Convenient Microsampling System for UV Resonance Raman Spectroscopy

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INTRODUCTION

We report here the development of a new microsampling device for UV Raman measurements which permits the use of sample volumes as small as 50 μL and which permits recirculation of the sample to minimize sample heating and the contribution of transient species. UV Raman studies with pulsed laser sources (typically ~10 ns duration) can cause transient heating during the laser pulse.[1,2] Unless the sample is exchanged between pulses, the Raman measurements will monitor previously heated sample volumes which may contain thermally or photochemically generated transient species. Given the ~10 ms intervals between laser pulses, we may observe photochemically generated transient species with decay times longer than ~1 ms. In addition, the long thermal diffusion times which exist for typical samples will result in the significant heating of a static illuminated sample volume.

A static sample illuminated by a pulsed laser can reach a very high steady-state temperature; this is the sample temperature at which the rate of sample heating by each pulse is compensated by the heat flow out between pulses. For example, at a 100-Hz repetition rate, a laser pulse energy of 0.1 mJ/pulse results in an average power of 10 mW. A single 0.1-mJ laser pulse will transiently increase the temperature of an aqueous 1-nL sample by ~24 K; a 1-nL sample would correspond to a sample of 0.1 mm thickness illuminated by a ~0.1-mm-spot-size laser pulse.

We can simply use Newton's cooling law,[3]

$$dQ/dt = hA\Delta T,$$

to estimate the steady-state temperature rise for this 1-nL sample illuminated by 0.1-mJ pulses at a 100-Hz repetition rate. We must determine the sample temperature which is necessary to achieve the cooling (dQ/dt) required to dissipate the incident 0.1-mJ pulse energy in the time interval between pulses (0.01 s). Although we could solve this problem exactly, we leave this to a future contribution, which will clarify the nature of the relevant thermal diffusion phenomenon. We here, for illustration, assume naively that the area A for heat flow is the external sample volume surface (6×10^{-4} cm^2) and that the heat transfer coefficient h corresponds to that for natural convection of water[3] (h = 570 J/m^2 K). Thus we assume that heat diffusion occurs across a temperature gradient corresponding to the temperature difference between the steady-state sample temperature and the ambient temperature. We calculate a temperature increase of 50 K, which represents a serious underestimate since a static sample will form a continuous temperature gradient which decreases the cooling rate.

To avoid this temperature increase, most liquid-phase resonance Raman measurements utilize a flowing sample in which the sample volume is exchanged between laser pulses. While this approach is effective, it requires relatively large sample volumes (~2 cc). In this report we demonstrate the development of a new microsampling system that easily reciprocates a small sample volume through the laser beam. This reciprocating volume is cooled prior to return to the incident beam. The steady-state temperature increase is minimized, and transient species can relax back to the ground state.

DISCUSSION

The microsampling system (Fig. 1) uses a tiny air pump to reciprocate the sample solution in a quartz capillary. A 50-μL sample solution from a syringe (**1**) is slowly injected into the 1-mm-i.d. quartz capillary (**2**) to give a cylindrical liquid sample segment (**3**) without any bubbles. Another syringe (**4**) is used to adjust the position of the sample by removing or injecting air. Teflon® tubing and compression fittings are used for connecting the components. A piece of 7-mm-i.d. rubber tubing (**5**), closed at one end, is used as a "bellows." The bellows is compressed by a reciprocating plunger (**6**) driven by a motor (**7**). The pumping force is controlled by varying the motor speed and by adjusting a hose clamp control valve (**8**). The sample is retrieved by the sample injection syringe (**1**).

