Spectrophotometry for Quantitative Analysis

Modern chemical analysis has routinely used spectrophotometry in agricultural, clinical, environmental, pharmaceutical, and quality control laboratories for over fifty years. Spectrophotometry is the study of absorption or emission of light by a chemical species. The versatility and ease of spectrophotometry makes it a cost-effective way to analyze large numbers of samples and even provide in-line quality assurance for the manufacturing of food, beverage, agrochemicals, and pharmaceuticals. For example, this technique is routinely used in the beverage industry to monitor phosphates, sugars, and coloring agents in soft drinks. The "Tools in the Laboratory" section "Spectrophotometry in Chemical Analysis" found in chapter 7 of Silberberg's Chemistry: *The Molecular Nature of Matter and Change* effectively introduces the ideas of spectrophotometry. This supplement will expand on the ideas of utilizing spectrophotometry as a tool for quantitative analysis.

The basis for using spectrophotometric measurements to quantitatively analyze a light-absorbing chemical species, generically called an analyte, in solution is the Beer-Lambert law:

$$A_\lambda = \varepsilon_\lambda b c$$

where A_λ is absorbance at a given wavelength, ε_λ is the molar absorptivity at that wavelength (formerly known as molar extinction coefficient), b is the distance the light travels through the solution (called the pathlength), and c is the concentration of the analyte in solution. The Beer-Lambert law simply states that absorbance is directly proportional to the concentration of analyte in the sample. One must know A_λ, ε_λ, and b to determine an unknown concentration, since:

$$c = \frac{A_\lambda}{\varepsilon_\lambda b}$$

Therefore, if the solution pathlength is defined by the sample compartment, often called a cuvette, and ε_λ is known, measuring A_λ for a solution allows the concentration of the absorbing species in solution to be calculated.

Absorbance is measured by a spectrophotometer as illustrated in figure B7.3 in the Silberberg text. Generally, simple spectrophotometers have a light source that emits light of all wavelengths (~190 – 1100 nm) in the visible and ultraviolet regions. The absorbance is quantified one wavelength at a time by use of a monochromator that selects the wavelength or series of wavelengths of interest. The light then passes through a cuvette which has a fixed pathlength, b. Finally, a detector measures the intensity of the light that has passed through the sample, I, and compares it to the intensity of light that passed through a 0.0 M solution, I_0. The ratio of I/I_0 is a measure of the fraction of light that passes through the sample and is called the transmittance. Absorbance is related to transmittance:

$$A_\lambda = -\log \frac{I}{I_0}$$

Imagine that a pharmacist finds the labels on two insulin prescriptions have fallen off the bottles. To conserve costs and not waste the medication, the pharmacist prepares samples by precisely diluting 1.000 μL from each vial to 10.000 ml water. With a 1.000 cm cuvette and the spectrophotometer set to detect at a wavelength of 280 nm, the pharmacist measures the absorbance of each sample. The A_{280} values are found to be 0.43 and 0.58. The published ε_{280}

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for insulin in aqueous solution is 5,510 L/mol•cm, the pharmacist can now determine the unknown concentration of each insulin vial. A basic application of the Beer-Lambert law followed by a $M_1V_1 = M_2V_2$ calculation can solve the problem. The known values:

$$A = \varepsilon bc$$

$$A_{280,vial\ 1} = 0.43$$

$$A_{280,vial\ 2} = 0.58$$

$$\varepsilon_{280} = 5510 \frac{L}{mol \bullet cm}$$

$$b = 1.000\ cm$$

Solving for the concentration gives:

$$c = \frac{A_{280}}{\varepsilon_{280}b} = \frac{0.43}{\left(5510\ \frac{L}{mol \bullet cm}\right)(1.000\ cm)} = 7.8 \times 10^{-5} M$$

$$M_1 = \frac{M_2V_2}{V_1} = \frac{\left(7.8 \times 10^{-5}\ \frac{mmol}{mL}\right)(10.000\ mL)}{1.000\ \mu L} \times \frac{1000\ \mu L}{1\ mL} = 0.78\ M$$

Similarly, for the second insulin vial:

$$c = \frac{0.58}{\left(5510\ \frac{L}{mol \bullet cm}\right)(1.000\ cm)} = 1.1 \times 10^{-4} M$$

$$M_1 = \frac{M_2V_2}{V_1} = \frac{\left(1.1 \times 10^{-4}\ \frac{mmol}{mL}\right)(10.000\ mL)}{1.000\ \mu L} \times \frac{1000\ \mu L}{1\ mL} = 1.1\ M$$

The pharmacist can now correctly relabel the insulin vials.

A continuous absorption spectrum for chlorophyll *a* is shown in figure B7.4 of the Silberberg text. Spectra such as these can be utilized to calculate ε_λ for the analyte from the known concentration in a particular solvent and measured absorbance, A_λ, at the wavelength of maximum absorption, since

$$\varepsilon_\lambda = \frac{A_\lambda}{bc}$$

Two areas of maximum absorption, 431 nm and 663 nm, A_{431} and A_{663}, are present in the continuous spectrum of chlorophyll *a*. This one spectrum can determine either ε_{431} or ε_{663} values, but more accurate values of either ε_λ can be determined by plotting A_λ versus *c* for a series of solutions. The equation $A = \varepsilon bc$ results in a straight line for εb when A is plotted versus *c*.

