Spectroscopy II

Introduction: Spectrophotometry and Fluorometry

In this experiment you will use the SpectroVis Plus (a small computer controlled Spectrophotometer + Fluorometer) to:

- Measure the Transmission and Absorbance spectra of some common liquids from 380-950 nm.
- Measure the Fluorescence spectrum of Olive Oil for excitation at 405 and 500 nm.

In the process you will learn about:

- Beer's law and verify its dependence on the number density of particles.
- Light scattering from small particles and molecules and how to detect it, as well as how to estimate the size of the scatterers.

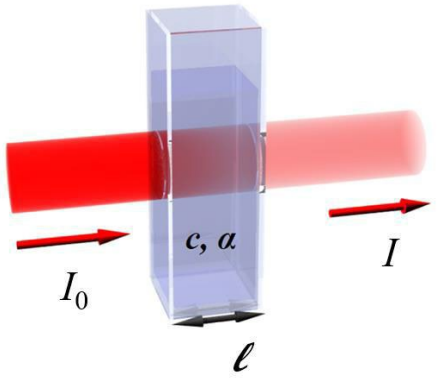
Absorbance, Transmittance and the Beer-Lambert Law

Transmittance

The Transmittance is the Intensity of light going through a sample as a function of wavelength, so if 10% of the light going into a sample comes out the other side, then the transmission coefficient T would be 0.1.

Mathematically the transmission coefficient *T* is given by the ratio of the transmitted final intensity *I* to the incident or initial intensity I_0 (often a percentage)

$$T = \frac{I}{I_0}$$



Absorbance

The Absorbance is basically the inverse of transmittance, whatever a material does not transmit, is absorbed.

The absorbance is written in terms of transmittance and Intensity by

$$A = \log_{10}\frac{1}{T} = \log_{10}\frac{I_0}{I}$$

Fluorescence

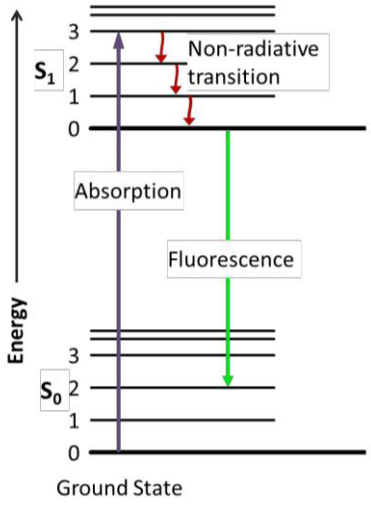


Fig : Jablonski diagram.

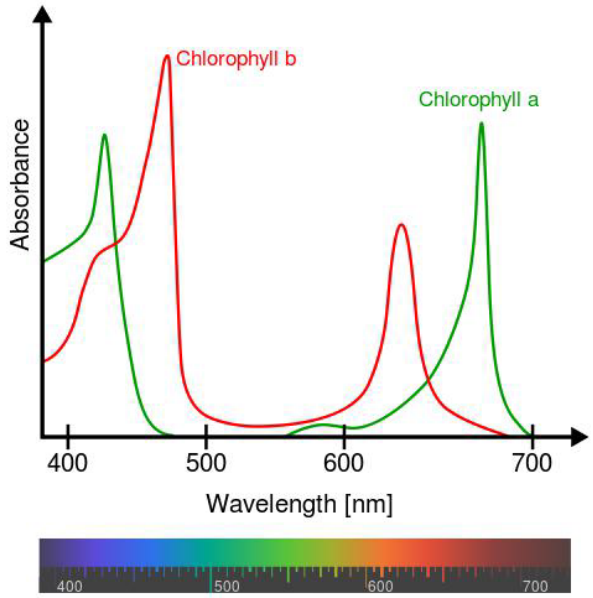
Fluorescent molecules are compounds that absorb light of one wavelength, then re-emit light at a longer wavelength. Fluorescence is this process.

For example if we shine a violet (405nm) laser into a fluoroscene mixture a bright green light will be transmitted. (Your instructor can demo this)

After an electron absorbs a high energy photon the system is excited electronically and vibrationally. The system relaxes vibrationally, and eventually fluoresces at a longer wavelength. In this lab you will observe the fluorescence of extra virgin Olive Oil and compare to its transmission and absorption spectrum.

Sample Colors

If we look at Absorbance over the visible spectrum we can determine what color an object will be, for example chlorophyll, a molecule found in plants, has the following absorption spectrum.



From this we see for chlorophyll that there is a dip in absorbance in 500-600nm range which means this range of light is what is being transmitted through the material to our eyes.

Question 1:

a.) What color should you expect chlorophyll a to be?

b.) What would be different about chlorophyll b?

Hint: Remember a peak in absorbance is what you DON'T see.

