UV Vis Absorption Experiment 1: Beer-Lambert Law and Identification of an Unknown Mixture

Overview

In the first part of this experiment, UV Vis spectra will be recorded for several, simple aromatic molecules in toluene solution. Beer Lambert plots will be constructed for each aromatic spices and the molar extinction coefficient determined. In the second part of the experiment, unknown samples will be investigated. Given the information derived in part one, UV Vis spectroscopy will be used *qualitatively* to identify the species, and then *quantitatively* to determine the amount of each component present in the unknown sample.

Version 1.0

Introduction

Ultraviolet-Visible (UV-Vis) spectroscopy[1] is concerned with measurement of the interaction of outer shell electrons with electromagnetic radiation[1] in the range 190 nm to 700 nm. Electrons of organic compounds with a high degree of conjugation or aromaticity, transition metal ions and charge transfer complexes are active in this part of the spectral region[1]. Compounds with the ability to interact with or *absorb* UV-Vis radiation are given the general name *chromophores*. UV Vis spectroscopy has many uses across science such as in monitoring the progress of a chemical reaction, through analysis of pharmaceutical substances and in biology, in investigation of proteins. This experiment is concerned with chemistry and analytical science and introduces the concepts of *qualitative* and *quantitative* analysis through use of UV-Vis spectroscopy.

Qualitative Analysis is defined as analysis in which substances are identified or classified on the basis of their chemical or physical properties, such as chemical reactivity, solubility, and in this experiment, UV Vis absorption characteristics. *Quantitative Analysis*, on the other hand, refers to analysis in which the amount or concentration of an analyte is determined. This experiment will demonstrate that *Quantitative Analysis* can also be performed by using UV Vis spectroscopy.

UV- Vis spectroscopy involves measurement of the fraction of incident electromagnetic radiation that is either absorbed or transmitted by a sample. The measurement can be performed at a single wavelength or across a broad range of wavelengths when a spectrum[1] is produced. Compounds can then be characterized *qualitatively* and identified by comparing the absorption spectrum produced with the spectra of known compounds.

The basis of UV- Vis spectroscopy is shown in Figure 1:

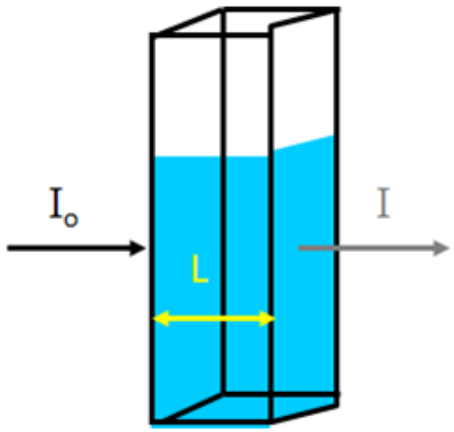


Figure 1 Basis of a uv vis measurement

This process can be described by The Beer Lambert Law[1] which relates the incident light intensity (I_o) at the front of a cell of length, L, (in centimetres) of a single absorbing species of concentration, c, to that which is transmitted by the sample [i.e., the intensity of light (I) which emerges from the cell]. ε is known as the molar extinction coefficient and is a measure of the probability of absorption of a photon[1] of light by a compound. The Beer Lambert law is expressed formally in equation 1:

$$\log_{10}(I_o/I) = -\log_{10}(\%\,T/100\%) = \varepsilon\, c\, L = A \qquad \text{Equation 1}$$

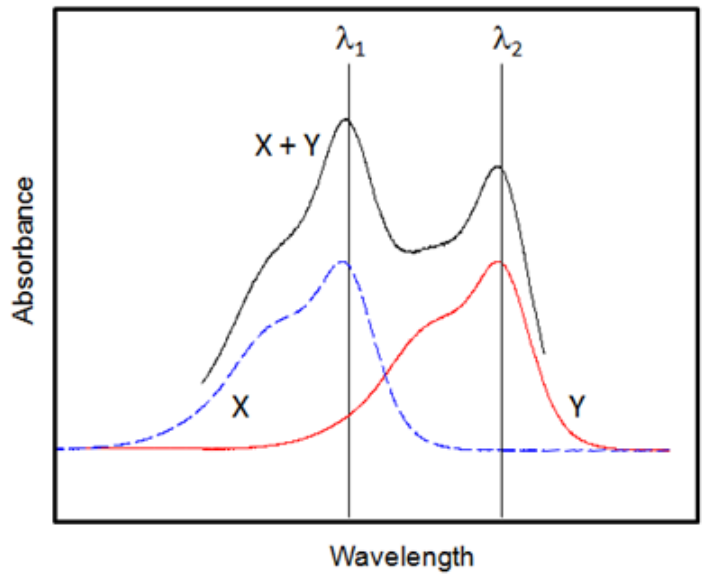


Figure 4: The spectrum produced by a mixture of components X and Y. Analyses wavelengths for the mixture, λ_1 and λ_2 are also shown.

