorting to surface enhancement or resonance approaches and is compatible with the typical peak width volumes eluted by microbore and minibore HPLC (packed 1–2-mm-i.d. columns). Detection limit enhancements of over 1000-fold have been achieved for the measurement of alcohols in the aqueous phase with the Raman cell utilizing liquid core waveguide technology. The liquid core waveguides demonstrated in this study were constructed using Teflon AF 2400 tubing with a refractive index of 1.29. The low refractive index of the polymer material allowed HPLC separations with Raman detection to be performed with an aqueous mobile phase. A calibration curve for aqueous solutions of 2-propanol was generated and a limit of detection (LOD) of 2 ppm was determined. The Raman waveguide detector is demonstrated for the HPLC analysis of alcohol test mixtures, with LODs in the low-ppm range at the detector. By coupling the temporal separation achieved by HPLC with the vibrational information gleaned from Raman detection, an information-rich multivariate data matrix is obtained that can be deconvoluted to provide chemical speciation even when the HPLC resolution is poor. In this paper, we will discuss the physical and optical design of the Raman waveguide detector and the demonstration of the detector for HPLC detection.

Raman spectroscopy is a powerful tool for providing structural information for qualitating individual compounds in a complicated matrix. However, the application of Raman spectroscopy as both a qualitative and quantitative analytical detector for high-performance liquid chromatography (HPLC) has been limited by its inherent lack of sensitivity. Raman scattering is a very weak phenomenon; approximately 1 in 10^7 photons is scattered at an optical frequency different from that of the source radiation.[1] Techniques currently used for enhancing the sensitivity of Raman

measurements include surface-enhanced Raman spectroscopy (SERS)[2,3] and resonance Raman spectroscopy (RRS).[4] Both of the these techniques are useful for increasing the sensitivity of Raman measurements; however, the methods are highly specific, difficult to apply for routine analysis, and often not suitable for quantitative analysis. While HPLC is a popular and robust analytical tool, the range of applications for HPLC is always limited by the chemical selectivity and sensitivity provided by the detector. The work described in this paper is aimed at addressing this limitation in the field of Raman detection for HPLC. The effective coupling of Raman detection to HPLC hinges on the proper design of a flow cell that is compatible with the peak width volumes of HPLC, and the need for an optimal cell geometry for Raman detection. Furthermore, the flow cell must be compatible with aqueous solutions, since many HPLC applications are performed in the reversed-phase mode, using water with organic modifiers as the mobile phase.

The use of a sample cell with increased sample path length for improved Raman signal intensity was first recognized by Wood in 1928.[5] Since then, much work has been done to develop liquid core waveguides for signal enhancement in UV–visible,[6,7] fluorescence[8,9] and Raman spectroscopy.[10,11] A liquid core waveguide can be made from any tubing in which the wall material has a lower refractive index than the core material. Under these physical conditions, it is possible for light launched into the core at the proper angle to propagate, or waveguide, down the tube by total internal reflection with minimal loss. Because of this waveguiding phenomenon, it is possible to design long path length optical cells which increase the number of molecules excited to improve Raman scattering efficiency and to facilitate collection.

Walfaren and Stone were the first to develop effective liquid core waveguides for the enhancement of Raman signal.[12,13] They

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reported Raman signal enhancements of 10^2–10^3 for various organic solvents including benzene and tetrachloroethylene, in 10–25 m of liquid-filled quartz tubing. The use of liquid-filled quartz fibers for Raman enhancement was also reported by Ross and McClain for the measurement of *trans*-stilbene in benzene.[14] Schwab and McCreery were the first to report the use of a fiber-optically coupled liquid core waveguide for enhancing the Raman signal of aqueous solutions.[15] Their 1-m-long polished quartz waveguide sensor incorporated a fiber-optic bundle (18 collection around 1 excitation) to achieve signal enhancements of 30–50-fold. Benoit and Yappert reported a 70-fold signal enhancement using a two-fiber (1 excitation/1 collection) polished quartz Raman waveguide for the analysis of aqueous solution.[16] One disadvantage of using polished quartz tube waveguides for aqueous measurements is that the waveguide is formed at the glass/air interface and not the glass/solution interface. This leads to wave propagation problems and light loss due to contamination of the glass surface (i.e., dust, fingerprints, etc.). Another disadvantage is that the laser is propagating through both the solution and the quartz tube, which leads to signal variability in a dynamic flowing system (due to changes in refractive index) and the generation of a large Raman signal due to the quartz tubing.

