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A Modular Raman Spectrometer for Solids

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Abstract: We describe a modular Raman spectrometer system capable of quickly collecting spectra from pellets of organic and inorganic substances, solid mineral samples, and solid biological samples. The system can be assembled from commercial components. This spectrometer system opens up the possibility of including solid-state Raman spectroscopy in the undergraduate physical and analytical chemistry laboratory curricula.

Introduction

Raman spectroscopy is a powerful form of vibrational spectroscopy capable of giving detailed information about molecular structure with a wide variety of samples; however, molecular Raman cross sections are weak, necessitating the use of relatively intense light sources and sensitive detection electronics. These constraints can lead to high instrumentation costs, creating a barrier to implementation of Raman spectroscopy in undergraduate programs. Recently, relatively low-cost laser light sources and detectors have made it possible to construct inexpensive Raman spectrometers suitable for undergraduate teaching applications. In 2002 DeGraff et. al. [1] described in this journal the assembly of a modular low-cost Raman spectrometer capable of producing good quality Raman or resonance Raman spectra with neat or concentrated liquid samples. Here we extend the work of DeGraff et. al. with a Raman spectrometer capable of producing spectra of solid samples.

Even before DeGraff et. al. published their design, Sommer and Stewart [2] gave a very simple design for a Raman microprobe for solid samples based on CCD detection.Sommer and Stewart's apparatus, like most Raman microscopes, involved an excitation beam path that travels along the same axes as the signal. This type of a design leads to the use either of a holographic beam splitter or two holographic notch filters. Holographic beam splitters are expensive and difficult to find, and the need for two holographic notch filters adds significantly to the cost of the apparatus. In addition, the microscope line up can be tricky. The design that we present here involves an oblique angle arrangement, which does not require a beam splitter or more than one notch filter.

We seek to demonstrate several possible applications for the spectrometer. First, we show that the spectrometer can be used to observe spectra of solid samples of inorganic salts. Comstock and Gray [3] have published a useful physical chemistry laboratory in which a series of aqueous solutions of salts of symmetric oxyanions are examined by Raman spectroscopy and the peak locations are compared to the results of computational modeling of normal vibrational modes. Observing solid samples removes the solution-making step and also allows one to demonstrate effects of lattice structure on vibrational modes.

The second application is the detection of structural detail in amino acids, which are only weak Raman scatterers. Raman spectroscopy has long been a key technique in research in structural molecular biology, especially as related to protein structure and function. For this reason we were interested in assembling a spectrometer that would be capable of producing spectra of amino acids and perhaps even spectra of peptides and proteins. Other groups have demonstrated that it is possible to construct homebuilt Raman spectrometers capable of gathering detailed information on proteomic analytes. For example, Ben-Amotz and colleagues [4] have constructed a relatively inexpensive micro-Raman system capable of detecting phosphorylation sites in peptides [5] and of identifying insulin variants [6]. Uzunbajakava et. al. [7] have imaged the protein distribution in individual human cells using a homebuilt confocal Raman microscope. One of our goals was to design a Raman system for teaching applications that was modular (in order to allow components to be used for other experiments in physical and analytical chemistry laboratories) and that involved even lower cost and less line-up than these previous systems.

Another application of solid-state Raman spectroscopy is found in geology. Minerals can be identified in a nondestructive way by their characteristic Raman spectra[8]. Raman spectroscopy can also be used to answer more detailed questions about the molecular structure of minerals. We wanted our spectrometer to be able to easily collect spectra of mineral samples.

A final application that we pursued was resonance Raman spectroscopy. In resonance Raman spectroscopy the intensity of the Raman signal is significantly enhanced due to an electronic resonance of the excitation light with the sample. Resonance Raman spectroscopy can be used to monitor the vibrational spectrum of one selected molecule (the resonant molecule) in a complex mixture containing other species that

are not electronically resonant with the excitation light. For this reason, resonance Raman spectroscopy can be used to study the behavior of specific molecules in vitro. DeGraff et.

