

Raman
Spectroscopy

Experiment 1: An Introduction to Raman Spectroscopy and Using the MacroRAM and LabSpec 6 Software



MacroRAM
Educational
Laboratory

Purpose

- ✚ To provide a brief introduction to Raman Spectroscopy
- ✚ To practice acquiring data with the MacroRAM and the LabSpec 6 software
- ✚ To learn and test the data processing features of the LabSpec 6 software
- ✚ To think about other relevant applications of Raman Spectroscopy

Background

A Brief Introduction to Raman Spectroscopy

Discovered by Sir C.V. Raman in 1928, Raman spectroscopy is a non-destructive spectroscopic technique based on light scattering. When monochromatic light is incident on a molecular system, most of the light is scattered at the same wavelength as the incident light (known as Rayleigh scattering). A small amount of the scattered light (typically less than 0.0000001%), however, will scatter at different wavelengths from that of the incident light. This is an inelastic scattering process known as Raman scattering. Although weak, Raman scattering is information-rich and can give insight into chemical structure and bonding, crystal phase and polymorphism, degree of crystallinity, intrinsic stress/strain, and molecular interactions within a system.

A typical Raman spectrum contains a number of peaks showing the intensity and wavenumber shift of the Raman scattered light. Each peak corresponds to a specific molecular bond vibration including individual bonds such as C-C, C=C, N-O, C-H, etc. and groups of bonds such as ring breathing modes, polymer chain vibrations, lattice modes, etc. Because of this, each Raman spectrum can be used as a fingerprint for identifying different molecules or materials.

Raman spectroscopy is an important analytical and research tool for many application fields, including, pharmaceuticals and cosmetics, forensic science, geology and mineralogy, polymers, thin films, semiconductors, and life science.










An Introduction to the MacroRAM

The MacroRAM is a fully automated analytical instrument used to measure and record the Raman spectrum of a sample. It consists of a 785 nm excitation laser source that passes through a narrow bandpass filter, which helps to “clean up” the spectral profile of the source. The light is then focused onto the sample, which interacts with the excitation photons and emits photons at wavelengths that are shifted to lower energy relative to the excitation source (Raman scattering). An edge filter is used to block the Rayleigh scattering (laser line) and the signal is then passed to a fixed spectrograph with CCD to record the Raman spectrum. The geometry of the MacroRAM system collects the Raman signal in the back scattered direction. The signal from the detector is sent to the host computer, where the data can be presented and manipulated using the LabSpec 6 software platform.

An Introduction to this Lab

In this lab, you will use the MacroRAM to acquire data for several samples such as glass/quartz cuvettes, water, methanol/isopropanol, polyethylene terephthalate (PET) soda bottles, and something metallic. Once the data are successfully collected, you will learn how to process the data using the LabSpec 6 software. This processing includes background subtraction, smoothing and filtering, adding/subtracting spectra, and peak analysis, which are some of the most common features available in LabSpec 6 that are used to process Raman data. As the lab progresses, you will also learn some common challenges and roadblocks to be aware of when acquiring Raman data from certain samples. Throughout this lab, the best resource is the MacroRAM user manual, which can be found on the desktop of the computer connected to the MacroRAM.

Supplies

-  MacroRAM cuvette sample holder
-  MacroRAM solid sample holder
-  Glass cuvette
-  Quartz cuvette
-  Water
-  Methanol or isopropanol
-  A clear PET water/soda bottle
-  A colored PET water/soda bottle
-  An object made of stainless steel or a piece of Al metal