Historically, the use of long path length optical cells for the measurement of aqueous solutions has not been possible due to a lack of materials with refractive indexes less than that of water (n_d = 1.33). However, in recent years a material has become available, Teflon AF, that has advanced the use of liquid core waveguides for aqueous phase optical spectroscopy. The material, Teflon AF (2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole), is an amorphous fluoropolymer with a refractive index of 1.29–1.31 that was developed and is manufactured by DuPont.[17] This polymer is unique because it has an extremely low refractive index, high gas permeability, and low thermal conductivity.

The use of a Teflon AF waveguide was first described by Gilby and Carson, who internally coated glass tubes with the fluoropolymer.[18] Hong and Burgess reported the use of polypropylene tubes internally coated with Teflon AF for the analysis of ammonia and trichloroethylene vapors diffusing into the waveguide core by UV–vis.[19] Altkorn et al. were the first to report the use of Teflon AF tubing for liquid core waveguides and describe their applications to Raman spectroscopy.[10] The recent commercial availability of tubing manufactured out of Teflon AF led to a surge in the development of liquid core waveguides for analyzing aqueous systems by UV–visible, fluorescence, and Raman spectroscopy.[11,20–24]

Previously, Raman analysis of HPLC eluents was performed by diverting flow to an off-line sampling cell.[25] In this static condition, a high-powered laser and long integration times were used to detect and identify xylene isomers at concentrations down to 10^{-5} mol/L. A detection cell for coupling a typical Raman (nonwaveguide) measurement with HPLC was demonstrated by Hong for the analysis of coeluting toluene and naphthalene.[26] Recently, Gooijer et al. reported on the use of a Teflon AF waveguide absorbance cell coupled directly to a commercial HPLC instrument that enhanced the detection limits by 30–50 times for various environmental contaminants in water.[24] Wider application of HPLC/Raman requires dynamic in-line sampling and real-time data acquisition using straightforward affordable instrumentation.

In this paper, we describe a novel waveguide detection cell for performing sensitive Raman measurements of flowing systems, in particular, for reversed-phase HPLC.[27,28] The quantitative performance of the Raman cell was demonstrated by comparing the Raman waveguide cell data to that of a typical Raman measurement for 2-propanol (IPA). Substantial signal enhancements and improved limits of detection (LODs) were achieved and will be reported for 2-propanol and other analytes. The qualitative performance of the Raman waveguide cell was demonstrated by coupling the detector to a minibore HPLC (2-mm-i.d. packed column) system. The coupling of an HPLC separation with real-time Raman spectroscopic detection allows for the positive identification and quantitation of complicated mixtures without the need for baseline separation. The HPLC/Raman analysis was relatively fast, selective, and sensitive and allowed for the collection of information-rich multivariate data.

EXPERIMENTAL SECTION

The experimental setup for the HPLC/Raman system is shown in Figure 1. The Raman spectra were collected with a Kaiser Optical Systems Hololab Series 5000 Raman instrument consisting of a Holoprobe transmission holographic spectrograph interfaced to an infinity-corrected, fiber-coupled microscope. The Raman system was equipped with a 785-nm stabilized external cavity diode laser (SDL Inc.) operating at an average power of 60 mW at the sample. A beam splitter in the collimated space between the Raman probe head and the microscope objective provided a real-time visual image of the waveguide tubing in the Raman flow cell to facilitate laser alignment. The Raman probe head was coupled to the microscope with an 8-μm-i.d. single-mode excitation fiber, and the scattered signal was collected using a 100-μm-i.d. multimode fiber. A 40× (0.65 NA) objective was used to both focus the laser and collect the scattered radiation in an epi-illumination configuration. The 40× objective was chosen for its large numerical aperture, which was useful to maintain full collection of the Raman scattered radiation from the waveguide during dynamic flowing measurements. All Raman spectra were collected using a 50-μm slit width and a detector temperature of −40 °C.