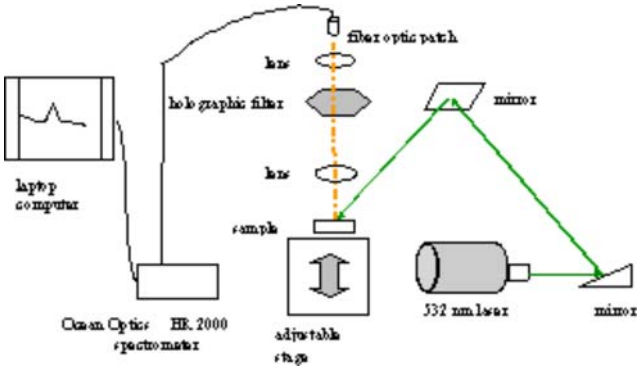

Figure 1. Raman apparatus schematic (side view). Not shown are irises, which are added as needed to block stray laser light. In addition, room light is blocked as much as possible with black cloth.

al. [1] were able to gather resonance Raman spectra of beta-carotene in aqueous solution; however, the signal was very dependent on the beta-carotene concentration in solution, or required a change in lineup to a back-scattering geometry. We wished to demonstrate a spectrometer that could more easily collect resonance Raman spectra of a single component in a complex biological mixture with a minimum of sample preparation.

Experimental Details

The experimental layout is shown in Figure 1. The beam from a 20-mW 532-nm diode-pumped doubled continuous wave YAG laser (Power Technology LCM-T-111-20) [9] was steered from the table onto the sample by two mirrors. The beam approached the sample from above at an approximate angle of 45° with the horizontal surface of the table. This angle was not measured but was simply adjusted to avoid being clipped by collection optics. The sample was on an adjustable stage in order to allow the height of the sample to be tuned. The adjustable stage was a small lab jack. A micrometer-driven stage would be an improvement that would allow for smoother adjustment and better reproducibility of sample height.

The signal was collimated and then refocused onto the fiber optic probe with two matched achromatic lenses with effective focal lengths of 14.00 mm (Edmund Optics) [10]. A holographic 532-nm notch filter (Kaiser Holographic SuperNotch Filter HSNF-532.1 – 1.0) [11] was placed between the two lenses in order to reject as much laser excitation light as possible. The Kaiser filter has a nominal spectral edgewidth of <150 cm^{-1}; however, the bandwidth is extremely angle-dependent and we did not try here to observe Raman lines less than 300 cm^{-1} from the Rayleigh line. Irises were also used as needed in various parts of the spectrometer to reject scattered light. The second lens focused the signal onto a 1-mm diameter fiber optic patch (Ocean Optics P1000-2-UV/VIS). The other end of the fiber optic cable was connected to a CCD-based spectrometer (Ocean Optics HR2000 high resolution miniature fiber optic spectrometer with an L2 collection lens and upgraded with two AgPlus mirrors), which contains a 50-mm slit and 2400-groove/mm grating (Ocean Optics H12 grating blazed to start at 540 nm) [12]. We tried to minimize room light reaching the detector by surrounding the set-up as much as possible with black cloth and by running experiments with the main room lights turned off.

The spectrometer is equipped with a USB connection that leads to a laptop computer. Ocean Optics software (OOIBase32) was used to collect and process the data. The spectra are collected as unitless channel counts. We did not correct spectra for wavelength variation of the CCD detector, so intensities are not quantitative. The grating and HR2000 spectrometer were factory-calibrated; however, we performed a further one-point wavelength calibration by using the 913-cm^{-1} dihydrogen phosphate peak location given in reference 5 as a calibration point. Using this calibration point, we set the center wavelength of our excitation laser and used this number to calculate Raman shifts.

