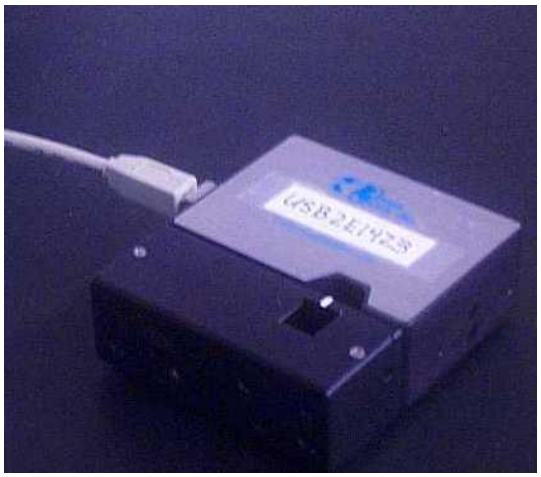
Diode Array Spectrometer

Jump to Spectrometer Operating Instructions

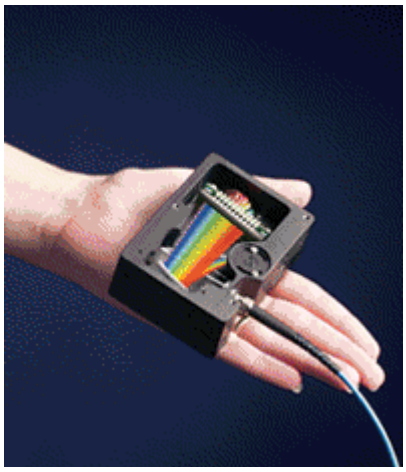
Visible light is electromagnetic radiation ranging in wavelength from about 400 to 750 nm. If a light source uniformly emits electromagnetic radiation in this range, the emitted light appears white. Some close approximations include light emitted from the sun and from incandescent light bulbs. White light is a composite of many wavelengths of light. This is readily shown by separating it into its component colors (wavelengths) by refraction or diffraction. Refraction or diffraction of white light produces the familiar rainbow of colors. (Indeed, a rainbow is produced by the refraction and dispersion of light by raindrops.)

A colored solution appears colored because something in the solution selectively absorbs visible light at one or more, but not all, wavelengths. Light that is not absorbed by the solution passes through–is transmitted by–the solution, and the solution takes on the color of the transmitted light.

When you observe a colored solution, ambient light serves as the light source that is incident on the solution and your eye detects the color of the solution. The CCD array spectrometer operates in a similar way, except that a tungsten lamp serves as the light source and a 2048 pixel element linear CCD array serves as the detector. Light from the source passes through a sample solution and into a monochromator. Here it is collimated and reflected onto a diffraction grating that separates it into its component wavelengths. The resulting diffracted light is projected onto the CCD detector. Data (photon flux) captured by the CCD array are transferred to a computer and displayed. Most surprisingly, the source and detector contain no moving parts and fit in the palm of your hand!



Diode Array Spectrometer with Integrated Visible Source and Sample Compartment



Cutaway View of Spectrometer

The detector is the same type of detector used in digital cameras and scanners. And much like a camera, the CCD array in the spectrometer takes a continuous real-time “picture” of light impinging on it. The associated spectrometer software calculates and displays this “picture” as a graph of light transmitted (or absorbed) as a function of wavelength.

The CCD array spectrometers used in lab can detect light from 200-1000 nm which includes the ultraviolet (UV), visible, and part of the near infrared (IR) regions of the electromagnetic spectrum. Hence, they can be used to quantitatively measure the amount of light at any wavelength in this range that is absorbed by a solution.

The most common display modes are transmittance and absorbance. Transmittance, T, is defined as the fraction of incident light of a particular wavelength transmitted by a solution:

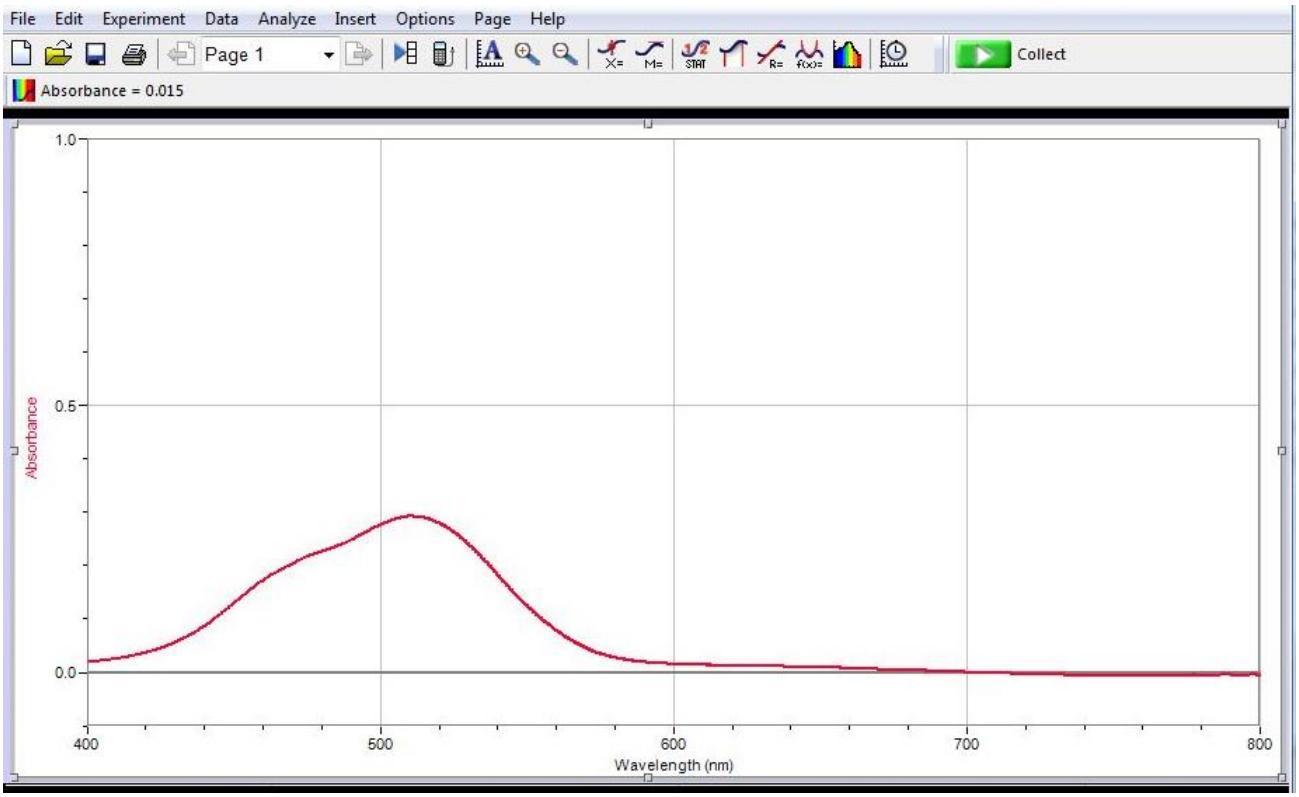
$$T_\lambda = (I)_\lambda/(I_o)_\lambda$$

Where $(I_o)_\lambda$ is the intensity of incident light at a particular wavelength on a solution and $(I)_\lambda$ is the intensity of light transmitted by the solution. Percent transmittance is simply the transmittance times 100.

Absorbance, A, is related to transmittance as follows:

$$A = -\log T = -\log (I)_\lambda/(I_o)_\lambda = \log (I_o)_\lambda/(I)_\lambda.$$

The absorbance spectrum of an aqueous solution of Co^{2+}over most of the visible region of the electromagnetic spectrum, 400-800 nm, is shown below:



Information about the electronic structure of a molecule or ion is revealed in its absorbance spectrum. Additionally, absorbance varies linearly with (is directly proportional to) the concentration of absorbing species in solution, and this is our primary interest in general chemistry. Hence, we will usually measure absorbance at the wavelength that corresponds to maximum absorbance, λ_{max}.

This relationship between absorbance and concentration is known as the Beer-Lambert Law which can be expressed as:

$$A = abc$$

where A is the absorbance, *a* is the absorbtivity which is a constant at a given wavelength and is characteristic of the absorbing species, *b* is the length of the absorbing medium, and *c* is the absorbing species concentration. Both *a* and *b* are constants and the product of these two values is also a constant, k; hence, the Beer-Lambert Law can be rewritten as:

$$A = kc$$

The most common application of the Beer-Lambert law is for concentration determination via an absorbance measurement which requires that *k* is known.

k is commonly determined by experiment in conjunction with the analysis of the sample(s) of interest. This is accomplished by preparing a series of standard solutions (of the species of interest) of known concentration. The absorbance of each standard solution is measured at λ_{max} since the absorbance and sensitivity of the measurement are greatest at this wavelength. Next, the data are graphed as absorbance versus concentration and a regression analysis performed to obtain a linear equation that corresponds to the best 'fit' of this data.

