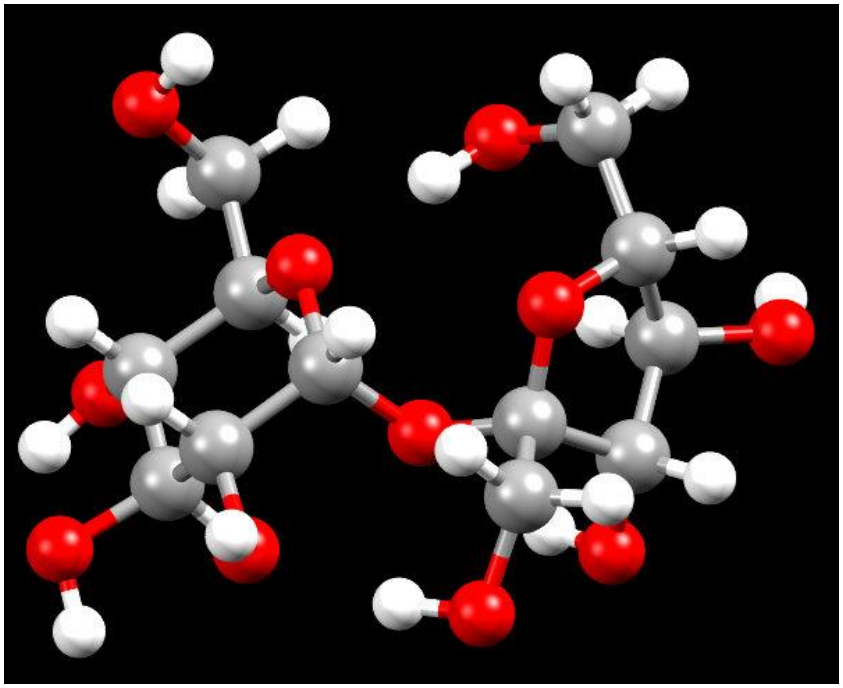
Spring 2021

LAB A

Polarimetry Measurement of the Inversion of Sucrose

TA: Alec Beaton



Pre-Lab Activities

1. Review the Polarimetry Overview document that is posted on the CHE357 Blackboard site under "Lab Procedures"
2. Review and do the exercises contained in the Computational Chemistry Lab Training Handout posted on the CHE357 Blackboard site under "Lab Procedures".
3. Review the TA notes and Excel Solver Notes on Blackboard that you will discuss with your TA during the lab.

Main Learning Objectives

- Demonstration of the fundamental composition of light (light polarization: plane, left-circular, right-circular)
- Molecular chirality and light interaction (optical activity)
- Use of ab initio simulations to predict and investigate the optical activity of a molecule
- Measurement of reaction kinetics

Graded Material

Your grade in each lab is based on your completion of the Post-Lab work and questions that are detailed at the end of each lab procedure.

Text-based questions need to be answered in a narrative style using complete sentences and full explanations. You will be evaluated on accuracy, clarity, and completeness. Keep in mind that adding more text does not necessarily mean you are providing a better answer.

Number-based questions need to be answered numerically with appropriate units, but must also include a clear statement or justification as to how the numbers were determined.

Graphs or plots submitted for grading must be easy to read, have properly labeled axes with units, an overall title, and a legend defining multiple traces. All graphs/plots must include a brief descriptive caption of the represented information.

Tables must be logically organized with column and row labels as appropriate. Units must be specified. All tables must include a brief descriptive caption of the represented information.

Images or figures must clearly and concisely convey information to the reader. Simple figures with clear labeling are most effective. All figures must include a brief descriptive caption of the represented information.

You need to demonstrate in your completed lab reports that you understand the answers you are providing to the questions being posed.

ABSTRACT

You will be using a Vernier Go Direct Polarimeter with a 589 nm light source to measure the angle of rotation of polarized light passing through solutions of sucrose in water. You will monitor the change in the angle of rotation as sucrose is broken down into glucose and fructose by the invertase enzyme. You will lastly use GaussView to analyze and compare the Optical Rotary Dispersion (ORD) of both D-lactic acid and L-lactic acid from completed Gaussian jobs (provided to you).

