Raman and resonance Raman spectroscopy laboratory

CH454 Physical Chemistry Lab #2

1.0 Introduction

Most undergraduate courses have limited treatment of Raman spectroscopy, or possibly no discussion at all. Therefore, you will need to learn some of the basics of the method, which will not be covered in the laboratory manual. Raman spectroscopy can be a turnkey experiment if you simply want a spectrum and you are not concerned about resonance, depolarization and other finer points. In fact, Raman spectroscopy has become very widely used because it can be adapted to fiber technology (fiber tip Raman probe), Raman microscopy and imaging, surface enhanced Raman spectroscopy, gas phase Raman (e.g. LIDAR) for detection of molecules from a great distance. The aspect that unites this disparate experiments is that Raman scattering is excited with a laser and so one can use the properties of laser light (high intensity and directionality) to advantage.

Raman scattering is an inelastic light scattering experiment. Energy is exchange during the scattering event so that the scattered light can give up some of its energy to the molecule. In this type of Raman scattering, known as Stokes Raman scattering, the molecule receives the energy of a vibrational normal mode. In the quantum mechanical picture the molecule is promoted from the $v = 0$ to $v = 1$ level. We say that the molecule receives one quantum of energy from the exciting radiation. For completeness, we should also add that the molecule can give energy to the scattering radiation if it starts out in an excited vibrational state, $v = 1$. In that case, known as anti-Stokes Raman scattering the molecule gives up energy as is demoted by one quantum from $v = 1$ to $v = 0$ and the scattered radiation increases in energy by an equal amount.

In this laboratory experiment you will learn how to think about the properties of a molecule and then to measure those properties experimentally. The two most important properties are the vibrational normal mode wave numbers (frequencies) and the electronic transition energies. A subset of the vibrational modes is Raman active and we can measure those using Raman spectroscopy. We can determine their symmetry using Raman depolarization. We can determine the nature of intermolecular interactions from shifts in the Raman spectrum as well. We can determine the properties of the excited state from the resonant Raman scattering.

1.1 Experimental principles

1.1.1 Basic principles

Our goal in this laboratory manual is to provide sufficient information for you to study the Raman effect on your own. While this is potentially a vast area of spectroscopy, our emphasis will be on certain basic principles. You should be able to understand the basic idea of inelastic scattering, the importance of polarization, and the meaning of resonance. The Raman effect involves a shift in the wavelength of scattered laser light because of an interaction with a molecule that alters the vibrational energy of the molecule. Most commonly the light gives energy to the molecule promotes it to a higher vibrational level.

1.1.2 The Raman depolarization ratio

Since the Raman effect involves two electric field interactions (the incident and scattered fields) the effect on the molecule can be expressed in terms of a transition polarizability. This is a significant difference when compared to infrared (IR) for example where the absorption of light depends on a transition dipole moment. Because of the tensor effect the depolarization ratio defined as:

$$\rho = \frac{I_{\perp}}{I_{||}}$$

depends on the mode frequency. Totally symmetric modes are polarized meaning that $\rho < 1/3$. For ideal totally symmetric molecule such as CCl_4 or CH_4 $\rho = 0$ for the totally symmetric modes. For a planar absorber such as benzene or a porphyrin $\rho = 1/8$ for the totally symmetric modes. For other molecules ρ can be as large as 1/3, but not larger for totally symmetric modes. For non-totally symmetric modes $\rho = ¾$. These differences make it possible to experimentally distinguish between totally symmetric and non-totally symmetric vibrational modes.

1.1.3 Resonance Raman spectroscopy

The resonance enhancement of the Raman effect is one of the most interesting phenomena in spectroscopy. If the laser wavelength is coincident with a molecular transition (i.e. an absorption wavelength) then the light is scattered “on resonance”, which means that the laser light can cause a transition to the excited state, but then instead of being absorbed it is scattered and the molecule returns to the electronic ground state (but to a different vibrational state). We can probe resonance Raman spectroscopy by studying a molecule with an absorbance maximum in the range 400 – 430 nm, which is the easy tuning range of the laser. Even if the absorbance maximum

is outside this range we can still see the resonance effect if there is a change in absorbance over this range.

1.1.4 Applications

Molecular vibrations are sensitive to the environment. Hydrogen bonding and other molecular interactions can cause shifts in the wave number of normal modes. A simple example can be found in H_2O. In the gas phase the symmetric and asymmetric stretching vibrations of H_2O are 3,825 and 3,935 cm^{-1}, respectively. In liquid water, where this is strong hydrogen-bonding these frequencies shift down to approximately 3,670 cm^{-1} and 3,760 cm^{-1}, respectively. This large shift in the stretching mode wave number is due to the polar interaction of the bond with the lone pair of a neighboring H_2O molecule. We will consider this in the equilibrium of an acid with its salt. The acid and salt differ because of protonation. We will prepare several different mixtures and then use Singular Value Decomposition (SVD) to study the spectral shifts.