Lineup is optimized by using a small piece of fluorescent paper placed on top of the sample. (We did note that small fibers from the fluorescent paper could contaminate the sample, so it was important to place the fluorescent paper on the sample as lightly as possible.) With the fluorescent paper in place, the height of the adjustable stage and location of the fiber optic patch can be adjusted to give the maximum signal strength. The fluorescent light can often be seen on the back of the Kaiser filter and used as an additional guide. Next the filter paper is removed from the top of the sample. One can now angle-tune the holographic notch filter by adjusting the angle to minimize laser bleed. We chose our grating to begin at 540 nm, with the intention of reducing bleedthrough from the Rayleigh line into our spectra, precluding using our spectrometer to directly monitor the Rayleigh line at 532 cm^{-1}; however, for many samples a small amount of laser bleed can still be observed near 540 nm, even at short integration times, making it relatively simple and quick to adjust the angle of the Kaiser filter to minimize laser light throughput.

The Ocean optics software allows facile variation of integration times, number of linear averages, and degree of boxcar averaging. We found that a good tradeoff between the goals of short collection times and good signal-to-noise ratios was obtained with integration times ranging from 300 ms (for strong Raman scatterers) to 2 seconds (for weaker Raman scatterers), number of linear averages ranging from 20 to 100, and boxcar average setting ranging from 2 to 4. With these parameters spectra could be collected in a few minutes. The Raman signal is weak in comparison to pixel noise, so it is essential that a dark spectrum (with laser light blocked) must be collected and subtracted from the overall signal. The dark spectrum must be collected using the same integration time and averaging as that of the spectrum.

The Ocean Optics HR2000 spectrometer configured with a 2400-groove/mm grating was chosen specifically for future high resolution applications, such as observing Raman spectra of whole proteins; however, Ocean Optics also sells 300-, 600-, 1200- and 1800-groove/mm gratings, so a larger spectral range and lower cost could be achieved by sacrificing resolution. For most of the spectra presented here, the high resolution is not necessary.

Inorganic solid samples were made into pellets with a pellet press. We did attempt to take spectra of unpelletized powders, but the powder spectra did not appear as clean as the spectra of pellets. Amino acid samples were also made into pellets. Pellets were about 0.3–0.5 cm in width. Changes in sample width from sample to sample necessitate adjustment of the sample stage. Mineral samples had irregular shapes and were placed directly onto the sample stage. Beta-carotene was placed onto the sample stage as a powder. The carrot sample was placed onto the spectrometer as a slice of about the same

thickness as the pellets that were used.

DeGraff et. al. [1] suggested that their Raman spectrometer could be assembled by undergraduate laboratory students in the timescale of a typical physical chemistry laboratory. The same may be true of the system described here;however, we caution that the upwardly-directed beam involved in this lineup, along with an upwardly-directed reflection from the sample, and the higher laser power, present more safety risks (see Safety). It might be wise to have the system essentially lined up for the students in a teaching laboratory setting and then let the students optimize the signal by adjusting sample stage height and notch filter angle.

Approximate prices for the major components are as follows: Power Technology LCM-T-111-20 diode-pumped YAG laser with power supply and heat sink ($1,800), Ocean Optics P1000-2-UV/VIS 1000-mm-diameter and 2-meter-length fiber-optic patch ($350), Kaiser HSNF-532.1–1.0 Holographic SuperNotch Filter ($1,400), two Edmund Optics M45-209 achromatic lenses with a 14-mm effective focal length ($100). We built our system with an Ocean Optics HR2000 high-resolution miniature fiber-optic spectrometer with

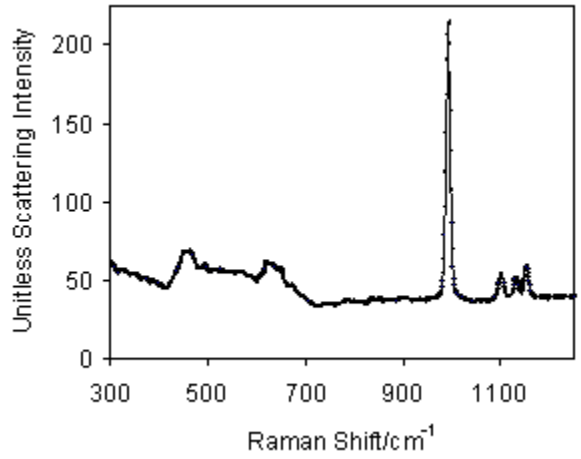

Figure 2. Raman spectrum of a solid pellet of sodium sulfate.