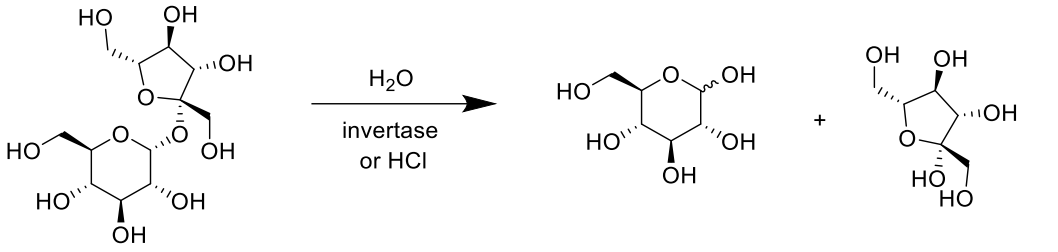
INTRODUCTION

The specific rotation of a sample is an intrinsic property at a specific wavelength and temperature. It may be determined for a given sample using a polarimeter and Biot's Law:

$$\alpha = [\alpha] l c$$

where α is the observed optical rotation in units of degrees, $[\alpha]$ is the specific rotation in units of degrees mL dm^{-1} g^{-1} (Note: these are the formal units of the specific rotation, but it is often referred to using just units of degrees), l is the length of the sample that occupies the polarimeter cell (in units of dm for this equation – be cognizant of the fact that polarimeter cell measures in units of cm), and c is the concentration in units of g mL^{-1}.

In Part I of the lab, you will use Biot's Law to determine the specific rotation of sucrose. In Part II of the lab, you will monitor the inversion of sucrose (see reaction below) as the invertase enzyme breaks sucrose down into glucose and fructose. The change in chiral chemical species that are present in your sample will result in a change in the observed optical rotation. By monitoring the optical rotation over the time course of approximately 40 minutes, you will be able to determine the rate of the reaction.



sucrose
[α] = 66.5 degrees

glucose
[α] = 52.7 degrees

fructose
[α] = -92.4 degrees

In the last part of the lab, you will analyze the Optical Rotary Dispersion (ORD) of L-lactic acid and D-lactic acid. These spectra demonstrate the change in the specific rotation as the wavelength of light changes.

PROCEDURE

Part I. Specific Rotation

1. Open the Vernier Instrumental Analysis software with the polarimeter connected to the computer via USB.
2. Choose 'Polarimetry' from the options that pop up.
3. Measure out 20 mL of deionized water and transfer to a Polarimeter Cell.
4. Carefully record the *height* of the solution by reading the graduated markings on the cell.
5. Insert the cell in the polarimeter and click 'Finish Calibration.' Click 'Done' once finished.
6. With **Optical Rotation** selected as the polarimetry mode (this is the default), click 'Collect' at the top. You will then begin collecting for water. This will take approximately 15 seconds. You will know the run has completed once the button changes from 'Stop' to 'Collect.'
7. Once finished, go to the top left corner where it says 'Untitled' and click here to open the file options – first save as Vernier Instrumental Analysis file, and then Export > CSV, and choose the appropriate decimal format.
8. Remove the polarimeter cell containing water.
9. Measure out 20 mL of 15% w/v sucrose solution and transfer to a polarimeter cell.
10. Carefully record the *height* of the solution by reading the graduated markings on the cell.
11. Insert the cell in the polarimeter and click 'Collect'
12. Once finished, save as Vernier Instrumental Analysis file *and* export as CSV.
13. Remove the polarimeter cell containing your sample.
14. Repeat steps 9 – 13 for 30% w/v sucrose.

Part II. Kinetics

Note: You will use the data collected in step 14 of Part I for the first time point of this kinetics experiment.

1. In your lab notebook, prepare a table of about 30 rows and 2 columns, with time in one column and optical rotation in the other column.
2. Switch the polarimetry mode to **Kinetics** – this will prompt you to specify a sample rate and time frame under Kinetic Collection Settings. Set a sampling rate of 1 sample/s and end collection after 40 – 50 min, depending on the time remaining in your section. You will want to be sure to delete all Data Sets that may be left over from previous runs.
3. Measure out 10 mL of 30% w/v sucrose from the appropriate polarimeter cell from Part I.
4. Measure out 10 mL of 15 mg/mL invertase solution.
5. Transfer the 10 mL of invertase to a 20 mL beaker, and then transfer the sucrose solution. The solution will turn slightly turbid. Carefully transfer this mixture to a polarimeter cell.
6. Insert the cell and begin collecting.
7. Record the value for the optical rotation every 2 minutes for 40 – 50 minutes (depending on the amount of time remaining in your section).
8. Once finished, save as Vernier Instrumental Analysis file and export as CSV.

POST-LAB

Part I.

1. Determine the observed rotation angle for 15% w/v sucrose, based on a cosine squared model (refer to the **How to use Solver: Cosine squared write up**). (10 points)
2. Plot intensity as a function of angle, and include both the experimental data as well as your fit with appropriately labeled axes and legends. (10 points)
3. Determine the observed rotation angle for 30% w/v sucrose, based on a cosine squared model. (10 points)
4. Plot intensity as a function of angle, and include both the experimental data as well as your fit with appropriately labeled axes and legends. (10 points)
5. Determine the specific rotation of sucrose based off of the observed rotation angles for each sample. Compare to the literature value. (10 points)

Part II.