1.2 Equipment

1.2.1 Excitation laser

The Raman apparatus in Room 175 of Partners III uses several different lasers to provide a number of fixed wavelengths for Raman excitation. We will use the Ar/Kr Ion Laser, which has wavelengths of 488 nm, 514.5 nm, 568 nm and 647 nm. It is important to understand that there are many other lines, which are less intense. A very weak laser line at a wavelength shifted slightly from the main line will show up as a peak in the spectrum. For this reason it will be necessary to measure a blank (consisting of a cuvette with water in it) at each wavelength where a small signal is measured. Since water has essentially no measurable Raman activity under the conditions we are working we can use the blank to determine what signals arise from additional laser lines. For solvents such as CCl_4 or C_6H_{12} , which have very intense scattering (hundreds or even thousands of counts per second on the CCD detector) we can ignore these very small laser additional lines. For the acetic acid studies it will also not be a large contribution. However, the final part of this study where we are looking at much weaker scattering from non-resonant Na_2SO_4 and from the dye malachite green it will be essential to obtain a spectrum of the blank.

The laser light is focused on the sample by a camera objective. This apparatus uses a back scattering geometry. This means that the light is focused in the sample and then the scattered light retraces the same path. There is a beam splitter which directs 50% of the incident laser light into the sample. Because of this beam splitter we also lose 50% of the scattered light. This may sound like a lot of losses, but the efficiency of the optics and the small spectrograph make this a very efficient design. The scattered

light is collected and focused onto a fiber, which conducts the light to a single-grating spectrograph.

1.2.2 Software

The Raman acquisition software is opened by clicking on the icon on the desktop. It may already be opened, in which case you may begin using. However, in either case you should make sure that configuration is RamanClass. If you do not see RamanClass at the very top of the GUI then you should look for a folder icon at a highest level on the menu and click on it. This folder should give a list of different configurations. Select RamanClass and you should see those words on the top of the menu. There are three sets of parameters that will need to alter as you conduct the experiments.

1. Acquisition time and number of frames (top menu)
2. File name and path for saving data (near the middle of the menu list)
3. Raman excitation wavelength and center wave number (bottom menu)

In 1. you will need to set to set the acquisition time in seconds that tells the program how long to permit the CCD to be irradiated by scattered light before it is read. Each time the CCD is read we can call this a frame. Then you will need to determine the number of frames. If you are doing real time observation of solvent bands to "tweak up" the signal then you should set both the time and number of frames to 1. In that case if you select Run above the spectral window you will see a new spectrum every second. If you want to acquire a Raman spectrum and save it then we recommend the following:

A. Solvents (e.g. CCl_4, C_6H_{12}) Frames 4, Time 5 seconds
B. Raman signals from relative weak scatterers (essentially all other acquisitions) Frames 4, Time 30 seconds.

With these settings you will obtain data in 2 minutes. If you would like better looking data you can always set the number of frames to a greater number. If we were trying to get publication quality data from this experiment, we might use 40 frames and a 20 minute acquisition. The signal-to-noise ratio would be better by approximately $\sqrt{N}$ where N is the number of frames so in this case it would $\sqrt{10}$ better. That is quite a lot!

The file name and path are fairly obvious. We have set up a folder in the Data directory that is called CH454. Please make a subdirectory in that folder to avoid a proliferation of names in the Data directory. It is a good practice to include the excitation wavelength in the name of the file. For example if you are exciting CCl4 at

514.5 nm you could call the file ccl4_514nm. It will generate a file with a SPE extension. When you are done collecting data you will need to export the data SPE data in the CSV (comma separated values) format. You will use the Data tab (upper left of the GUI window). Then you can selected "Recently acquired" and drag down and select all of the files you just obtained. Be careful when exporting since you must input the excitation wavelength (hence the reason it is a good idea to put that wavelength in the file name). You may export multiple files, but you will want to make sure that they are in groups that all have the same excitation wave length.

Finally, the control over the positioning of the grating in the spectrograph is in the bottom menu. This is not obvious. You need to click on the small cm-1 that is in the center of that menu. Then you will obtain a menu that asks you for the excitation wavelength. The only one that is not a whole number if 514.5 nm. You type in the number of the wavelength. Then you need to type in the center cm^{-1} of the Raman window. This will typically be a number between 700 and 1300 cm^{-1}. The width of the window is approximately 1200 cm^{-1} (using the 1800 groove per mm grating which is set by the RamanClass configuration). Therefore, a center of 700 cm^{-1} will give you approximately 100 cm^{-1} – 1300 cm^{-1}. This is a good window for low frequency modes. It will work for the solvents CCl_4 and C_6H_{12}. However, acetate and acetic have interesting modes that are slightly higher (near 1600 cm^{-1}) so you will need to set the center wave number to 1200 cm^{-1} for those experiments. For the Na_2SO_4 and malachite green resonance Raman experiment a similar wave number is probably best. Note that most of the vibrational modes are between 200 – 1700 cm^{-1}. There is a normal mode desert from about 1700 – 2800 cm^{-1}. Above 2800 cm^{-1} one can observe the X-H stretching modes (X =C, O, N etc.). These are typically weak in Raman. We focus our efforts on the modes below 1700 cm^{-1}. We leave some discretion for the student to play around a bit and find a good window for taking data. The idea is to capture as many modes as possible. We will not be using the information of modes below 300 cm^{-1} (except for CCl_4).

