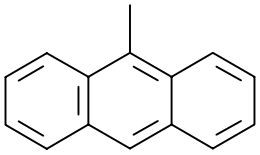
MULTICOMPONENT ANALYSIS USING UV/VIS SPECTROPHOTOMETRY

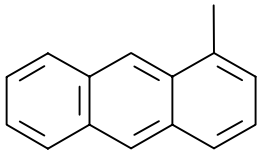
Introduction

The goal of this experiment is to determine the concentrations of two hydrocarbon isomers, 1-methylanthracene and 9-methylanthracene, in a solution using absorbance measurements in the ultraviolet region of the spectrum. Both compounds are *polycyclic aromatic hydrocarbons* (PAHs), compounds that are generated during the incomplete combustion of organics. PAHs are ubiquitous environmental organic pollutants, and many are suspected carcinogens.

The structures of the analytes is given below:



9-methylanthracene



1-methylanthracene

Since the compounds are so similar, it might be expected that there is considerable overlap of absorption spectra. In this experiment, you will demonstrate that it is possible to measure the concentrations of both isomers in a mixture using spectrophotometry.

Background

Molecular Absorption

Spectroscopy is the study of the interaction of light and matter; in *spectrochemical analysis*, this interaction is used as the basis for chemical analysis. Spectrochemical methods (which include methods such as IR and NMR spectroscopy) are perhaps the most widely used group of techniques in analytical chemistry; they are used for structure analysis, compound identification and quantitative analysis.

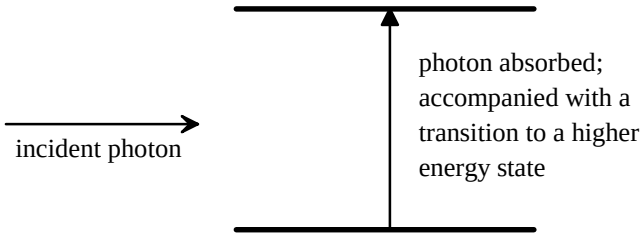
Almost all spectrochemical methods of analysis depend in some way on the *absorption* of light energy by atoms or molecules. Light can be considered to consist of energy packets called *photons*, each with energy

$$E = h\nu = h\frac{c}{\lambda}$$

where h is Planck's constant, c is the speed of light, ν is the frequency of the light, and λ is the wavelength of the light.

Recall that the energy possessed by an atom or molecule is *quantized*. Let's imagine an encounter between a photon and an atom, molecule or ion. If the energy of the photon exactly matches the energy

difference between two energy levels in the chemical species, then there is a chance that the photon may be absorbed by the species, with a corresponding increase in energy:

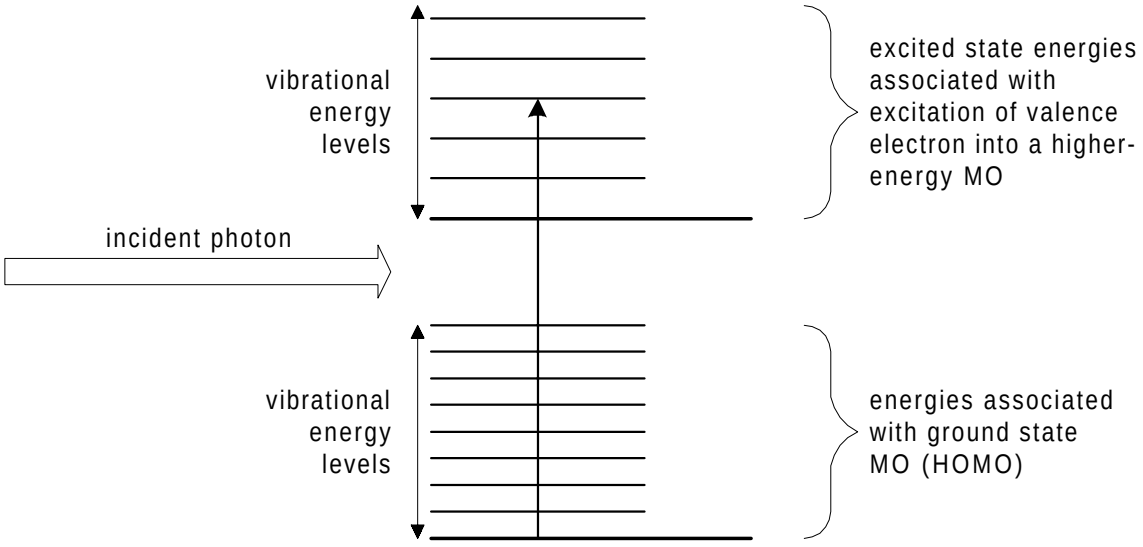


The probability that a photon is absorbed when it encounters an atom, ion or molecule is called the *transition probability* of the chemical species.