1. Enter your tabulated data from lab into an Excel sheet. Be sure to use the optical rotation determined in step 9 of Part I **for 15 % w/v sucrose** as your value at t = 0 min. (10 points)
2. Plot the angle of rotation vs time, and determine the rate constant for this reaction with respect to sucrose (refer to **How to use Solver: Kinetics write up**). (15 points)
3. Compare the rate constant to literature value of 0.0749 min^{-1}. (5 points)

Part III.

The Gaussian software is able to calculate the optical rotation values of chiral molecules using density functional theory methods. These simulations replicate the interaction of light with molecules by the application of a varying electric field and monitoring the subsequent response of the overall electron density (polarizability). This computational approach can reproduce the optical activity at various wavelengths of light and ultimately yield optical rotation dispersion plots. These types of calculations are time consuming and require a great deal of computer memory and storage to run, so having students run these simulations is not practical. Therefore, Gaussian output files will be provided to you.

1. Using GaussView, create an illustrative figure demonstrating the mirror image chirality of D-lactic acid and L-lactic acid using the provided optimized structures (note you are not running any Gaussian calculations). (10 points)
    a. NOTE: Use “File: Open” to open the provided files. Once you have manipulated a molecule to your preferred position, you can use “Edit: Image Capture” to copy the molecule display to the Windows clipboard (for pasting into Word, etc.) or you can use “File: Save Image” to save the molecule display for later use.
2. Export the ORD plot for each output file (using “Results: ORD”, then right-clicking on the ORD plot window to Save Data to a file). Then plot the ORD for each within a single graph in Excel. Are the enantiomers easier to distinguish at longer or shorter wavelengths of light? (5 points)
3. Using GaussView and the output files for D-lactic acid and L-lactic acid, compare their molecular energies and their vibrational frequencies. Treat energy differences of ≤ 0.01 cm^{-1} as indistinguishable. What do you notice in the comparison that emphasizes the value of optical rotation measurements for studying chiral compounds? (5 points)