1.2.3 Collection and dispersion of the scattered light

To measure a Raman spectrum we will use a collection optic, a spectrograph and a CCD detector. The spectrograph is a triple monochromator, which consists of two stages. The filter stage is a double monochromator and the main spectrograph is single 0.6 meter monochromator. There are two slits. The most important slit is the entrance to the spectrograph stage, which is shown in Figure 3. This slit can be set between 30-100 microns depending on the requirement for resolution and light level. A typical value is 60 microns. The spectrograph has a grating that disperses the light onto the CCD detector.

A Charge-coupled device (CCD) is a detector that can collect an image of the dispersed light. Actually a CCD is just a more sensitive version of the chip used in a digital camera. The CCD is usually binned in vertical strips so the light dispersed over a narrow range can be resolved as shown in Figure 3.

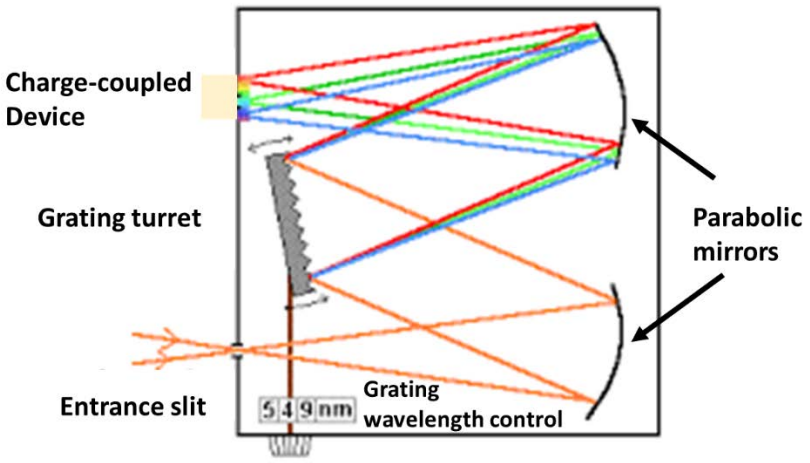

Figure 3. Illustration of a spectrograph. The grating disperses the light that enters through the entrance slit. The dispersed light is refocused using a parabolic mirror. However, the focal point of the light is slightly behind the plane of the CCD so that the light is dispersed across the CCD.

The output is digitized using the computer software and stored as a file that contains the number of counts per pixel. We must calibrate the window of the CCD for each setting of the monochromator. The calibration will tell us how the pixels correspond to the Raman shift in cm^{-1}. The simplest way to calibrate the CCD is to use one or more standard molecules (see Section 1.3).

1.3 Calibration

A Raman spectrum can be calculated in the same way as the laser line (Rayleigh line). This is quite a bit of work since such a calibration will obtain the Raman spectrum in terms of wavelength. We can then subtract the Rayleigh line and convert to cm-1. In practice a much simpler way to obtain the Raman shift is to record the Raman spectra of several solvents using the same monochromator settings used to obtain the data. For example, we can use toluene as shown in Figure 4.

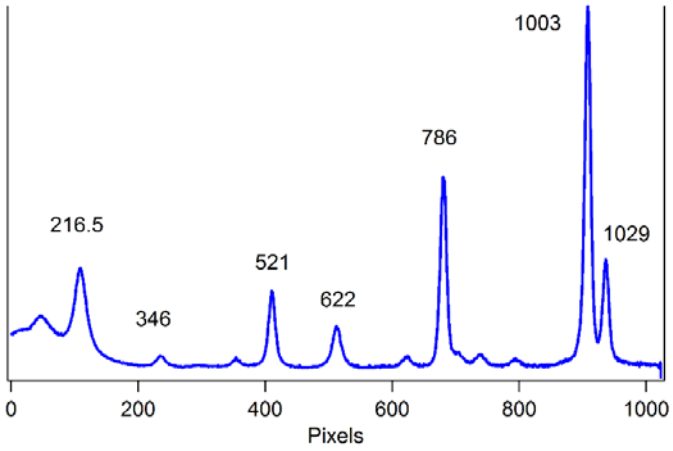

Figure 4. Uncalibrated Raman spectrum of toluene as it appears on the CCD. The CCD has 1024 pixels and the data are plotted using these units as the x-axis. Each Raman peak is labeled with its appropriate wave number from the known calibration of toluene.

We can record the peak values of toluene in terms of pixels and then create a table using the known Raman shifts. The table has one column for pixels and one for wave numbers (Raman shift). Table 1 reports the actual values from Figure 4.

Pixels	Peaks
109	216.5
236	346
354	465.5
410	521
512	622
681	785.8
908	1003
936	1029

A fit of these values to a line gives us the calibration line. The line is shown in Figure 5. It should be quite a good line. If not, then there is probably an error in the assumption about the peaks.

