Department of Chemistry

Laboratory Manual
Physical Chemistry
M. Sc. I year

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Contents

1 Introduction to the physical chemistry laboratory

This section gives you the general guidelines that are followed in the physical chemistry laboratory and the format for your lab notebook.

1.1 General Guidelines

1. First and foremost, ATTENDANCE IS MANDATORY. Absence is only permitted for medical reasons. You will be docked 25% of the total marks for every experiment that you are absent. No repeat turn is granted for an experiment missed due to absence.
2. You are expected to be in the lab ON TIME.
3. Wearing a lab coat, safety goggles, and shoes is MANDATORY.
4. Completed lab notebooks are due before class the following week (except during Minors).
5. An important part of the laboratory is the oral examination on the objective, the theoretical basis, the experimental method, the expected results, or, in other words, anything even remotely related to the experiment. You will be examined at random on the experiment that are performing or may have performed earlier. Background reading from a physical chemistry textbook in addition to reading the handout is definitely required. In addition, knowledge about the chemistry and instrument related to the analysis is extremely beneficial.
6. You will be graded for your pre-lab preparation, your effort, your laboratory skills, and your laboratory report.

1.2 Laboratory Report Format

The format for the lab report is given below.

1. Title and Date
2. Introduction: The introduction contains three important pieces of information. (1) The objective of the experiment or in other words "What is being investigated in this experiment?" (2) The theoretical basis of the experiment and (3) a description of the method that will be used to achieve this objective. Please do not copy this from the lab manual or from your predecessor. Write in your own words. Here is an example.

   In this experiment we study the solubility of KIO_4, a sparingly soluble salt and the effect of ionic strength on it. The equilibrium under consideration is

   $$KIO_4(s) \rightleftharpoons K^+(aq) + {IO_4}^-(aq)$$

   for which the equilibrium constant may be written as

   $$K = \frac{a_{K^+} a_{{IO_4}^-}}{a_{KIO_4}},$$

   where a stands for the activity. The activities are related to the concentrations as $a = \gamma c$ and the γ's in turn are related to the ionic strength, at least in some limiting situations, by the relation

   $$\log \gamma_{\pm} = -A z_+ z_- \sqrt{I}.$$

The experiment involves measuring the concentration of IO_4^- in 5 saturated solutions of KIO_4 with varying amounts of an inert salt, $NaNO_3$, which serves to change the ionic strength. The IO_4^- is estimated in each case by an iodometric titration against $Na_2S_2O_3$ after liberating the iodine present quantitatively by the addition of potassium iodide in acid medium.

THESE TWO SECTIONS ARE TO BE COMPLETED BEFORE YOU COME TO CLASS.

3. Data and Observations: This section will be written as you perform the experiment; the data you collect and the observations you make are recorded here. If necessary, make a flow chart (or any other device to jog your memory) of the procedure to be followed. View this is as an opportunity to organize your thoughts and not to reproduce the procedure in the handout. Perhaps the easiest way to collect your data and observations is to paraphrase the procedure as you go through the steps and then enter your data and observations for that step. Simply enter a one- or two-line statement to describe the operation and then your observations for that step. To do this you must have read the procedure more than once, discussed with people who have done the experiment before you and most importantly, made a mental picture of how you intend to proceed and the types of information you will be collecting. Most importantly, for every step in the procedure answer for yourself the question "Why am I doing what I am doing?" We discourage you from looking at the procedure in the handout during the course of the experiment. This is a sure sign that you have not done the pre-lab preparation.

   Write all the observations and data for the experiment in this section. Data and observations will only be recorded IN PEN. As you record your data and observations this section may become messy and unorganized which is to be expected. If you make a mistake score it off and make a note of the error made. Avoid overwriting. No fairing of your rough data and observations is allowed or required.

   Here is an example. Procedure: 1. Prepare 50 mL of a stock solution of 100 ppm quinine bisulphate in 4 M sulfuric acid. 2. Make 10 solutions, each of 10 mL, of quinine bisulphate with concentrations varying from 1 to 10 ppm. 3. Measure the fluorescene intensities with the 10 ppm solution as standard. 4. Measure the decrease in fluorescence intensity for th 10 pmm solution on the addition of increasing volume of 0.1M NaCl.

   Preparation of 50mL of 4 M sulphuric acid: Provided conc. sulphuric acid. Take x mL and make it up to 50 mL.

   Preparation of 100 ppm quinine sulfate: Done in two steps. Prepare 1000 ppm and dilute. Weighed y mg and diluted to 100 mL.

   And so it goes.

   Recording data or observations on parts of your body, scraps of paper, back of your notebook, etc. is not tolerated.

   THIS SECTION IS DONE DURING THE LAB PERIOD. At the end of the lab period the laboratory instructor will sign and date your observation sheets.

4. Calculations and Results: If your data and observation has become cluttered or disorganized, this is the place to clean it up and present it with clarity. Use tables, if and when required.

   In a table, the independent variables (if they have been identified) go in the left hand columns, the dependent variables on the right. Any column heading should have all the information needed to define the table's meaning. A categorical variable should include a description of the class. A discrete or continuous variable should identify units and any

multipliers (e.g., hundreds of people, millions of dollars, milliliters, micromolars). A title summarises what the table is showing. When investigating, the order of the entries is arbitrary. When reporting results, they should be sorted into an order.

Show all set-ups for each type of calculation: be explicit. If you have to perform the same calculation more than once you do not have to write the set-up for each one, but it should be clear as to which set-up correlates to which calculation. An example follows.

The solubility experiment discussed earlier requires you to calculate I, the ionic strength. The ionic strength was calculated from the expression $I = \sum_i c_i z_i^2$ where the contributing species are K^+, IO_4^-, Na^+, NO_3^-. The first two are known from the estimation and the last two from the amount of added salt. In this case, your report would say "The activity coefficient was calculated using the expression ...."

Be sure to include the final results of all your calculation and to highlight the final answers in some fashion. Support your results with appropriate graphs. Unfortunately, many of you (yes, you) don't know how to draw a good graph. Or even know what a good graph is supposed to look like. HERE ARE SEVEN SIMPLE STEPS TO DRAW A GOOD GRAPH.

Step 1: Give your graph a title When you title your graph, the only reasonable title looks like this: *The effect of independent variable on dependent variable.* The independent variable in an experiment is the variable that you manipulate, and the dependent variable is the outcome of that manipulation.

Step 2: Find the right axis for your graph The x-axis of your graph (the one on the bottom) should always represent your independent variable. The y-axis on your graph (the one that goes up and down) should represent your dependent variable. If you're not sure which variable is which, either look at what you wrote in step 1, or ask yourself "What did I actually do in this experiment?" (x-axis) and "What did I measure in this experiment?"" (y-axis).

Step 3: Label each axis If you don't label the axis of a graph, nobody will have any idea what you're talking about. As a result, it's important that you label each axis with the name of the variable (time, heat, etc.) and the unit you used to measure the variable (seconds, degrees Celsius, meters, etc.)

Step 4: Always use a line graph You've probably seen many types of graph in your life. Bar graphs are common in economics books, pie charts are common in the Times of India, but in science, we always use line graphs. This is because graphs are intended to help us to figure out the trends that develop between the independent and dependent variables. For example, if we have a good line graph that covers how temperature has changed from time = 0 seconds to time = 240 seconds, we can use it to figure out what the temperature should be at any time between these points, even if we didn't take data there. In other words, line graphs are good for describing causal relationships, where one thing causes another to happen.

Step 5: Never connect the dots! Remember, the idea of a graph is to figure out how two variables are related to one another and to make it possible to predict what will happen at any given point. If you connect the dots, this won't be possible.

Step 6: Fill all available space If you make a graph with the goal of showing how something changes over time, it doesn't do anybody much good if the graph is too small to see. Choose a scale so that the graph fills most, if not all of the page.

Step 7: Don't leave anything out One of everybody's favorite tricks is to make a graph look cooler by leaving out the data.

With a spreadsheet it is much easier to prepare graphs, but it is also much easier to produce a poor quality graph. TO ASSURE THAT YOUR GRAPHS ARE CORRECTLY PREPARED

(E. G. , LOOK GOOD AND ARE EASY TO UNDERSTAND BY THE READER), FOLLOW THESE STANDARD PROCEDURES:

- Most of the graphs that you will prepare in a chemistry class are called XY Scatter plots in Excel. The other formats will be used sparingly because they are generally not useful in chemistry.
- When creating the graph, make the chart occupy a new sheet; do not create it in the worksheet containing the data.
- Make all lines (the axes, the tick marks, the box around the plot area and any data lines) black with medium intensity (third choice from the top under Weight in the format boxes).
- The plot area should have no fill color (not the default setting) and no box around it.
- Turn off all gridlines (not the default setting), unless told otherwise by your instructor.
- Legends showing what a given symbol means are useful, but not always needed (e. g., in a formal laboratory report this information is given in the caption that accompanies the figure). Be sure that your selection of lines and legend titles clearly distinguish between multiple data sets and fits.
- A title should be placed at the top of the graph if the graph is to be placed in the laboratory notebook. This helps the reader immediately know what the graph is. The title should be a concise description of what is being graphed (e. g., "Pressure as a Function of Temperature for Nitrogen"). Usually you do not need to describe in the title the units used in the graph, but there are some instances where this is necessary. Avoid using the words "versus", "vs." and "plotted" in the title. The title is omitted when the graph will be used as a figure in a publication or formal laboratory report. This is because the information normally put in the title will be included in the figure caption.
- Watch your tick marks. The spreadsheet automatically selects the spacing, which may not be appropriate for your graph (see General Considerations).
- Be careful with the use of color to distinguish different data sets on the same graph. Yellow and light blue do not show up very well when printed either on color or black and white printers. Do not distinguish different data sets by color if you do not have a color printer. In general, it is best to dispense with color entirely and make all lines and symbols black (or at least a uniform dark color).
- Data are always shown as symbols and fits to the data are shown as lines or curves. Do not connect the data points with lines. This will confuse the reader as to whether these lines represent a fit, or not.
- For graphs that will be placed in a notebook, you can include the equation of a best-fit line and the R^2 value for the fit in a legend (but remember that this information should also be written in the notebook as part of the graph's description, in case the graph is removed). However, for graphs that will be submitted for publication or used in a formal laboratory report, this information is not shown on the graph itself. Rather, it is placed either in the figure caption or in the body of the text itself.
- Sometimes a fit is not required, or cannot be done, but you still want to show a trend in the data. In this case a smooth line that passes through the data as an `aid to the eye` is used, and is so indicated in the text accompanying the graph.
- A 16-point font for axis labels and graph titles and a 14-point font for the tick mark labels are better choices for general work than the default settings. The font used for the title should match that used for the axis labels.

- Depending on the final size of the graph, you might want to increase the size of the symbols to 9 point.
- Most spreadsheets automatically select the number of decimal places on the tick labels from the data, but not always correctly. For example, if your data were between 50 and 100, you would want tick labels of 50, 60, 70, 80, 90, and 100. But if the data in the spreadsheet are set to two decimal places, most spreadsheets would make the labels 50.00, 60.00, 70.00, 80.00, 90.00 and 100.00. Although this is not necessarily incorrect, the convention is to show the minimum number of decimal places on the tick labels.

Most importantly, REMEMBER THAT THE DEFAULT SETTINGS IN A SPREADSHEET MAY NOT FIT YOUR NEEDS. It is critical that you know how to change the settings.

Use a spreadsheet (Microsoft Excel is the world's favorite!) or any other program of your choice to perform the least squares fits of your data wherever required.

Finally, state your results to the question you set out to answer in the objective. Please keep in mind the significant figures in the results that you report - do not blindly write the number that your calculator or Excel spits out. This is one of the most baffling things that chemistry students run into. There is a difference if you write a number as "100 g" or "100.0 g." Your calculator doesn't know the difference, so why should you worry about it? There are good reasons that you should be concerned with the number of digits that a number has.

Significant figures help you to understand how precise your data are. Here is an example. What do the three numbers: "100 g", "100.0 g", and "100.0 g" mean?

The value "100 g" indicates that the instrument you used to take the measurement could only take data to the nearest hundred g. As a result, objects weighing both 55 g and 130 g will both read "100 g" on the balance. The value "100.0 g" indicates that the instrument could take data to the nearest gram. If such an instrument says "100.0 g", the actual answer must be between 99.5 and 100.4 g. If you see "100.0 g", your instrument is taking measurements to the nearest 0.1 g. In this case, the actual weight of the object is measured to be between 99.95 and 100.49 g.