Procedure

1. Review Chapter 5 (Data Acquisition) of the MacroRAM user guide in order to become familiar with the Lab Spec 6 software layout and data acquisition with the MacroRAM.
2. Use the MacroRAM to acquire data for each sample above, including the empty glass and quartz cuvettes. Also, acquire a spectrum with no sample in the sample holder. Be sure to place any liquids in a quartz cuvette and to clean the quartz cuvette after each liquid is measured. Use various laser power and acquisition time/accumulation settings to find the ideal selections for each sample. You may want to set the spike filter to “multiple accumulations” to avoid any unwanted spikes in the data, but when doing so, make sure you are acquiring at least two accumulations. Experiment with the various settings and see how the results vary. The ultimate goal is to have a clean spectrum with good signal to noise (without saturating the detector or burning the material) for each material. Make sure you save each spectrum!
 - a. Spectrum collection basics: Place your sample in the sample holder within the MacroRAM. In the LabSpec 6 software, click on “Start Real Time Display RTD” (button at the top that looks like a ‘play’ button) to preview the spectrum. In the acquisition tab on the righthand side of the window, adjust the acquisition time and number of accumulations. To determine an appropriate acquisition time, find the intensity of the highest peak being shown in the spectrum preview. This intensity will be multiplied by the number of seconds chosen for the acquisition time, so select a number that, when multiplied by the highest intensity value, will result in a product that is less than 50,000 (the signal becomes saturated around 50,000 counts). You may also adjust the laser power in the same tab; a higher laser power will typically increase the signal-to-noise ratio. Once this is done, click on “Start spectrum acquisition” (circle button at the top of the window, next to “Start Real Time Display RTD”).

Analysis

1. Use the “Baseline Correction” function under the “Processing” tab in LabSpec 6 to subtract a proper baseline from all samples.

- a. Play with the “Degree” and “Max Points” values to find a combination that creates a more uniform baseline. You may also directly add points on the baseline adjustment by clicking the third button up from the bottom on the left-hand side of the window and clicking on the spectrum.

After a baseline correction, the spectra should have a zero, flat baseline (see Chapter 8 (Data Processing and Analysis) of the MacroRAM user guide for more information regarding baseline correction). If you make any mistakes, you can simply press “Ctrl-Z” to undo them. What is the origin of the background that you subtracted? Do some samples seem to have a higher background? Which ones have a higher background, and why do you think this is (consider the type of material, the color of the material, any fillers, etc.)?

2. Compare the Raman spectra of the empty glass and quartz cuvettes. Why would quartz be a better choice than glass when measuring the Raman spectra of liquids? Could there still be issues when using a quartz cuvette? Why? Keep this in mind for all future labs where liquids are measured.
3. Compare the spectrum for the quartz cuvette filled with water to the spectrum for the empty quartz cuvette. Pick one of these spectra and adjust the smoothing & filtering parameters, which are found under the “Processing” tab in LabSpec 6 (see Chapter 8 (Data Processing and Analysis) of the MacroRAM user guide for more information about the smoothing & filtering functions), until you have a clean, smooth spectrum. Test out different functions and see how they change the spectrum but be careful as too much smoothing can result in a loss of peaks or peak definition. Then, find smoothing and filtering parameters that work best for the other spectrum. Finally, overlay both spectra and note the differences. Which peaks correspond to the water? (Note: ideally, you can subtract the cuvette spectrum from the water/cuvette spectrum to obtain the pure spectrum of just water)
4. Display a spectrum of methanol or isopropanol. Use the “Data Range” function under the “Processing” tab in LabSpec 6 to extract the region of the spectrum that contains the most intense peak. Use the “Peaks” function under the “Analysis” tab in LabSpec 6 to fit this peak to an appropriate oscillator shape (see Chapter 8 (Data Processing and Analysis) of the MacroRAM user guide for more information about peak fitting). Before fitting the peak, you should make sure the baseline of the spectrum is at 0. Which oscillator did you choose, and why? Which alcohol did you choose, and what is the Raman shift (in cm^{-1}) of the most intense peak? Under the “Peaks” function, display the “Peak Table” and under “Show Options,” select to view the values for position, amplitude, width, and area. What are these values? Repeat this same process and answer the same questions for the series of peaks located between 2800 and 3100 cm^{-1} , but be careful as there are multiple peaks present in this region!
5. In order to obtain the pure Raman spectrum of water, you would have to subtract out the Raman spectrum from the quartz cuvette. Do you think you would have to do the same for the alcohol? Why or why not?
6. Compare the Raman spectrum of the clear PET bottle to the Raman spectrum of the colored PET bottle. Why might these two spectra look different? Based off these results, what might be some of the challenges to using Raman spectroscopy for certain samples?
7. Compare the spectrum for the stainless-steel object (or the piece of Al metal) with the spectrum taken when no sample was present. What do you notice? Do you think Raman is an ideal technique for measuring or distinguishing metallic samples? Why or why not?
8. Can you think of any other samples or experiments that might be ideal for study with Raman spectroscopy?

References

- ✚ MacroRAM User Manual
- ✚ LabSpec 6 Reference Manual



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