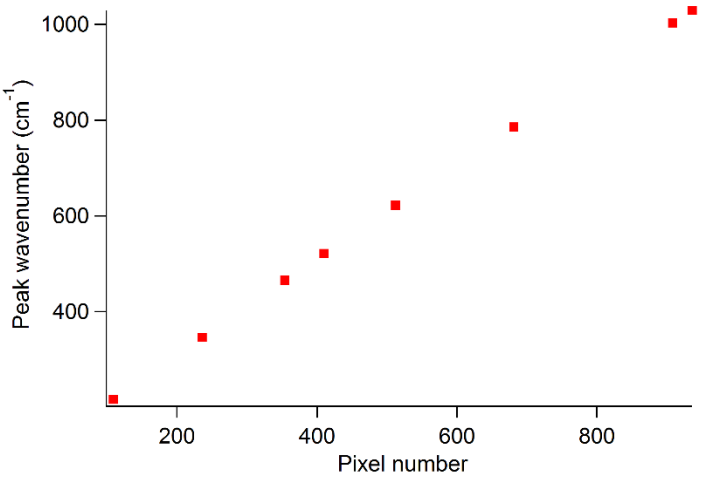

Figure 5. Calibration line derived from the data for toluene shown in Figure 4.

In this case the fit to the line is given by the equation y = mx + b, where m = 116 $\pm$ 3 and b = 0.98 $\pm$ 0.01. Using these values we can make a calibration for the x-values of the above Raman spectrum for toluene. To show how this is implemented we replot the data above using the calibration line as the x-value in Figure 6. Notice that now the values of the peaks corresponds to their x-values. The spectrum is calibrated.

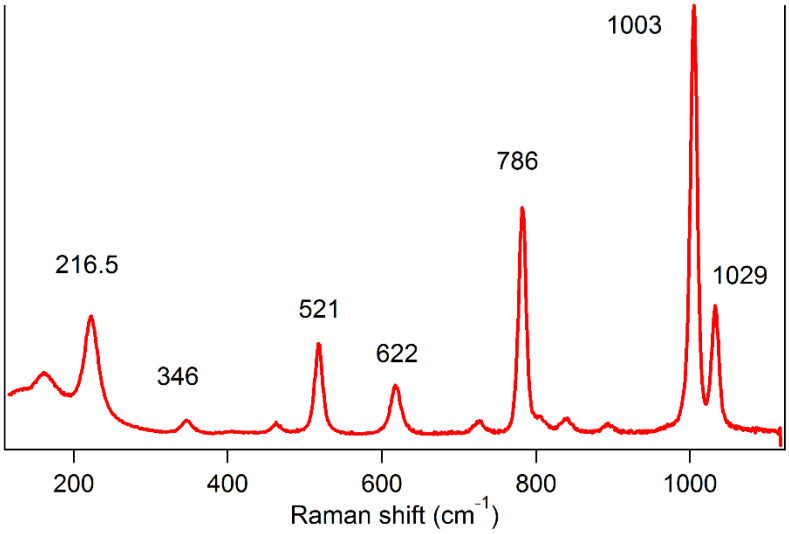

Figure 6. Calibrated Raman spectrum of toluene.

1.4 Singular value decomposition

Singular value decomposition (SVD) is a method used to analyze data in a matrix format. This method can be used to obtain a global fit to spectral data that depend on some other parameter such as time, concentration, pH, temperature, electric field etc. The idea behind SVD is to reduce the data to a set of orthogonal basis spectra in each of the dimensions. For example, if we have a data matrix in the dimensions of wavelength, λ, and time t, that has the form A(λ,t), we can decompose that matrix into three matrices as follows:

$$\boldsymbol{A}(\lambda, t) = \boldsymbol{U}(\lambda, n)\boldsymbol{W}(n, n)\boldsymbol{V^T}(n, t)$$

Where n is the number of components and the matrices have the following definitions:

$$\boldsymbol{U}(\lambda, n) \equiv Spectral\ matrix - component\ basis\ spectra$$

$$\boldsymbol{V^T}(n, t) \equiv Time\ course\ matrix - component\ time\ courses$$

$$\boldsymbol{W}(n, n) \equiv Eigenvector\ matrix$$

The eigenvector matrix ranks components and assigns them a relative weight. Typically, only the first 2 or 3 components are important in a spectral decomposition. The spectral matrix consists of the following components:

$$\boldsymbol{U_1} \equiv grand\ mean\ of\ the\ data\ set$$

$$\boldsymbol{U_2} \equiv difference\ grand\ mean$$

$$\boldsymbol{U_3} \equiv second\ difference\ grand\ mean$$

And so on. $\mathbf{U_1}$ is the first component and consists of the grand mean, which is simply the average of all of the spectra. The second component $\mathbf{U_2}$ is the average of all of the spectra after subtracting off component $\mathbf{U_1}$. So it is a grand mean of the difference spectrum. Each successive Un spectrum is the grand mean of the (n-1)th difference spectrum. These spectra are orthogonal to one another. A time course corresponds to each spectrum. One can carry out global fitting using the appropriate number of time courses (usually 2 or 3) for the fitting. This fitting procedure is much more robust than the alternative of fitting each wavelength individually.

