**DIY Spectrophotometer and Determination of Quinine in Tonic Water** (adapted from Dr. Richard Keithley, Roanoke College, Salem, VA)

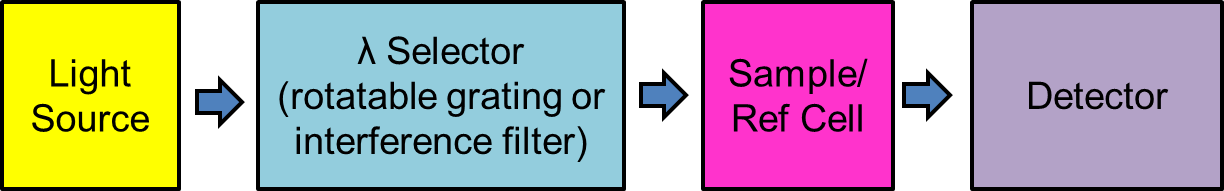
***Introduction***

The absorption of light is one of the fundamental physical properties that scientists take advantage of for qualitative and quantitative analyses of molecules. A spectrophotometer is an instrument that separates light into individual wavelengths (i.e., energies) and quantifies the degree to which molecules absorb those specific energies. Today, we will build a simple, yet powerful, spectrophotometer and use it to determine the amount of quinine in bottled tonic water.

***Single- and Dual-Beam UV-VIS Spectrometers Found in Research Laboratories***

In a single beam instrument, a light source shines onto a wavelength selector that directs a small portion of the incident light onto a sample cuvette before the light is measured by a detector. Most light sources create light through the process of incandescence (like older filament-style light bulbs found in your grandparent’s home), emitting a broad spectrum of white light over a relatively constant range of energies; this necessitates the use of a wavelength selector, like a rotatable grating or interference filter (**see Figure 1**).

Dual-beam instruments are like single-beam instruments, except they split the light coming out of the wavelength selector to pass through a sample and reference cuvette at the same time.



**Figure 1. A block diagram of a single-beam UV-VIS spectrometer.**

The wavelength selector allows a narrow bandwidth (1-2 nm) of light to shine upon the sample. This wavelength can be changed by the user over the entire UV-VIS region to suit the experiment. However, these wavelength selectors must be mounted on a rotating platform which pivots to direct a certain wavelength of light onto the sample. This creates the need for moving parts and gears of extreme precision, something that is both more expensive to manufacture and will eventually wear down.

***Light Emitting Diodes (LEDs)***

In today’s experiment you will be using an inexpensive LED to generate light. Instead of incandescence, LEDs use a voltage to facilitate electron transport within a semiconductor material. When electrons, made mobile by an applied voltage, transition from the higher energy conduction band of the semiconductor into the lower energy valence band, energy is released in the form of light.

Energy levels of electrons depend upon the chemical makeup of the semiconductor material. Therefore, the light that is emitted by an LED is usually only one color and has a very narrow (10-25 nm) bandwidth. Changing the molecular structure of the semiconductor material allows for the creation of different types of LEDs, which scientists can use to their advantage. For example, LEDs made of aluminum gallium arsenide produce red light, LEDs made of gallium (III) phosphide produce green light, and LEDs made of aluminum gallium nitride produce a narrow band of ultraviolet light (the light source for our spectrophotometer).

The primary use of LEDs is to generate light. However, the process can work in reverse - incident light can generate a measurable voltage difference in an LED that’s proportional to the intensity of the incident light. How this works is very complicated and will not be discussed here. However, we will take advantage of this effect to create a simple single-beam UV-VIS instrument which utilizes an LED as a light source and a second LED as a detector.

***Absorbance Determination***

Regardless of the instrument setup, a user records the number of photons incident upon a detector for both a sample and a blank. The absorbance (Abs) is then calculated using the equation below

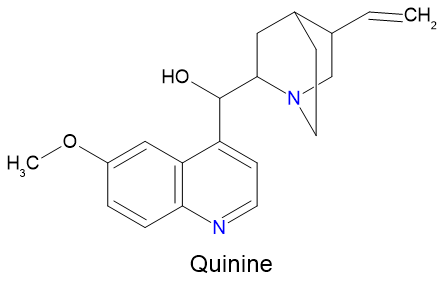
(EQ 1)

where P is the photon intensity registered by the detector after light has passed through the sample and Po is the photon intensity registered for the blank. This intensity will be measured as voltage in our setup.