Inversion of Sucrose

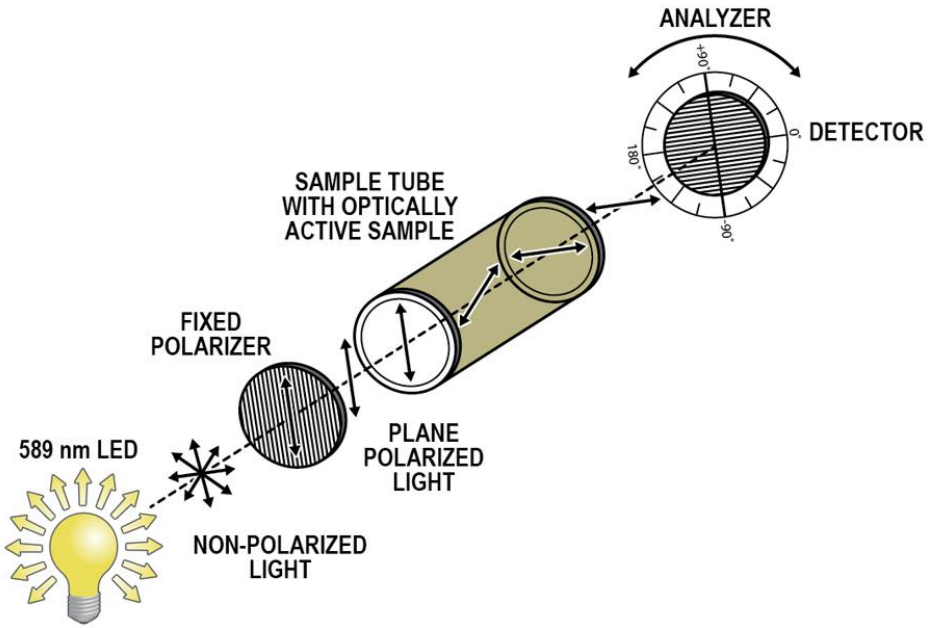
TA Notes

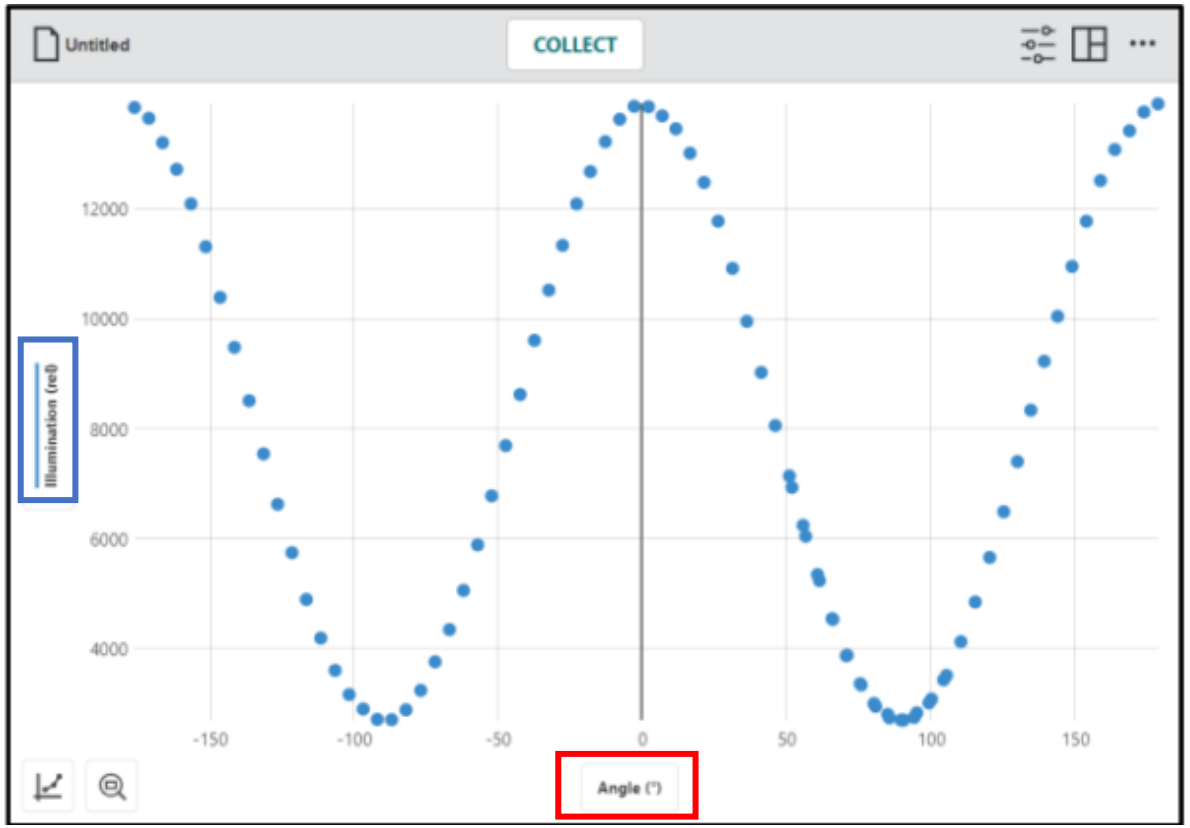
Alec Beaton

CHE 357

Part I. Specific Rotation

cosine squared function





$$Illumination = A\ \cos^2(B \cdot \theta + C) + D$$

Observed optical rotation

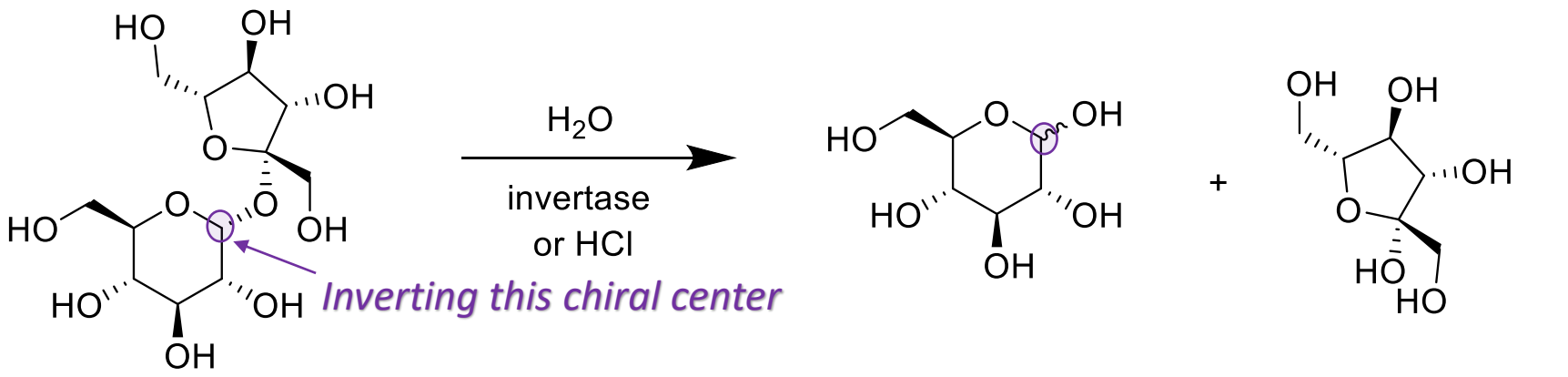
Part I. Specific Rotation

$$Illumination = A\ \cos^2(B \cdot \theta + C) + D$$

$$\alpha = [\alpha]\, l\ c$$

Specific rotation

Part II. Kinetics



sucrose
[α] = 66.5 degrees

glucose
[α] = 52.7 degrees

fructose
[α] = -92.4 degrees

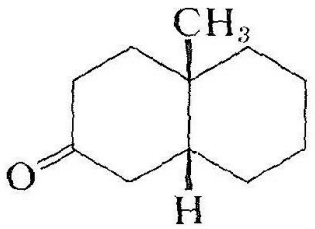
We will measure the *optical rotation* as a function of *time*