From a practical point of view we can carry out SVD in IgorPro. The hardest part of this procedure is constructing the matrix. The trick is to create a table with the correct dimensions. Then you can just paste your data into that matrix and type one command (matrixSVD) to get the result. For example, suppose that you have a data matrix of

1000 wavelengths and 200 time points, A(1000,200). You need to create a matrix will 200 columns and 1000 rows. Use the command

$ make /n=(200,1000) A

$ edit A

This syntax automatically makes A a two-dimensional matrix. You can open the table using the edit command. Then you can paste your data set into the table. Once the entries in the table are filled you return to the command line and type:

$ matrixSVD

In IgorPro the components are found automatically in the matrices **U_M**, **VT_M** and **W_M**. You can examine the components to determine how many components are useful. The **W** matrix gives you important information as the eigenvalues. The eigenvalue of the second component may be 10% as large as the first (very significant) or smaller. The third component is typically in the range of 1% or smaller. The decision to include two, three or more components is subjective. However, the quality of the components often makes it clear which components are worth including. If you fit the components of the VT matrix to a model then you can use that model to estimate the magnitude of an unknown.

2. Materials and Methods

This laboratory experiment consists of three stages. First, you will perform a calculation of the vibrational normal modes of the molecules of interest. You will also estimate its electronic transition energy for the purpose of understanding resonance in the Raman experiment. Second, you will perform a Raman experiment on a solvent molecule (e.g. CCl_4), a molecule with different chemical states (e.g. a perchlorate salt and perchloric acid mixtures at different pH values), and one of two experiments.

2.1 Calculation of the normal modes.

The normal modes of molecules can be calculated from first principles using density functional theory (DFT). In this class we will not ask you to do the calculations, but we will provide you with the output files from DFT calculations so that you may compare these to experiment. This will help you to make assignments and also give a feeling for the accuracy of DFT calculations. It is also a useful exercise in how to interpret somewhat complex data sets. In the case of CCl_4 there are only 4 Raman active modes. Given the high symmetry of the molecule it is quite an easy task to identify each of the modes in the Raman spectrum and to determine the

accuracy of the DFT calculation. You can also compare the depolarization to the symmetry of the calculated mode with ease. Cyclohexane (C_6H_{12}) is much more complicated. It has a total of 48 normal modes of vibration. Some of these are C-H stretching modes. These are not very intense in the Raman spectrum and they are clustered at around 2,900-3,200 cm^{-1}. You can use group theory and DFT output to determine how many of these modes exist and then you do not need to consider them further. However, it is worthwhile to compare the Raman spectrum from the lowest possible value (between 200 – 300 cm^{-1}) and 1600 cm^{-1}, which is where the highest wave number skeletal modes are observed.

2.2 Raman experiment

The basic Raman experiment for this laboratory has the following steps.

1. Obtain the Raman spectrum of CCl_4 and cyclohexane for parallel and perpendicular polarization. Determine the depolarization ratio for the Raman bands and compare these to the predicted Raman spectra based on calculation and group theory. You may use the output from DFT calculations provided as supplementary material for your discussion and analysis.
2. Obtain the Raman spectra of an acetic acid/acetate buffer solution at 5 pH values 4-10. Analyze these using SVD to understand the shifts in the bands in a comprehensive manner. Explain the shifts in the Raman bands in terms of the vibrations of acetic acid and sodium acetate. Determine the pH of an unknown based on the correlation.
3. Obtain a basic Raman excitation profile (REP). This means that we will monitor the Raman intensity of vibrational modes as a function of the excitation wave length. In practice, since we only have three wave lengths we will compare the scattering from a dye (resonant molecule) and Na_2SO_4 salt (non-resonant ions) in order to understand the significant of resonant Raman spectroscopy.

2.2.1 Procedure to obtain the Raman spectrum of CCl_4 and cyclohexane. To obtain a Raman spectrum you will need to determine the correct range for the monochromator. The monochromator must be moved far enough from the Rayleigh line that there is no “tail”. The Rayleigh line is so strong that you will need to be about 10 nm away from before you can obtain any meaningful Raman signal. However, you want to be close of enough to the Rayleigh line that you can measure low frequency modes. Toluene is a good test case since it has a mode at 220 cm^{-1}. You should be able to see this mode. Once you have set the monochromator and the filter to the right range you will want to ensure that the signal is optimized. You can tweak the beam steering mirrors in order to get the best signal. Once this has been achieved you may obtain a Raman spectrum of CCl_4 and one of cyclohexane. Then

put the polarizer in the beam path right before the first slit. Obtain the spectrum for parallel and perpendicular polarization as well for both CCl_4 and cyclohexane. Each of these spectra can be obtained in a short time. Record 6 spectra with an acquisition time of 5 seconds.