That's why significant figures are important: They tell you whether your instrument is measuring to a lot of decimal places or just to a few.

When working with significant figures, never write too many because if you do, your data will show that you've got amazing super-duper precision even though you do not. And if you do not write enough, your data will show that your measurements are not as precise as you really are. The biggest rule of science is to tell the truth, and significant figures are one way of making sure you do that.

Significant figures tell you how precise a measured value is. A measurement of "430 g" is precise to the nearest ten grams, as indicated by significant figures.

Precision is a measurement of how reproducible an answer is with some piece of equipment. If an instrument is precise, it will give you an answer that's more detailed than one that is not because it's assumed that the instrument will give you the same answer each time.

Accuracy is a measurement of how correct a measured value is. If something weighs 150 g and you measure the weight as 130 g, it is not an accurate measurement. Precise measurements need not to be accurate, but accurate measurements must be precise. If an answer is not reproducible, you're stuck with the question of which value you should treat as the real one. There is a mistaken impression that digital instruments give more accurate or precise data than analog ones. An old-style analog machine is not less awesome because it does not have a digital readout. It's just older.

Always see if measured values pass the idiot test. If you find that the mass of a textbook is 4300 kg, it is entirely possible that you have written down exactly what the balance told you. It is also 100% certain that the answer is wrong. It is easy to make mistakes, and life is easier if you spot them before you finish the experiment.

Numbers not associated with actual measurements do not have significant figures. For example, if somebody says "How many significant figures does "340" have?", there is no good answer to that. Without knowing that it is a measured value, you can not determine which (if any) of the digits are significant, or if it is simply meant to be a theoretical construct.

Similarly, many conversion factors can be thought to have an infinite number of significant figures. When you say that there are "100 cm in 1 meter", you do not need to worry about how many significant figures "100 cm" has because one meter is defined as 100 cm. It is not that one meter is about 100 cm - it is exactly, to infinite precision, 100 cm!.

Here are some rules to figure out the number of significant figures in measured values:

Rule 1: Any digits that are not zeros in a measured value are significant. For example, the number "229 cm" has three significant figures and is assumed to be precise to the nearest centimeter. After all, why would you ever write those numbers unless they meant something?

Rule 2: Any zero that is in front of all of the nonzero digits is not significant. For example, if you find that something has a mass of "0.0134 g", there are only three significant figures, and the value is precise to the nearest 0.0001 g. This rule might appear arbitrary. Though the zeros in front of the "134" in this measurement are meaningful, including them as significant figures would screw things up when doing stuff with scientific notation.

Rule 3: Any zero after all of the nonzero digits is only significant if a decimal is shown. By this I mean that any zero after all of the nonzero digits is only significant if you actually see a little point written somewhere in the number. If you do not actually see a dot drawn in the number, the zeros afterward are not significant. As a result, "210 cm" has two significant figures and is precise to the nearest 10 cm, while "210.0 cm" has three significant figures and is precise to the nearest centimeter.

Rule 4: The number of significant figures for a number written in scientific notation is that of the part in front of the exponent. For a measured value of "2.10×10^4 cm", you have three significant figures and the number is precise to the nearest 0.01×10^4 cm (or 10 cm).

Imagine that you want to find the density of styrofoam. Using a kitchen balance we find that the mass is 10 g. Also, that the volume of the piece of styrofoam is 90 mL. Plugging these values into the calculator we find that the density of styrofoam is 0.111 111 11 g mL.

What does this mean? One thing it means is that styrofoam is not very dense, which explains why it floats so well. More importantly, this answer shows that I was able to use really bad equipment to find the density of Styrofoam to the nearest 0.000 000 01 g mL. Wrong!

So, how do we really show the answers to chemistry problems with the correct precision? It depends on the type of calculation you're performing.

The rules for calculations with significant figures:

Rule 1: When you add or subtract numbers from each other, the answer should be written to the number of decimal places of the least precise number. For example, if you're going to add 45 gram (which is precise to the nearest gram) and 29.1 g (which is

precise to the nearest 0.1 g), the answer will be "74 g" because the actual number of 74.1 g rounds to the nearest gram because of the first number. If you were to add 45 g and 29.6 g, you'd write the answer as "75 g because 74.6 rounded to the nearest whole number is 75.

Rule 2: When you multiply or divide numbers, the answer should be written with the same number of significant figures as the number with the fewest significant figures. In our example, our answer should be written as "0.1 g mL" because both 10 g and 90 mL have one significant figure. Even if we had the best instrument in the world for finding volume and saw that the volume of the Styrofoam was 90.023 011 133 002 234 488 23 mL, the answer would still round to 0.1 g mL because "10 g" only has one significant figure.

Here are a few more examples:

Example 1 If you have one object that weighs 300 g and another that weighs 23.2 g, the mass of both the objects put together is "300 g." Though this appears to be incorrect, it makes sense when you consider that saying "300 g" is essentially saying "somewhere between 250 and 349 g." With that lousy level of precision, the 23.2 g addition is not meaningful.

Example 2 The density of a 91 g object with a volume of 31.03 mL, the density of the object would be 2.9 g mL (rounded from 2.936 g mL because "91 g" has only two significant figures.

Example 3 If an object that weighs 83 g is divided into four parts, the average mass of each part is 21 g. Though the number "83 g" has two significant figures and "4" has one, we only worry about the significant figures in 83 g because we know we'll have exactly four pieces.

THIS SECTION IS TO BE COMPLETED EITHER IN THE LABORATORY, IF THE EXPERIMENT FINISHES EARLY, OR AT HOME.

5. Discussion and Conclusions: The discussion is used to explain your results you presented in the previous section. Ask yourself provocative questions like: Do your results make sense? Is there some data missing? Did your results conform to your expectations? If the data from an experiment is straightforward and self-explanatory this section may be brief. However, most of the time you will have to explain to the reader why you obtained a particular result, especially if your result is different from that expected. Discuss you results in the context of what you stated in the Introduction.

   Explain any sources of error and how they affect your results. Discuss any improvements that may be made to the experiment or procedural errors in the laboratory manual.

   Answer any during-lab questions that may have been raised by the instructor.

   THIS SECTION IS TO BE DONE EITHER IN THE LABORATORY, IF THE EXPERIMENT FINISHES EARLY, OR AT HOME.

2 Intercalation of sodium into vanadium oxide and potentiometric estimation of extent of intercalation

Intercalation of sodium ions into layered vanadium oxide through soft chemistry approach and its structural characterization using powder X-ray diffraction. Demonstration of potentiometric titration for the analysis of the mixed-valent solid $NaxV_2O_5 \cdot yH_2O$.

2.1 Theory

Vanadium pentoxide (V_2O_5) crystallizes into a layered structure and serves as a host for the intercalation of several inorganic and organic cations. Crystal structure of V_2O_5 consists of layers of VO_5 square pyramids that share edges and corners with the apical V−O bond (much shorter than the four other distances and considered as V=O).

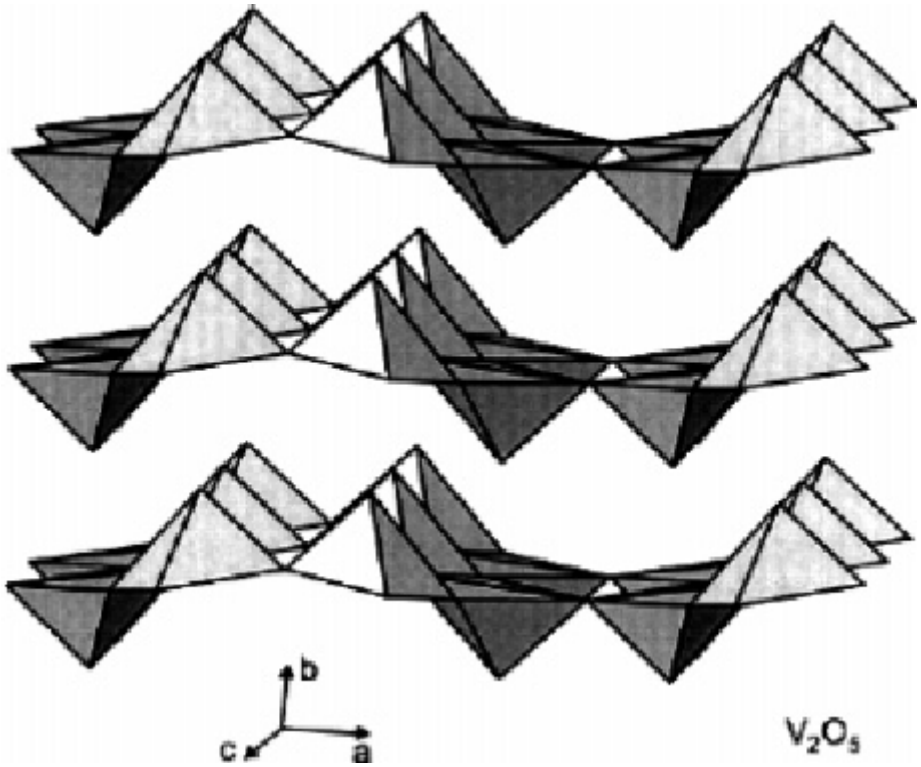

Figure 2.1: Structure of V_2O_5.

The layered structure of V_2O_5 illustrates a two-dimensional character for this material with a layer separation ≈11.5 Å; the structural anisotropy also enables the material to be able to insert guest species (protons or alkali ions and/or water) in between the layers. The host-guest intercalation materials show interesting electronic, optical and magnetic properties owing to the presence of mixed-valent vanadium in the solid state structure. Powder X-ray diffraction pattern (PXRD) is an essential technique to establish the crystalline nature of the host-guest solid and the phase purity. PXRD is used to establish the incorporation of guest species in between the layers. During intercalation, the two cell parameters of the orthorhombic system remains almost similar suggesting the preservation of structural integrity of the layers, the third axis increase depending on the size of the guest molecule. This type of reaction generally occurs around room

temperature and is referred as intercalation reaction. V_2O_5 is Lewis acidic and the vanadium is in fully oxidized state (5+). The most popular soft chemistry approach (*chemie douche*) exploits acid-base or redox reaction as the major driving force to incorporate a variety of organic and inorganic species in between the vanadium oxide layers. Examples include organic bases like pyridine, aniline, polyaniline and metal based reducing agents like $LiAlH_4$, $NaBH_4$, Zn or Sn metal etc.

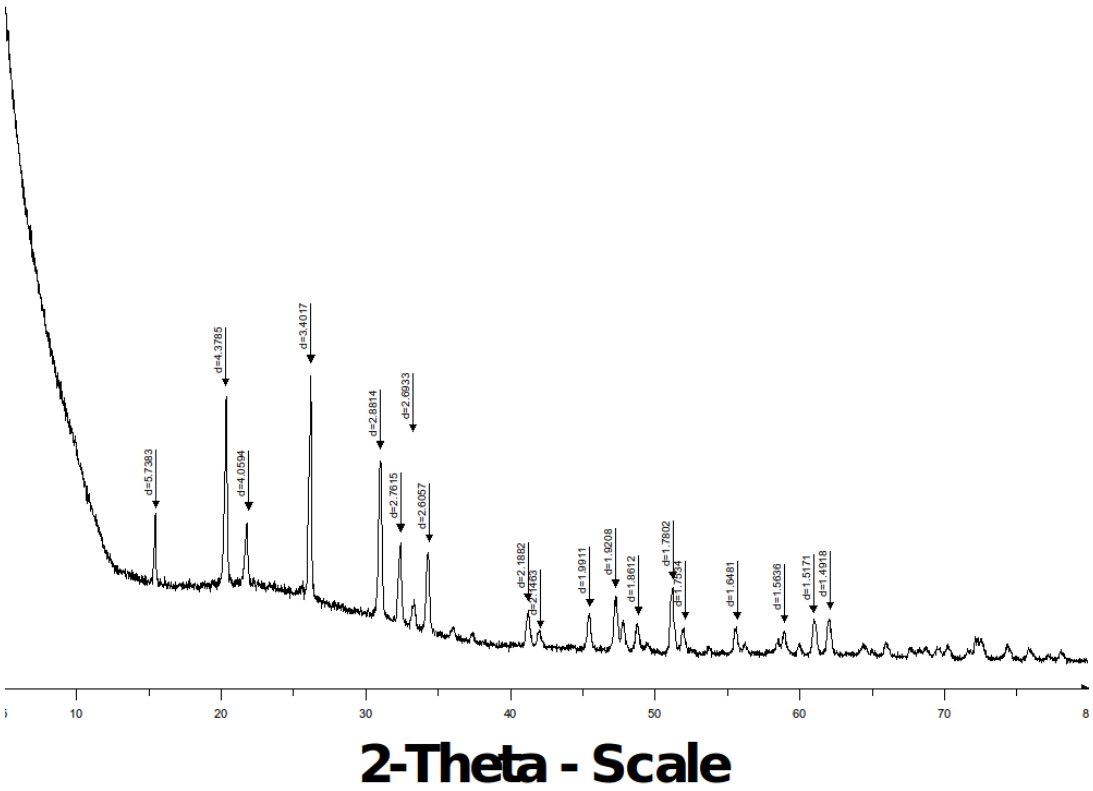

Figure 2.2: Powder X-ray diffraction pattern of V_2O_5.

