

# Small-Volume Raman Spectroscopy with a Liquid Core Waveguide

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A liquid-core waveguide based on a new fluoropolymer tube is proposed as the basis of a novel, small probe-volume Raman-scattering-based detector. Illumination is transverse to the axis of the tube, with end-on detection through a transparent window at the tube terminus. Using this approach and a ~6-nL probe volume, we have measured benzene, carbon tetrachloride, and aqueous solutions of 2-propanol (in 5–100% concentration) as test compounds. We show successful collection of Raman spectra to shifts of ~200 cm<sup>-1</sup> collected *without* a holographic notch filter. Additionally, the apparatus naturally discriminates against Raman signals from the capillary material. This permits simplified detection of the liquid sample without the need to subtract the otherwise omnipresent contribution of the capillary material. A simple, multiple-pass, laser illumination scheme is found to enhance the Raman signal intensities; the gain in S/N increases slightly less than linearly with the number of passes. In the case of 2-propanol, experiments were conducted in both static and in a flow injection mode. With a correction applied for RI change due to the solute, the Raman intensity was linearly dependent on the analyte concentration.

Raman spectroscopy, in common with infrared (IR) spectroscopy, is a powerful tool that can provide compound-specific structural information. It has the advantage over IR spectroscopy that aqueous solutions pose no special problems and exotic materials are not required to fabricate cells or other optical components. However, Raman scattering is an inefficient process, only 1 out of 10<sup>10</sup>–10<sup>4</sup> incident photons undergoes Raman scattering. Even with focused laser sources and modern electronics, it is generally not a very sensitive technique.

Walrafen<sup>1,2</sup> pioneered Raman spectroscopy in long-path liquid core waveguide (LCW) cells. Fused silica or glass tubes can act as LCWs because total internal reflection (TIR) takes place at the silica/air interface. Using this technique, large enhancements in limits of detection were observed and even some new spectral features of benzene and tetrachloroethylene were discovered.<sup>2,3</sup> With bare glass or silica tubes as waveguides, one must be

extraordinarily careful to maintain a clean, dust-free exterior. Walrafen's experiments with a polytetrafluoroethylene (PTFE) tube as a LCW cell were less successful, and large losses were encountered.<sup>4</sup> More than a decade later, Schwab and McCreery<sup>5</sup> revisited this system with a drawn thin-wall glass tube as the LCW and a fiber bundle attached to it for remote spectroscopy. One central fiber brought in the excitation beam while the backscattered Raman signal was collected by 18 fibers surrounding the transmitter fiber. More recently, Benoit and Yappert<sup>6</sup> showed the utility of a simple two-fiber (one transmitter, one receiver) configuration for such a system. Relatively inexpensive Raman spectrometers based on diode laser excitation and detection with a linear CCD array have since become commercially available.<sup>7</sup>

Although Walrafen's results, with a Teflon PTFE tube as an LCW, were not especially encouraging, if the refractive index (*n*) of the sample is higher than that of PTFE (RI, 1.35 for Na D line), it will behave as an efficient LCW. Aust et al.<sup>8</sup> successfully studied the curing of epoxy polymers (which display an RI greater than that of PTFE) in a PTFE-based LCW. As pointed out in a previous paper,<sup>9</sup> the major advance in LCW-based spectroscopy of aqueous solutions in recent years has been the introduction of a new amorphous fluoropolymer, Teflon AF. Teflon AF has a refractive index as low as 1.29, less than that of water (1.33). Tubes made from Teflon AF or fused-silica tubes coated with Teflon AF are both commercially available. The use of such LCWs for Raman spectroscopy, with axial launching of the excitation beam and the detection of the Raman signal in the backscattering geometry, is the subject of a patent.<sup>10</sup> Such LCWs are of considerable utility in enhancing Raman signals and this has been discussed in detail.<sup>11–14</sup>

While the LCW cells above have contributed much to enhancing Raman signals, the total effective probe volume is large for many applications. If, for example, Raman spectroscopy is to be