PART 1: Olive Oil Spectra

In the first part of the experiment, you will measure the Transmission, Absorbance, and Fluorescence spectra using 405nm and 500nm excitation light of a sample of Extra Virgin Olive Oil and compare the various features of each type of spectrum.

A.) Before you do anything you must calibrate the experiment and learn how to use it, jump to **Calibration and Equipment** and follow the steps.

Procedure

B.) After calibrating get the Olive Oil from your instructor and insert it into the spectrometer.

C.) Open logger pro and navigate to Experiment > Change Units > Transmittance and hit collect to take a transmittance spectrum

D.) Be sure the entire graph is shown, you will likely have to rescale your y axis, make sure the graph doesn't go off screen.

Question 2)

a.) Make a rough sketch of the graph you see

b.) List any significant peaks or troughs for the graph shown

c.) What does each peak/trough physically mean?

E.) Repeat step **C and D** for Absorbance (Change Units -> Absorbance)

Question 3)

a.) Make a rough sketch of the graph

b.) What is the relation between this graph and your graph of Transmittance?

c.) What is the physical meaning of a peak on this Absorbance Graph?

F.) Now repeat **B** and **C** for 405nm and 500nm fluorescence (Again go to change units). Be sure to hit collect again each time, the graphs should all look very different from transmittance, if ANY of them look the same call over your instructor.

Question 4)

a.) Make a rough sketch of both of the graphs.

b.) What color is going into the sample? (Take a look in the spectrometer while its on fluoresce)

c.) Based on the graphs you see what color is coming out of the sample?

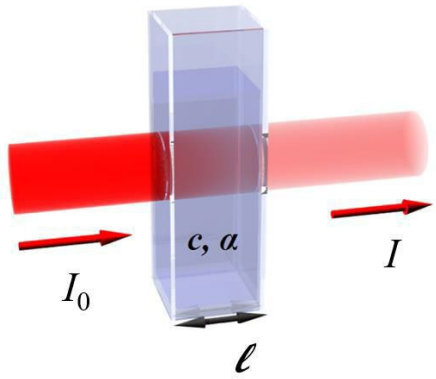
G.) Obtain a sample of red wine vinegar or other sample from your instructor and take data for Transmittance and fluorescence as before to answer the following question 5.

Question 5)

a.) Comment on the Transmittance graph, is there anything you didn't expect? Is it exactly what you expected?

b.) Does the red wine vinegar fluoresce with either of the wavelengths? How do you know?

Part II Beer-Lambert Law



For the Beer-Lambert Law we have the relation of the final intensity to the initial intensity is

$$I = I_0 e^{-\alpha l}$$

where α is the absorption coefficient (Specific to the material) and *l* is the path length in the absorbing/scattering medium.

The absorption coefficient can be written in terms of the number density n (number of particles/volume of material) and the absorption/scattering cross section σ which we'll discuss in part 2. The absorption coefficient is

$$\alpha = n\sigma$$

leaving the absorption as

$$A = \log_{10} \frac{I_0}{I_0 e^{-\alpha l}} = n\sigma l(\log_{10} e)$$

$$A = (0.4343\ \sigma\ l)n$$

The importance of this last result is that it tells us that the absorbance is proportional to path length *l* and the number of particles per volume, n. The standard cuvette length we will use is 1cm.

We can also use this in a linearization, substituting for *n=N/V* we have

$$A = \frac{(0.4343\ \sigma\ l)N}{V},$$

$$\frac{1}{A} = m * V.$$

So if we plot 1/A against the volume of the dilution, which will be given to you, we should see a linear graph, with a slope equal to $m = 1/0.4343\sigma lN$.

Procedure 2:

To test Beer's law we will measure the absorbance of a solution of Tea and decrease the number density by diluting the solution by specific amounts. Hence for one specific wavelength through this material we should see a linear relationship between the number density and the measured absorbance.

Start by measuring the absorbance spectrum for each dilution of tea, to do so do the following.

A.) Start by calibrating your system to the water cuvette at your station.
B.) To take an Absorbance spectrum go to Experiment->Change Units-> Absorbance
C.) Insert the Cuvette labeled "1" and click "run". Allow the program to run long enough to get a stable graph and completed data table (about a minute.) Click "Stop" and carefully remove the cuvette.
D.) Repeat for cuvettes 2-5

Hint: When you click on "Run" again, you can choose to "store latest run" and it will conveniently put all the data on one graph.