***Quinine***

Quinine, derived from the bark of the cinchona tree, has a history as a potent antimalarial drug. Renowned for its bitter taste, quinine has also found an application in drinks like tonic water, imparting the distinct bitter flavor enjoyed in adult beverages like gin and tonic. While modern antimalarial drugs have largely supplanted quinine in medical practice, it remains a vital treatment in regions where drug-resistant strains of malaria persist. Additionally, ongoing research is exploring its use in treating other diseases, such as leg cramps and certain cardiac arrhythmias.

Quinine belongs to a class of organic compounds called alkaloids, characterized by their nitrogen-containing heterocyclic rings. The chemical formula of quinine (C20H24N2O2) indicates its molecular composition, but the structural arrangement of atoms (**shown below in Figure 2**) gives it its unique properties.

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**Figure 2. Structure of quinine.**

***Today’s Experiment***

In today’s experiment, you will be performing several tasks. First, you will assemble a single beam UV-VIS instrument out of Lego’s, and UV LEDs. Then you will use that instrument to quantify quinine in commercial tonic water. If your classroom has a research UV-VIS instrument (like a Spec20), your teacher may also have you use it to quantify quinine and compare those results to our simple, yet powerful device.

***Procedure***

This lab requires you to multitask. You need to build an instrument, take a bunch of measurements, and graph the data (on paper or in Excel). You may want to work with a partner to ensure you finish on time.

***Single Beam Spectrometer Assembly***

**What you need:** *Fourteen 2x3 rectangular bricks, two couch-looking bricks (colored white), four 1x2 bricks with through holes, an 8x8 baseplate to assemble the work on, two 365 nm LEDs, and a cuvette.*

***All parts are included in the Lead-to-Gold Publishing LLC DIY Spectrophotometer****.*

Using the pictures on the next page as a guide, work though and assemble this device. Keep in mind you may have different colored LEGO bricks, but the design should still be the same.

Some important information as you assemble this device:

1. When you place the LEDs into the brick with the hole, use some light pressure to insert the head into the LEGO brick. When you are finished, it should not move out of the front piece (the back brick though is loose around the two metal legs).
2. Each LED has two leads, one of which is longer than the other. Keep track of which leads are which. You will need to connect the red (positive) lead to the longer lead of the LED.

***Recording Absorbance Data Using the LEGO Spectrophotometer***

**What you need:** *A battery pack with three batteries, an 18 ohm / 1W resistor, a bread-board, four cables with alligator clips, a piece of red hook-up wire with the red plastic coating removed from both ends, and a voltmeter with leads.*

***All parts are included in the Lead-to-Gold Publishing LLC DIY Spectrophotometer.***

1. The system uses two LEDs, one which acts as a light source and the other as a detector. It does not matter which one you use for the light source or the detector, if you hook up the leads correctly.
2. You will use a total of four cables with alligator clips on the end – one RED, one BLACK, one GREEN and one WHITE. Use the GREEN and WHITE alligator cables to hook up the battery pack to the source LED. Attach the positive end of the battery terminal (RED) to the breadboard. Add the resistor and the RED hook-up wire to the breadboard. **Look at the photo below and make sure the resistor and leads are on the same side of the breadboard and in the same rows.** Then, connect one end of the GREEN alligator cable to the red hook-up wire and the other end to the longer lead of the source LED. Attach the negative end of the battery terminal (BLACK) to one end of the WHITE alligator clip and the other end of the WHITE alligator clip to the shorter lead of the source LED. You may need to bend the two LED leads to ensure the alligator clips or the leads themselves don’t touch. If your wires touch, you can start a small fire due to uncontrolled current flow. Be careful!
3. Allow the LED to equilibrate for ~10 minutes.

If you do not see light coming from the LED, something is wrong – most likely the leads are reversed. If you reverse the leads and you still don’t see a blue light, remove the batteries from the battery pack and check with your teacher.