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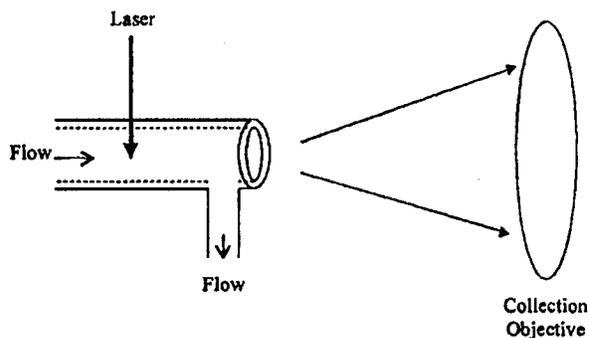
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successfully used as a liquid chromatographic detection technique, other innovations are necessary. Much of the recent rejuvenation of interest in Raman spectroscopy as a practical analytical tool can be attributed to the development of holographic notch filters (HNFs).<sup>15,16</sup> The use of optically fast Raman systems, incorporating a HNF and multichannel detection, results in high signal-to-noise spectra with short integration times. Additionally, the use of a single-grating monochromator over the traditional double-grating systems results in a significant cost reduction. The combination described is potentially powerful for examining liquids flowing through a conduit by Raman spectrometry.

Direct backscattering is a widely used mode that permits a small detection volume conducive to chromatography and other applications. However, in this geometry, the Raman spectrum of the conduit material, typically glass or silica, overlaps that of the analyte signals and can greatly complicate tasks of interpretation. This is the same problem encountered in optical fiber-based Raman spectroscopy, especially where the same fiber is used for both excitation and reception of the Raman signal, as is common with LCW cells.<sup>10-12</sup> In principle, it is possible to overcome the problems associated with Raman scattering by the capillary walls by using a transverse illumination configuration. In this setup, the flow conduit is illuminated from its side and the Raman signal is collected axially. However, this requires that the illuminated volume be very close either to the detector itself or to an optical fiber that carries the signal to the detector. Such an arrangement also increases interference from proximally scattered and reflected excitation light.

In a previous paper, we have shown the utility of Teflon AF-based LCW cells for fluorescence detection using the transverse illumination geometry.<sup>17</sup> Efficient rejection of the excitation light and collection of the fluorescence emission, with the emission monitoring point (a transparent window or an optical fiber) axially distant from the illumination window could be achieved. Extending this method to Raman spectroscopy may significantly enhance future application of this concept. This configuration *optically* discriminates against the spectrum of the conduit. This is because any light scattered within the LCW material will undergo one of several fates. Light scattered outside the TIR acceptance angle will simply emerge from the fiber walls, far from the detection optics. The light scattered within the capillary TIR cone emerges principally from the open aperture end. A smaller amount is guided within the wall of the capillary conduit, this portion is originally rich in the Raman signal originating from the conduit material. However, this portion also undergoes randomizing scattering within the capillary walls and is significantly attenuated by the several centimeter passage through the conduit material. Most importantly, it is convenient to adjust the imaging optics such that, at the detection end, it is the light emanating from the core liquid and not that from the thin conduit wall periphery that is imaged onto the detector. Finally, stray light is also discriminated against since only the weak Rayleigh scattering at near-right angles to the illumination experiences TIR to reach the detection system. In this initial study, we show that it is in fact



**Figure 1.** Diagram of the sample chamber illumination setup. The incident laser light was focused by a spherical lens or a cylindrical lens (line focus) or was unfocused.

possible to collect spectra to  $\sim 200 \text{ cm}^{-1}$  with a small probe volume, *without* the use of a HNF.

## EXPERIMENTAL SECTION

The transverse illumination LCW cell arrangement is shown in Figure 1. A Teflon AF 2400 tube, 0.79-mm i.d., 1.04-mm o.d. (BioGeneral, San Diego, CA),  $\sim 30 \text{ cm}$  long, was provided with a L-joint at one end, with the joint end being open (functionally equivalent to a T-joint with one arm cut off). A thin microscope slide cover slip was cemented on the open aperture and functioned as the optical window to the spectrometer. Liquid samples were injected through the distal end of the tube and exited through the vertical arm of the L-fitting. The tube was illuminated from the side, at a distance of 1–3 cm with an Ar<sup>+</sup> ion laser (Coherent 306) operating at 488.0 nm with an approximate continuous power of 50 mW incident on the capillary. The laser illumination was polarized transverse to the tube axis (parallel to the entrance slit of the spectrometer). Focused spot ( $\sim 100 \mu\text{m}$ ), unfocused spot ( $\sim 0.5 \text{ mm}$ ), cylindrical lens ( $\sim 3 \text{ mm}$ ), and line illumination (using a ruled plastic spot-to-line converter) of 7-mm illumination length were investigated. A multiple-pass illumination system (unfocused beam), using plane mirrors on each side of the capillary, was used to achieve higher illumination levels of the liquid core. Unless otherwise stated, the results presented here for carbon tetrachloride and benzene are for focused spot illumination.