In this experiment, you will adopt a soft chemical (*chemie douche* technique) approach to incorporate sodium ions into orthorhombic V_2O_5. A suspension of vanadium oxide solid in aqueous ethanol is treated with solid $NaBH_4$ at room temperature. The color changes from reddish orange to dark green (Remember the reaction is not dissolution followed by precipitation.). Interpretation of the color of a solid is quite complex. Record the diffuse reflectance spectrum, FTIR, and PXRD patterns of both V_2O_5 and the dark green product (TAs will provide you a copy of the spectra and the diffraction pattern). Based on these data, comment on the structure of the dark green product. Rationalize the color change using a simplified band theory. What structural information do you derive from FTIR spectra? What do you infer from PXRD patterns of the host and host-guest solid? [Hint: use the data available in powder diffraction database for V_2O_5]. Is the product crystalline? Do you find any evidence for the incorporation of sodium ions into the structure? [Hint: $NaBH_4$ is unstable in aqueous solution and hydrolyses to produce nascent hydrogen. It is quite likely that protons can also incorporate into the structure along with water molecules].

Potentiometric titration for vanadium quantification in the mixed-valent solid

The second objective of this experiment is to appreciate the underlying physical chemistry principles involved in a potentiometric titration(rather than the quantitative analysis).

During the intercalation reaction, part of vanadium(V) in the solid state undergoes reduction to vanadium(IV) with simultaneous incorporation of sodium ions in between the vanadate layers producing a solid with approximate composition, $NaxV_2O_5 \cdot yH_2O$.

Can you write an appropriate equation for the formation of $NaxV_2O_5 \cdot yH_2O$ from the reactants? Is this a solid state reaction or a reaction between solid and solution? $NaBH_4$ is known to undergo hydrolysis in aqueous solution forming nascent hydrogen. If so, protons can also incorporate into vanadium oxide. How do you rationalize the presence of protons inside the lattice?

The potentiometric titration exploits the well separated potentials between three sets of equilibrium reactions.

$$Ce^{4+} + Fe^{2+} \longrightarrow Ce^{3+} + Fe^{3+}$$

$$VO^{2+} + Ce^{4+} \longrightarrow {VO_2}^{+} + Ce^{3+}$$

$${VO_2}^{+} + Fe^{2+} \longrightarrow VO^{2+} + Fe^{3+}$$

The amount of vanadium(IV) estimated may be considered equivalent to amount of sodium intercalated. A single potentiometric titration enables us to estimate both the amount of vanadium(IV) and total vanadium.

First, you will perform a titration between ceric and ferrous ions using an electrochemical cell consisting of a platinum indicator electrode (inert electrode - a potential is developed only when the electrode is in contact with a solution containing a redox couple) and a reference electrode. Here you will employ a standared calomel electrode ($E_0 = 0.2444\,\mathrm{V}$ vs standard hydrogen electrode (SHE)). At the equivalence point, an abrupt change in potential of $\approx 800\,\mathrm{mV}$ occurs.

Can you rationalise the logarithmic nature of the titration curve? A potentiometric titration is performed against a standard ferrous sulphate solution prepared using 2M acid. Why? Chloride is avoided. Explain with appropriate numbers.

In a redox titration the oxidised form of an ion (e. g. Ce^{4+}) is reduced by the addition of a reductant (e. g. Fe^{2+}). In a potentiometric titration the stoichiometric point of a redox titration is detected by monitoring the potential of the cell formed by a platinum electrode and a reference electrode (calomel electrode in this experiment) in electric contact with the mixture. There is a sharp change in the cell potential at the stoichiometric point when exactly enough reductant has been added to reduce all the oxidised form.

In a second experiment, we establish the mixed-valency of vanadium in sodium intercalated vanadium oxide. For this, a known amount of the intercalated solid is dissolved in a known excess of cerium(IV) solution and again titrated against ferrous sulfate. In this titration you will observe two equivalence points: the first one corresponds to a change from $Ce^{4+}|Ce^{3+}$ ($1.61\,\mathrm{V}$) to ${VO_2}^{+}|VO^{2+}$ ($1.00\,\mathrm{V}$) while the second one is due to change from ${VO_2}^{+}|VO^{2+}$ to $Fe^{3+}|Fe^{2+}$ ($0.77\,\mathrm{V}$). Notice the color change from orange to blue during the titration. The end point (different from equivalence point!) is a blue solution containing all vanadium in +4 oxidation state. Unlike the blank titration, the equivalence points are less sharp as the change in potential for the two curves are comparatively less ($\approx 600\,\mathrm{mV}$ and $230\,\mathrm{mV}$ respectively).

2.2 Procedure

Preparation of $NaxV_2O_5 \cdot yH_2O$

Weigh approximately $2\,\mathrm{g}$ of ${V_2O_5}$ and suspend it in water. Add excess of sodium borohydride, stir it till a dark green colour solid separates out (make sure the brown colour solid disappears). Allow the precipitate to stand for $15\,\mathrm{min}$ to $30\,\mathrm{min}$. Filter, wash with alcohol and dry the solid in air.

Standardization of the ceric solution by potentiometric titration

Pipet out 25 mL of 0.01 M ceric sulfate solution into a 50 mL beaker. Add just enough distilled water to cover the cell assembly consisting of a Pt and reference electrodes, if needed. Note down the potential of the solution before adding ferrous solution. Titrate the ceric sulfate solution against ferrous solution. Add 0.5 mL of the titrant each time in the beginning and smaller volumes closer to the endpoint. After each addition, stir well and measure the cell potential.

Potentiometric titration of the intercalated material

Dissolve a known amount of the intercalated solid (20 mg) in 25ml ceric solution and titrate against the ferrous sulphate solution potentiometrically. Adopt the same procedure you did while standardizing the ceric solution. [Note: Warm the solution, if necessary, for dissolving the solid. Note the colour of the solution. If required, add distilled water so that the electrode is immersed into the solution. Before adding the ferrous solution note the potential]. Note the colour changes in the solutions at different points of the titration.

2.3 Calculations and Results

1. Plot the emf (mV) against the volume of ferrous (II) sulphate (mL) for both the standarization and estimation. Also, plot $\delta E/\Delta V$ against v. Sketch a graph of emf versus $\log [Fe^{2+}]/[Fe^{3+}]$.

2. Deduce the equivalence point and calculate the formal redox potential of $Ce^{4+}|Ce^{3+}$, $VO^{2+}|VO^{2+}$, $Fe^{3+}|Fe^{2+}$ from the titration curves.

3. Interpret the titration curves on the basis of Nernst's equation.

4. From the titration curve, determine the concentration of vanadium(IV) and consequently the amount of sodium intercalated. Calculate the ratio of V(IV)/V(V).

5. Rationalise the colour of the solution at different points of the titration in terms of the aqueous chemistry of vanadium with suitable equations.

3 Kinetic study of the esterification of an alcohol by NMR Spectroscopy

Reaction of trifluoroacetic acid with an alcohol (ROH) yields the corresponding ester

$$CF_3COOH + CH_3CH_2OH \longrightarrow CF_3COOCH_2CH_3 + H_2O \tag{3.1}$$

In this experiment we will follow the time course of this esterification reaction by ^{1}H NMR. It exploits the deshielding effect of the trifluoroacetyl group on the hydrogens of the carbon adjacent to it. The effect is a considerable shift of the signal for methylene protons to lower field (higher δ values) compared with it in alcohol. One can thus monitor the progress of this reaction by recording the appearance and disappearance of those hydrogens. In the case of ethanol, this signal is a quartet.

An important feature NMR is that areas of the peaks, i. e. the integrated intensities, are approximately in the same ratio as the number of hydrogens responsible for resonance. Integration of the area under the alcohol signal with respect to the integrated area of the ester signal gives the concentration of the species present. The rate for the reaction is given by

$$\text{Rate} = k[\text{acid}]^x[\text{alcohol}]^y \tag{3.2}$$

where x and y are the reaction orders. Under the conditions of a large excess of acid (pseudo first order conditions) the rate equation simplifies to

$$\text{Rate} = k_{\text{obs}}[\text{alcohol}]^y \tag{3.3}$$

The integrated rate equation for a first order reaction, as is the case here, is

$$\ln[\text{alcohol}] = -k_{\text{obs}}t + \ln[\text{alcohol}]_0 \tag{3.4}$$

3.1 Procedure

1. Using the variable temperature accessory on the NMR, set the desired temperature and allow the probe to stabilize for several minutes.
2. Pipette exactly 1.00 mL of TFA into the NMR tube.
3. Using a microlitre syringe add 1.00 mmol (58.3 microlitre) of alcohol and start the stopwatch.
4. Cap the NMR tube, shake vigorously, and then carefully wipe the exterior.
5. Immediately load the sample into the NMR and begin collecting data at regularly timed intervals. The reaction is analyzed after every 5 min.
6. The time in seconds for the analysis should be determined from the stopwatch when the recorder pen reaches a point between the two absorptions
7. Follow the reaction for 35 minutes.
8. Integrate the absorptions for both ester and alcohol. Adjust the integrator amplitude so that the height of the trace is at least 40mm.

3.2 Calculations

Concentration of alcohol at time t can be calculated from the integral unit for alcohol, which is denoted as [alcohol](t). The sum of the integral units of the alcohol and ester at any time is a conserved quantity and is equal to initial alcohol concentration, $[\text{alcohol}]_0$. If the spectrum has more than one peak (for any alochol other than methanol), use the better resolved peak for integration.

Tabulate, t, [alcohol](t), ln[alcohol](t). Plot ln[alcohol](t) vs. t from which the rate constant is obtained as the slope.

4 Effect of ionic strength on reaction rate

The reaction between potassium peroxodisulphate and potassium iodide may be represented as

$$S_2O_8{}^{2-} + 2\,I^- \longrightarrow 2\,SO_4{}^{2-} + I_2 \tag{4.1}$$

During the reaction, iodine is liberated and the progress of the reaction can be followed by titrating the liberated iodine in V ml of the reaction mixture against standardised thiosulphate solution at different intervals of time. The titre values are directly proportional to iodine liberated and therefore, the amount of peroxodisulphate which has disappeared by reaction from V mL of reaction mixture. The titre values at any time t will be proportional to the amount of iodine liberated, i.e. to the amount of KI oxidized or the amount or potassium peroxodisulphate used up in oxidation; hence it gives the value of x at that time.

Increase in ionic strength I of a solution influences the specific reaction rate k according to the sign of charge on the reacting substances A and B. If the charges on A and B are represented by Z_A and Z_B, the relationship between the rate constant and the ionic strength, $I(= \frac{1}{2}\sum_i c_i Z_i^2)$ is given by the Bronsted equation

$$\log k = B + 1.018 Z_A Z_B \sqrt{I} \tag{4.2}$$

where B is a constant depending on temperature. Accordingly, if Z_A and Z_B are both positive or both negative, $\log k$ will increase as $\sqrt{I}$ increases. It will decrease if only one of the Z values is negative. It will remain unaffected if one of the reacting species is uncharged. In the present experiment we find the effect of changing the ionic strength by adding different amounts of a neutral salt and of adding different salts, keeping the ionic strength the same.

