**Directions for Using the Cary 60 Spectrophotometer**

***Obtaining an Absorbance Spectrum***

1. Open the "Scan" program by double clicking the shortcut on the desktop. This application may already be open.

2. Click "Setup" to set the scanning parameters

 -- On the "Cary" tab set the scan range using the "Start" and "Stop" windows. Set

 range for 650 to 400 nm. Under "Scan controls" choose the "Medium" sample

 rate.

 -- On the "Baseline" tab click the "Baseline correction" radio tab

 -- On the "Auto store" tab click the "Storage off" radio tab

 -- Click "Ok" to exit this window

3. Insert a cuvette containing DI water into the sample holder. The instrument will obtain the absorbance spectrum for water, store it on the hard drive, and then subtract it from your other spectra so that you have the absorbance of your sample only.

 \*\*\*Note the cuvettes have a clear side and a frosted side. Be sure to place the cuvette into the sample holder so that the clear side is facing left and right while the frosted side faces front to back. The beam of light being measured runs in this direction.

4. Click the "Baseline" button and then "OK" when prompted.

5. Prepare your sample by adding your most concentrated (stock) solution to a new cuvette. Place the new solution to be measured in the sample holder and then click "Start". You will be prompted to insert a sample name but just click "OK" to start the scan. You should now see the absorbance spectrum of your sample appear on the screen. When prompted to insert a sample name again simply click "Finish"

6. Determine λmax by using the crosshairs to hover over the wavelength giving the highest absorbance. The X,Y coordinates will be displayed at the bottom of the window. Note this absorbance for the next portion of the experiment.

7. Save this graph for later use by clicking "File → "Save Data As…" Name the graph appropriately and save it in the "D:\Beer's Law" folder. ***Be sure to save the file as a Spreadsheet Ascii (\*.CSV) file***.

***Measuring Your Samples for the Calibration Curve***

1. On the desktop click on the "Simple Reads" shortcut icon. When the application loads click on the "Connect" button.

2. Click "Setup" to set the collection parameters.

 -- Set the wavelength to the λmax as determined from the absorbance vs.

 wavelength spectrum.

 -- Click "OK" to exit this screen.

3. Insert the cuvette filled with DI water into the sample holder and click "Zero". This will zero the instrument at this specific wavelength.

4. Remove the cuvette filled with DI water and replace it with the cuvette containing your most concentrated (stock) solution. Click "Read" at the top of the window to obtain the absorbance of the solution at this specific wavelength.

5. Determine the absorbance of all your dilutions using this same cuvette. To do this empty the cuvette into the waste container and refill with the new solution to be measured and then pour this out as well. This essentially replaces the old solution with the new one. Finally, refill with the new solution, insert the cuvette into the sample compartment and click "Read". You should see the absorbance of this solution appear below the previously measured one. ***Do not forget to measure the absorbance of your unknown solution!!***

6. When finished measuring all of your samples you will need to save this data in the same location as you saved the absorbance vs. wavelength spectrum. Click on "File → Save Data As…" and save the file in the "D:\Beer's Law" folder with an appropriately labeled file. ***Be sure to save the file as a Rich Text Format (\*.RTF) file***.

7. Remove the cuvette from the sample holder, pour all dilutions down the drain and place your dirty test tubes in the dirty test tube container. Close all applications so that the next group can start fresh.