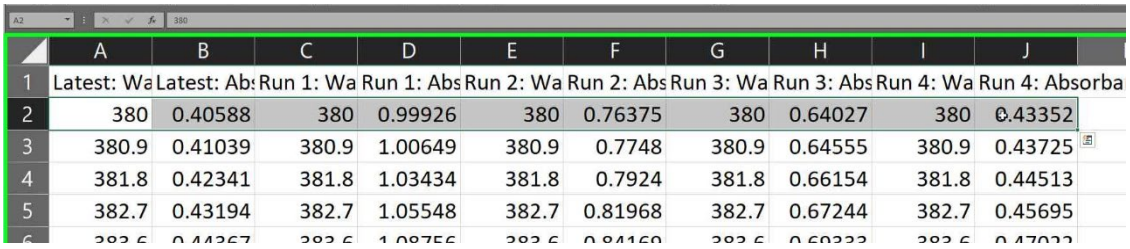
Data Analysis

Table

A.) Open the course excel sheet and go the Spectro2 Absorbance Tab.
B.) Export the values from LoggerPro, then copy paste them into the proper location in the Spectro2 Absorbance tab.
   a. **To do this** Export your Logger Pro file to Excel (*.csv) by selecting file->export->csv and save the file.
   b. Open that csv then click the first wavelength value (Don't include the titles)

	A	B	C	D	E	F	G	H	I	J
1	Latest: Wa	Latest: Ab	Run 1: Wa	Run 1: Abs	Run 2: Wa	Run 2: Abs	Run 3: Wa	Run 3: Abs	Run 4: Wa	Run 4: Absorba
2	380	0.40588	380	0.99926	380	0.76375	380	0.64027	380	0.43352
3	380.9	0.41039	380.9	1.00649	380.9	0.7748	380.9	0.64555	380.9	0.43725
4	381.8	0.42341	381.8	1.03434	381.8	0.7924	381.8	0.66154	381.8	0.44513

   c. While holding click, drag across the top column letters to select all 10 columns.



	A	B	C	D	E	F	G	H	I	J
1	Latest: Wa	Latest: Ab	Run 1: Wa	Run 1: Abs	Run 2: Wa	Run 2: Abs	Run 3: Wa	Run 3: Abs	Run 4: Wa	Run 4: Absorba
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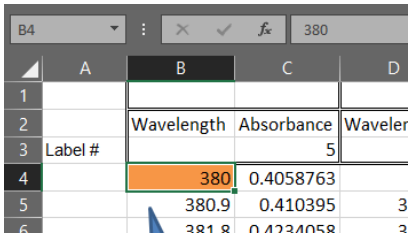
4	381.8	0.42341	381.8	1.03434	381.8	0.7924	381.8	0.66154	381.8	0.44513
5	382.7	0.43194	382.7	1.05548	382.7	0.81968	382.7	0.67244	382.7	0.45695

   d. On your keyboard hold ctrl+shift then press the down arrow, this will select all values.
   e. Copy what you have selected (ctrl+c)

f. Switch to the course excel sheet and click the first wavelength value.
g. Paste (ctrl + v) those 10 columns into the proper locations in the excel sheet. (Highlighted orange here)
h. Check that the graphs to the right have consistently increasing values.



Note that the order that logger pro will output is 5 1 2 3 4, since it stores the most recent one at the farthest left, then appends previous to the farthest right. You shouldn't need to reorder anything.

Here's an example.

The examples used 428.8nm offpeak, and 397nm for on peak, but use whatever works best for your data.

(off peak)	A	label #	V(relative)	1/A
lamda(nm)	0.809	1	2	1.236
467.1	0.563	2	4	1.776
	0.484	3	6	2.066
	0.305	4	8	3.279
	0.112	5	10	8.929

(peak)	A	label #	V(relative)	1/A
lamda(nm)	1.186	1	2	0.843
402.3	0.855	2	4	1.170
	0.754	3	6	1.326
	0.491	4	8	2.037
	0.218	5	10	4.587

Graphs

A.) In the excel sheet you'll now see three graphs.
   a. The one to the left is the raw data, it should match your collected data.
   b. One graph to the right displays the data from your Off Peak table.
   c. The other graph shows data from the On Peak Data

B.) The off-peak wavelength is fit with a linear trend line with the option "set intercept = 0" ENABLED. (this is a trendline option which forces the intercept to be zero).

Question 6:
   a. What are the slope and correlation coefficient of your off peak graph?
   b. Based on these values does your data for the off peak value reflect the Beer-Lambert law? Explain your answer.

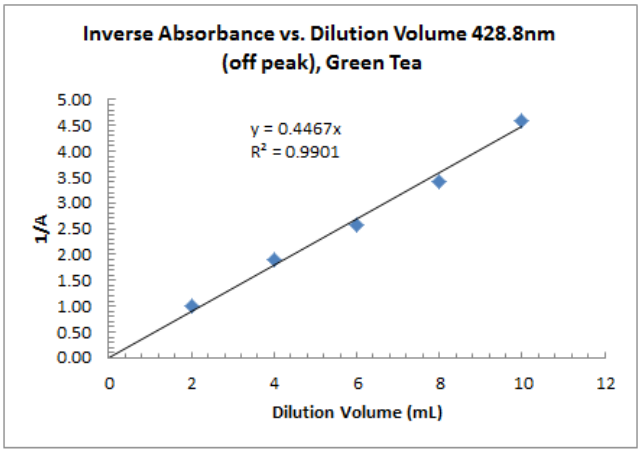
C.) The on-peak wavelength is fit a linear trend line with the option "set intercept = 0" DISABLED. (this is a trendline option, default does not force the intercept to zero).