The formation of a single, highly charged ionic complex from two less highly charged ions is favoured by a high ionic strength because the new ion has a denser ionic atmosphere and interacts with it more strongly. Conversely, ions of opposite charge react more slowly in solutions of high ionic strength. Now the charges cancel and the complex has a less favourable interaction with its atmosphere than the separated ions.

4.1 Reagents

0.06 M $K_2S_2O_8$, 0.12 M KI, 0.9 M KCl, 0.9 MNaCl, 0.3 M $MgCl_2$, 2.0 M CH_3COOH and 0.01 M $Na_2S_2O_3$, starch solution, and crushed ice.

4.2 Procedure

1. Prepare a series of solution as given in table below

Set	$K_2S_2O_8$ Vol. mL	KI Vol. mL	KCl Vol. mL	NaCl Vol. mL	$MgCl_2$ Vol. mL	H_2O Vol. mL	CH_3COOH mL
1	25	25	0	0	0	100	2.5
2	25	25	10	0	0	90	2.5
3	25	25	25	0	0	75	2.5
4	25	25	50	0	0	50	2.5
5	25	25	75	0	0	25	2.5
6	25	25	0	75	0	25	2.5
7	25	25	0	0	75	25	2.5

2. For each set keep the $K_2S_2O_8$ solution separate and mix the other components in a conical flask.
3. Keep the two conical flasks in a thermostat for 5-10 mins.
4. Mix the two solutions. Start the stop watch simultaneously.
5. At intervals of 5 mins pipette out 20 mL of the above mixed solution into a conical flask containing crushed ice.
6. Titrate the iodine liberated against sodium thiosulfate using starch as the indicator.
7. Take at least 5 readings for each set.
8. Repeat the procedure for other sets.

4.3 Calculations and Results

Calculate the rate constant for each set. Comment on effect of ionic strength of rate constant from sets 1, 2, 3, 4 and 5. Also comment on the effect of the nature of cation from sets 5 and 6 and on the rate observed for sets 6 and 7.

5 Polarimetric study of the kinetics of mutarotation of dextrose

Optically active compounds rotate the plane of polarized light by a specific angle. This angle of rotation can be found out by using a polarimeter. Specific rotation of a substance is the observed rotation of the plane of polarization of a plane polarized light passing through a cell of unit length containing the solution of the substance at unit mass concentration.

$$[\alpha] = \frac{\alpha}{cl}$$

The quantity $[\alpha]$ depends on the temperature, wavelength of light used, the nature of solvent, and the nature and concentration of the solute.The temperature and the wavelength are written as superscript and subscript of $[\alpha]$ respectively. Thus, $[\alpha]_D^{298}$ means the specific rotation of light from sodium D line at 298 K.

When a monosaccharide is dissolved in water, the optical rotatory power of solution gradually changes until it reaches a constant equilibrium value. For example, a freshly prepared D-glucose solution has a specific rotation of 110 which on standing falls to a constant value of 52.5.This is due to first order change of α-D-glucose and β-D-glucose. This change in the specific rotation is termed as mutarotation.This equilibrium can be reached quickly by heating the solution or by adding a catalyst (acid or base).

The first order reaction of mutarotation of dextrose can be written as :

$$\alpha-\text{D}-\text{glucose} \rightleftharpoons \beta-\text{D}-\text{glucose}$$

At equilibrium the rate of forward reaction is equal to rate of backward reaction, that is

$$k_1[A]_{\text{eq}} = k_{-1}[B]_{\text{eq}},$$

where A and B refer to the α and β form of glucose respectively. A parameter y which is the displacement of the reaction from equilibrium is defined.

$$y = \frac{\mathrm{d}[A]}{\mathrm{d}t} = -\frac{\mathrm{d}[B]}{\mathrm{d}t} = k_1[A] - k_{-1}[B] \tag{5.1}$$

Thus

$$\frac{\mathrm{d}y}{\mathrm{d}t} = k_1\frac{\mathrm{d}[A]}{\mathrm{d}t} - k_{-1}\frac{\mathrm{d}[B]}{\mathrm{d}t} \tag{5.2}$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = -k_1\frac{\mathrm{d}[B]}{\mathrm{d}t} + k_{-1}\frac{\mathrm{d}[A]}{\mathrm{d}t}, \tag{5.3}$$

which implies that

$$\frac{\mathrm{d}y}{2\,\mathrm{d}t} = (k_1 + k_{-1})\left(\frac{\mathrm{d}[A]}{\mathrm{d}t} - \frac{\mathrm{d}[B]}{\mathrm{d}t}\right) \tag{5.4}$$

Substituting for $\frac{\mathrm{d}[A]}{\mathrm{d}t}$ and $\frac{\mathrm{d}[B]}{\mathrm{d}t}$ from equation 5.1 we get

$$\frac{\mathrm{d}y}{\mathrm{d}t} = -(k_1 + k_{-1})y \tag{5.5}$$

The solution of this equation is

$$y = y_0\exp(-kt) \tag{5.6}$$

where $k = k_1 + k_{-1}$ is the mutarotation coefficient and y is the displacement from equilibrium.

The optical rotation α at time t is of the form

$$\alpha = a[\mathrm{A}] + b[\mathrm{B}] \tag{5.7}$$

where a and b are instrument dependent constants at a particular temperature. When the reaction is complete ($t = \infty$), we have

$$\alpha_\infty = a[\mathrm{A}]_\infty + b[\mathrm{B}]_\infty \tag{5.8}$$

Subtracting equation 5.7 from 5.8, we get

$$\alpha - \alpha_\infty = a([A] - [A_\infty]) + b([B] - [B_\infty]) \tag{5.9}$$

Because the total concentration of glucose in both forms is conserved, we have $[A] + [B] = [A]_\infty + [B]_\infty$, which yields

$$\alpha - \alpha_\infty = (a - b)([A] - [A_\infty]) \tag{5.10}$$

Similarly, from equation 5.1 we have

$$y - y_\infty = (k_1 + k_{-1})(A - A_\infty) = k(A - A_\infty) \tag{5.11}$$

At equilibrium, $y_\infty = 0$, which yields

$$y = \frac{k(\alpha - \alpha_\infty)}{a - b} \tag{5.12}$$

Substituting in equation 5.6, we get

$$\frac{k(\alpha - \alpha_\infty)}{a - b} = y_0 \exp(-kt) \tag{5.13}$$

Taking logarithms,

$$\ln(\alpha - \alpha_\infty) = \ln\left(\frac{y_0(a - b)}{a - b}\right) - kt \tag{5.14}$$

At $t = 0$, $\alpha = \alpha_0$ so that

$$\ln(\alpha_0 - \alpha_\infty) = \ln\left(\frac{y_0(a - b)}{a - b}\right), \tag{5.15}$$

which if used in equation 5.14 yields the final equation

$$\ln(\alpha - \alpha_\infty) = \ln(\alpha_0 - \alpha_\infty) - kt \tag{5.16}$$

The mutarotation coefficient can be found out from the slope of plot of $\ln(\alpha - \alpha_\infty)$ vs. t. However, in the above method the whole experiment is dependent upon the single reading of α_∞, which if it is in error can be cause an error in the determination of k. Thus one resorts to the Guggenheim method for the analysis.

The mutarotation of glucose is catalyzed by acids, bases, and amphoteric solvents such as water. Mutarotation of an aqueous solution is catalyzed by H_2O, H_3O^+, and OH^- ions. The mutarotation coefficient may be written as

$$k = k_{\mathrm{H_2O}}[\mathrm{H_2O}] + k_{\mathrm{H_3O^+}}[\mathrm{H_3O^+}] + k_{\mathrm{OH^-}}[\mathrm{OH^-}] \tag{5.17}$$

In aqueous solutions the concentration of water is high and constant, while in acidic medium the base catalyzed reaction is small and may be ignored, and thus the mutarotation coefficient can be written as

$$k = k'_{\mathrm{H_2O}} + k_{\mathrm{H_3O^+}}[\mathrm{H_3O^+}] \tag{5.18}$$

A plot of k as a function of acid concentration yields the acid catalyzed rate constant and water.

5.1 Reagents

Glucose and 0.2 M HCl

5.2 Procedure

1. Switch on the light source and set up the polarimeter.
2. Wash and dry the polarimeter tube.
3. Measure the optical rotation of dextrose solution in H_2O of the same concentration as in the two kinetics experiments.
4. Weigh 10 g of dextrose and dissolve it in distilled water in a 25 mL beaker. Transfer it to a volumetric flask, add 5 mL of 0.2 M HCl to the solution. Make up the volume to 50 mL and note the time.
5. Fill the polarimeter cell with the solution. Take care to exclude air bubbles.
6. Record the angle of rotation at 5 mins intervals.
7. Repeat the procedure with 10 mL of 0.2 M HCl solution.

5.3 Calculations

Calculate k by the Guggenhiem method discussed below. Calculate k_{H_2O} and $k_{H_3O^+}$ using $k = k'_{H_3O^+}[H_3O^+] + k_{H_2O}$ from the k's obtained at the two different $[H_3O^+]$.

Guggenheim's method for analysis of kinetic data

When carrying out kinetic studies it is frequently difficult or impracticable to measure the concentration of reactant at the start of a reaction or after equilibrium is attained. Guggenheim outlined a useful method for computing the reaction rate coefficient for a first-order process.

The final and (in some cases) the initial concentrations in a first-order process may be estimated from the extremely simple extrapolation procedure outlined below. The treatment is for a gas reaction obeying the law,

$$(p_\infty - p) = (p_\infty - p_0)\exp(-kt) \tag{5.19}$$

although its adaptation to any first order process is obvious.

If pressure readings p_1, p_2, ..., p_n are made at times t_1, t_2, ..., t_n, and a second series p'_1, p'_2, ..., p'_n are made at times $t_1 + T$, $t_2 + T$, ..., $t_n + T$ then

$$(p_\infty - p_n) = (p_\infty - p_0)\exp(-kt_n) \tag{5.20}$$

and

$$\left(p_\infty - p'_n\right) = (p_\infty - p_0)\exp\left(-k\left(t_n + T\right)\right) \tag{5.21}$$

Taking the difference of equation 5.21 and equation 5.20, we get

$$\left(p_n - p'_n\right) = (p_\infty - p_0)\exp\left(-kt_n\right)\left(\exp\left(-kT\right) - 1\right) \tag{5.22}$$

Taking logarithms, we get

$$\ln\left(p_n - p'_n\right) = \ln\left(p_\infty - p_0\right)\left(\exp\left(-kT\right) - 1\right)) - kt_n \tag{5.23}$$

The first term is a constant because T is a constant, and hence a plot of the LHS vs. t_n yields the rate constant as negative of the slope.

6 Linear free energy relationship from the electrochemical reduction of substituted nitro benzenes

In this experiment the $E_{1/2}$ is measured for a range of nitrobenzenes which shows irreversible waves. We expect the rate of reduction to be determined by inductive and mesomeric effect of the substituents in the molecule. The results of these effects on any reaction of a substitutedd benzene derivative canbe estimated quantitatively by means of Hammett σ parameter. In general we find

$$\log k_1^0(\mathrm{X}) = \log k_1^0(\mathrm{H}) + \rho\sigma, \tag{6.1}$$

where $k_1^0(\mathrm{H})$ is the rate coefficient for the unsubstituted case, ρ is a constant whose value depends on the reaction in question, and σ is a value for the substituent. The value of σ is also a function of where the substituent is located, that is meta or para. If we assume that $E_{1/2}$ is independent of σ, then

$$E_{1/2} = \frac{2.303RT\rho\sigma}{\alpha z F} + \text{constant}, \tag{6.2}$$

where α is the transfer coefficient which is close to $1/2$ in many cases.

6.1 Procedure

1. Make 250 mL of 0.1 M KCl solution that will be used as supporting electrolyte solution. Transfer this solution into properly cleaned (rinse the cell with distilled water after cleaning) electrochemical cell.

2. Purge N_2 gas through electrolyte solution taken in the electrochemical cell for 5 min. before putting electrodes in it.

3. Clean the working electrode (glassy carbon) and make the appropriate connections. (White-Reference Electrode and Red-Counter Electrode).