A blue and white building blocks

Description automatically generatedA building blocks on a table

Description automatically generatedA blue and white building blocks

Description automatically generatedA group of blue and white building blocks

Description automatically generatedA group of white square objects with wires

Description automatically generatedA blue and white building blocks

Description automatically generated

**Step 5**

**Note:** The front blue brick has been removed so you can see the “couch bricks.”

**Note:** The longer lead is the positive one

**Step 4**

**Step 2**

**Step 3**

**Step 1**

A yellow digital multimeter with red and blue wires

Description automatically generatedA group of batteries connected to a building block

Description automatically generated

1. Use the other set of alligator cables (one RED and one BLACK) to hook up the voltmeter to the detector LED. Attach the positive end of the voltmeter to the longer LED lead and the negative end of the voltmeter to the shorter LED lead. This will ensure all voltages measured are positive.
2. Turn on the voltmeter and set it to the 2000 setting. If you detect negative values, your hookups are backwards.

**You should now have a working system!**

1. Open the plastic vial labeled “deionized water” (your blank), fill the cuvette to ~3/4 of the way full, and place it into the LEGO spectrophotometer. **Note: Make sure the arrow on the cuvette is pointing toward the LED source, the cuvette is fully inserted into the couch-looking white pieces, and it is standing straight up.**
2. Cover the system with the box the spectrometer came in, making sure not to knock any leads loose. Record the voltage value on a piece of paper – ideally, in your lab notebook. This will be Vo in your calculations.
3. Return the deionized water to its plastic vial and tap the cuvette gently on a paper towel to remove any excess solution. Now, find the vial labeled “10 ppm” and place that solution into the cuvette. Again, cover the system with the box and record the voltage (VX). Repeat these steps for all calibration solutions (going from low concentration to high) and your quinine unknown, remembering to write down the voltage each time.
4. When finished, unhook the leads and take out the batteries from the battery pack.
5. Disassemble the device, placing everything back where you got it. You may need to use a pair of pliers to pull the LED out of the brick with a hole.

***Waste Disposal***

All solutions can be reused if care is taken to return them to their proper containers. If your teacher instructs you to dispose of the solutions, they can be safely disposed of down the sink.

***Data Analysis***

Absorbance is calculated with the LEGO spectrophotometer using the voltages you collected above, exactly like it is calculated using photon intensity of light. The voltage from the detector LED is proportional to the photons that hit it. Thus, voltages are used in the equation instead of light power (i.e. Vo and Vx rather than Po and Px). To calculate the absorbance of any solution X (Absx), use the equation below, where Vo is the voltage measured for the blank and Vx is the voltage measured for solution X.

(EQ 2)

Included with your Lead-to-Gold DIY Spectrophotometer is a paper graph. You can also plot the data using the Excel file at [www.Pb2AuPublishing/DIY-Spectrophotometer](http://www.pb2aupublishing/DIY-Spectrophotometer).

A graph with lines and a line

Description automatically generated with medium confidenceIf you are asked to plot the data on paper, be careful to identify which is the x-axis (concentration) and which is the y-axis (absorbance). When you are done, you should have a straight line. Use a ruler to draw a line that best fits the points – don’t connect the points, rather draw a single, continuous straight line. Now, find the absorbance of the unknown on the y-axis. Use the ruler to draw a horizontal line from the value to the line you just drew. Then, draw a vertical line down to the x-axis – this is the unknown concentration. See the photo below for an example.

If you are asked to plot the data in Excel, download the workbook, open it, and fill in the cells highlighted in yellow. Your data will be plotted automatically, and the unknown concentration calculated.

**Questions for the Student**

1. What is the difference between an incandescent light source and an LED?
2. What is the purpose of the voltmeter? Why are we measuring voltage and not absorbance?
3. What is the difference between a single beam and double beam instrument?
4. What are two uses for quinine outside of the lab?
5. Which value is plotted on the x-axis, and which is plotted on the y-axis?
6. **Bonus:** Look up the Beer-Lambert Law on Google or Bing. How does it relate to today’s experiment?