Representative resonance Raman spectra obtained by this microsampling system are shown in Figs. 2 and 3. These resonance Raman spectra of N-methylacetamide and diglycine (DGL) were excited at 220 nm by using a Raman spectrometer which is described in detail elsewhere.[4,5] The samples were excited by a XeCl excimer

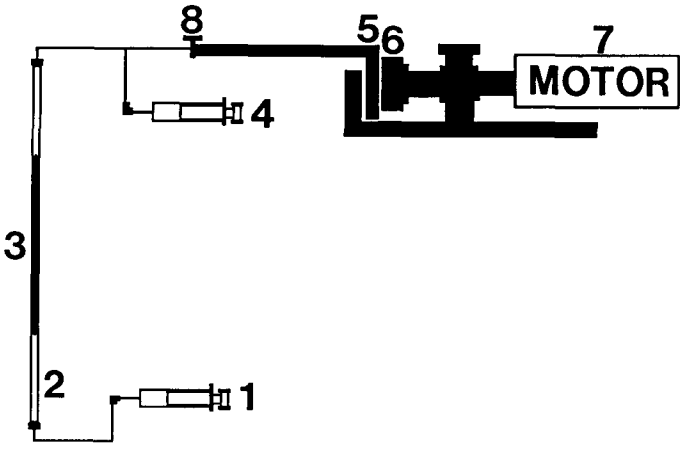

FIG. 1. Microsampling system. (**1**) Sample syringe; (**2**) quartz sample capillary; (**3**) sample volume; (**4**) pressure adjustment syringe; (**5**) rubber bellows; (**6**) plunger assembly; (**7**) motor; (**8**) control valve.

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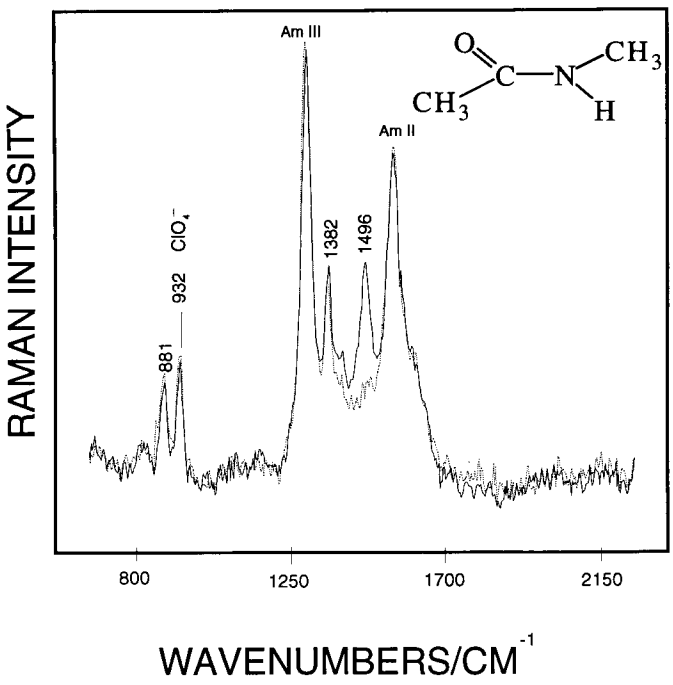

FIG. 2. Resonance Raman spectra of a 100-μL sample of 0.1 **M** N-methylacetamide excited at 220 nm. Pulse energy flux density was ~5 mJ/cm^2 pulse. Samples contain 0.4 **M** $NaClO_4$. (Dashed curve) Illuminated sample volume exchanged between pulses. (Solid curve) Illuminated sample volume completely exchanged between approximately 14 pulses (~140 ms).