Data workup. Read the data into Excel (or other similar program). Using CCl_4 first we will want to establish the calibration factor needed to obtain accurate depolarization ratios using the Raman apparatus. Plot the parallel and perpendicularly polarized data as shown below in Figure 7.

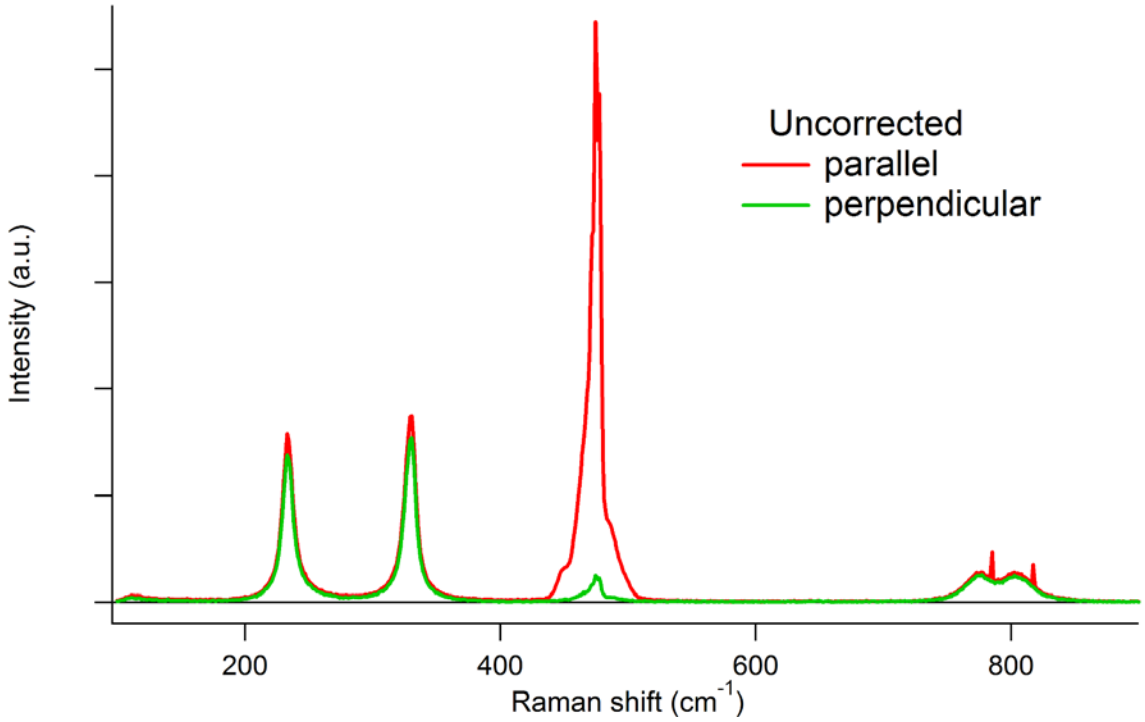

Figure 7. Raw Raman data for CCl_4 overlaid.

CCl_4 is a nearly ideal molecule because of its high symmetry. Actually, we should be aware of the fact that there are two isotopes of Cl (^{35}Cl and ^{37}Cl) in CCl_4. For this reason the depolarization ratio of the totally symmetric stretch at 462 cm^{-1} (Figure 7) is not exactly zero, as theory would predict for a molecule with spherical symmetry (i.e. tetrahedral, octahedral or icosahedral). However, the remaining non-totally symmetric bands should have $\rho = ¾$. Since they do not have this value and they all deviate by nearly the same amount we will assume that the reason is that the spectrograph is not perfectly balanced in its throughput of parallel and perpendicular light. We will determine that appropriate factor needed to multiply the perpendicularly polarized Raman spectrum in order to obtain the theoretical $\rho = ¾$. Once we have determined this correction fact we will use it for cyclohexane (C_6H_{12}) or any other

depolarization ratio measurement. Once we have applied a correction of 0.856 to the perpendicular channel we obtain the Raman spectrum shown in Figure 8.