4. Record linear sweep voltammetry (LSV) curves from 0.0 V to 1.0 V at a scan rate of 5 mV/s.

5. Remove the supporting electrolyte solution from the bottom of the cell. Make four different solutions of various nitrobenzenes in 0.1 M KCl solution.

6. Record LSV curves using the above mentioned parameters. Do not forget to clean your working electrode and cell before you use next solution of nitrobenzene.

6.2 Calculations

1. For each of the nitrobenzenes, obtain the $E_{1/2}$ from the experiment.

2. Tabulate the $E_{1/2}$ together with the appropriate Hammett σ value, some of which are tabulated below (see Chem. Rev. **91**, 165 (1991) for extensive tabulation).

3. Plot a graph of $E_{1/2}$ against σ and draw a straight line and find the value of ρ.

X	σ_m	σ_p
COOH	0.35	0.44
CHO	0.35	0.42
OMe	0.12	−0.27
Cl	0.37	0.24
SO_3^-	0.30	0.35
NH_2	−0.16	−0.66
OH	0.12	−0.37

7 Determination of the formation constant of iron (III) salicylate complex

In this experiment you will determine, by Job's method, the stoichciometry of the complex formed between iron (III) and salicylate ion and determine the equilibrium constant for the formation of the complex.

7.1 Theory

The reaction we consider here is the reaction of $Fe_3{}^+$ with salicylate ion to form a violet colored complex.

$$Fe^{3+} + nsal^- \longrightarrow Fe(sal)^{(3-n)+}$$

The complex is formed by the anion of salicylic acid complexes with ferric ion, and thus the stability constant of the complex varies with pH. This experiment is carried out at pH 2.6 to 2.8 at which phenolic group of acid is undissociated and the carboxcylic acid partly so. This pH range is achieved by using Fe^{3+} solution and Salicylic acid in 0.0025 M HCl.

The empirical formula of the complex may be found using Job's method. When equimolar solutions of the reactants are mixed in varying proportions, the maximum amount at equilibrium is formed when the two reactants are present in the same mole ratio as required for the complex formation. Equimolar solutions are made up and mixed in volume ratios 1:9, 2:8, ... 9:1. The total reaction concentration is therefore the same in each case. The maximum absorbance of each solution is plotted against the mole fraction of one of the components to give a Job's plot.

The experiment employs a UV-visible spectrophotometer. A spectrophometer is a device which detects the percentage transmittance (%T) of light radiation when light of certain intensity and frequency range is passed through the sample. The instrument compares the intensity of the transmitted light with that of the incident. The source of radiation in such an instrument is usually hydrogen or deuterium lamp. The intensity is measured using a phototube. The use of reference or blank solution (0.0025 M HCl) compensates for reflection losses at the air glass and glass solution interface so that only absorption due to the sample is measured.

The optical density/absorbance (A) is given by Beer- Lambert's Law

$$A = \log(I_0/I) = \epsilon c L$$

where I and I_0 are the incident and transmitted light intensities, ϵ is the extinction coefficient of complex and L is the path length.

7.2 Experimental Procedure

1. Prepare M/400 Fe^{3+} in M/400 HCl from the solution of Fe^{3+} that you are provided.
2. Prepare M/400 salicylic acid in M/400 HCl from the solution provided.
3. Make 9 solutions, each of total volume 10 mL, containing x mL of Fe^{3+} and $(10 - x)$ mL of HCl with $x = 1, 2, 3, \ldots 9$ mL. In the 10th solution take 10 mL of M/400 HCl. This will serve as the reference solution or blank.
4. Record UV-visible spectra of the 9 above solutions.

7.3 Data and Calculations

1. Record the absorbance measured at $\lambda_{\max}$ from the spectrum of each solution. Tabulate the absorbance, X, Y, XY/A for each of the nine solutions.

2. For each of the nine solutions whose absorbance were measured plot the absorbance versus the mole fraction of the Fe(III). Draw a smooth curve through the nine points and determine the value of mole fraction of Fe(III) where the absorbance is a maximum. From this, determine the nearest integer value of Fe(III) to sal ratio.

3. The equilibrium for the formation of a ferric salicylate complex, may be written as

$$\mathrm{Fe}^{3+} + \mathrm{nSal}^{-} = [\mathrm{Fe(Sal)}]^{(3-\mathrm{n})+}$$

The stability constant or equilibrium constant K is defined as

$$K = \frac{\left[\mathrm{Fe(Sal)}^{(3-\mathrm{n})+}\right]}{\left[\mathrm{Fe}^{3+}\right]\left[\mathrm{Sal}^{-}\right]^{n}} \tag{7.1}$$

Let X be the concentration of Fe^{3+} and Y be the concentration of salicylic acid as in Table I. From Beer-Lambert law

$$C = \left[\mathrm{Fe(Sal)}^{(3-\mathrm{n})+}\right] = A/\epsilon L$$

which yields

$$K = \frac{\frac{A}{\epsilon L}}{\left(X - \frac{A}{\epsilon L}\right)\left(Y - \frac{A}{\epsilon L}\right)} \tag{7.2}$$

Rearranging 7.2 we get

$$K = \frac{\frac{A}{\epsilon L}}{XY - \frac{A}{\epsilon L}(X+Y) + \frac{A^2}{\epsilon^2 L^2}} \tag{7.3}$$

Taking the reciprocal of 7.3 gives

$$\frac{1}{K} = \frac{\epsilon L(XY)}{A} - (X+Y) + \frac{A}{\epsilon L}, \tag{7.4}$$

which may be rewritten as

$$\frac{XY}{A} = \frac{\frac{1}{K} + (X+Y)}{\epsilon L} - \frac{A}{(\epsilon L)^2} \tag{7.5}$$

The total concentration $(X+Y)$ is constant, so a plot of $\frac{XY}{A}$ vs. A yields the value for ϵL from the slope. Obtaining ϵL from the slope, K can be calculated from the intercept.

8 Thermodynamics of micellization

In this experiment we will calculate the thermodynamic parameters of micellization of a surfactant from conductivity measurements.

8.1 Theory

Surfactants are amphiphilic substances, which consists of hydrophilic head and a long hydrophobic hydrocarbon tail. Sodium dodecyl sulphate(SDS) [$NaOSO_3C_{12}H_{25}$] is an anionic surfactant. When it is dissolved in water, several bulk properties (such as viscosity, surface tension, conductivity etc.) are significantly changed but abrupt changes in these properties do not occur until a certain minimum bulk properties SDS concentration is reached. This concentration is known as the critical micelle concentration (CMC). Below the CMC, the surfactant exists mainly as solvated monomeric species whereas above the CMC these monomers undergo self-organization to form interesting structures called micelles. Here conductivity at different concentration is measured and plotted against concentration of SDS. The CMC thus calculated is used further to calculate the thermodynamic parameter $\Delta H^\circ_{\text{mic}}$, $\Delta G^\circ_{\text{mic}}$, $\Delta S^\circ_{\text{mic}}$from the formulae given below.

$$\Delta G^\circ_{\text{mic}} = 2.303RT(1+\alpha)\log CMC$$

$$\Delta H^\circ_{\text{mic}} = -2.303RT^2\left[\frac{\partial(1+\alpha)\log CMC}{\partial T}\right]$$

$$\Delta S^\circ_{\text{mic}} = (\Delta H^\circ_{\text{mic}} - \Delta G^\circ_{\text{mic}})/T$$

Here α is the fractional micellar ionization and measures the micellar charge neutralized by the counterions included in the micelle.

8.2 Procedure

1. Switch on the conductometer. Record the room temperature.
2. Prepare 50 mL of a 50 mM stock solution of SDS in distilled water.
3. Use 20 steps of the step-by-step dilution-extraction method outlined below to go from this concentration to a final concentration of 1 mM to 2 mM. The step-by-step dilution-extraction proceeds as follows. To V mL of solution of concentration C_0 is added v mL of solvent. The concentration of the resulting solution is $C_1 = C_0(1 - v/(V + v))$. This procedure is then repeated by adding v mL of solvent to V mL of solution of concentration C_1 and so on. If you define a dimensionless volume factor $V_F = 1-v/(V+v)$ then the final concentration after n such dilutions is $C_n = C_0V_F^n$. Choose a V_F of ˜0.85. For example, if V is 25 mL you could use a v of 5 mL.
4. Measure the conductivity of each of the solutions.
5. For each solution repeat the conductivity measurement at a higher temperature (preferably 10-15 ℃ higher than room temperature). Use the thermostat provided to control the temperature.

8.3 Observations

1. At room temperature, tabulate the concentration of the SDS solution and the corresponding conductance.

2. Repeat at the second temperature.

8.4 Calculations

1. Plot the conductance vs. [SDS]. There should be two linear regions to this plot. At low concentrations the slope of the line is higher than at higher concentrations. (If the two regions are not clear then plot conductance vs. $\sqrt{[SDS]}$ and verify.) Fit these two regions to different straight lines and obtain their slopes. The intersection point of the two lines is the CMC.

2. The ratio of the slopes of the line at higher concentration to that at lower concentration gives you the micellar ionization, α. From a knowledge of α calculate the relevant thermodynamic parameters of micellization.

8.5 Bibliography

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9 Intermolecular hydrogen bonding in benzyl alcohol using infrared spectroscopy

In the condensed phase, molecules that contain certain functional groups, e. g. , hydroxyl, can associate with one another by means of intermolecular hydrogen bonds. Well known examples of compounds that show this behaviour are water and hydrogen fluoride. Hydrogen bonding is responsible for many interesting properties like the high boiling point of water.

In this experiment you will investigate hydrogen bonding in benzyl alcohol by infrared spectroscopy. At low alcohol concentrations in carbon tetrachloride, the IR spectrum shows a unique peak at the characteristic O−H stretching frequency ($3300\,\mathrm{cm^{-1}}$ to $3600\,\mathrm{cm^{-1}}$). However, at higher concentrations, the IR specatrum shows a second peak that is slightly red shifted. This peak corresponds to species formed by the association of a few benzyl alcohol molecules through hydrogen bonds. Such aggregates coexist with completely solvated free molecules and which lead to IR absorption at the frequency characteristic of the O−H functionality. The frequency of the associated form still belongs to the O−H valence stretch, although it is slightly perturbed by the hydrogen bond - the frequency does not correspond to the hydrogen bond.

This experiment features two parts: a) To determine the number of molecules in the associated form, and b) to determine the equilibrium constant for the association. This is performed by following the variation of the IR absorption intensity with concentration. The usual method of determining the degree of association is cryoscopy. The present method can reach the same goal, taking advantage of an instructive case of an apparent deviation from Beer's law of absorption.

9.1 Procedure

1. Prepare a stock solution (0.4 M) of benzyl alcohol in CCl_4 in a 25 mL volumetric flask.
2. Prepare a series of benzyl alcohol solutions by diluting the stock solution so that the concentration varies from 0.001 M to 0.1 M.
3. Record their IR spectrum.

9.2 Results

The free O−H stretching vibration occurs near $3600\,\mathrm{cm^{-1}}$ and has a narrow width. The O−H stretching vibration in O−H...O (formed when concentrations of the benzyl alcohols solutions exceed 0.001 M) is red shifted by about $120\,\mathrm{cm^{-1}}$ and is quite broad. The absorbance maxima of the C−H, free O−H, and associated O−H are plotted against concentration. The values of the C−H absorbance follows Beer-Lambert's law, whereas those for free and associated O−H show opposite deviations at higher concentrations. The absorption band for the free molecule exhibits a negative deviation while that for the associated molecule shows positive deviation. This indicates that an equilibrium displacement to the right hand side, as expected from Le Chatelier's principle, of the following equation with increasing benzyl alcohol concentration.

$$n\mathrm{C_6H_5CH_2OH} \rightleftharpoons (\mathrm{C_6H_5CH_2OH})_\mathrm{n}$$

At low concentrations, the free O−H absorption band satisfies the Beer-Lambert's law because the equilibrium is far to the left.

