Experiment 11

Spectroscopic studies on phenolphthalein derivatives

There are two phenolphthalein derivatives which are used extensively in biological labs, phenol red and bromophenol blue. Phenol red is used as a pH indicator for cell culture media. At neutral pH it is a reddish-pink color. As the cells consume the nutrients in the medium and produce acidic waste products, the color of the indicator changes to an orange or yellow color. Bromophenol blue is used as a loading dye for SDS-PAGE, among other uses. The purpose of this experiment is to record the absorbance spectra of these compounds at different pH values and determine the extinction coefficients and isosbestic points.

Materials

- 0.04% (w/v) Phenol red (FW = 354) solution
- 0.04% (w/v) Bromophenol blue (FW = 670) solution
- 0.1 N HCl (pH 1.0)
- 0.1 N NaOH (pH 13.0)
- 0.1 M Sodium phosphate buffer, pH 4.0
- 0.1 M Sodium acetate buffer, pH 6.0
- Microcentrifuge tubes
- Plastic cuvettes
- UV/visible spectrophotometer with spectral recording capabilities

Procedure

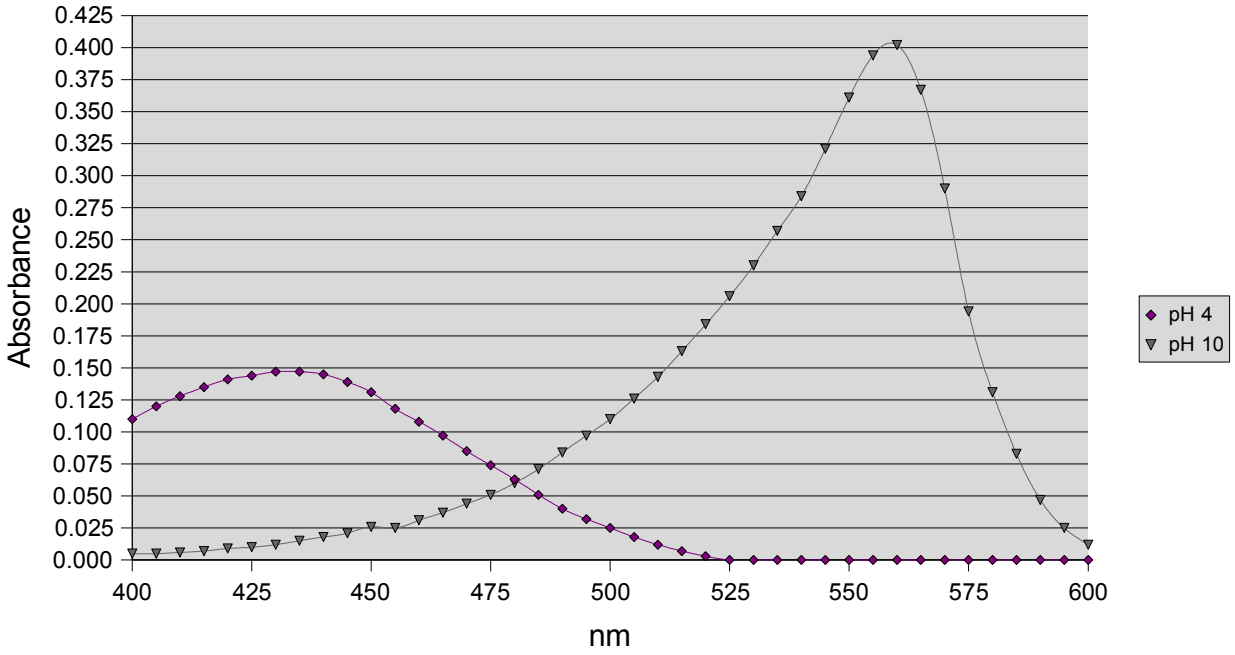
The class will be divided into four groups, two to three students in each group. Two groups will be working with bromophenol blue and two will be working with phenol red. Each group will prepare two tubes of phenolphthalein-derivative indicator, one at each of two pH values. The following steps will be performed by each student:

1. Make up either the phenol red or bromophenol blue solutions from the table below. You should be able to see visually that the color varies as a function of pH.

Bromophenol blue	Phenol red	0.1 N HCl	0.1 M phosphate buffer, pH 4.0	0.1 M acetate buffer, pH 6.0	0.1 N NaOH
10 ul		990 ul			
10 ul				990 ul	
	5 ul		995 ul		
	5 ul				995 ul

2. Record the full absorbance spectrum of each tube, from 400 to 600 nm at an increment of 5 nm. There are two different recording spectrophotomers available, and the procedure will vary somewhat depending on which instrument you use. Use water as a blank. Make sure that the peaks are within the linear range of the instrument (less than A = 0.8).

3. Plot the points on a common graph for both pH conditions. You should have something like what is shown below:



4. The isosbestic point should be immediately obvious. Make note of the wavelength in your notebook. Also note λ_{max} for each pH value. Calculate the extinction coefficient at three points, one for each pH peak and one at the isosbestic point. You will need to take into consideration the formula weights given in the Materials section in order to perform the calculation. Consult a chemistry textbook if you have any difficulty with this. **Answer this question in your notebook: of what possible value would knowing the extinction coefficient at the isosbestic point be?**