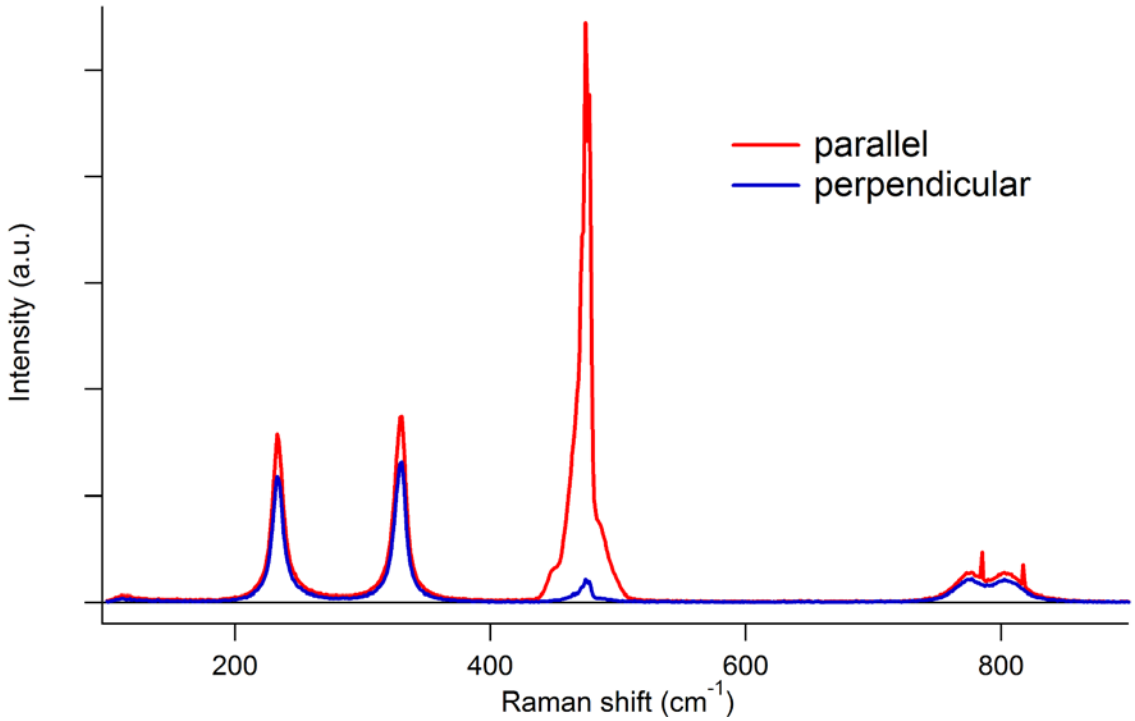

Figure 8. Corrected Raman spectra showing parallel and perpendicular polarizations.

Note that after correction the depolarization ratio of the totally symmetric stretch is $\rho = 0.039$, which is in reasonable agreement with the literature value of $\rho = 0.046$.

2.2.2 Procedure to obtain the Raman spectrum of acetic acid and sodium acetate. Make at least 4 solutions in the range from pH 4-10. Place these solutions in NMR tubes so that there is at least 0.5 mL of solution in the tube. Label them according to their pH. The Raman spectrum of each of these will take a few minutes. Record 6 spectra with an acquisition time of 20 seconds. If the signal-to-noise ratio is not ideal record for a longer period of time.

Data workup. Read the data in IgorPro and calibrate the data using the procedure described above. Report the Rayleigh wave number, the spectra and depolarization ratios for CCl4 and cyclohexane. Relate the depolarization ratio to the predicted symmetries of the modes based on quantum chemical calculations and group theory. Finally, create a data matrix of the acetic acid/acetate spectra. Obtain the SVD components of this data matrix. There should be two dominant SVD components in the data set. These should represent the grand mean of the data and the shift of the data over the pH range. This spectral analysis permits you to estimate the pH of an

unknown sample. Come up with a method to do this and estimate the pH of the unknown that were given.

2.2.3 Laboratory experiment: Raman excitation spectrum

For this portion of the laboratory experiment we will compare a non-resonant and resonant molecule. The available laser has only 4 wavelengths that permit us to conduct a Raman excitation profile. Therefore, the absorption band must be sufficiently broad that these wavelengths can map out the enhancement by observing the different intensity patterns from the resonant molecule and the non-resonant molecule. We will use a dye molecule known as malachite green as a dye molecule (Figure 9). Malachite green has a prominent absorption band peaked at λ_{max} = 620 nm. You should obtain the absorption spectrum of the dye molecule to observe this for yourself.

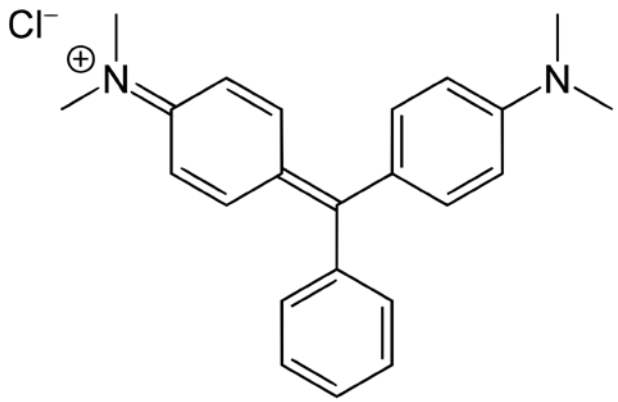

Figure 9. Chemical structure of Malachite Green (MG).

Using the Raman apparatus in room 175 we can study the system of a dye solution containing 200 mM Na_2SO_4 as an internal standard in comparison with a solution containing only the internal standard. The internal standard has no absorption at any of the excitation wavelengths and SO_4^{2-} is a tetrahedral ion with a relatively large Raman cross section. The symmetric stretching (A_1 breathing) mode of SO_4^{2-} is at 982 cm^{-1}.