The concentration of absorbing species in an unknown is readily calculated by substituting its measured absorbance into the linear equation obtained from the regression analysis and solving this equation for concentration.

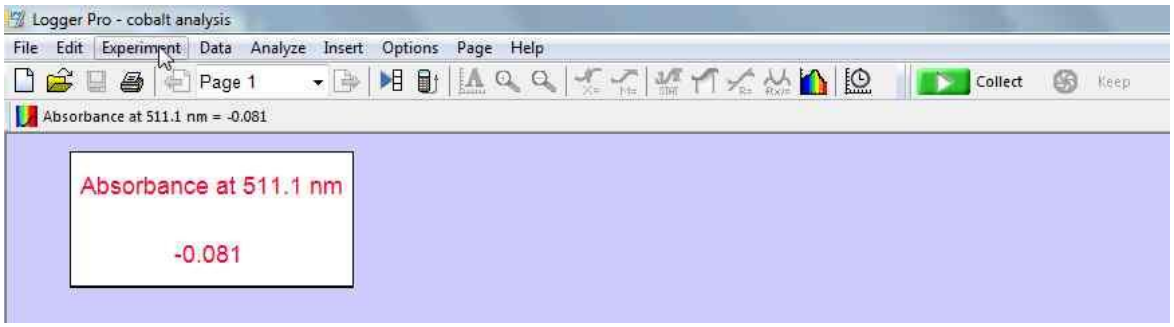
Absorbance Measurements Using the CCD Array Spectrometer

Obtaining an absorbance reading involves two steps: 1) calibrating the spectrometer using a blank—typically the solvent used to dissolve the substance of interest;2); measuring the absorbance of the sample of interest at its λ_{max}.

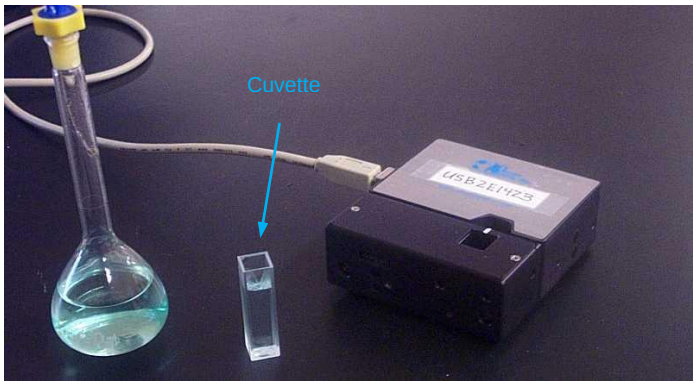
A detailed protocol follows. A condensed protocol is given in the ***Procedure*** section of experiments which utilize this instrument

<u>Making Absorbance Measurements</u>

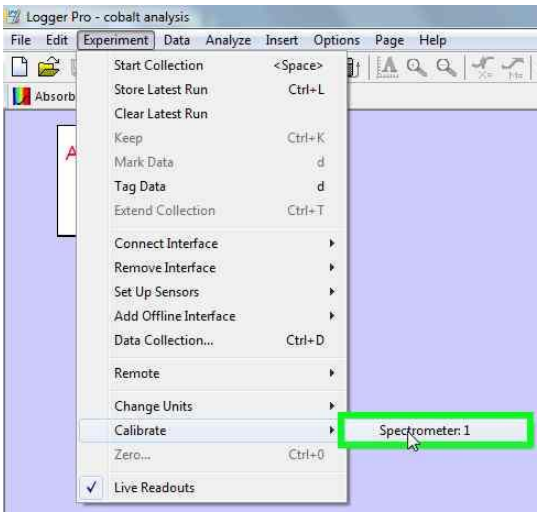
1. Bring solutions, deionized water wash bottle, and 250-mL beaker to a nearby instrument.
2. The computer and spectrometer should be on, and the spectrometer software, Logger Pro, should be open. If so a box displaying the absorbance wavelength and absorbance value will be displayed. The absorbance is continually displayed (updated) in real-time:

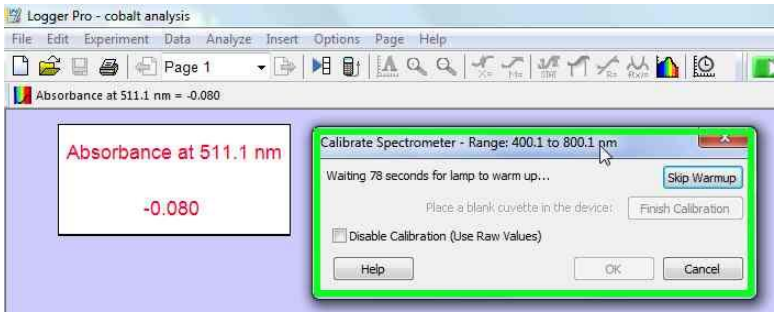


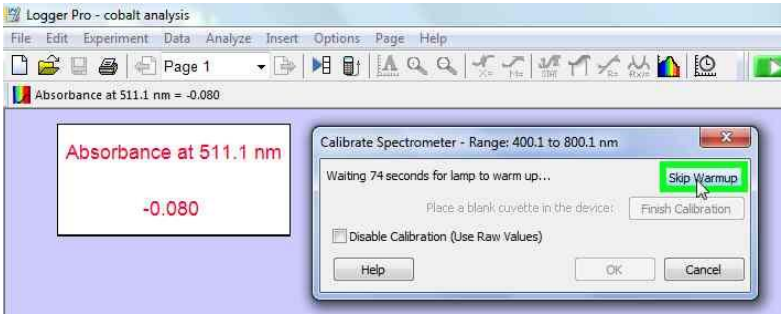
3. Obtain a rectangular cuvette. Two sides of the cuvette are frosted and two are transparent. Handle the cuvette by the frosted sides only. Glass cuvettes are usually provided in lab and these have a G (for glass) inscribed on one of the transparent sides.
4. Fill the cuvette two-thirds full with deionized water. Always wipe the transparent sides with a Kimwipe prior to inserting the cuvette into the sample compartment.
5. Insert the cuvette into the sample compartment and orient it so that the G on the cuvette window and the white line on the sample compartment are aligned. Press carefully but firmly to ensure that the cuvette is properly seated: the top quarter-inch will protrude from the sample compartment.

