Teaching Spectrophotometry and Graphing Using Red Cabbage Extract and pH Buffers

Frances G. R. Kennedy

Biology Department
Agnes Scott College
Decatur, Georgia 30030
(404) 638-6267

Since students often come to an introductory science course with an insufficient math or science background, we found it useful to teach an exercise where students generate data that they use to construct a table and graph. Cabbage anthocyanins change color at different pH values and we introduce students to this effect of change of pH on these pigments. We also teach use of the Spectronic 20 spectrophotometer as a means of data acquisition, in this case with regard to the electromagnetic spectrum and visible colors. This exercise is performed early in the semester as preparation for later discussions and experiments regarding the effects of changes in pH relative to the action of enzymes during metabolic processes.

The first part of the exercise generates data over the same portion of the visible spectrum for each of four pH solutions. A composite graph is prepared in order to compare the absorption maxima for each of the different colored solutions.

The second part of the exercise deals with the serial dilution of a pigment solution. In this case, absorbance is plotted against pigment concentration relative to the initial amount in solution to demonstrate the linear relationship of pigment concentration to absorbance reading.

Shredded cabbage is boiled in water until grey in color, cooled, and filtered through cheesecloth to remove the cabbage pieces. The purple liquid that results may be diluted with water if the color is too intense. Buffer solutions are prepared from powder capsules and 3 ml of cabbage extract is added to 5 ml of each buffer solution used to prepare color standards. Students are instructed in the proper use of a Spectronic 20. They take a reading for each buffer solution at 40-nm intervals beginning with 400 nm and ending with 600 nm readings. In this process they should realize that different colors absorb more or less light at the same wavelength. The data are collected into table format. Graphing of data is discussed and each student makes a composite graph of the data collected. This task introduces the concept of dependent and independent variables and how to determine one from the other.

Students then select a pH solution and consider the 600 nm reading as the full concentration of pigment in solution. A series of three 1:2 dilutions are made with a reading taken of each dilution as it is made. The independent variable must again be determined and is a bit more subtle. If done correctly, the graph should show the linear relationship between concentration and absorbance at a given wavelength.