9.3 Treatment of the results

To obtain the equilibrium constant of the above reaction, we represent the degree of association n as a constant over the concentration range studied. In other words, only one type of aggregate is believed to exist. With this assumption, we get

$$c_{\text{free}} = c - x$$

where c_{free} is the free alcohol concentration, c is the initial concentration of the alcohol, and x is the unknown amount that formed the associated species. The oligomer concentration is

$$c_{\text{assoc}} = \frac{x}{n}.$$

Thus, the equilibrium constant is

$$K = \frac{x}{n(c-x)^n} \tag{9.1}$$

The Beer-Lambert relation $c_i = A_i/\epsilon_i L$ allows the rewriting of the equilibrium constant expression in terms of absorbance. This yields

$$K = \frac{A_{\text{assoc}} \left(\epsilon_{\text{free}}\right)^n L^{n-1}}{\epsilon_{\text{assoc}} \left(A_{\text{free}}\right)^n} \tag{9.2}$$

where K, n, ϵ_{assoc} are unknown; and ϵ is the extinction coefficient, which is independent of concentration.

If the assumption that only one type of oligomer is present is correct, the equilibrium constant should make sense. Representing all the concentration independent terms as C, a constant, equation 9.1 becomes

$$(A_{\text{assoc}}(c))^{1/n} = C A_{\text{free}}(c) \tag{9.3}$$

It is possible to verify the hypothesis by plotting the LHS of equation 9.3 vs. the RHS. According to equation 9.3, a straight line through the origin is obtained when using the correct association degree n.

Once the degree of association is determined, Beer-Lambert's law for the oligomer

$$A_{\text{assoc}} = \epsilon_{\text{assoc}} L c_{\text{assoc}}$$

can be rearranged using

$$c_{\text{assoc}} = \frac{x}{n} = \frac{1}{n}\left(c - c_{\text{free}}\right) = \frac{1}{n}\left(c - \frac{A_{\text{free}}}{\epsilon_{\text{free}} L}\right)$$

to yield

$$A_{\text{assoc}} = \epsilon_{\text{assoc}} L \frac{1}{n}\left(c - \frac{A_{\text{free}}}{\epsilon_{\text{free}} L}\right) \tag{9.4}$$

The determination of ϵ_{assoc} is obtained from the slope of the LHS of 9.4 vs. $\left(c - \frac{A_{\text{free}}}{\epsilon_{\text{free}} L}\right)$ at low concentrations (less than 0.006 M). The equilibrium constant is then determined from equation 9.2.

10 Raman Spectroscopy

Spectroscopy is at the heart of physical chemistry. It provides us with tools to determine the structure and dynamicsof molecules and molecular association, for analytical applications, and to test theory. Vibrational spectroscopy is particularly relevant, since the pattern of vibrational bands can be analyzed to gain information on the composition and structure of a compound and also provides a characteristic fingerprint for chemical analysis. Vibrational frequency shifts can give information about the local environment of a vibrating group, which can be used to study intermolecular bonding and association.

Raman and IR spectroscopy complement each other. Each method has its own merits, making the method of choice different for different applications. Raman spectroscopy is distinguished by different selection rules, which, for example, lead to greater sensitivity to nonpolar functional groups, and it is also distinguished by minimal sample preparation and nondestructive analysis, ability to easily access low-frequency vibrations, and low sensitivity to water allowing convenient measurements of wet samples or aqueous solutions. It is desirable that students are exposed to both methods in their undergraduate studies. In this experiment[1] you will study the Raman spectra of a few liquids, analyze them, and correlate the observed features with their point groups.

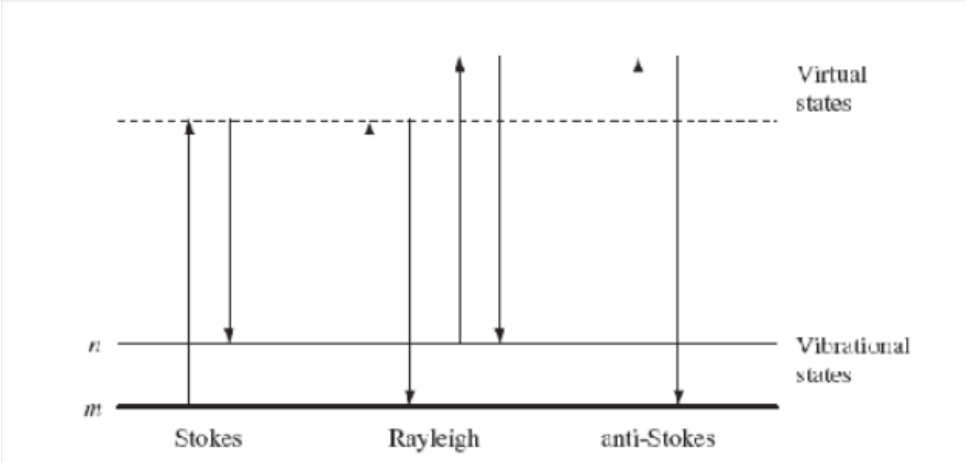

Figure 10.1: Schematic of the Rayleigh and Raman scattering processes. The lowest energy vibrational state m is shown at the foot with states of increasing energy above it.

Figure 10.1 shows the basic processes which occur for one vibration. At room temperature, most molecules, but not all, are present in the lowest energy vibrational level. Since the virtual states are not real states of the molecule but are created when the laser interacts with the electrons and causes polarization, the energy of these states is determined by the frequency of the light source used. The Rayleigh process will be the most intense process since most photons scatter this way. It does not involve any energy change and consequently the light returns to the same energy state. The Raman scattering process from the ground vibrational state m leads to absorption of energy by the molecule and its promotion to a higher energy excited vibrational state (n). This is called Stokes scattering. However, due to thermal energy, some molecules may be present in an excited state such as n in Figure 10.1. Scattering from these states to the ground state m is called anti-Stokes scattering and involves transfer of energy to the scattered photon. The relative intensities of the two processes depend on the population of the various

[1]Contributed by P. K. Chowdhury, Department of Chemistry, IIT Delhi

states of the molecule. The populations can be worked out from the Boltzmann equation but at room temperature, the number of molecules expected to be in an excited vibrational state other than any really low-energy ones will be small.

Figure 10.2 shows a typical spectrum of Stokes and anti-Stokes scattering from cyclohexane separated by the intense Rayleigh scattering which should be offscale close to the point where there is no energy shift. However there is practically no signal close to the frequency of the exciting line along the x-axis. This is because filters in front of the spectrometer remove almost all light within about $200\,\mathrm{cm}^{-1}$ of the exciting line. Usually, Raman scattering is recorded only on the low-energy side to giveStokes scattering but occasionally anti-Stokes scattering is preferred.

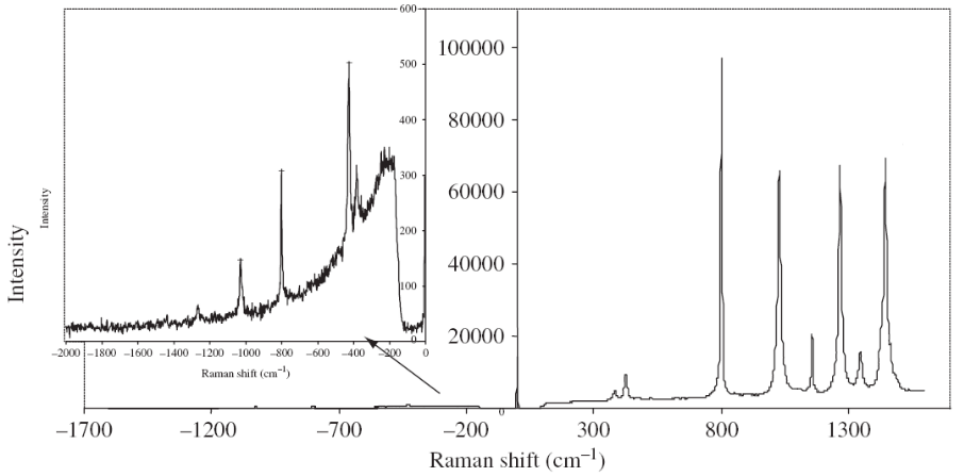

Figure 10.2: Stokes and anti-Stokes scattering for cyclohexane. To show the weak anti-Stokes spectrum, the y-axis has been extended in the inset.

Figure 10.1 illustrates one key difference between infrared absorption and Raman scattering. As described above, infrared absorption would involve direct excitation of the molecule from state m to state n by a photon of exactly the energy difference between them. In contrast, Raman scattering uses much higher energy radiation and measures the difference in energy between n and m by subtracting the energy of the scattered photon from that of the incident beam (the two vertical arrows in each case).

The cyclohexane spectrum in Figure 10.2 shows that there is more than one vibration which gives effective Raman scattering (i.e. is Raman active); the nature of these vibrations will have to be looked up in any basic spectroscopy book. However, there is a basic selection rule which is required to understand this pattern. Intense Raman scattering occurs from vibrations which cause a change in the polarizability of the electron cloud round the molecule. Usually, symmetric vibrations cause the largest changes and give the greatest scattering. This contrasts with infrared absorption where the most intense absorption is caused by a change in dipole and hence asymmetric vibrations which cause this are the most intense. Not all vibrations of a molecule need, or in some cases can, be both infrared and Raman active and the two techniques usually give quite different intensity patterns. As a result the two are often complementary and, used together, give a better view of the vibrational structure of a molecule.

One specific class of molecules provides an additional selection rule. In a centrosymmetric molecule (has an inversion centre), no band can be active in both Raman scattering and infrared absorption. This is sometimes called the mutual exclusion rule. In a centrosymmetric molecule, reflection of any point through the centre will reach an identical point on the other side (C_2H_4 is centrosymmetric, CH_4 is not). This distinction is useful particularly for small molecules where a comparison of the spectra obtained from infrared absorption and Raman scattering can be used to differentiate *cis* and *trans* forms of a molecule in molecules such as a simple azo dye or

a transition metal complex.

Figure 10.3 shows a comparison of the infrared and Raman spectra for benzoic acid. The x-axis is given in wavenumbers for which the unit is cm^{-1}. Wavenumbers are not recommended SI units but the practice of spectroscopy is universally carried out using these and this is unlikely to change. For infrared absorption each peak represents energy of radiation absorbed by the molecule. Raman scattering is presented only as the Stokes spectrum and is given as a shift in energy from the energy of the laser beam. This is obtained by subtracting the scattered energy from the laser energy. In this way the difference in energy correspondingto the ground and excited vibrational states (n and m in Figure 10.1) is obtained. This energy difference is what is measured directly by infrared. The scattering is measured as light detected by the spectrometer and the maximum amount of light detected is the highest point on the trace.

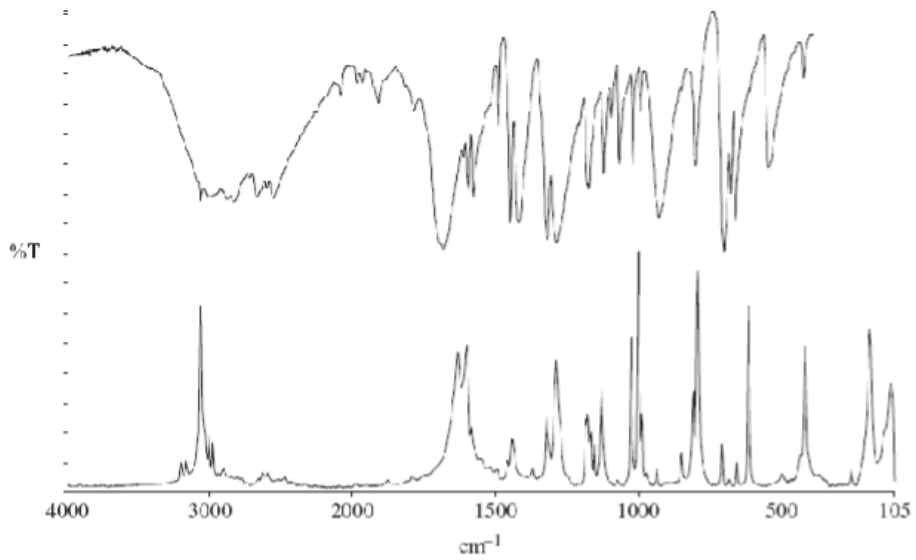

Figure 10.3: Infrared and Raman spectra of benzoic acid. The top trace is infrared absorption given in % transmission (%T) so that the lower the transmission value the greater the absorption. The lower trace is Raman scattering and the higher the peak the greater the scattering.