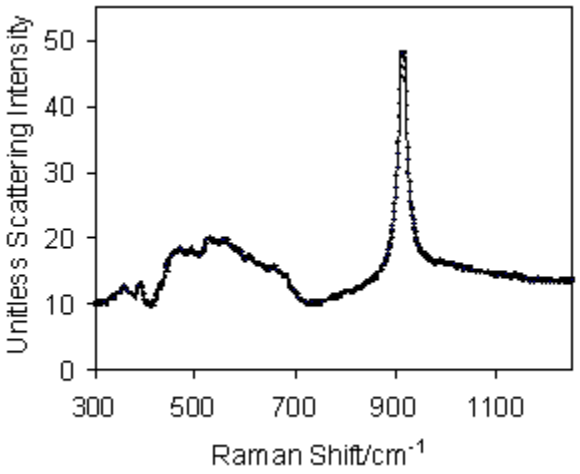

Figure 3. Raman spectrum of a solid pellet of potassium dihydrogen phosphate.

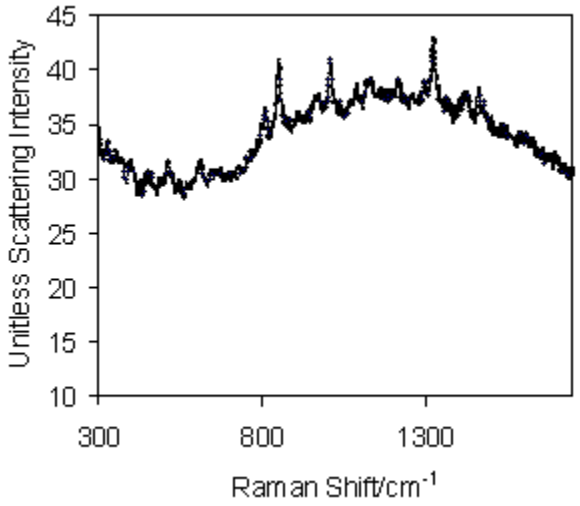

Figure 4. Raman spectrum of a solid pellet of the amino acid serine.

AgPlus mirrors, L2 detector collection lens, 50-mm slit and H12 grating (combined cost ~$3,700); however, Ocean Optics has discontinued the HR2000 spectrometers and replaced them with HR4000 spectrometers. An HR4000 spectrometer with the AgPlus mirrors, an L4 detector collection lens and a 50 mm slit and H12 grating costs ~$4,600. Ocean Optics SPECTRASUITE cross-platform spectroscopy operating software (replacing OOIbase32 software) is available for ~$200. We estimate that the total cost of the mirrors, iris, and mounting hardware is between $1000 and $2000, depending on the exact configuration that is chosen. Significant savings on mirrors and mounting hardware can be found at dealers of surplus equipment. Appropriate safety goggles must also be purchased. Our spectrometer was built on an optical table, whose price has not been included; however, we have previously used the Ocean Optics HR2000 in a different configuration on an 8-in-by-8-in aluminum breadboard to observe Raman spectra of liquids and we obtained good signal-to- noise ratios, so we believe that the solid state Raman spectrometer described here could also be assembled on a breadboard.

Results and Discussion

Figure 2 shows the spectrum of a solid pellet of sodium sulfate. The sulfate spectrum shows (in order from lower to higher wavenumber) the n_2, n_4, n_1 and n_3 modes given by Herzberg for tetrahedral XY4 molecules [13]. In the solid sample of sodium sulfate we observe a splitting of the n_3 mode, which is described in Herzberg as a triply degenerate vibration, nicely demonstrating crystal lattice structure effects, which are predicted to break the degeneracy of some degenerate modes [14]. Figure 3 shows the spectrum of a solid pellet of potassium dihydrogen phosphate. Here only the strong totally symmetric n_1 peak is visible above the background.