For example, suppose the percentage by mass of chlorophyll *a* in the algae of a local lake needs to be determined. After chlorophyll *a* is extracted from the algae with 90% acetone and

diluted to a known value, the absorbance can be measured and compared to known concentrations of chlorophyll a in the experimental solvent. First, a series of six known concentrations of chlorophyll a are prepared in 90% acetone solutions and analyzed for absorbance values to give the following data:

Solution	Concentration of Chlorophyll a in 90% Acetone	Absorbance at 663 nm
1	1.80×10^{-6} M	0.144
2	3.60×10^{-6} M	0.259
3	5.40×10^{-6} M	0.442
4	7.20×10^{-6} M	0.564
5	9.00×10^{-6} M	0.717
6	1.08×10^{-5} M	0.843

The molar absorptivity of chlorophyll a can be determined from the following plot of absorbance, A_{663} versus concentration:
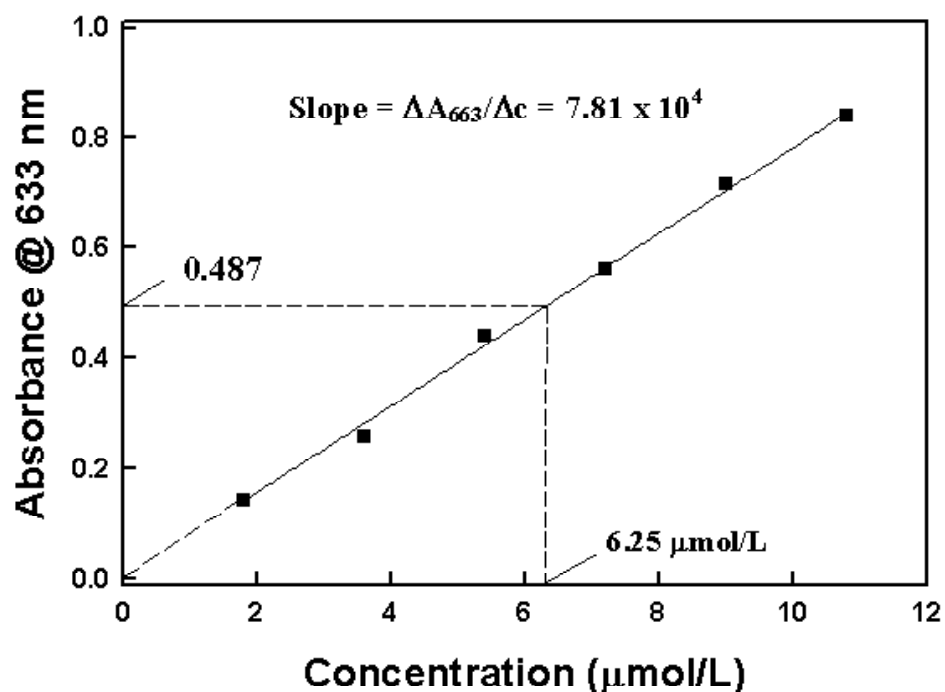
Via linear regression of the data, the slope of the line, $\Delta A_{663}/\Delta c$, is 7.81×10^4 L/mol and represents the product $\varepsilon_{663}b$. The pathlength, b is defined at 1.000 cm by the cuvette, therefore, ε_{663} is 7.81×10^4 L/mol•cm.

Chlorophyll a is extracted from a 0.2105 g sample of the dried algae into approximately 50 mL solution of 90% acetone by soaking the mixture for 1 hour. The mixture is filtered and rinsed with more 90% acetone. The resultant solution is then diluted to 1.000 L in a volumetric flask. Finally, A_{663} is measured on a portion of the solution with a spectrophotometer and found

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to be 0.487. The Beer-Lambert law allows the chlorophyll *a* concentration of the solution to be calculated:

$$c = \frac{A_{663}}{\varepsilon_{663}b} = \frac{0.487}{7.81 \times 10^4 \ L/mol} = 6.24 \times 10^{-6} \ mol/L = 6.24 \ \mu mol/L$$

The graph can also be used to directly find *c* by interpolating the concentration that corresponds to $A_{663} = 0.487$. The dashed lines on the graph show this interpolation and yield an approximate value $c = 6.25 \ \mu$mol/L. This value agrees with the value obtained above.

The extraction and dilution to 1.000 L of a 0.2105 g sample of algae was used to determine the chlorophyll *a* content. The determined concentration can be used to calculate the total number of moles of chlorophyll *a* in the algae:

$$\text{mol of chlorophyll } a = 1.000L \times 6.25 \ \frac{\mu mol}{L} = 6.25 \ \mu mol$$

The mass of chlorophyll *a* present in the algae is:

$$6.25 \ \mu mol \times \frac{1 \ mol}{1 \times 10^6 \ \mu mol} \times \frac{893.5 \ g}{mol} = 5.58 \times 10^{-3} \ g$$

The percent chlorophyll *a* by mass in the 0.2105 g sample of algae is:

$$\frac{5.58 \times 10^{-3} \ g}{0.2105 \ g} \times 100\% = 2.65\%$$

As demonstrated by these typical examples, spectrophotometry is a valuable tool in quantitative analysis. Generally, these analysis procedures include the following steps:

1. A series of solutions with known concentrations are used to measure absorbance of the analyte and prepare a calibration plot (Beer-Lambert law plot).
2. The absorbance is measured for the solution of unknown concentration.
3. The unknown concentration is determined by using the calibration plot.