Strictly speaking, Raman scattering should be expressed as a shift in energy from that of the exciting radiation and should be referred to as cm^{-1} but it is often expressed simply as cm^{-1}. Although different energy ranges are possible, the information of interest to most users is in the $3600\,\mathrm{cm}^{-1}$ to $400\,\mathrm{cm}^{-1}$ (2.8 micron to 12 micron) range in infrared spectroscopy and down to $200\,\mathrm{cm}^{-1}$ in Raman spectroscopy since this includes most modes which are characteristic of a molecule.

10.1 Procedure

Record the Raman spectra of carbon tetrachloride, CCl_4; chloroform, $CHCl_3$; dichloromethane, CH_2Cl_2; benzene, toluene and aspirin (Synthesized pure Aspirin and commercially available as part of a drug). A schematic of the Raman spectrometer on which you will record the spectra is given below.

Instructions on how to operate the instrument will be provided separately. Make sure that the spectrum is noise free. Also collect the FTIR spectra of the liquid samples for comparison.

Figure 10.4: Schematic of the inside of the Raman spectrometer that you are using. The wavelength of the laser is 532 nm and the detector is a CCD detector having a linear array of 3648 pixels. The position labeled 4 corresponds to that of a monochromator with the grating of 1200 grooves/mm.

10.2 Analyses of Raman spectra

This section of the lab will need significant application on your part. It not only includes vibrational assignments of the bands you see in the acquired spectra but also requires you to gain idea about the point group symmetry of molecules (in particular the liquid samples you are analyzing). This task might be a bit challenging for you and hence you are not required to submit the lab report immediately. Please consult with your instructor when you need to submit this report. At the end, you will realize that this exercise has been a highly fruitful one and would provide enough insights about many aspects of vibrational spectroscopy.

1. Assign the vibrational modes to the spectra for each of the samples you have collected. If needed, you can look up books on spectroscopy (some are provided in the reference below) to help you with the band assignment.

2. What obvious difference do you see in the Raman spectra (i) amongst the chlorinated solvent family (ii) in between benzene and toluene. For this you will need to know the point groups of the molecules which are as follows:

Sample	Point Group
CCl_4	T_d
$CHCl_3$	C_{3v}
CH_2Cl_2	C_{2v}
C_6H_6	D_{6h}
Toluene	C2v

   Explain the differences you see. You might need to look at the last two columns of the character tables of the aforesaid point groups to reach your answer.

3. Compare the spectra of the two types of aspirins and mention any difference you come across.

Calculation of force constants:

Based on the valence force model, the potential energy of the CCl4 molecule can be written as:

$$V = \frac{1}{2}k\left(r_1^2 + r_2^2 + r_3^2 + r_4^2\right) + \frac{1}{2}k_\delta\left(\delta_{12}^2 + \delta_{13}^2 + \delta_{14}^2 + \delta_{23}^2 + \delta_{24}^2 + \delta_{34}^2\right) \tag{10.1}$$

where the force constant k corresponds to the change in the bond length r_i of the C-Cl bond and the force constant k_δ corresponds to the change in angle δ_{ij} between the bonds i and j.

From classical mechanics one obtains the following relations (l being the C-Cl distance)

$$4\pi^2\nu_1^2 = \frac{k}{m_{\mathrm{Cl}}} \tag{10.2}$$

$$4\pi^2\nu_2^2 = \left(\frac{3}{m_{\mathrm{Cl}}}\right)\frac{k}{l^2} \tag{10.3}$$

$$4\pi^2\left(\nu_3^2 + \nu_4^2\right) = \left(1 + \frac{4m_{\mathrm{Cl}}}{3m_{\mathrm{C}}}\right)\frac{k}{m_{\mathrm{Cl}}} + \left(1 + \frac{8m_{\mathrm{Cl}}}{3m_{\mathrm{C}}}\right)\left(\frac{2}{m_{\mathrm{Cl}}}\right)\frac{k}{l^2} \tag{10.4}$$

$$16\pi^4\nu_3^2\nu_4^2 = \left(1 + \frac{4m_{\mathrm{Cl}}}{3m_{\mathrm{C}}}\right)\left(\frac{2}{m_{\mathrm{Cl}}}\right)\frac{k}{l^2}\frac{k}{m_{\mathrm{Cl}}} \tag{10.5}$$

Since there are two force constants and four frequencies, one can test the quality of the valence force field by determining k and $\frac{k_\delta}{l^2}$ separately from equations 10.2 and 10.3 and equations 10.4 and 10.5.

Find the force constants for the carbon tetrachloride molecule in the units of $\mathrm{N\,m^{-1}}$.

10.3 Questions to be answered:

1. Apart from the basics of instrumentation, what is the principle difference between Raman and IR from their underlying theories?

2. (a) What are the three different types of lines that are observed in a Raman spectrum?
   (b) In general, the low frequency lines, that is, with frequency lower than that of the exciting laser, are higher in intensity than the Raman shifts at higher frequency. Can you explain why this is the case?
   (c) Can one use Raman spectroscopy to estimate the temperature of your liquid sample? How?

3. CCl_4 has often been used to demonstrate Raman spectroscopy. (a) What according you is the feature that makes this molecule so commonly used? (b) If one acquires an IR spectrum of CCl_4, would these Raman bands be also observed? You can either collect the FTIR spectrum of CCl_4 in the instrumentation lab or look up books/web to see what the IR spectrum looks like.

4. Looking at the schematic of the instrument provided above, write a few lines about the different components that have been mentioned in the figure caption, that is, mention about their function and significance as a part of the Raman spectrometer. In addition to the components shown, most Raman spectrometers also include a laser clean-up filter called a notch filter. What is a notch Filter?

10.4 Turning in your lab report

Along with the necessary spectra, band assignments, calculations and questions to be answered, your introduction to the report should include a brief mathematical treatise on Raman based on classical mechanics. This must also have the derivation of the equation that simultaneously shows the existence of the three different kinds of frequencies in a Raman spectrum.

10.5 Bibliography

1. P. Larkin, Infrared and Raman Spectroscopy; Principles and Spectral Interpretation
2. G. Herzberg, Molecular Spectra and Molecular Structures; Infrared and Raman Spectra of Polyatomic Molecules, Vol. 2, Van Nostrand, Princeton, 1945.
3. E. Smith and G. Dent, Modern Raman Spectroscopy-A Practical Approach, Wiley.
4. D. C. Harris, M. D. Bertolucci, Symmetry and Spectroscopy: An Introduction Vibrational and Electronic Spectroscopy, Dover Publications.
5. Any other book on spectroscopy.

11 Thermodynamics of Denaturation of Bovine Serum Albumin

The aim of this experiment[1] is to study the thermodynamics of urea induced denaturation of a protein, bovine serum albumin, by using fluorimetry.

11.1 Theory

The denaturation of many globular proteins have been found to closely approach a two-state mechanism

$$\mathrm{N} \rightleftharpoons \mathrm{D}$$

in which only the native state, N, and the denatured state, D, are present at significant concentrations. The equilibrium constant for this reaction is defined as

$$K = \frac{[D]}{[N]}$$

From thermodynamics we know that the equilibrium constant is related to the standard Gibbs energy change for the reaction, that is,

$$\Delta G^\circ = -RT \ln K \qquad \text{or} \qquad K = \exp^{-\frac{\Delta G^\circ}{RT}}$$

Unfolding is studied by introduction of a perturbant (e.g. a temperature change, a pH change or an increasing concentration of denaturant) which incrementally decreases the stability of the native state, or increases the stability of the denatured state. Any observed signal, y, that changes upon increasing perturbation may be expressed as a weighted sum of contributions from the native, y_N, and of the denatured state, y_D,

$$y = f_\mathrm{N} y_\mathrm{N} + f_\mathrm{D} y_\mathrm{D}.$$

For the two-state model, the protein is present in either the native or denatured state, that is $f_\mathrm{N} + f_\mathrm{D} = 1$. Combining these equations, $f_\mathrm{D} = (y - y_\mathrm{N}) / (y_\mathrm{D} - y_\mathrm{N})$ and $f_\mathrm{N} = (y_\mathrm{D} - y) / (y_\mathrm{D} - y_\mathrm{N})$. Thus, an equilibrium constant, K, and the standard Gibbs energy of unfolding, ΔG°, can be calculated using

$$K = \exp^{-\frac{\Delta G^\circ}{RT}} = \frac{f_\mathrm{D}}{f_\mathrm{N}} = (y - y_\mathrm{N}) / (y_\mathrm{D} - y)\,. \tag{11.1}$$

11.2 Procedure

Preparation of tris buffer

Calculate the amount of tris buffer required to prepare 100 mL of 10 mM solution of tris buffer. Check the pH of this solution after dissolution - the solution should be alkaline with a pH around 9.7 to 10. Bring down the pH of this solution to 7.5 by adding HCl dropwise. This will be the pH at which you will carry out the experiments.

[1] Contributed by S. Saha and S. Deep, Department of Chemistry, IIT Delhi

Preparation of stock solution of urea

Weigh the amount required to prepare 10 mL of 8 M urea solution. Transfer this to a falcon tube. This should take up an enormous volume of the tube, close to 10 mL. Slowly add the tris buffer (pH 7.5) to the urea in the falcon tube. Urea should start dissolving. Continue this till you have made up the volume of the solution to 10 mL. If you find undissolved urea at this stage, you may keep the tube in a hot water bath. If the urea still does not dissolve, add a little more tris buffer, even though the volume exceeds 10 mL. Check the refractive index of your buffer and the urea solution, find out the actual concentration of the urea that you have prepared.

Preparation of BSA solution

Prepare 1 mL of BSA of approximate strength 500 µMin an Eppendorf, and label it as 'A'. Take another fresh Eppendorf, and add 20 µL of the above BSA solution to a 980 µL of the tris buffer, to make the total volume to 1 mL. Label this Eppendorf as 'B' and keep it aside for the time being.

Set the baseline in the UV-visible spectrophotometer in the wavelength range of 500 nm to 200 nm with buffer in both the cuvettes. Transfer the content of 'B' into the cuvette of the sample cell holder, after discarding the buffer in this cuvette. Take a spectrum of this sample in the above wavelength range.

Calculate the concentration of this solution using the Beer's law, with $\epsilon_{280\,\mathrm{nm}} = 43\,824\,/\mathrm{M/cm}$. Multiply the concentration obtained with the dilution factor (which is 50 in this case) to obtain the actual concentration of stock solution you had prepared by dissolving BSA in tris ('A')

Preparation of the ANS solution

In a manner similar to that for preparation of the BSA solution, make a solution of ANS in water, and check its exact concentration using the UV-visible spectrophotometer. Set the wavelength range from 700 nm to 200 nm. Calculate the concentration using the value of $\epsilon_{350\,\mathrm{nm}} = 5000\,/\mathrm{M/cm}$. Stock solution (A1) should be nearly 2000 µM, and solution used for checking the exact concentration (B1) should be diluted nearly 20 times)

Preparation of samples for the denaturation experiments

Label 17 eppendorfs starting from 0 M to 8 M in steps of 0.5 M. This denotes the concentration of urea. The concentration of BSA in each Eppendorf should be 10 µM, and that of ANS should also be 10 µM. In your notebook, prepare the following table:

Urea concentration (M)	Volume of solutions (µL)				
	Total	Urea	ANS	BSA	Buffer

Prepare the solutions according to the table. Add BSA in the end, after all other components have been added.

1. Equilibrate all the samples for at least half an hour.

2. Take the fluorescence spectrum of all these samples using the following parameters (two spectra for two fluorophores, for each sample). To probe tryptophan use an excitation wavelength of 295 nm and emission wavelength of 350 nm, while for ANS use an excitation wavelength of 350 nm and emission wavelength of 472 nm

11.3 Calculations

1. Plot the values of the fluorescence intensities at 350 nm and 470 nm against the concentration of urea to obtain the denaturation profiles.

2. Fit the linear regions of the denaturation curves to determine y_{N} and y_{D} and hence determine ΔG at each denaturant concentration using 11.1.

3. Plot the ΔG obtained in step 2 as a function of the urea concentration and obtain $\Delta G^{\mathrm{H_2O}}$.

12 Quantum confinement in CdTe nanocrystals

In this experiment[1] we will synthesize nanoparticles of CdTe and study quantum confinement.