Figure 4 shows the spectrum of the amino acid serine. Raman peaks can be clearly observed above a fluorescent background that is due to small amounts of impurity. Most peaks in the solid state Raman spectrum of serine have not been assigned in the literature [5]; however, features of the spectrum in Figure 4 agree with those found by Ben Amotz's group[5]. The large peak at 856 cm^{-1} is particularly interesting because it has been shown to redshift by 40 cm^{-1} upon phosphorylation of serine [5], an important step in many cellular functions .

We were, in fact, also able to obtain spectra of other amino acids, such as phosphoserine and tyrosine; however, impurities contributed large fluorescence backgrounds when samples were used without purification. Figure 4 is presented to show that it is possible to quickly collect Raman spectra of amino acids with our set up. It is worth noting that the Ocean Optics HR2000 spectrometer was sensitive enough to collect the Raman spectra of amino acids with relatively short integration times. For example, the spectrum in Figure 4 was achieved with an integration time of 1 second, 20 linear averages, and a boxcar setting of 2 on the Ocean Optics software. We are not sure whether this impressive sensitivity would or would not be possible without the upgrade of the AgPlus mirrors, because we did not have the capacity to do the control experiment without the mirrors; however, it is safe to say that the spectrometer with the AgPlus mirrors showed excellent sensitivity.

Ben Amotz's group was able to detect phosphorylation of specific amino acids in peptides and proteins by developing a technique called drop coating deposition Raman (DCDR), in which a protein or peptide sample is concentrated onto a slide substrate [15, 16]. With our configuration we were unable to

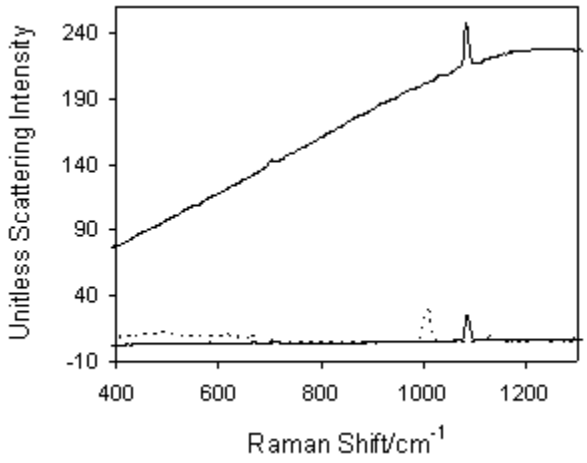

Figure 5. Raman spectra of solid samples of the minerals aragonite (solid line on top), calcite (solid line on bottom) and gypsum (dotted line).

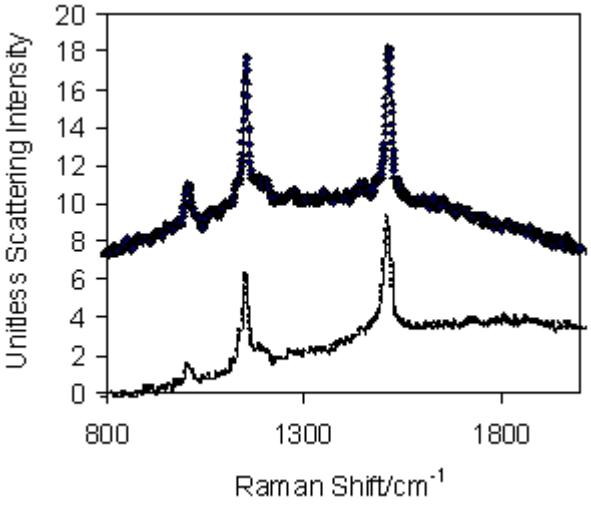

Figure 6. Raman spectra of a solid piece of carrot (top line) and powdered beta-carotene (bottom line). Spectra have been offset for visibility.

obtain good DCDR spectra. One improvement that might lead to success with the DCDR technique might involve focusing the laser tightly onto the sample; however, we did find that the 532-nm light led to some sample degradation for peptide and protein DCDR samples; focusing the light could exacerbate this problem.

Figure 5 shows the spectra of calcite, aragonite, and gypsum. Calcite and aragonite are both $CaCO_3$ but have different crystal lattice structures. The large peaks near 1083 cm^{-1} in the calcite and aragonite spectra are assigned to carbonate ion vibrations [8]. White has used Raman spectroscopy to distinguish between calcite and aragonite by noting differences in low wavenumber bands attributed to vibrations of the ionic arrangement within the crystals [8]; however, in our spectra the only visible low-wavenumber vibration is the one near 712 cm^{-1}, and this is practically hidden in the background. Our samples were obtained from a labeled collection used for teaching geology classes, but the purity of the samples was not known.

Although our apparatus was not able to distinguish between our calcite and aragonite samples, it could clearly distinguish gypsum ($CaSO_4 \cdot 2H_2O$) from the calcium carbonate minerals. The intense gypsum peak near 1000 cm^{-1} is due to the symmetric stretch of the SO_4^{2-} and its location agrees well that reported by White [8].

Figure 6 shows the resonance Raman spectra of beta-carotene from a slice of carrot and a sample of betacarotene powder. The overlapping peaks in the two spectra give an illustration of the power of resonance Raman spectroscopy. The 532-nm excitation light is resonant with beta-carotene, but not with the other components in a carrot, which is why the beta-carotene peaks can be seen clearly above the background in the carrot sample. Collecting the resonance Raman spectra was quite simple with this set up and required very short integration times (300 msec integration time, 50–100 linear averages, and a boxcar setting of 3).

Conclusions

We have demonstrated that it is possible to assemble from commercial components a modular Raman spectrometer that can be lined up relatively easily and that can quickly collect Raman and resonance Raman spectra from a variety of solid samples. Key components of the system are a spectrometer based on CCD detection, a narrow-band pass holographic filter and a small low-cost solid state laser. This system opens up applications in solid state chemistry, biochemistry, and geology for undergraduate teaching laboratories.

A price comparison can be made with commercially available Raman systems. Ocean Optics [12] and Delta Nu [17] both sell low-cost Raman spectrometers with 532-nm excitation, capable of measuring spectra from liquids or solids, for between $15,000 and $20,000. The assembled system described in this paper can potentially offer savings of several thousand dollars in comparison to these commercial Raman systems, especially if components of the system are already available in the laboratory. On the other hand, the commercial systems are turnkey systems offering great ease of use, and can easily switch between measuring the spectra of solid and liquid samples. In addition, commercial systems are available that are truly portable. A main advantage of the modular system described here is its flexibility. At GVSU we use components of this Raman system for other spectroscopic experiments. For example, we use the YAG laser to excite I_2 emission for a classic physical chemistry laboratory experiment [18], as well as to collect liquid phase Raman spectra using the set-up described by DeGraff et. al. [1]. There are also pedagogical advantages to a modular system because students can more easily see, handle, and understand the functions of the various components.

Safety

The 20-mW diode-pumped YAG laser is a Class IIIb laser, capable of causing blindness or serious eye injury if the beam or specular reflections are viewed directly. Appropriate laser safety goggles must be worn when operating the laser. The lineup involves beam paths at oblique angles to the table surface (including an upwardly-directed beam and an upwardly-directed reflection from the sample), so great care must be taken during lineup and operation to protect eyes from exposure to the laser beam and specular reflections. One helpful safety tactic is to reduce the beam intensity during line up by using an iris and/or an optical filter. Reference 19 is a source of detailed guidance about laser safety.

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