You should make two samples:

1. Malachite green in 0.2 M Na_2SO_4
2. Control containing 0.2 M Na_2SO_4

You will observe Raman bands of each sample using Ar ion laser excitation at the wavelengths 568 nm, 514 nm and 488 nm. There are other bands in Na2SO4 besides

the 982 cm^{-1} totally symmetry stretch, but we will only need to keep track of them to distinguish them from bands of the malachite green that are resonantly enhanced by excitation at the lowest energy (i.e. 568 nm in this case). Unfortunately, we do not have a laser at 620 nm in the laboratory so we cannot excite on resonance with the peak of the absorption band of malachite green. However, the resonance Raman signal approximately follows the contour of the band for such broad absorption bands. Therefore, we can see enhancement at 568 nm and even to a small extent at 514 nm. When the Ar ion line at 488 nm is used the malachite green bands should be very weak or not observable. However, the internal standard should still be observable. Question: what is the frequency dependence of the non-resonant Raman bands of the internal standard? Hint: look at the basic Raman equations (and even Rayleigh scattering equations) in a textbook such as Atkins.

Protocol for comparison of resonant and non-resonant Raman spectra

1. Be sure to measure the power of the laser at each wavelength using the power meter.
2. Determine the Raman spectrum of each sample at 568 nm, 514 nm and 488 nm. Use a detection window on the CCD camera with a center at 800 cm^{-1}. Use the Run with 1 acquisition at 5 seconds to make sure that the signal looks reasonable. You should be able to see a noisy band at 982 cm^{-1}. Then acquire a spectrum consisting of 4 frames, each with a 30 second acquisition time.
3. You can change the laser wavelength by tuning the upper left most knob of the Ar ion laser. Your instructor can help you with this aspect. You need to change the bandpass and cutoff filters to the appropriate wavelength as well.

Data workup

Identify the 982 cm^{-1} band of Na_2SO_4 in each spectrum. Determine the number of counts at the peak of this band relative to the baseline counts. Identify at least two bands from malachite green and make a similar determination of the peak counts relative to the baseline. Make a table showing each excitation wavelength and include the laser power and the number of counts of each peak. NOTE: we are making a crude approximation for the integrated intensity of the peaks by simply comparing their peak counts. This is fine for our purposes, but you should be aware that a more precise experiment should be based on integration of the peak areas for this comparison.

Excitation	Power	Na_2SO_4	MG 1	MG 2
488 nm				
514 nm				
568 nm				

3. Analysis and Laboratory report

This laboratory has two phases and so it is appropriate to report each of these in a separate document. The first report should focus on the basics including the calibration, depolarization ratio and application of Raman spectroscopy to a pH determination. The second report will focus on either the shifted excitation technique to detect a Raman spectrum on a fluorescent background or a Raman excitation profile of a MTPP or deoxy Mb molecule. The reports should contain an Introduction, Materials and Methods, Results, Discussion and Conclusion. You should go into appropriate detail regarding the computations used for calibration (report 1) and SVD analysis (both reports). Report 1 should contain a section the quantum chemical calculations and normal coordinate analysis of the molecules. Since you will be provided with the output files, the goal of this section for you to organize the relevant portions of the output and present the modes and their properties (calculated and measured wave number, symmetry and depolarization ratio) in tabular form. Clearly, some of the modes may not be Raman active. You may note this in the table by NA for any category that depends on the experiment. Report 2 should go into detail appropriate for each topic. For the detection of modes on a fluorescent background you should do a statistical analysis of the result and report a limit of detection. In this case, the significant parameters are both units of concentration and relative counts on the CCD detector. The REP experiment should focus on the differences in REPs of modes of varying symmetry. You will want to obtain a depolarization ratio (only necessary at one excitation wavelength) to determine which modes are totally symmetric and which modes are non-totally symmetric. The interesting point here is to determine the differences in their behavior. This may be subtle, but there should be differences between the A_{1g} modes and the B_{1g} or A_{2g} modes (which the dominant non-totally symmetric modes in the D_{4h} point group). Be sure to include references in both laboratory reports. The report should have the form of a journal article.

References

1. Gaynor, J.D.; Wetterer, A.M.; Cochran, R.M.; Valente, E.J.; Mayer, S.G. "Vibrational Spectroscopy of the CCl4 v(1) Mode: Theoretical Prediction of Isotopic Effects" J. Chem. Ed. 2015, 92, 1081-1085
2. Wang, L.; Mizaikoff, B.; Kranz, C. "Quantification of Sugar Mixtures with Near-infrared Raman Spectroscopy and Multivariate Data Analysis A Quantitative Analysis Laboratory Experiment" J. Chem. Ed. 2009, 86, 1322-1325
3. Vickers, T.J.; Pecha, J.; Mann, C.K. "Raman spectroscopy with a fiber-optic probe and multichannel detection" J. Chem. Ed. 2001, 78, 1674-1675

4. Sanford, C.L.; Mantooth, B.A.; Jones, B.T. "Determination of ethanol in alcohol samples using a modular Raman spectrometer" J. Chem. Ed. 2001, 78, 1221-1225