$$\alpha = Ae^{-B \cdot t} + C$$

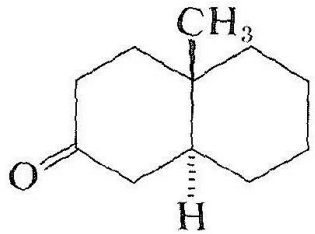
Rate constant

Part III. Optical Rotary Dispersion

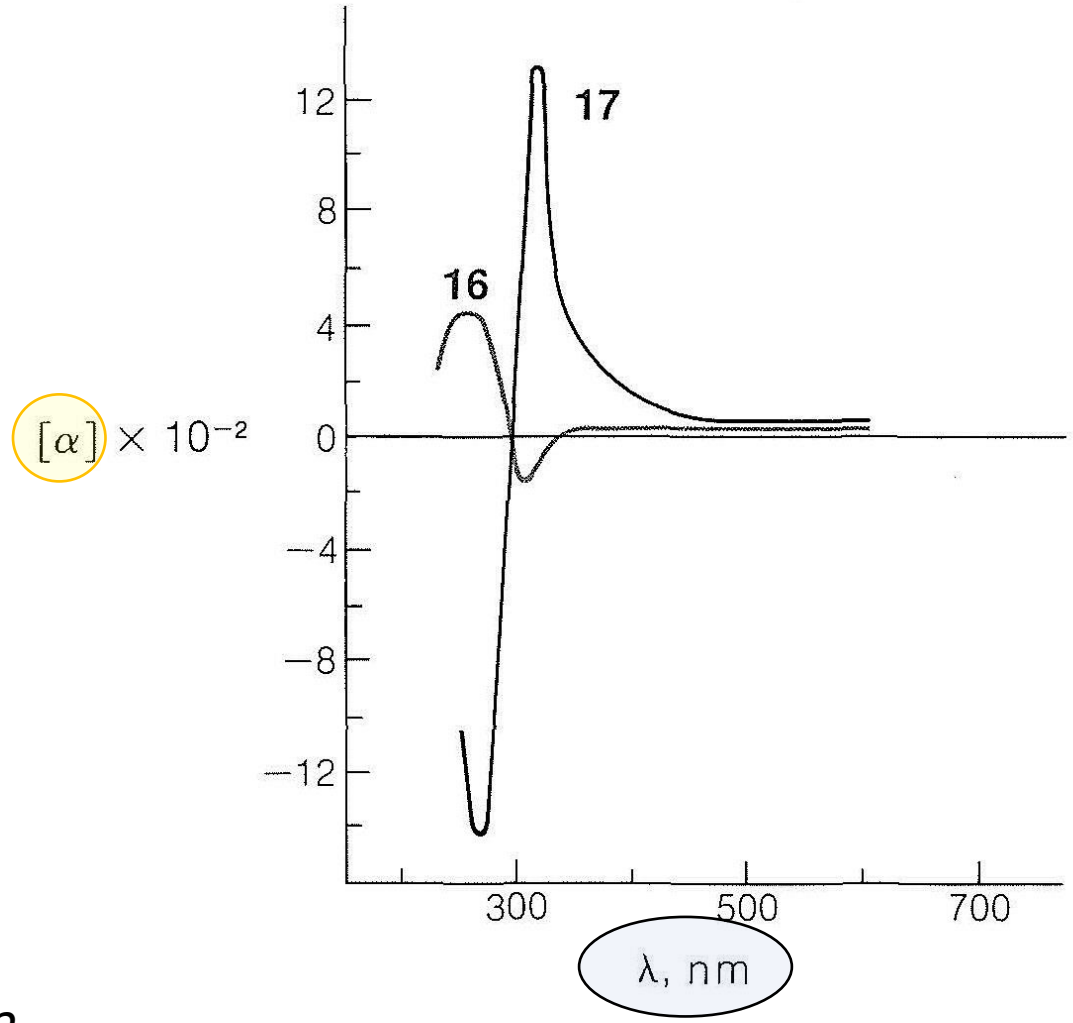
Specific rotation is a function of wavelength



16



17



*C. Djerassi, O*ptical Rotatory Dispersion*, McGraw-Hill Book Co., New York, 1960.*

Notes on Post-lab Calculations

- You will use Excel for all three parts of the lab
- Parts I and II will use Excel Solver which may be new to you. There are handouts for how to use Solver which you should attempt to follow first.
- Part III will require use of GaussView to export the ORD, which you will then plot in Excel.