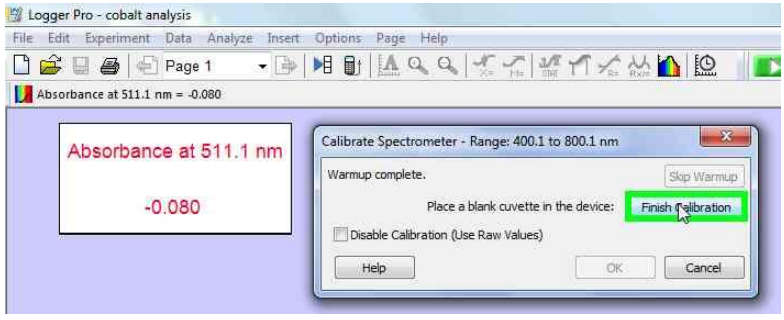


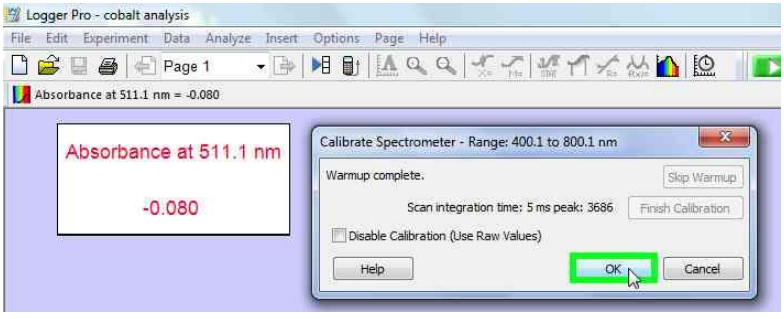
6. Calibrate the spectrometer: select Experiment on the main toolbar then select Calibrate: Spectrometer 1. On the pop-up window press Skip Warm Up then Finish Calibration. Wait for the calibration to finish—about 5 s—then press OK. The displayed absorbance should read 0.000, but like the electronic balances, there will likely be some fluctuation in the last digit. The screen shots on the next page show this sequence of operations.
7. Remove the cuvette from the sample compartment, discard the water, and shake out the excess onto a Kimwipe. Rinse the cuvette with a small portion of the solution of interest—the most dilute if sequential measurements are being made. Discard the rinse. Fill the cuvette two-thirds full with a fresh portion of this solution. Wipe it with a Kimwipe and place it in the sample compartment. Make certain the inscribed G is oriented in the same direction as it was in step 3.
8. Record the displayed absorbance.
9. Return the solution in the cuvette to its original container and shake out the excess onto a Kimwipe. Rinse the cuvette with the next solution of interest and discard. Fill the cuvette two-thirds full with a fresh portion of this solution and determine its absorbance.
10. Determine the absorbance of remaining solutions by repeating the previous step.
11. When you finish, take all your items with you. Leave Logger Pro running. Do not exit this program or turn the computer off.

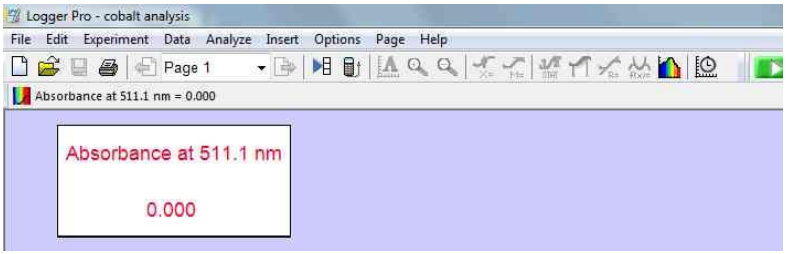

Logger Pro - cobalt analysis
File Edit Experiment Data Analyze Insert Options Page Help
Start Collection <Space>
Store Latest Run Ctrl+L
Clear Latest Run
Keep Ctrl+K
Mark Data d
Tag Data d
Extend Collection Ctrl+T
Connect Interface
Remove Interface
Set Up Sensors
Add Offline Interface
Data Collection... Ctrl+D
Remote
Change Units
Calibrate
Zero... Ctrl+0
Live Readouts
Spectrometer: 1


Logger Pro - cobalt analysis
File Edit Experiment Data Analyze Insert Options Page Help
Page 1
Absorbance at 511.1 nm = -0.080
Absorbance at 511.1 nm
-0.080
Calibrate Spectrometer - Range: 400.1 to 800.1 nm
Waiting 78 seconds for lamp to warm up...
Skip Warmup
Place a blank cuvette in the device:
Finish Calibration
Disable Calibration (Use Raw Values)
Help
OK
Cancel


Logger Pro - cobalt analysis
File Edit Experiment Data Analyze Insert Options Page Help
Page 1
Absorbance at 511.1 nm = -0.080
Absorbance at 511.1 nm
-0.080
Calibrate Spectrometer - Range: 400.1 to 800.1 nm
Waiting 74 seconds for lamp to warm up...
Skip Warmup
Place a blank cuvette in the device:
Finish Calibration
Disable Calibration (Use Raw Values)
Help
OK
Cancel


Logger Pro - cobalt analysis
File Edit Experiment Data Analyze Insert Options Page Help
Page 1
Absorbance at 511.1 nm = -0.080
Absorbance at 511.1 nm
-0.080
Calibrate Spectrometer - Range: 400.1 to 800.1 nm
Warmup complete.
Skip Warmup
Place a blank cuvette in the device:
Finish Calibration
Disable Calibration (Use Raw Values)
Help
OK
Cancel


Logger Pro - cobalt analysis
File Edit Experiment Data Analyze Insert Options Page Help
Page 1
Absorbance at 511.1 nm = -0.080
Absorbance at 511.1 nm
-0.080
Calibrate Spectrometer - Range: 400.1 to 800.1 nm
Warmup complete.
Skip Warmup
Scan integration time: 5 ms peak: 3686
Finish Calibration
Disable Calibration (Use Raw Values)
Help
OK
Cancel


Logger Pro - cobalt analysis
File Edit Experiment Data Analyze Insert Options Page Help
Page 1
Absorbance at 511.1 nm = 0.000
Absorbance at 511.1 nm
0.000