12.1 Introduction

Nanoparticles are the particles with the size ranging from 1 nm to 100 nm which act as a bridge between bulk materials and atoms/molecules. Quantum dots are semiconductor nanoparticles as these are small enough to produce quantization of electronic energy levels by quantum confinement effect. This effect results in size dependent optical and electronic properties of nanoparticles.(1, 2)

Quantum Confinement Effect: The energy levels in a bulk semiconductor are very close and hence continuous. The region of forbidden energy level is called band gap. When light having energy greater than the band gap is incident upon the semiconductor, electrons get excited to conduction band leaving a hole in the valence band. This electron-hole pair is called an exciton. Excitons have average separation between the hole and the electron, referred to as Bohr's exitonic radius (r_B). The size of a bulk semiconductor is much greater than the Bohr's radius. However, when the size of the semiconductor particle approaches that of Bohr's radius, the electron and hole are forced to stay closer than they naturally would. We would say they are confined. You will study this effect of confinement in your course on Quantum Chemistry. In essence what you will learn is that larger the dimension of a box, closer are the energy levels; the separation increasing as the box keeps getting smaller. For very small particles, the energy levels no longer remain closely spaced, but they rather become discrete. This is observed in sizes below about 10 nm for most of the materials. Due to discrete energy levels addition or deletion of few atoms has marked effect on the band-gap and hence leads to size-dependent band gaps, consequently resulting in tuneable electronic and optical properties.

Effective Mass Approximation: QDs are in the strong confinement regime, when radius of nanocrystal is smaller than Bohr exciton radius. Brus (3) proposed the following size dependent energy expression,

$$E(R) = \frac{hc}{\lambda(R)} = E_g + \frac{h^2}{8R^2}\left(\frac{1}{m_e} + \frac{1}{m_h}\right) - 1.76\frac{e^2}{\epsilon R}, \tag{12.1}$$

where E_g is the bulk band gap. The second term is the kinetic-energy term containing the effective masses, m_e and m_h, of the electron and the hole, respectively. The third term arises due to the Coulomb attraction between the electron and the hole. The energy levels of a quantum dot can be approximated with the particle-in-a-box model. The lowest energy for an electron in a 1-D potential wall is given as $E = h^2/8mL^2$, where h is Planck's constant, m is the mass of electron, and L is the length of the box. Hence, decrease in the size of semiconductor leads to increase in the separation of energy levels.

12.2 Materials required

Cadmium perchlorate hexahydrate ($CdClO_4 \cdot 6\,H_2O$), mercaptopropionic acid (MPA, 99%), zinc telluride (ZnTe, 100 mesh, 99.99 %), sodium hydroxide pellets (NaOH) and hydrochloric acid (HCl).

[1] Contributed by M. Mittal and S. Sapra, Department of Chemistry, IIT Delhi

12.3 Procedure

1. Prepare the cadmium solution dissolving $Cd(ClO_4)_2 \cdot 6\,H_2O$ (0.1 mmol) and MPA (0.13 mmol) in 20 mL MilliQ water with continuous stirring in a 50 mL round bottom (RB) flask.
2. Maintain the pH of the solution to 12.0 by addition of 1 M NaOH solution. Use a pH meter.
3. Connect one end of this RB flask with another four-necked 25 mL RB flask and other end to the NaOH trap (Dissolve the NaOH pellets in the distilled water) through the PVC pipes and pass the inert gas (Argon or Nitrogen) through the solution for about 10 min.
4. Pass the H_2Te gas through the solution which can be generated by adding HCl solution to ZnTe in a four necked 25mL RB flask, along with inert gas for about 15 min to 20 min till you get a dark brown solution.
5. CdTe nanocrystals (NCs) precursors are formed at this stage which is accompanied by change in color. The precursor solution becomes acidic; again maintain its pH to 12.0.
6. Reflux the solution at 100℃ under open-air conditions with a water condenser.
7. The size of CdTe NCs is controlled by duration of reflux and monitored by recording absorption spectra. Collect absorption spectra at regular intervals to check for the growth. It is a good idea to check every 1 min to 2 min . Use Milli Q water for the blank cuvette. The solution should be very mildly colored for absorption measurements.
8. Finally, precipitate the CdTe NCs with acetone and redisperse in Milli Q water for further characterization.

12.4 Observations and Calculations

S. No.	Time	Excitonic peak (nm)	Excitonic peak (eV)	ΔE_g (eV)	Size (nm)

Use the approximation $\lambda(\text{nm}) = 1240/\text{eV}$. Paste the absorption spectra along with the report.

12.5 References

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13 Electronic structure calculation of IR and Raman frequencies

In this experiment[1] we will determine the optimized energy, IR and Raman frequencies of some compounds with quantum chemistry computation software package Gaussian 09.

13.1 Introduction

Ab initio molecular orbital theory is concerned with predicting the properties of atomic and molecular systems. It is based upon the fundamental laws of quantum mechanics and uses a variety of mathematical transformation and approximation techniques to solve the fundamental equations.

Basis Set

A basis set is the mathematical description of the orbitals within a system (which in turn combine to approximate the total electronic wave function) used to perform the theoretical calculation. Larger basis sets more accurately approximate the orbitals by imposing fewer restrictions on the locations of the electrons in space. In the true quantum mechanical picture, electrons have a finite probability of existing anywhere in space; this limit corresponds to the infinite basis set expansion. Standard basis sets for electronic structure calculations use linear combinations of Gaussian functions to form the orbitals. Gaussian offers a wide range of pre-defined basis sets, which may be classified by the number and types of basis functions that they contain. Basis sets assign a group of basis functions to each atom within a molecule to approximate its orbitals. These basis functions themselves are composed of a linear combination of gaussian functions; such basis functions are referred to as contracted functions, and the component gaussian functions are referred to as primitives. A basis function consisting of a single gaussian function is termed uncontracted.

Minimal Basis Sets

Minimal basis sets contain the minimum number of basis functions needed for each atom. For examples, H is represented by the $1s$ basis function, while C is represented by $1s$, $2s$, $2p_x$, $2p_y$, $2p_z$ basis functions.

Minimal basis sets use fixed-size atomic-type orbitals. The STO-3G basis set is a minimal basis set (although it is not the smallest possible basis set). It uses three gaussian primitives per basis function, which accounts for the "3G" in its name. "STO" stands for "Slater-type orbitals," and the STO-3G basis set approximates Slater orbitals with gaussian functions.

Split Valence Basis Sets

The first way that a basis set can be made larger is to increase the number of basis functions per atom. Split valence basis sets, such as 3-21G and 6-31G, have two (or more) sizes of basis function for each valence orbital. For example, H is represented by the $1s$, $1s'$ functions, while C uses the $1s$, $2s$, $2s'$, $2p_x$, $2p_y$, $2p_z$, $2p'_x$, $2p'_y$, and $2p'_z$ for its representation. Here the primed and unprimed orbitals differ in size.

The double zeta basis sets, such as the Dunning-Huzinaga basis set (D95), form all molecular orbitals from linear combinations of two sizes of functions for each atomic orbital. Similarly,

[1] Contributed by A. Gupta and H. K. Kashyap, Department of Chemistry, IIT Delhi

triple split valence basis sets, like 6-311G, use three sizes of contracted functions for each orbital-type.

Polarized Basis Sets

Split valence basis sets allow orbitals to change size, but not to change shape. Polarized basis sets remove this limitation by adding orbitals with angular momentum beyond what is required for the ground state to the description of each atom. For example, polarized basis sets add d functions to carbon atoms and f functions to transition metals, and some of them add p functions to hydrogen atoms. For example, 6-31G(d). Its name indicates that it is the 6-31G basis set with d functions added to heavy atoms. This basis set is becoming very common for calculations involving up to medium-sized systems. This basis set is also known as 6-31G*. Another popular polarized basis set is 6-31G(d,p), also known as 6-31G**, which adds p functions to hydrogen atoms in addition to the d functions on heavy atoms.

Diffuse Functions

Diffuse functions are large-size versions of s- and p-type functions (as opposed to the standard valence-size functions). They allow orbitals to occupy a larger region of space. Basis sets with diffuse functions are important for systems where electrons are relatively far from the nucleus: molecules with lone pairs, anions and other systems with significant negative charge, systems in their excited states, systems with low ionization potentials, descriptions of absolute acidities, and so on. The 6-31+G(d) basis set is the 6-31G(d) basis set with diffuse functions added to heavy atoms. The double plus version, 6-3l++G(d), adds diffuse functions to the hydrogen atoms as well. Diffuse functions on hydrogen atoms seldom make a significant difference in accuracy.

Charge

The charge is a positive or negative integer specifying the total charge on the molecule. Thus, 1 or +1 would be used for a singly-charged cation, -1 designates a singly-charged anion, and 0 represents a neutral molecule. For example, H_2O has 0 charge.

Spin Multiplicity

The spin multiplicity is given by the equation $2S + 1$, where S is the total spin for the molecule. Paired electrons contribute nothing to this quantity. They have a net spin of zero since an α electron has a spin of $+1/2$ and a β electron has a spin of -1/2. Each unpaired electron contributes $+1/2$ to S. Thus, a singlet - a system with no unpaired electrons - has a spin multiplicity of 1, a doublet (one unpaired electron) has a spin multiplicity of 2, a triplet (two unpaired electrons of like spin) has a spin multiplicity of 3, and so on.

IR and Raman Modes

In order to obtain the spectroscopic signature of the molecule, the computational calculations can be performed for frequency analysis. The molecule, will have some symmetry, consisting of n number of atoms, so it will have $3n - 6$ number of normal vibrational modes (for non-linear molecule) ($3n - 5$ for linear molecule). Some of them modes will be IR active and some will be Raman active. Some of them will be both IR and Raman active and some will neither be IR active nor Raman active.

Rule of Mutual Exclusion

The rule of mutual exclusion states that no normal modes can be both IR and Raman active in a molecule that possesses a centre of symmetry.

13.2 Procedure

1. Open Gaussview software and make H_2O molecule. Alternatively one can also use Avogadro software to make molecule.
2. Setup the Gaussian calculation now. Set Job Type as "Opt + Freq" and make Compute Raman as "Yes" with method as "Hartree-Fock" (HF) "restricted" with "6-31G" "+" "d" "p" basis set.
3. "Submit" this input file to Gaussian software which will ask you to "save" the file as Gaussian input file (example: H2O.com) and run the calculation.
4. After the calculation is finished, open the "file name".chk file.
5. Note down the energy in table from the results summary button.
6. Visualise the frequencies from Vibrations button and note down the observations in the table in given format.
7. Repeat steps 1-6 whole process with "B3LYP" theory in place of "Hartree-Fock" theory.
8. Repeat steps 1-7 for CO_2, $CHCl_3$ and CCl_4.

13.3 Observations

Molecule	Level of theory	Energy	Frequency of IR modes		Frequency of Raman modes	
			Observed	Scaled	Observed	Scaled
H_2O	HF/6-31+G(d,p)					
	B3LYP/6-31+G(d,p)					
CO_2	HF/6-31+G(d,p)					
	B3LYP/6-31+G(d,p)					
$CHCl_3$	HF/6-31+G(d,p)					
	B3LYP/6-31+G(d,p)					
CH_2Cl_2	HF/6-31+G(d,p)					
	B3LYP/6-31+G(d,p)					
CCl_4	HF/6-31+G(d,p)					
	B3LYP/6-31+G(d,p)					

13.4 Calculation

Use scaling factor for HF/6-31+G(d,p) as 0.904 and for B3LYP/6-31+G(d,p) as 0.964.

13.5 Results and Discussion

1. Compare the energies you get from two theories for a molecule.
2. Discuss about the IR and Raman active modes and tell which frequency belongs to which mode of vibration (stretching: symmetric, anti-symmetric; bending: out-of-plane, in-plane, etc.)

13.6 References

1. I. J. D. Ebenezar, S. Ramalingam, C. R. Raja, V. Helan Precise Spectroscopic [IR, Raman and NMR] Investigation and Gaussian Hybrid Computational Analysis (UV-Visible, NIR, MEP Maps and Kubo Gap) on L-Valine. J. Theor. Comput. Sci. 1:106, 2013

2. J. B. Foresman and A. Frisch, Exploring Chemistry with Electronic Structure Methods, Second Edition.

3. D. A. McQuarrie, Quantum Chemistry, Second Edition; University Science Books, 2008; pp 410-435.

4. CCCBDB listing of precalculated vibrational scaling factors. `http://cccbdb.nist.gov/vibscalejust.asp`