The internal energy of a molecule or a molecular ion is partitioned between various forms:

- *electronic* energy: energy possessed by electrons in their molecular orbitals, due to electrostatic interaction with the protons in the atomic nuclei and with the other electrons
- *vibrational* energy: energy due to periodic changes in distances (the "vibrations") between the atoms in the molecule;
- *rotational* energy: energy due to the "tumbling" motion of the molecule

Two distinct energy levels in a molecule may correspond to different amounts of electronic, vibrational and rotational energy. Each of these forms of energies are quantized into discrete energy levels. Thus, photon absorption by a molecule is frequently represented by a diagram such as the following:

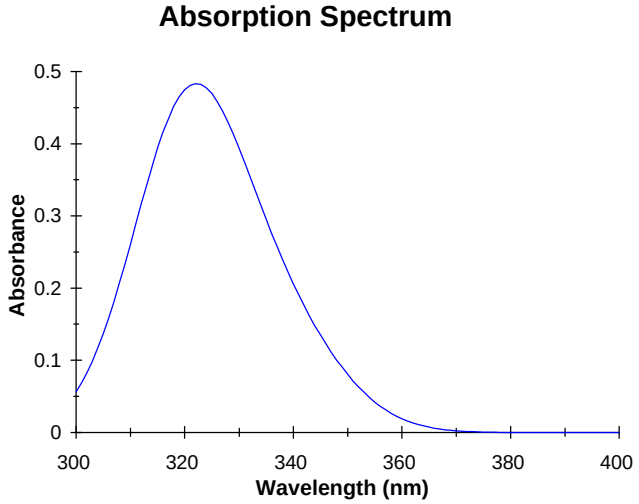


This figure shows molecular energy levels associated with two molecular orbitals, the ground state (HOMO) and an excited state. The longer, heavier horizontal lines represent the lowest vibrational energy levels associated with an MO, while the shorter lines represent different excited vibrational energy levels for the molecule when the valence electron is in these orbitals. Note that the distance

between these levels is not necessarily the same when the molecule is in the HOMO or LUMO energy states; this is because the bond energies are likely to be different in these two states.

Almost all molecules at room temperature occupy the lowest energy vibrational energy level of the HOMO state. Photon absorption will promote an electron to a molecular orbital of higher energy (the LUMO, or an even higher-energy orbital); some of the photon energy may also cause the molecule to vibrate more rapidly (i.e., to occupy an excited *vibrational* energy level). This situation is shown in the previous figure, where photon absorption causes the molecule to make a transition from the lowest energy vibrational level in the HOMO to an excited vibrational energy level in the LUMO state. Such transitions are called ***vibronic*** (“*vibr*ational and electr*onic*”) transitions.

I’m sure (!) you are asking yourselves, “why are the rotational energy levels ignored in the figure?” Certainly, if the molecules where in the gas phase we must also consider the quantized rotational energy levels associated with each MO. However, in solutions interactions of the solute molecule with surrounding solvent causes a “blurring” of rotational energy levels, resulting in overlap of the energy levels of different rotational states. This causes broader, more featureless absorption spectra for the solvated molecule compared to the free molecule in a gaseous state, such as the one in the following figure.



As shown here, an absorption spectrum is a plot of *absorbance* against photon wavelength. The absorbance, *A*, of a chemical sample is defined by

$$A = -\log\left(\frac{I}{I_0}\right) = -\log(T)$$

where I_0 is the intensity of the light incident upon the sample and I is the intensity of the light after it passes through the sample. These intensities are measured by the spectrophotometer; the difference between I and I_0 will be due to light absorption of chemical species in the sample. The ratio $\frac{I}{I_0}$ is called the *transmittance* of the sample; it is the fraction of light that is not absorbed. When expressed as a percentage, this quantity is referred to as the “percent transmittance.” Note that two measurements (for I and I_0) are necessary to determine the absorbance of any sample.

Let’s imagine that we have a solution that contains a single analyte solute species. The absorbance of the solution will depend primarily on four important parameters:

• the *wavelength* of the light incident upon the solution;

• the *identity* of the absorbing solute species;

• the *concentration* of the solute species;

• the *pathlength* that must be traveled by the photons through the solution.

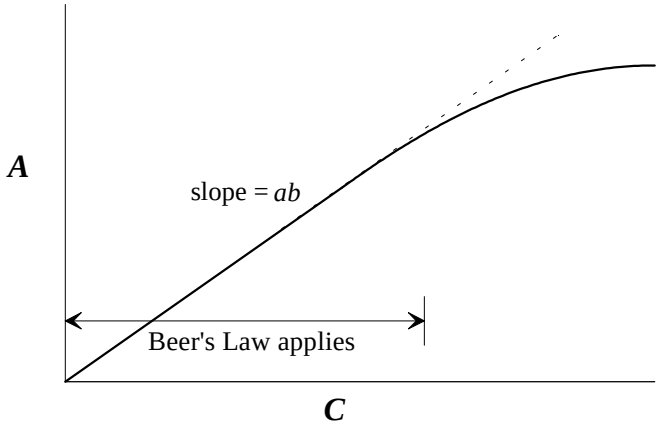
All of these parameters are collected in ***Beer's Law***, which states that

Beer's Law $$A_\lambda = a_\lambda b C_A$$

where a_λ is the ***absorptivity*** of the solute at wavelength λ, b is the pathlength and C_A is the concentration of analyte in the solution. The absorptivity is sometimes also called the *extinction coefficient*. Most commonly, the pathlength, b, is expressed in cm and the concentration, C, in M; in this case, the absorptivity is referred to as the ***molar absorptivity*** and given the symbol ε_λ.

