A SIMPLE PHOTOMETER

Instruments are extensions of our senses. They allow us to perform measurements often more sensitively, reproducibly, and conveniently than we could unaided. In this experiment you are going to build a simple photometer. As you know, the intensity of light that passes through a solution containing an absorbing substance is related through Beer's Law to the concentration of the absorber.

\[ A = a b c \]

where \( a \) is the molar absorptivity of the solution, \( b \) is the path length of the cell, and \( c \) is the molar concentration. In place of your eye, we will use a phototransistor to gauge the intensity of the light. With most modern instrumentation, the measured parameter (in this case, the light intensity) is converted to an electrical parameter, such as current or more commonly voltage.

In general, a transistor might be thought of as a sort of electrical valve or faucet. Transistors control the flow of current. A faucet regulates flow according to how far you have turned the handle. A transistor controls the current between two wire leads (known as the "emitter and collector") by the amount of current flowing into the base lead. In a phototransistor the base current is proportional to light intensity. The current that flows from the emitter to collector is proportional to, but is much bigger than, the base current. Hence, a transistor can amplify a signal.

Rather than measure the current directly with an ammeter, we will convert the phototransistor current signal to a voltage, which will be directly proportional, but easier to measure than the current. We do this by letting the current pass through a resistor. Ohm's Law tells us that the voltage that will develop across the resistor is equal to the product of the current and the resistance (\( V = IR \)). We use a digital voltmeter (DVM) or the mV mode of a pH meter to measure this voltage as in the diagram below.
The phototransistor, voltage source, resistor, and DVM act as our detector. If we add a light emitting diode as a light source, a cuvette, and sample, we have the main components of a photometer or colorimeter. The voltage that we read on the DVM is proportional to the light intensity, or power (P), hitting the phototransistor. So, if we compare the light power (P) passing through our sample at a given wavelength, to the light power (P₀) passing through a cuvette with water or buffer under the same conditions, we can calculate the transmittance (T) and absorbance (A):

\[
T = \frac{P}{P₀} \quad \text{and} \quad A = -\log\left(\frac{P}{P₀}\right)
\]

where P and P₀ are the voltages measured for the sample and the water "blank", respectively. We should also note that a small current will pass through the transistor even when no light is hitting the device. This error is known as the "dark current". We must subtract the voltage that develops across the resistor when the transistor is in the dark from the other measured voltages in order to correct for this error.

\[
T = \frac{(P-P_{\text{dark}})}{(P₀-P_{\text{dark}})} \quad \text{and} \quad A = -\log\left(\frac{(P-P_{\text{dark}})}{(P₀-P_{\text{dark}})}\right)
\]

For stable readings, the voltage source must be kept constant across the photodiode. We will use a 9V voltage regulated wall transformer for this purpose.

Light emitting diodes, LEDs, are cheap and bright and relatively narrow wavelength (±20 nm). Our two LEDs we will use emit at 470 nm in the blue and 660nm in the red. The current through the LED must be held constant for constant light levels. Our circuit will use the 9V constant voltage source with a current limiting resistor for this purpose. The maximum current for our LEDs is 35 mA. We will use a 360 ohm resistor that results in a LED current of 20 mA. The blue photodiode can also be used to excite fluorescence, so the photometer will also be useful as a simple fluorometer.

**Fiber Optics**

To make the photometer very flexible for subsequent experiments, we will transmit the light from the photometer to the sample and collect the light from the sample using fiber optics. Fiber optics will make it easy to connect the photometer to different size cuvette holders and to a flow cell.

Fiber optics operate through total internal reflection. The light transmitted through the fiber reflects off of the internal surface of the fiber. The fiber therefore allows the light to be guided around corners. Total internal reflection is possible when the light reflects from a boundary between a higher index of refraction material and a low index of refraction material. The plastic core of these fibers in PMMA, polymethylmethacrylate. The fiber is coated with a thin layer of a fluoropolymer with a lower index of refraction. You can't actually see this layer, because in our fibers a black protective cladding is added to avoid damaging this fluoropolymer layer.
PROCEDURE

1. Set up the phototransistor circuit and arrange the components for the photometer and flow cell. Detailed directions will be supplied in the laboratory.

2. Measure and record the "dark current signal", i.e., the voltage when no light is hitting the phototransistor. Take the flow cell apart in the center section to block the light path.

3. Record the spectrum of the stock solution of one of the supplied food dyes using the HP diode array spectrophotometer. The light emitting diodes we use have a maximum emission at 470 or 660 nm. Note the absorbance at these wavelengths in relation to the peak wavelengths in the sample. Calculate the molar absorptivity at 660 nm.

4. Prepare four standards by dilution of the stock standard solution using volumetric glassware. Use a syringe to pull samples into the flow cell. Start with water. Measure the dark-current corrected P₀. Then measure the dark-current corrected P for each of the four standards. You will use this data to construct a calibration curve (Absorbance vs. concentration).

5. Plot the absorbance of your standards vs. concentration. Determine the linearity of your standard curve by determining the correlation coefficient. Calculate the molar absorptivity of the compound from the slope of your calibration curve. Remember to measure the cell path length. Compare the molar absorptivity from your photometer to that calculated from the spectrum using the HP diode array system. How linear is the calibration curve?

6. Test the blue diode as a source for measuring the fluorescence from a solution of 1.0x10⁻⁴ M rhodamine-B. You will just hold up the two fibers next to a flask of the dye in a darken area.