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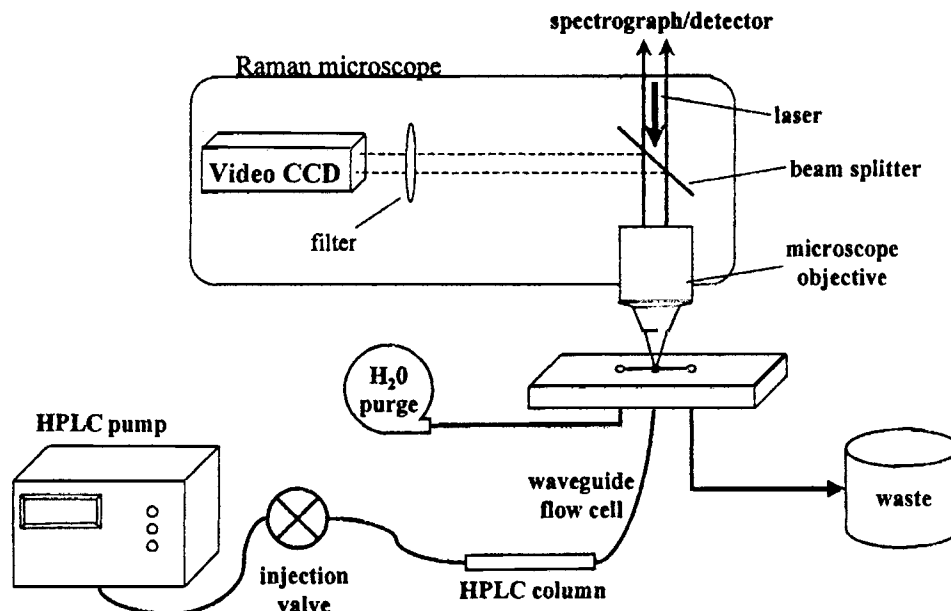
Figure 1. Schematic diagram of the HPLC/Raman waveguide detector apparatus.
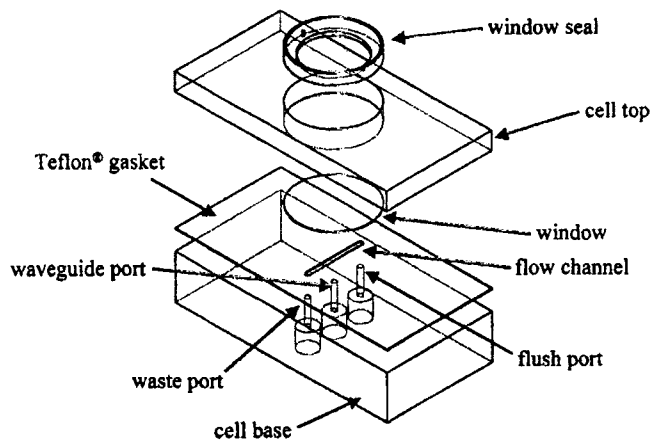

Figure 2. Detailed diagram of the Raman waveguide detector flow cell.

Raman Waveguide Detector. A novel flow cell was designed and optimized for performing sensitive Raman measurements of flowing liquid samples (Figure 2). The cell consisted of a length of polymer waveguide tubing and a stainless steel block of the same footprint as a typical microscope slide (3 in. × 1.5 in.) to facilitate use on a microscope stage. The cell was equipped with three fluid ports on the underside: one port for removal of waste, another to allow purging of air bubbles, and the third, center port, for coupling the liquid core waveguide. The three ports were connected on the surface of the cell by a channel that was laser cut into an FEP fluoropolymer gasket. The channel dimensions of the gasket were 20 × 1.6 × 0.25 mm ($L \times W \times D$). The gasket was placed between the stainless steel top plate and the base to form a liquid-sealed cell. A 25 mm o.d. × 250 μm fused-silica window is then secured over the center port to allow optical access to the liquid core waveguide. The Raman waveguide detector cell was interfaced directly onto the microscope stage with the fluid connections routed under the stage. Once the cell was secured and aligned on the microscope, the excitation laser was focused with a microscope objective through the fused-silica window into the bore of the waveguide tubing in an epi-illumination configu-

ration. The sample is pumped into the waveguide from the distal end and flows toward the laser and exits to waste through the channel in the gasket.

The Raman waveguide detector was formed from Teflon AF copolymer tubing. The tubing material is transparent from 200 to 2000 nm and can be purchased in various internal diameters from Biogeneral Technologies Inc. (San Diego, CA). The liquid core waveguide used in the Raman waveguide detector was 1 m in length and had a 50-μm core diameter. The total volume of the liquid core waveguide was 1.9 μL. The 50-μm core diameter was chosen for two fundamental reasons. First, it provided an optimal optical match to the diameter of the collection fiber in the Raman probe thereby maximizing signal collection. Second, it was compatible with the elution volumes of a microbore or minibore HPLC experiment. To further stabilize the optical properties of the waveguide, the tubing was coiled and cast into a polymer disk at a constant bend radius of 1.9 cm for protection from the environment and to reduce optical effects due to variable bend radius.