While Raman scattering occurs in all directions, only the portion within the acceptance angle undergoes TIR and travels down the lumen. The acceptance angle  $\theta_a$  is given by

$$\theta_a = 90^\circ - \theta_c \quad (1)$$

where  $\theta_c$  is the critical angle for total internal reflection, given by

$$\sin \theta_c = n_{\text{clad}}/n_{\text{core}} \quad (2)$$

where  $n_{\text{core}}$  and  $n_{\text{clad}}$  are the refractive indexes of the liquid and the Teflon AF, respectively.

Light emerging from the capillary will have a numerical aperture (NA) of

$$\text{NA} = \sqrt{(n_{\text{core}}^2 - n_{\text{clad}}^2)} \quad (3)$$

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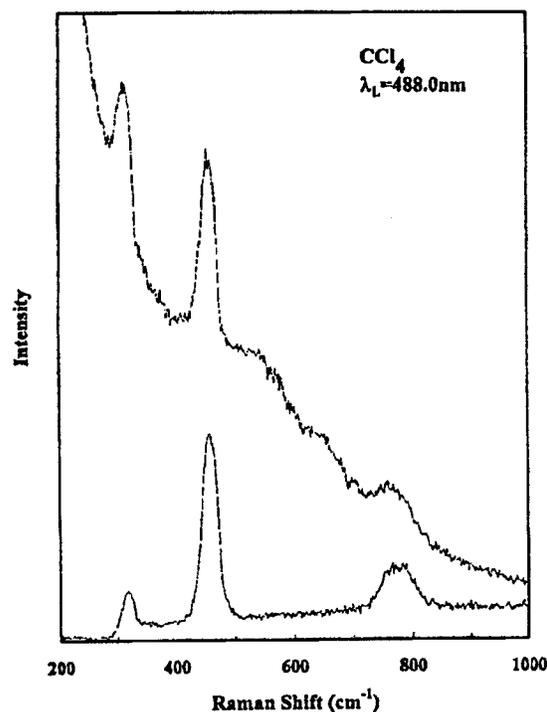
Light from the LCW was collected using two schemes. The first approach, used for the carbon tetrachloride and benzene measurements, used a camera objective of NA 0.5, conditioned to match the spectrometer  $f$ number, and imaged on the entrance slit of a 0.5-m Spex 500M spectrometer ( $f/0.22$ ). When a HNF (Kaiser Optical Systems) was used, it was positioned between the collection objective and spectrometer. Light dispersed by the spectrometer was detected by a cryogenically cooled, charge-coupled device (CCD) array (Princeton Instruments, TEK 512  $\times$  512 TKB). Integration times ranged from 1 to 60 s. For comparative purposes, a micro-Raman instrument (Spex Industries), with the same spectroscopic setup (necessarily including the notch filter),<sup>14-18</sup> was used to acquire the spectra from the liquid-filled tube in the direct backscattering mode with illumination transverse to the axis of the capillary (excitation beam enters through the capillary walls and backscattered Raman intensity measured).

Measurements of 2-propanol were carried out using a modification of the micro-Raman instrument. In this case, the capillary was situated with the end window in the focal plane of the microscope objective ( $f/0.5$ ). This system gave us somewhat narrower Raman bands ( $\sim 2/3$  the width of those obtained using the camera-objective collection optics) based on direct comparisons of carbon tetrachloride. In the microscope setup, laser illumination was focused into the capillary using a cylindrical ( $f = 7.5$  cm) lens. No significant change was observed in the scattering intensity measured these two ways. A cylindrical mirror was also used, in conjunction with the cylindrical lens, to direct transmitted light back onto the capillary. This produced an enhancement of  $\sim 1.8$  in the measured Raman intensities as discussed in more detail in the results section. No notch filter was used for these measurements. The remainder of the Raman system was identical to what was described above. Spectra for 2-propanol were acquired at various dilutions (concentrations are quoted in percent v/v) both under static conditions and in a flow injection analysis setup. Water served as carrier, flowing at  $\sim 110$   $\mu\text{L}/\text{min}$ , into which 70  $\mu\text{L}$  of the 2-propanol sample was injected. Spectra were collected sequentially at 6-s intervals (5-s integration time). This allowed us to monitor the Raman intensity vs time.