Please follow the How to Use Solver procedures carefully, and start with the cosine squared procedure – if you have issues, do not hesitate to contact me. I should reply within 24 hours.

HOW TO USE EXCEL SOLVER: FINDING OPTICAL ROTATION

Note: Once you have opened your CSV file from your lab section, please go to the top left corner of Excel, File > Save As and save this as an ***Excel Workbook***. If you do not do this, the work/equations you use may not save properly.

This data is supposed to follow a cosine squared model:

$$Illumination = A\ \cos^2(\ B\ \cdot \theta\ + C) + D$$

in order to fit the data in Excel to this equation, we must use the **Excel Solver**.

In our situation, we will give Excel an educated guess as to the values of A, B, C, and D. We know θ will be the x-axis (angle values) and the y-axis will therefore be a fit, or prediction, based on the values we choose for A, B, C, and D. **Solver** will then optimize all four of those variables (A, B, C, and D) so that the predicted values best match the real data values. **Solver** will do this by ***minimizing the sum of the square of the difference*** between the *real* values and the *predicted* values. Bear in mind that this is just a single number. We must therefore create one more cell, which contains this single number, and then we will be ready to use **Solver**.

1. In your spreadsheet, create two new columns: one for **fit** data values, and one for the **difference squared**.

A	B	C	D	E	F
Data Set 1	Data Set 1:Optical Rotation(Â°)	Data Set 1:Illumination(rel)	Data Set 1:Angle(Â°)	**Fit Values**	**Difference Squared**
0	10.12	47198	-10.7		
0.02	10.12	50069	-5.6		
0.04	10.09	51893	-0.6		

2. Off to the side (in my case, columns G and H from row 2 to 6), create a table of parameters which we will feed to **Solver**.

G	H
A	
B	
C	
D	
SSQD	

3. We must guess at the values for these parameters, which are commonly called the **initial values**. The quality of the result (i.e., how good the fit is) we get from **Solver** will depend on the quality of your initial values, so always be aware of this. The following suggestion for initial values for each parameter should work well: **A** = 30000, **B** = 1, **C** = 10, **D** = 5000.

4. Next, we will use these initial values to generate a *fit* to the data. These values will go into the **Fit Values** column. Click on cell E2, then enter (carefully)

   `=$H$2*COS(RADIANS($H$3*D2-$H$4))^2 +$H$5`

   This may look familiar to you – it is the righthand side of the formula presented above, but in 'Excel' format.

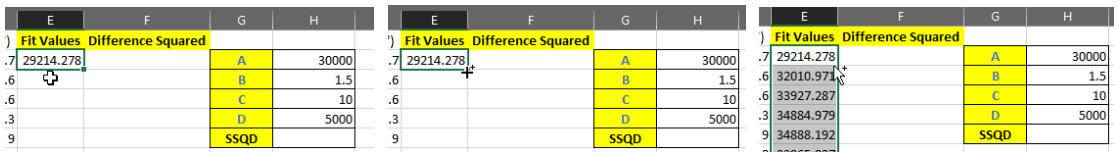
   Note: In our case, `H2`, `H3`, `H4`, and `H5` each represent the variables **A**, **B**, **C**, and **D**, respectively (the entries in the small table we generated off to the side). We include the dollar signs (e.g., `$H$2`) to tell Excel to keep re-using the contents of this cell when applying this formula to additional cells (which you will see done below). The variable `D2` represents θ which is the x-axis in our dataset from the polarimeter.

   Note: Excel expects any argument to its trig functions to be in units of radians, rather than degrees, so we use the Excel function `RADIANS` to carry out this conversion.

   Note: You may have noticed Excel automatically color-coded the cells you typed in and may have even shown you a corresponding colored box around the cell in question. This is a feature of Excel which allows you to double-check you are using the specified cell as intended, and one which you should use to your advantage.

5. Once the above code is entered, hit enter. To apply to all subsequent cells, be sure to use Excel's auto-fill method.

   Note: In order to use the auto-fill method, click on the cell (in our case, cell E2), then hover your cursor over the small green square in the bottom right corner. Your cursor should turn from a white crosshairs to a black crosshairs. With black crosshairs hovering over the dark green square in the bottom right, left-click twice and this will apply the equation in cell E2 to the rest of the cells in column E, for all the values in column D (since the column D is right next to E and is the only varying variable in the equation as we defined it). For questions on this or regarding the syntax, do not hesitate to ask me!