Liquid Chromatography System. The HPLC system consisted of an Isco SFC-500 syringe pump (Isco Inc., Lincoln, NE), a 10-port injection valve (Valco Instruments Co. Inc., Houston, TX), and a Brownlee Cyano Spheri-5 column (Perkin-Elmer Corp., Norwalk, CT). The column specifications were 100 × 2.1 mm column with cyanopropyl stationary phase bound to 5-μm silica particles (approximate dead volume of column is 175 μL). The column was used in-series with a 2-μm precolumn filter (Upchurch Scientific, Oak Harbor, WA) to remove any particulate matter that could plug the waveguide. The mobile phase was deionized water (Milli-Q system) flowing at 50–80 μL/min. A 10-μL sample injection loop was used for the flow injection analysis and a 6-μL injection loop was used for the HPLC/Raman separations. All analytes used in these experiments were of analytical grade (Sigma-Aldrich Chemical, Milwaukee, WI) and are listed in Figure 6c. The cyanopropyl stationary phase was selected because it has proven especially effective in rapid and efficient separations of aromatic and aliphatic alcohols using a water mobile phase, at

ambient temperature.[29] The use of 100% water mobile phase for reversed-phase HPLC is an emerging technology that is gaining interest due to reducing chemical waste, enhancing detection, and expanding the applicability of HPLC.[29-31] Keep in mind that the analytes and HPLC conditions chosen are for demonstration purposes only and that the HPLC/Raman system has broad applicability to many reversed-phase mobile phases, such as, methanol/water and acetonitrile/water. The use of organic modifiers can actually improve the performance of the HPLC/Raman analysis compared to a pure water mobile phase. To be effective, the Raman spectrum of the mobile phase cannot interfere with the Raman spectrum of the analyte of interest, as the intensity of the mobile-phase spectrum will be dominant.

Furthermore, the 1-m length of Teflon AF tubing was selected for the Raman waveguide detector to maximize detection cell path length without introducing chromatographic band broadening. The 1.9-μL detection volume was compatible with the peak width volumes of this system, which ranged from 35 (methanol) to 185 μL (benzyl alcohol). Peak band broadening was not introduced either through volume effects or through retention effects from the Teflon AF tubing.

RESULTS AND DISCUSSION

To illustrate the signal enhancement made possible using the Raman waveguide detector, two Raman spectra of 10% IPA aqueous solutions are shown in Figure 3. The higher intensity Raman spectrum of IPA, shown in Figure 3a, was collected in a 1 m × 50 μm i.d. Teflon AF waveguide under flow injection analysis (FIA) conditions. In FIA mode, a 10-μL sample plug was injected into the water mobile phase flowing at a constant rate of 50 μL/min. The lower intensity IPA spectrum, shown in Figure 3b, was collected vertically through a fused-silica cover slip placed over 1-cm-i.d. glass vial to reproduce the same sampling conditions. Both spectra in Figure 3 were the result of one 4-s integration with the identical microscope excitation and collection optics. Note the different vertical scales for the two spectra. The intense Raman peak at 817 cm^{-1} (in-phase C−C−O stretch)[32] was used to evaluate the performance of the Raman waveguide cell vs the vial measurement. The Raman signal intensity from the spectrum of 10% IPA in the waveguide cell was greatly enhanced over the spectrum collected in the vial. The intensities of the Raman peaks at 817 cm^{-1} were calculated after background correction was performed to remove Raman scatter due to the water and fused-silica window. A comparison of the two spectra in Figures 3 shows that all features of the vial spectrum are both present and enhanced in the waveguide spectrum. Note, there are also no extraneous peaks in the waveguide spectrum from the Teflon AF tubing. When the laser is properly launched into the core of the waveguide, there is minimal Raman scatter collected from the Teflon AF waveguide cladding.

The inset spectra in Figure 3 shows the detection of IPA spectra nearing the LOD for both the vial and the waveguide
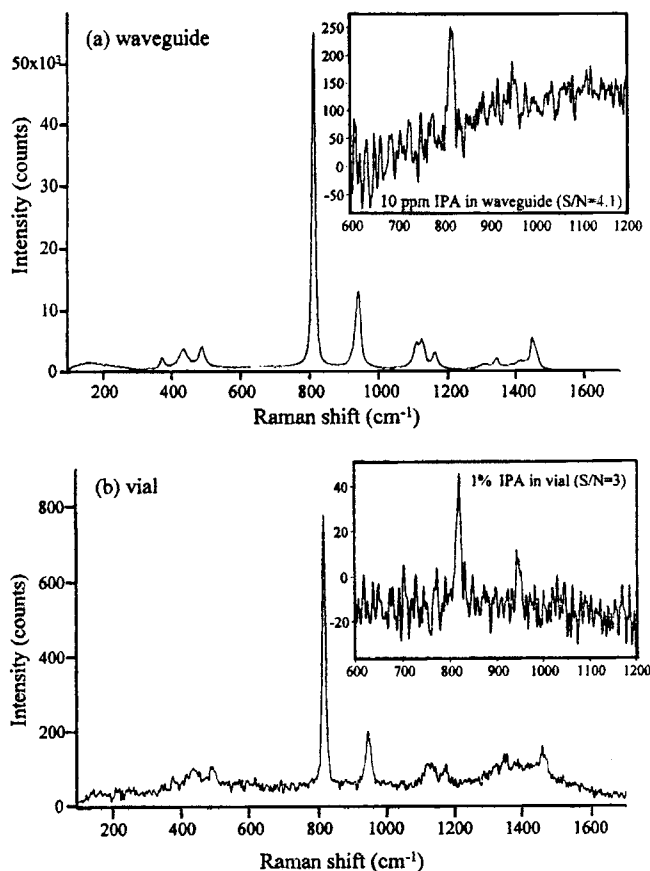
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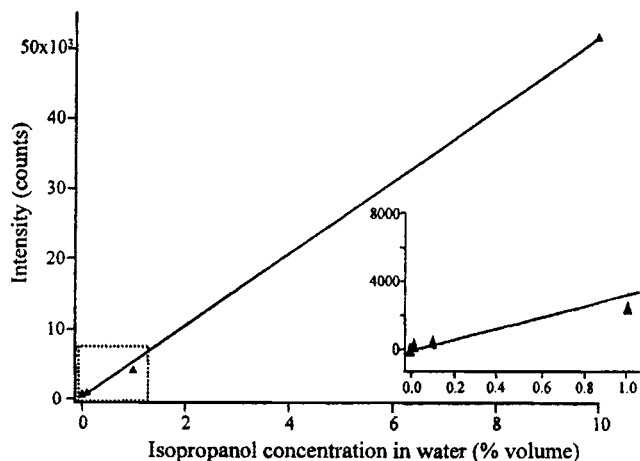
Figure 3. (a) Raman spectrum of 10% IPA in a 1 m × 50 μm i.d. Raman waveguide detector; (inset) Raman spectrum of 10 ppm IPA in the same waveguide (S/N = 4.1); (b) Raman spectrum of 10% IPA in a glass vial; (inset) Raman spectrum of 1% IPA in a vial (S/N = 3.2)

between 600 and 1200 cm^{-1}. The inset spectrum in Figure 3a is the result of one 4-s integration of a 10 ppm aqueous solution of IPA in the 1 m × 50 μm i.d. waveguide detector. The calculated signal-to-noise ratio (S/N) of the 10 ppm IPA spectrum was 4.1, where the noise is defined as three times the standard deviation of the mean between 630 and 680 cm^{-1}. The inset spectrum in Figure 3b is the result of one 4-s integration of 1% IPA in a vial. The calculated S/N of the 1% IPA spectrum was 3.2. Notice that there is an improvement in detection limits of over 1000 times for measurements performed in the Raman waveguide cell in comparison to a typical vial measurement. The sloping background and negative signal intensities in the inset spectra are due to the removal of the water background.

A calibration curve for aqueous solutions of IPA ranging in concentration from 10^{-3} to 10% (v/v) is shown in Figure 4. The data were obtained using the same FIA conditions as in Figure 3. The intensity of the 817-cm^{-1} Raman peak of IPA was used for calibration. Each point on the curve is the result of one accumulation of 4 s. The calibration curve appears linear over this large dynamic range; however, upon closer inspection, the slope of the curve decreases at lower concentrations. Some of the phenomena that contribute to the nonlinearity in the waveguide are the near-infrared absorption of the laser excitation (by water) and the absorption of the Raman scattered radiation by the mobile phase or analyte. As a result, one cannot simply extrapolate from the high concentration signals to infer what the concentration detec-

Figure 4. 2-Propanol calibration curve showing the intensity of the background-corrected 817-cm^{-1} Raman peak vs the concentration of IPA in water (v/v %) (concentration range, 1×10^{-3} −10%). The spectra were acquired using the 1 m × 50 μm i.d. Raman waveguide detector.

tion limit might be. Therefore, the LOD was calculated according to the formula, LOD = 3σ/m, where σ is the standard deviation of the noise around the mean between 630 and 680 cm^{-1} and m is the slope of the calibration curve over the low concentration range. The LOD for IPA in water at 785-nm excitation (60 mW) in a 1 m × 50 μm i.d. waveguide was determined to be 2 ppm. This concentration LOD is readily applicable to HPLC detection as will now be demonstrated.

The performance of the Raman waveguide detector for HPLC is illustrated in Figure 5, which shows the HPLC/Raman chromatogram obtained for a mixture of 1% injected methanol, ethanol, 2-propanol, and 1-propanol dissolved in water. Figure 5a shows a three-dimensional (3D) plot of a Raman chromatogram of the four alcohols each at a concentration of approximately 1000 ppm at the detector. Figure 5b shows the same data as Figure 5a but presented in a topographical contour plot format for clarity. The contour plot displays changes in signal intensity as a series of more tightly spaced lines; the smaller the spacing between lines the larger the Raman signal. Resolution of the Raman spectra along the chromatographic time axis was determined by the data collection rate, one 2-s integration required 4.3 s of total acquisition time. The data acquisition rate was limited by a 2.3-s data-transfer step that was inherent to the Raman system. The total run time for the HPLC/Raman analysis of the four alcohols was less than 6 min. The contour plot in Figure 5b more clearly demonstrates the power of coupling Raman spectroscopy to HPLC. The use of Raman detection helps to fully resolve peaks in two dimensions, where there would be partial overlap if viewed from the chromatographic axis only, as is the case with 1-propanol and 2-propanol in Figure 5b. To demonstrate the power of the HPLC/ Raman waveguide detector to identify overlapped peaks, a two-dimensional (2D) chromatogram was calculated from the data in Figure 5b and is shown in Figure 5c. The chromatogram in Figure 5c was calculated by graphing the maximum Raman intensity value for each spectrum in time over the spectral range from 600 to 1200 cm^{-1}. Notice in Figure 5c the overlap of the 2-propanol and the 1-propanol peaks when the data are displayed in a typical HPLC detector format. The four alcohols may not be completely separated in time (Figure 5c); however, their distinct spectral
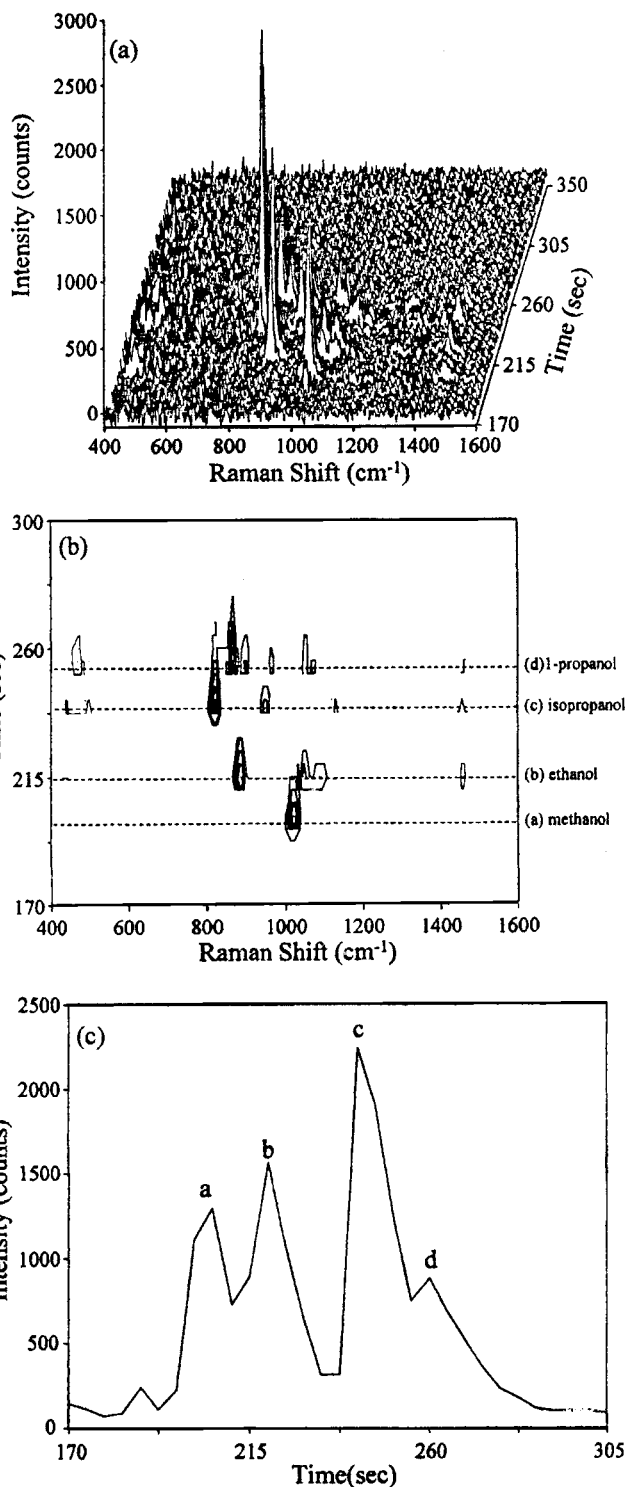