## RESULTS AND DISCUSSION

Figure 2 shows Raman spectra for  $\text{CCl}_4$  using our LCW Raman system. Spectra in Figures 2 and 3 were each acquired with a 1-s integration time. The lower spectrum is taken with the HNF in place. We see three features near 315, 460, and 775  $\text{cm}^{-1}$ . Upon removing the HNF, the background rises revealing a strong Rayleigh tail. The Raman bands also gain in intensity by  $\sim 50\%$  (spectra are rescaled in Figure 2 so that the principal Raman band near 460  $\text{cm}^{-1}$  has approximately the same intensity). This spectrum is remarkable, because it is generally difficult, even impossible, to obtain laser-induced Raman spectra with a single-grating monochromator without the use of a HNF. In a standard configuration with a single-grating monochromator and no HNF, the detector is saturated by the Rayleigh tail and the weaker Raman bands are not detected.

For comparison, Figure 3 shows the spectrum of  $\text{CCl}_4$  in the same tube in the backscattering configuration. In addition to the Raman bands of  $\text{CCl}_4$ , a surfeit of Raman lines attributable to the

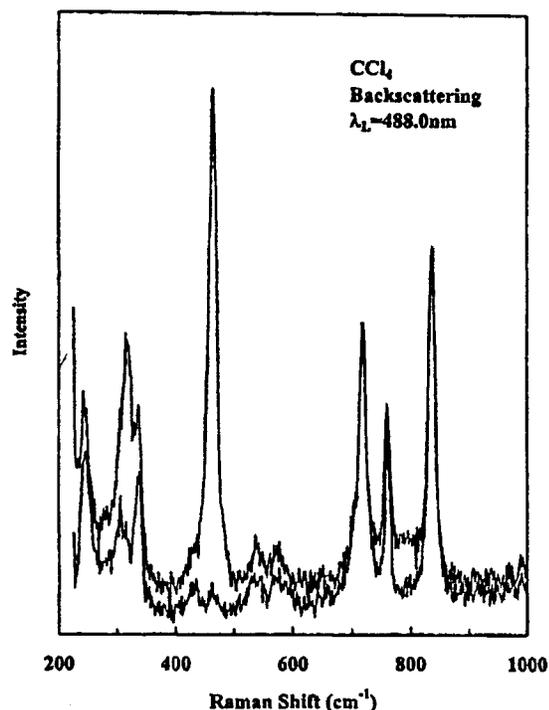


**Figure 2.** Raman spectra of  $\text{CCl}_4$  using the LCW setup depicted in Figure 1. The upper spectrum was collected without the aid of a HNF and exhibits a Rayleigh tail. The lower spectrum was taken with the addition of the HNF. The absorption cutoff for the latter appears just below the low-wavenumber feature near 200  $\text{cm}^{-1}$ .

tube material is readily discernible. This is confirmed in the lower spectrum, obtained with an empty tube (no  $\text{CCl}_4$ ). The spectra in Figure 3 were obtained in the presence of a HNF; without such a filter, no Raman bands could be discerned. The results shown in Figure 3 clearly illustrate the problems associated with overlapping lines from the sample container material. In the present instance, vibrational bands from the liquid and capillary wall are comparable in intensity. Thus, without the spectrum from an empty capillary available for comparison, even though  $\text{CCl}_4$  has a strong Raman spectrum, it would not be obvious as to what bands are attributable to  $\text{CCl}_4$ . Even with known band assignments, the 460- $\text{cm}^{-1}$  Raman band is the only feature with sufficient intensity to attempt quantitative analysis on, the 315- $\text{cm}^{-1}$  band is marginal, and the 775- $\text{cm}^{-1}$  band is hopeless without sophisticated signal-processing efforts. For liquids exhibiting Raman signals weaker than what we show in Figure 3 for  $\text{CCl}_4$ , it would not be possible to perform identification based on these spectra alone. A comparison with Figure 2 clearly indicates the advantage of the transverse illumination LCW Raman spectroscopy that successfully rejects the signals from the sample containment material.