6. Next, we must calculate the square of the difference between the *fit* values and the *real* (or experimental) values. The experimental values in our case are in column C, as Illumination(rel). To calculate the difference squared, enter:

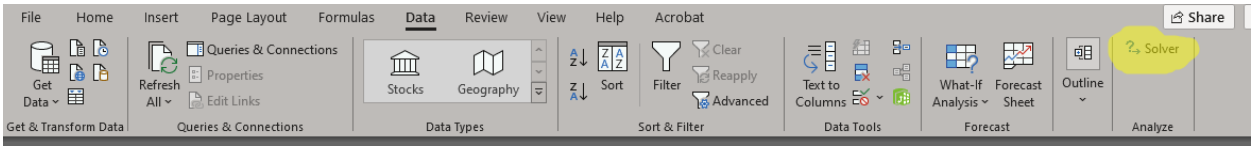
   `= (C2-E2)^2`

   then auto-fill. The parentheses are very important and are not optional.

7. At this point, we are nearly ready to use **Solver**. We must, finally, *sum* the square of the differences. Do this in the cell for SSQD – if you are following along exactly with my example and copied the table in Step 2, then this will be in cell H6:

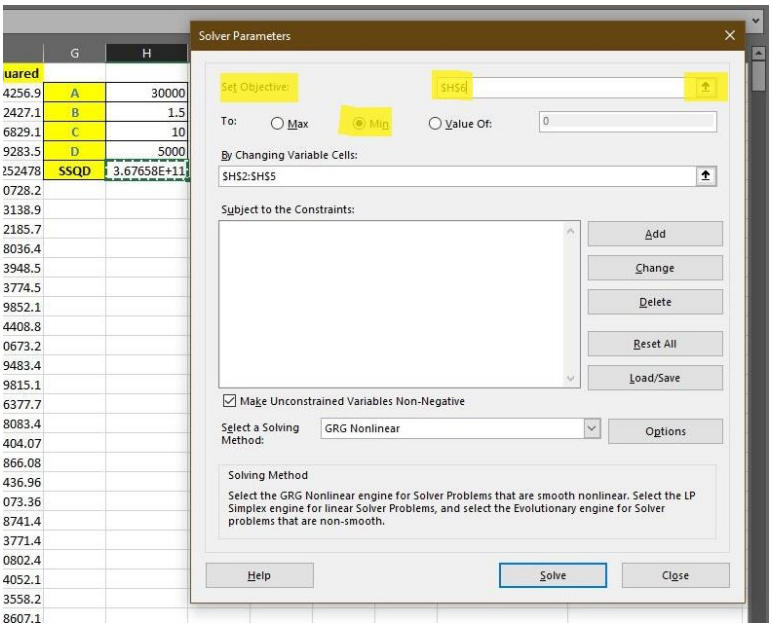
   `= sum(F2:F752)`

   The key step here is to start typing `=sum(` in cell H6 and then clicking on the *first cell* of the column that you wish to take the sum along (in our case, that is column F, and the first data point of interest resides in cell F2) – from here, you would then hold down the **Ctrl** and **Shift** keys (Windows) or **Command** and **Shift** keys (Mac) at the same time, then select the **down arrow** button. This will highlight all the values in this column (given that each consecutive cell contains a number). If there was a blank space somewhere in the column, between numbers, it would only copy up until the cell containing the blank space. You may find this to be a very useful "hot key" for this lab course, and any time you are using Excel to graph something.
8. We now have everything we need to use **Solver**! You may need to tell Excel to install **Solver**, depending on how Excel was installed, which you can do by going to the search bar at the top of the Excel window, typing 'Browse Add ins' and then typing **Solver** in the search bar of the window that pops up. Click the option for **Solver**, click the Add button and then proceed to install **Solver**. After this is completed, you can exit from any pop-up windows and you should be able to go to the Data tab of your toolbar and find **Solver** somewhere in the toolbar there. Click on the **Solver** button.



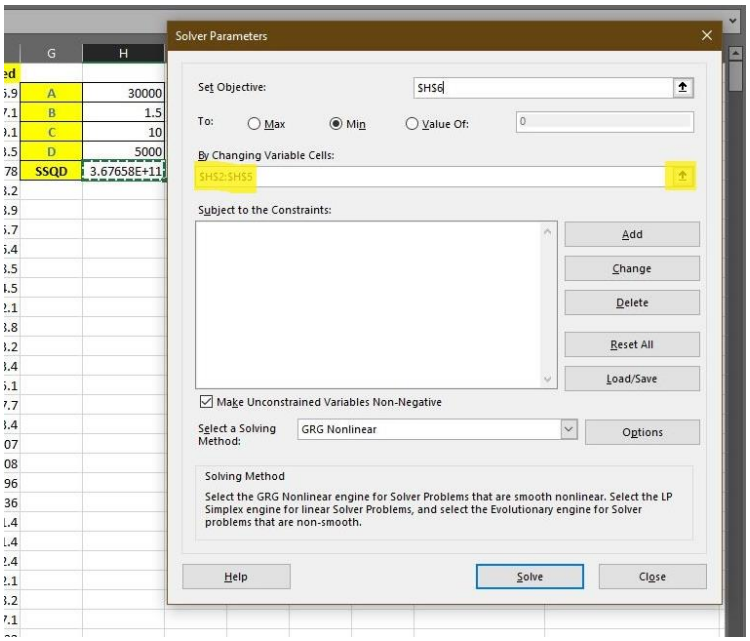
9. Clicking on the **Solver** button will open a dialog box called **Solver Parameters**. In that box, we want to specify the cell containing the sum of the square of the differences, which is the item we seek to minimize – do this by entering that cell number where it says 'Set Objective' and be sure to click the Min button in the row below.