Since the total absorbance (A_{total}) of the solution at a given wavelength is equal to the sum of the absorbances of the individual components (as in Equation 2), it is possible to analyse the individual constituents of a mixture even when their spectra overlap.

$$A_{total} = ABS_1 + ABS_2 \qquad \text{Equation 2}$$

Clearly from Figure 4, there is no wavelength we can select that does not contain a contribution of absorbance from both X and Y. To overcome this problem, we must first choose wavelengths which are well separated from one another (i.e., λ_1 and λ_2 in Figure 4.)
The absorbance of a solution containing two chromophores, X and Y will then be equal to the sum of the absorbances of each of the components at λ_1 and λ_2:

$$\lambda_1 : A^1 = \varepsilon_X{}^1 L C_X + \varepsilon_Y{}^1 L C_y \qquad \text{Equation 3}$$
$$\lambda_2 : A^2 = \varepsilon_X{}^2 L C_X + \varepsilon_Y{}^2 L C_y \qquad \text{Equation 4}$$

The four molar extinction coefficients, $\varepsilon_X{}^1$, $\varepsilon_X{}^2$, $\varepsilon_Y{}^1$ and $\varepsilon_Y{}^2$ can be derived from standard solutions containing only X or Y. Then, if the absorbances of the mixture are measured at λ_1 and λ_2, the concentration of the individual components can be calculated by solving the two simultaneous equations (Equations 3 and 4).

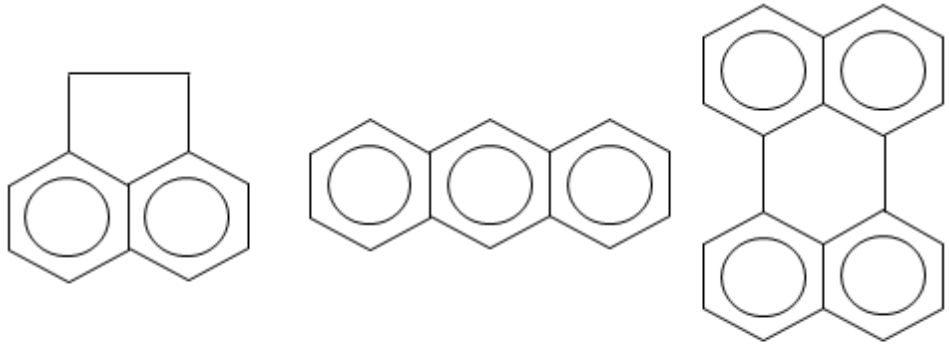
It follows, in general, for a solution containing *n* components that

$$A_{total} = ABS_1 + ABS_2 + \ldots ABS_n \qquad \text{Equation 5}$$

If the optical path length and the molar extinction coefficients for each component are known, the concentration of each chromophore can be determined by measuring the total absorbance of the solution at *n* wavelengths and solving *n* simultaneous equations.

Aims

In this experiment, you will prepare samples containing different concentrations of a simple aromatic compound, acenaphthene, (see Figure 5 for the structure) in toluene. You will then record UV Vis absorption spectra for the various acenaphthene, samples. You will subsequently construct a Beer Lambert plot and determine the molar extinction coefficient for acenaphthene in toluene. You will repeat this procedure and obtain spectra for anthracene and perylene, respectively (see Figure 5 for the structures) in toluene. You will then construct Beer Lambert plots and determine the molar extinction coefficient for anthracene and perylene, respectively, in toluene. In the second part of the experiment, you will be given an unknown sample. Given your experience and the information derived in part one you will use UV Vis spectroscopy *qualitatively* to identify the species present, and then *quantitatively* to determine the amount of each component present in the unknown sample.



acenaphthene *anthracene* *perylene*

Figure 5 Structures of aromatic chromophores

Experimental Procedure:

Part 1: Absorption Behaviour of Simple Aromatic Chromophores

References

1. Sim Pack 4: Download Notes on UV-Vis Spectroscopy & the Beer Lambert Law, Copyright 2012, sim4t.com.