It is noteworthy that the Raman bands of  $\text{CCl}_4$  at 315 and 460  $\text{cm}^{-1}$  are nearly twice as broad in the LCW configuration (Figure 2) than in the comparison backscattering experiment (Figure 3). This is due to a difference in the imaged area registered on the spectrometer slit. Although the same spectrometer system and slit settings (200  $\mu\text{m}$ ) were used in both cases, the micro-Raman system generates a 100  $\mu\text{m}$  wide image at the entrance slit. The LCW system actually has an extended image (transversely and

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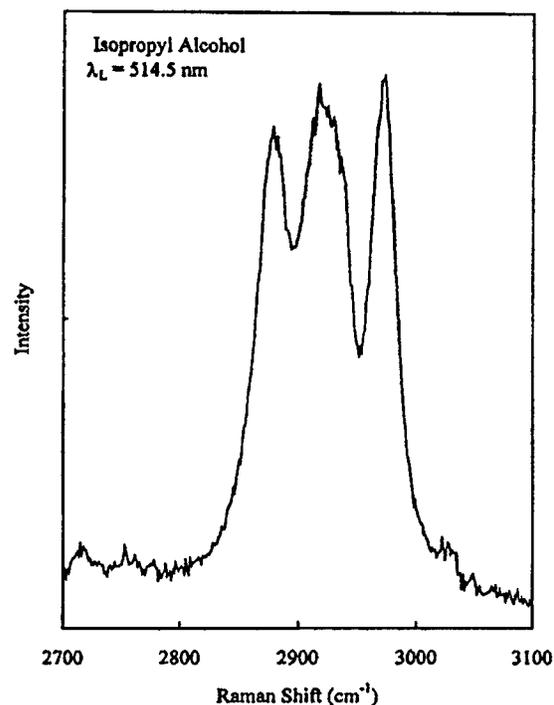


**Figure 3.** Raman spectra of  $\text{CCl}_4$  using a micro-Raman apparatus in the direct backscattering configuration. In addition to the features from the carbon tetrachloride, we also see strong scattering from the Teflon AF capillary. A spectrum of the latter material (no  $\text{CCl}_4$ ) is shown in the lower trace for comparison.

longitudinally) greater than that of the slit. The total response function is therefore slightly larger than the limit imposed by the slit, producing twice the bandwidth. It would be possible to eliminate this simply by using a smaller diameter capillary.

No significant improvement in S/N was found with the use of a spot-to-line converter. Although a larger number of molecules is irradiated, the incident radiation flux is also decreased. We also tested the utility of a simple multiple-pass arrangement to improve the signal intensity. In a single pass experiment, relative to the tightly focused (0.1-mm spot diameter) illumination, an unfocused beam (~0.5-mm spot diameter) leads to a signal that is 2–3 times lower in intensity. This intensity reduction is due to greater reflection losses from the curved surface and the fact that the unfocused laser has an optical spot size comparable to the conduit diameter. These factors result in a much lower power density within the capillary liquid when an unfocused laser is used for excitation. Nevertheless, for these initial multipass experiments, a defocused spot diameter of ~0.5 mm (probe volume per pass ~150 nL) was used because it was difficult to maintain tight focus on multiple passes. For the multiple-pass illumination, the signal enhancement was found to be 1.0:1.9:2.3 for one, two, and three passes without a concomitant increase in noise. The fact that the gain in the signal intensity is sublinear can be attributed to beam divergence, reflection losses, and, to a lesser extent, absorption by the LCW assembly. On the basis of this observation, we are currently setting up a more sophisticated illumination system to take advantage of the improved signal measured with multiple passes which will also allow maintaining low illumination densities.

We also successfully conducted Raman measurements on benzene in the 1200–2000- $\text{cm}^{-1}$  range of Raman shift. However,

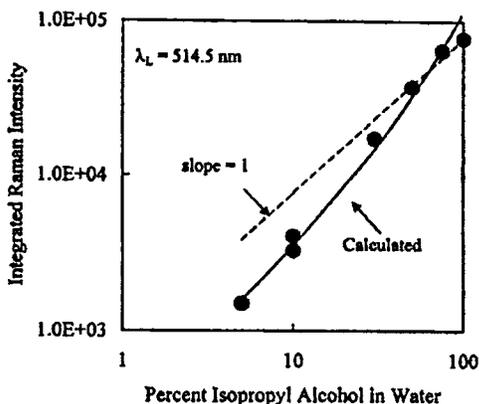


**Figure 4.** Raman spectrum of 2-propanol obtained in the transverse illumination micro-Raman setup. The bands correspond to C–H stretching vibrations.

the carbon tetrachloride study requires a lower wavenumber shift measurement, much closer to the excitation wavelength and therefore provides a more stringent test. Benzene spectra are therefore not separately shown.

