

will use the provided information to discover at what wavelength bromophenol blue absorbs the most light, the *optimum wavelength* for measuring light absorption by the dye. You will then construct a *standard curve* for bromophenol blue, using the wavelength of peak absorbance. In this way, you will use your standard curve to estimate the concentration of a sample of bromophenol blue whose concentration you do not know.

#### Traditional Absorption Spectrum Lab Procedure

1. Obtain 2 CLEAN test tubes, also called cuvettes.
2. Bring a small beaker to the front of the room, where there is a bromophenol blue solution that has already been prepared in a concentration of 0.01 mg/ml. Using a graduated cylinder, measure 50 ml of the blue dye, transfer it to the beaker and bring it back to your work station.
3. Fill one cuvette with distilled water; you will use this as a 'blank' to zero the spec. Fill the other cuvette with bromophenol blue solution; this is the sample you will assay (measure).
4. Following your TAs instructions, blank the spec. Then, take absorbance readings of the dye sample at the following wavelengths. (There are no true units for absorbance.)

Table 1.4 Absorbance of Bromophenol Blue

Wavelength (nm)	Absorbance
500	0.228
520	0.304
540	0.273
560	0.421
580	0.656
600	0.782
620	0.209

5. Using the data provided in the Exercise #1 PowerPoint, what is the peak absorbance of bromophenol blue?

The highest peak is found at wavelength  
600 nm.

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