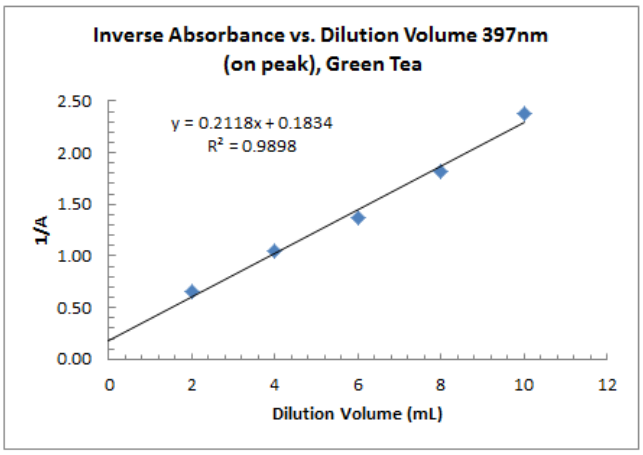
Question 7:
   a. What are the slope and correlation coefficient of your on peak graph?
   b. Based on these values does your data for the on peak value reflect the Beer-Lambert law? Explain your answer.

Question 8:

What types and specific deviations may have affected the quality of your analysis for questions 6 and 7? List at least two deviations and explain.

Hints for Q6,7,8:





Off peak wavelength obeys Beer's law over this range of concentration. Note the forced fit with intercept of zero fits the data nicely

On peak wavelength deviates noticeably from Beer's law over this range of concentration. Note the non-zero intercept required to fit the data. Fit with zero intercept gives lower R^2 value with poor fit.

Comments

So the off peak wavelength produces a good fit to Beer's law, while the peak wavelength requires a non-zero intercept, although the concentration dependence was still linear.

There are several factors which can produce deviations from Beer's law.Under certain conditions Beer-Lambert law fails to maintain a linear relationship between absorbance and concentration of analyte. These deviations are classified into three categories:

- Real Deviations - These are fundamental deviations due to the limitations of the law itself.
- Chemical Deviations- These are deviations observed due to specific chemical species of the sample which is being analyzed.
- Instrument Deviations - These are deviations which occur due to how the absorbance measurements are made.

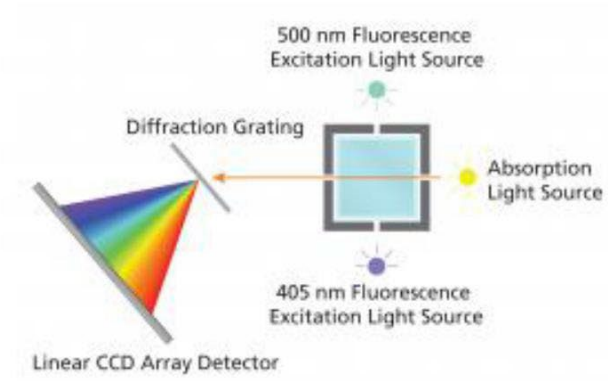
Calibration and Equipment

Equipment Info and Use

The Equipment you will use is the Vernier SpectroVis Plus Spectrophotometer + Fluorometer shown below

Vernier **SpectroVis Plus** Spectrophotometer + Fluorometer

How does it work?



The figure on the right shows what's going on inside the spectrophotometer. To work for absorption light from the LED and tungsten bulb light source passes through a solution. Emerging light goes through a high-quality diffraction grating, then the diffracted light is collected and sorted by the CCD array detector.

To measure Fluorescence , fluorescent light is scattered at right angles (RA fluorescence) to the excitation light sources

(LED's) to minimize light detected at the excitation wavelength(s). Basically we try to make sure all the light being measured is coming from the molecule, not the source.

***Note that for this to work you must insert the flat face of the cuvette toward the absorption light and source, the grated side should face the two fluorescent lights top and bottom.**

Calibration

To calibrate the device insert a blank cuvette into the SpectroVis as directed above. Then do the following :

1) Open Logger pro, if the device is properly connected you should see a graph overlayed with the visible spectrum of light.
2) In the top menu go to Experiment > Calibrate > Spectrometer 1
3) Run the calibration, the first time you must wait the 90s for the warm up, then hit finish calibration, after thefirst time you can skip this warmup. The device is ready.