Figure 4 shows the Raman spectrum of 2-propanol, taken in the C–H stretching range, using the microscope-based transversely illuminated LCW system. This spectrum shows a grouping of strong features, which are best fitted by four Lorentzian bands at 2878, 2916, 2936, and 2972  $\text{cm}^{-1}$ . The first two fitted bands have line widths (full width at half-maximum) of 28  $\text{cm}^{-1}$ , while the latter two have line widths of ~22  $\text{cm}^{-1}$ . These line widths are comparable to our measurements of  $\text{CCl}_4$  with the identical setup. Moreover, the spectrum shown is comparable to that obtained using a conventional higher resolution (spectral band-pass of 5  $\text{cm}^{-1}$ ), double-monochromator setup. It is therefore evident that this geometry is capable of yielding good Raman spectra.

The analyte concentration dependence of the observed Raman spectrum, using conditions identical to those used for Figure 4, was studied for 5–100% 2-propanol, using a 60-s integration time. In Figure 5 the experimental data for the integrated Raman intensity in the C–H stretching region are shown as points plotted on a log–log scale. The dashed line is a theoretical response curve that assumes zero intercept and direct proportionality between the measured Raman signal and 2-propanol concentration. Note that the experimental data largely fall below the dashed line. This occurs because around room temperature, pure 2-propanol has a refractive index of 1.374, while that of water is 1.334. The use of different concentrations of 2-propanol as analyte results in different refractive indexes of the liquid core of the LCW. Hence, the exit numerical aperture of the LCW will decrease with a lower analyte concentration, resulting in a decreased Raman signal, the light



**Figure 5.** Dependence of the integrated Raman intensity of 2-propanol (area under the bands in Figure 4) on concentration of the aqueous 2-propanol. The dashed line has a slope of unity, corresponding to linear, zero intercept dependence between the measured intensity and the analyte concentration. The solid curve is calculated taking into account variations in the refractive index with dilution.

throughput being related to the square of the numerical aperture. Additionally, the acceptance angle for total internal reflection will depend on the refractive index of the liquid core. The solid curve in Figure 5 (labeled "Calculated") takes into account these two factors and results in excellent agreement with the experimental data. This agreement also establishes that the actual Raman intensity (before modification by the involuntary changes in numerical aperture) is strictly linear with the 2-propanol concentration. Note that the refractive index for pure 2-propanol is such that the measured Raman intensity may be marginally lower than that expected because this elevated NA is close to exceeding the NA of the collection optics (or downstream optics). In such a case, the entire throughput of the LCW will not be processed by the Raman detection system, resulting in a (small) reduction in the observed intensity.

The quantitative reproducibility of the results shown in Figure 5 was very good, suggesting that the present approach will be well suited as a quantitative technique. The lower limit of detection using the microscope optical setup was low single-digit percentile levels of 2-propanol. Comparative studies with the macro-objective used for the carbon tetrachloride and benzene indicate an order

of magnitude gain in S/N. We are in the process of improving this further, by an alternative setup which increases the illuminated volume – illumination flux product.

An obvious application for a low-probe-volume Raman spectrometry system is as a detector in liquid chromatography. While this would be difficult in the presence of an organic solvent background, it should be possible to do this with only water as the eluent, an especially weak Raman scatterer. Practical chromatography with water as eluent has now been successfully accomplished by us and others.<sup>14,19,20</sup> We have now tested the proposed flow-through Raman detection scheme using a water carrier in a flow injection mode. Despite the limitations of the present system, the profile of a typical flow injection peak (ascending to a maximum, descending more slowly to baseline) was readily observed as a bolus of injected 2-propanol (estimated concentration at peak maximum 20–30%) moved through the illuminated volume.

In conclusion, we have successfully set up and implemented Raman scattering from a liquid based on transverse illumination and total internal reflection within the liquid. This LCW approach has several key advantages for examining a small probe volume. First, illumination can be at least several centimeters (3–5 cm in our experiments) from the capillary end without significant compromise to the measured intensities. A second advantage is that *no* spectral features are seen from the conduit material (Figure 2). In contrast (Figure 3), the more traditional backscattering mode and observing the contents of the tube through its walls often shows large signals from the conduit material resulting in overlapping spectra. The third advantage of this approach is that Raman spectra can be obtained without the use of a HNF, which is observed to reduce measured intensities by a factor of ~2. This is demonstrated in this paper to Raman shifts of ~200  $\text{cm}^{-1}$ . The detection of an injected bolus in a water carrier has been shown to be feasible. Ongoing work is aimed at improving the limits of detection, so that low concentrations of dissolved solutes within a carrier liquid can be studied by Raman spectroscopy in a low-probe-volume, flow-through configuration.

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