   Note: You can easily enter the cell number by clicking on the upward arrow on the right side of the text box, clicking on the cell of interest (H6) and then clicking the button on the right side of the text box again (this time it will be a downward arrow).

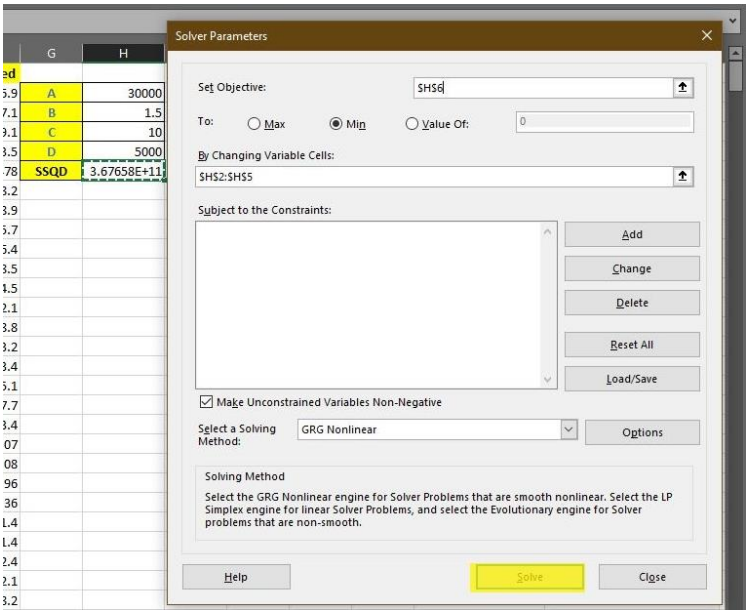


10. Next you want to tell **Solver** which variables to use in order to optimize the fit. Once we do this, **Solver** will proceed to find the best fit by changing all the variables in such a way that it minimizes the quantity we specified in step 9. We want to tell **Solver** to use all 4 parameters A, B, C, and D, which are in cells H2, H3, H4, and H5, respectively.

    Note: As in step 9, we can easily indicate the cells we want to use by clicking the arrow on the right side of the input prompt, and this time left-click and drag from cell H2 to cell H5, which will have the effect of highlighting (and therefore selecting) all the cells from H2 to H5. When you have selected your cells, hit the button on the right side of the text box again (this time it will be a downward arrow).



11. With your objective specified as well as your variables to change specified in **Solver Parameters**, you can now hit the **Solve** button, which will run **Solver**. If it succeeds, you should see the variables you highlighted as well as the **SSQD** value change in place, along with the numbers in the **Fit Values** and **Difference Squared** columns. You may be shown a pop-up window asking you to accept the solution. You can hit **OK** to accept the solution **Solver** has found.



12. The value of interest in our fit is the parameter **C**, which is the optical rotation (the phase shift of the cosine squared function). This is the value you will want to use to calculate the specific rotation.

    Note: To assess the performance of the fit found by **Solver**, you should graph the values in the **Fit Values** column on the y-axis and **Angle** on the x-axis as one dataset, and **Illumination** on the y-axis and **Angle** on the x-axis as another dataset, to compare fit to experimental, respectively.

Lab A: Polarimetry Measurements of the Inversion of Sucrose

Student: XXX

Part I. Specific Rotation (50 pts)

1. (x / 10 pts)
2. (x / 10 pts)
3. (x / 10 pts)
4. (x / 10 pts)
5. (x / 10 pts)

Total: (x / 50 pts)

Part II. Kinetics (30 pts)

1. (x / 10 pts)
2. (x / 15 pts)
3. (x / 5 pts)

Total: (x / 30 pts)

Part III. Optical Rotary Dispersion (20 pts)

1. (x / 10 pts)
2. (x / 5 pts)
3. (x / 5 pts)

Total: (x / 20 pts)

Report Total: (x / 100)

General Comments: