Experiments
Учебен експеримент в природните науки

AN ANALYTICAL EXPERIMENT USING BEER'S LAW FOR A MIXTURE OF TWO DYES

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Abstract. This is a short communication of an inexpensive and safe experiment used in Analytical Chemistry. The experiment uses the idea of Beer's law from a Freshman Chemistry Laboratory course for one material, where the absorption of light changes with the amount of material. However, in this experiment, it is expanded for two materials. Plus, since both materials are dyes and usually readily available in a College or University, it makes it both affordable and easy to use.
Keywords: Beer's law; analytical laboratory; visible spectroscopy

Introduction

Simply stated, Beer's law for one absorbing material is A = ecl, where A is the absorbance, e is the molar absorptivity which is wavelength dependent, c is the concentration, and l is the path length. Typically, one uses a simple Visible spectrometer to measure the absorbance as a function of wavelength. One can also use a Spec 20 spectrometer, where the absorbance is measured at a unique wavelength. Now, for two absorbing materials (X and Y), one has to use an expanded version of Beer's law, with two different wavelengths (Eqs. (1) and (2)), and allowing the length l = 1, which is typical for most laboratories.

$$A_1 = \varepsilon_{1x}(X) \quad + \quad \varepsilon_{1y}(Y) \qquad (1)$$
$$A_2 = \varepsilon_{2x}(X) \quad + \quad \varepsilon_{2y}(Y) \qquad (2)$$

where A_1 represents the absorption at wavelength 1, e_{1x} represents the molar absorptivity constant for material x at wavelength 1 and e_{1y} is the molar absorptivity constant for material y at wavelength 1, and (X) and (Y) are the concentrations, respectively. Eq. (2) is the same, but at the second wavelength. There are a few ways to solve for these two equations. Using linear algebra or a computer program will work (Christian, 2004; Silbey et al., 2001). The other way is to directly solve for X and Y, and then one obtains

$$(X) = A_1\ \varepsilon_{2y} - A_2\ \varepsilon_{1y}\ /\ \varepsilon_{1x}\ \varepsilon_{2y} - \varepsilon_{1y}\ \varepsilon_{2x} \qquad (3)$$

and

$$Y) = A_2\, \varepsilon_{1x} - A_1\, \varepsilon_{2x} / \varepsilon_{1x}\, \varepsilon_{2y} - \varepsilon_{1y}\, \varepsilon_{2x} \quad (4)$$

In order to use Eq. (3) and (4), you must measure the absorption for each material separately at a given wavelength (Eq. (1) or Eq. (2)) at different concentrations in order to determine their e values. Once this is done, you can measure a sample of the two unknowns, and obtain the concentrations of X and Y.

Laboratory

For our experiment, we used two commercial dyes, one, a very common food dye, Red 40 (Spectrum Laboratory Products) and the other, a very common dye used for staining in a microbiology laboratory, Crystal Violet (Fisher). These two were picked as they are well known, inexpensive, easy for disposal, and have a nice spectral overlap, as shown in Fig. 1. It is critical, that the two absorbing materials have a sizable overlap, and have an absorbance no larger than one, otherwise results will not be reproducible. For our experiment, we used a Molecular Devise Spectra Max M2^{e} Visible spectrometer in the range of 350 – 750 nm for the total Visible spectrum. This could have been used for the students' data, but the students preferred to us a Genesys Spectronic 20 (Spec 20) spectrometer for the data collection, as it was easier to obtain the data for further analyses, such as plotting and putting into an Excel spreadsheet. For this experiment, the two wavelengths used were 500 and 550 nm, as both dyes absorb at those wavelengths, as seen in Fig. 1.