Figure 5. HPLC/Raman analysis of four alcohols (methanol, ethanol, 2-propanol, and n-propanol). (a) Three-dimensional plot of the four alcohols. (b) Contour plot of the four alcohols; the temporal elution position of each alcohol is indicated by the dashed line. (c) Calculated HPLC chromatogram from the data in (b). The letters above the peaks correspond to the analytes shown in (b).

features allow for positive identification using Raman spectroscopy (Figure 5b). The HPLC first simplifies the original matrix of compounds by a separation step prior to the spectroscopy. The Raman spectroscopy complements the separation step by allowing for the identification of specific analytes on the basis of their molecular signature, without the need to rely on retention time
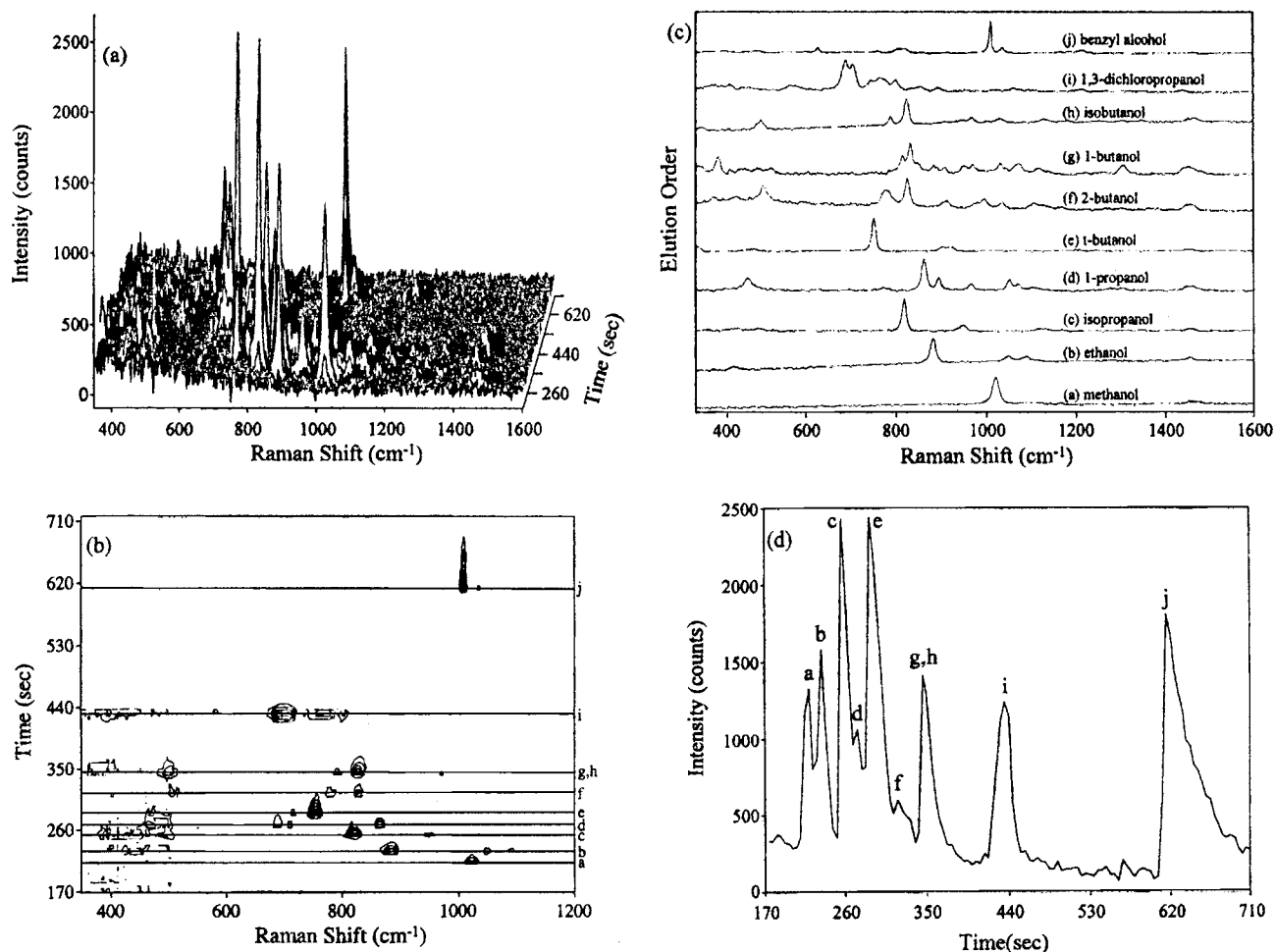
Figure 6. HPLC/Raman analysis of 10 alcohols. (a) Three-dimensional plot of the 10 alcohols injected at 0.5% (v/v). (b) Contour plot of the 10 alcohols; the temporal elution position of each alcohol is indicated by the dashed line. The letters a—j correlate to the standard spectra found in (c). (c) Standard spectra for each of the 10 alcohols injected at 1% (v/v) in the order that they elute from the column (bottom to top). (d) Calculated HPLC chromatogram from the data in (b). The letters above the peaks correspond to the analytes shown in (c). Note, the coeluting compounds g and h at 360 s (1-butanol and isobutanol).