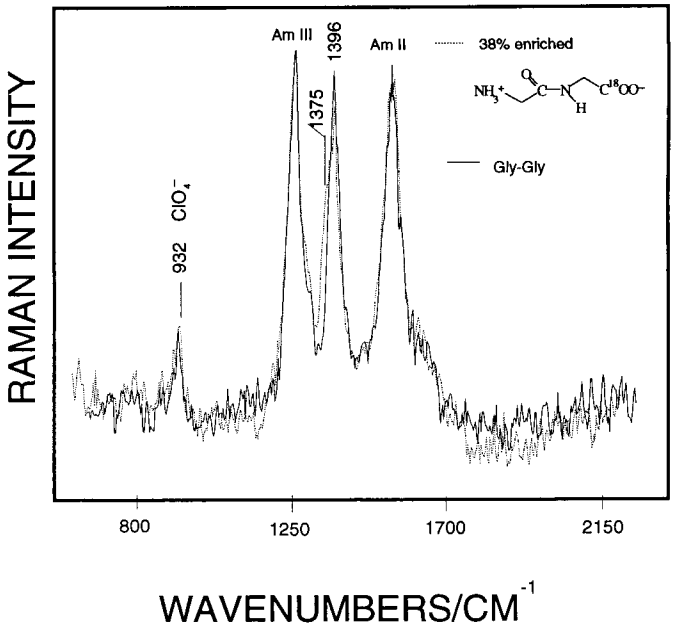

FIG. 3. Resonance Raman spectra of 0.1 **M** diglycine solution at neutral pH. Samples contain 0.4 **M** $NaClO_4$. Excitation wavelength is 220 nm at a pulse energy flux of ~5 mJ/cm^2 pulse. (Solid curve) DGL in normal water. (Dashed curve) DGL in ^{18}O enriched water. The ^{18}O exchange reaction occurred over 3 days with acidic catalysis.[8] Mass spectral data show 38% ^{18}O enrichment of Gly-Gly after evaporation of H_2O.

laser system in which the excimer pumped a dye laser which was frequency doubled to achieve the UV excitation wavelength. The pulse energy flux density at the capillary was estimated to be 5 mJ/cm^2 pulse.

Figure 2 compares spectra of a 100-μL sample of 0.1 **M** N-methylacetamide (NMA) measured for two different sample recirculation rates. The dashed curve was for a recirculation rate that replenished the sampled volume between pulses (within 10 ms), while the solid curve was for a slower recirculation rate that recirculated the sample after approximately 14 pulses (within 140 ms). The UV Raman spectra of NMA have been extensively studied[1,6,7] over the last few years, and the unusual NMA photochemistry has been well characterized; normally *trans* NMA is easily thermally and photochemically converted to the *cis* form. The 932-cm^{-1} band in the spectra derives from the internal intensity standard perchlorate. The *trans* NMA amide II and amide III bands are labeled in the spectra. The 1382-cm^{-1} band is a methyl umbrella motion[1] of *trans* NMA. At the slow recirculation rate, a small intensity decrease of the *trans* bands occurs, while a large peak appears at 1496 cm^{-1} which derives from the *cis* form[1] of NMA. Given the 10-ms period between pulses, it is most likely that the formation of the *cis* form derives from sample heating in the slowly reciprocated sample. The utility of the microsampling system is clear from the fact that the *cis* peak is weak in the quickly recirculating sample; at room temperature approximately 1.5% of NMA is in the *cis* form.[1]

Figure 3 shows the utility of the microsampling system for a precious 100-μL sample of ^{18}O enriched DGL. The solid curve is the Raman spectrum of a 0.1 **M** natural isotopic abundance sample of DGL in water, while the dashed curve shows the spectrum of 0.1 **M** DGL in 50% ^{18}O enriched water at neutral pH; the carboxyl group oxygen exchanges.[8] The small frequency shift for the 1397-cm^{-1} band of Gly-Gly-$^{18}O_1$ is easily distinguished. A detailed analysis of these data demonstrates that the 1397-cm^{-1} band contains an overlapping symmetric COO- stretching band.[9]

Thus, we show here that this microsampling system makes it possible to obtain UV resonance Raman measurements of tiny liquid (and presumably gas) samples, without significant thermal heating. The signal-to-noise ratios achieved with this sampling system are identical to those obtained with macro flowing sampling systems. This sampling system will always be superior to the macro systems when the sample illumination time is not limited by irreversible photochemical damage.

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