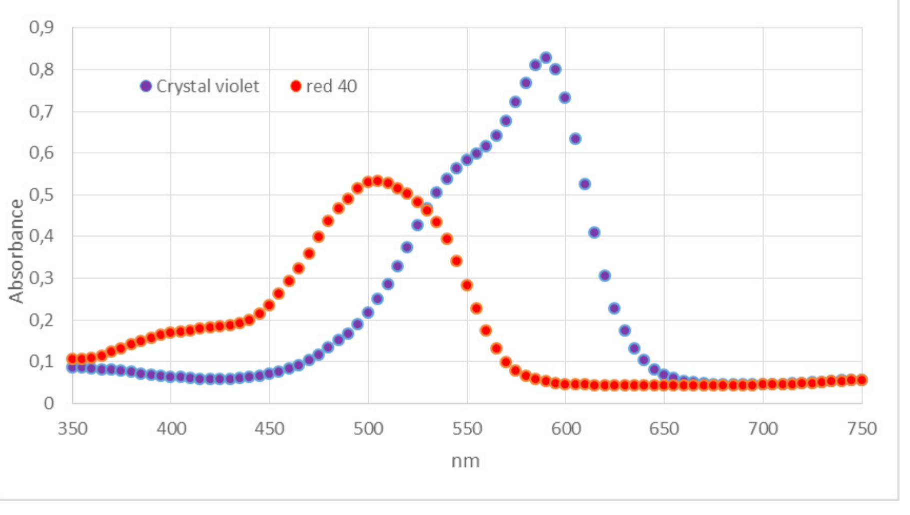

Figure 1. The absorbance of Crystal Violet and red 40 vs wavenumber

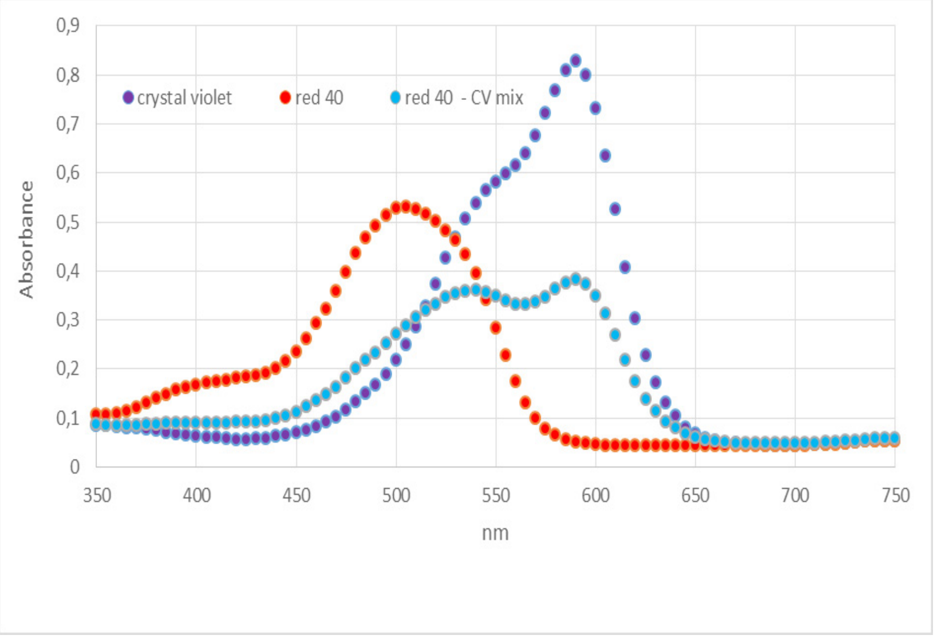

Figure 2. The absorbance of the two dyes and the mixture vs nm

Six concentrations or more of each dye were used, and data were obtained at each wavelength. Students were then required to plot A vs c for both dyes, at both wavelengths, in order to obtain the molar absorptivity constants, as well as show the statistical results. This is fairly easily done on a standard formatting program such as Excel. Then, each student, or group of students would measure the absorption on an unknown, a mixture of the two dyes, and determine the percent of each dye in the mixture. For our lab, we had 4 groups of students, each determining two unknowns, and all the results were within 15% or better. In addition, students would obtain a spectra of the mixture, just to give them a better perspective, as shown in Fig 2.

Conclusions

This experiment is a nice way to use a Visible spectrometer, learn and be able to use Beer's law for two samples, and obtain experience on an Excel spreadsheet. The lab is safe, fun, and allows students to see how well they can perform. Options to the experiment might include statistics of the unknown data if there are enough students, different wavelength use, and different spectrometers.

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