precision. The resultant analysis is fast, selective, and sensitive and it allows for the collection of information-rich multivariate data.

To further demonstrate the effectiveness of the Raman waveguide as a detector for HPLC, a more complicated mixture of 10 similar alcohols was investigated. Figure 6a shows the 3D HPLC/Raman chromatogram obtained for a mixture of 10 alcohols injected at a concentration of 0.5% in water. The total run time for this HPLC/Raman chromatogram was less than 12 min. In a complicated matrix, such as the mixture of 10 alcohols, it is very difficult to distinguish individual spectral features from a 3D plot (Figure 6a). However, a contour plot of the same 10 alcohols, shown in Figure 6b, is much easier to interpret. The contour plot is useful for identifying the individual compounds in the HPLC/Raman chromatogram as a function of their spectral and temporal positions. Figure 6c shows the standard spectrum, collected in FIA mode, for each of the 10 aqueous alcohols injected at a concentration of 1%. The distinctive molecular fingerprint of each of the alcohols is evident from Figure 6c. In Figure 6c, the analyte name is labeled with a letter to indicate the position of that analyte in Figure 6b and d. The butanol compounds were included to evaluate the use of Raman spectroscopy for identification of compounds that coelute along the chromatographic time axis, namely, 1-butanol and isobutanol. The calculated chromatogram

in Figure 6d is shown to further demonstrate the enhanced temporal resolution gained from coupling Raman detection to HPLC. Notice the lack of baseline resolution in the calculated chromatogram, especially peaks d and f—h. The peaks g and h corresponding to 1-butanol and isobutanol, respectively, are completely overlapped in the 2D chromatogram (Figure 6d). However, because the sensitivity enhancements gained from the Raman waveguide detector allow for the identification of many smaller Raman peaks, even at injected concentrations of 0.5%, positive identification of coeluting analytes can be accomplished. For example, comparing the spectra of 1-butanol and isobutanol in Figure 6c, the standard spectra of the two analytes show unique spectral features in the $400-600-cm^{-1}$ region and as well in the $700-900-cm^{-1}$ region.

Some issues pertaining to the use of Raman waveguide detection that are currently being addressed in our laboratory are more fundamental in nature. These investigations include analyzing individual optical waveguides to predict and ensure reproducible analytical performance among cells. These measurements are meant to identify variations in core diameter, physical inclusions in the tubing walls, and possible microvoids in the walls, all of which will affect the waveguiding performance of both the excitation and scattered radiation. Another issue is whether

different tubing materials may be better suited to specific applications for reasons of optical matching (refractive index or numerical aperture). Issues pertaining to the laser excitation wavelength and the mobile phase are currently being investigated to improve the sensitivity and selectivity of HPLC/Raman analysis.

CONCLUSION

The continued development and optimization of Raman waveguide sampling and detection should facilitate the application of Raman spectroscopy to continuous on-line monitoring. The HPLC separation increases the utility of Raman spectroscopy by separating complex mixtures for easier identification and also by isolating fluorescing contaminants from the matrix to allow for analysis. Further investigation of the various interactions between excitation wavelength, absorption, and scattering phenomenon is needed to select optimal conditions for performing Raman measurements in liquid core waveguides.