The absorptivity is directly proportional to the transition probability of the analyte molecules, which depends on the wavelength. The use of the subscript λ in Beer's law emphasizes the dependence of absorptivity, a_λ, and absorbance, A_λ, on photon energy (i.e., wavelength).

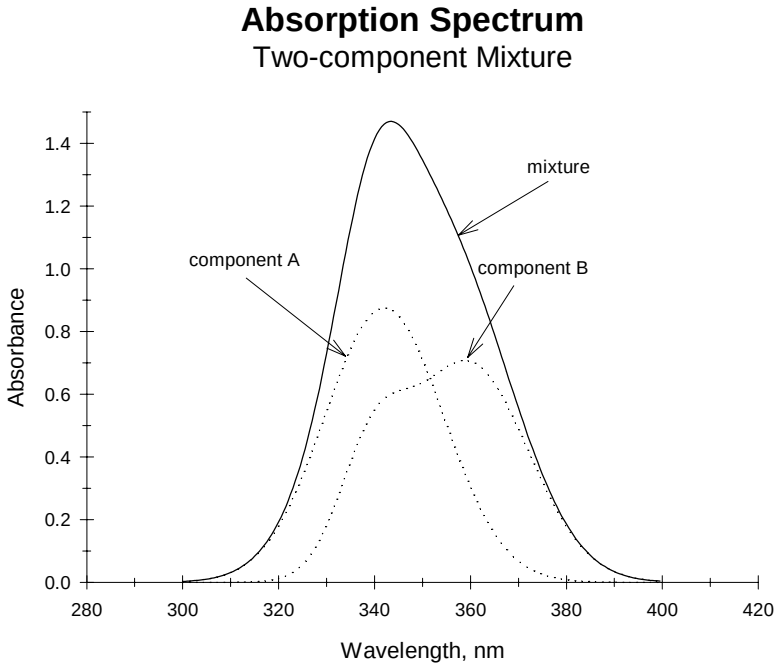
From Beer's law, if the cell pathlength and concentration of the compound are known, it is possible to calculate the molar absorptivity at any desired wavelength. Before doing so, however, it is a good idea to check for deviations from Beer's law. Beer's law is usually only followed over a certain concentration range of absorbers. One way to evaluate the range over which Beer's law is valid is to obtain absorption measurements of different concentrations of analyte. The value of a compound's absorptivity can be calculated from the slope of a plot of absorbance at the wavelength *vs* concentration. Such a plot is called a **Beer's Law Plot**. The slope of a Beer's Law plot is the product of absorptivity and pathlength, $a_\lambda \times b$, as shown in the following figure.



A number of compilations of molar absorptivities (ε_λ) exist, so it is customary when reporting absorptivities to convert the value obtained from a Beer's Law plot to units of $cm^{-1}M^{-1}$.

Multicomponent Analysis using Molecular Absorption

Beer's law is valid simultaneously for all absorbers in a solution. Let's imagine that we have two analyte species, *A* and *B*, present in a solution. The absorbance due to the two individual species will usually add to give the total absorbance of the solution, as shown in the following figure:



In the situation shown in this figure, it is not easy to measure the absorbance of one component without the other component interfering in the measurement. The absorbance at any wavelength is due to the absorbance of each species:

absorbance at wavelength λ = absorbance by species *A* + absorbance by species *B*

Beer's Law apoplies to each absorbing species independently; thus, we may express the absorbance at any wavelength as

$$A_\lambda = a_\lambda^{\mathrm{A}} b C_{\mathrm{A}} + a_\lambda^{\mathrm{B}} b C_{\mathrm{B}}$$

where a_λ^{A} is the absorptivity of species A at wavelength λ.

We may use this additive property of absorbance to determine the concentration of both components of a binary mixture by measuring the absorbance at two different wavelengths. For example, in the situation shown in the figure, we might measure the absorbance at λ = 340 nm and λ = 360 nm to obtain the following equations:

$$A_{340} = a_{340}^{\mathrm{A}} b C_{\mathrm{A}} + a_{340}^{\mathrm{B}} b C_{\mathrm{B}}$$

$$A_{360} = a_{360}^{\mathrm{A}} b C_{\mathrm{A}} + a_{360}^{\mathrm{B}} b C_{\mathrm{B}}$$

Usually the pathlength, *b*, is known; thus, we can solve these simultaneous equations for the concentrations of species A and B *if the absorptivity coefficients of both species at both wavelengths are known*. In this experiment, the absorptivity coefficients of each PAH isomer will be determined at two different wavelength by Beer's Law plots. The absorbance of the sample mixture will then be obtained for the same two wavelengths; solution of simultaneous equations will thus enable us to obtain estimates of the PAH concentrations.

References

• Skoog 13A-B, 13D, 14D

• Harris 19.1-19.5

MULTICOMPONENT ANALYSIS: *PROCEDURE*

The solvent for this experiment is cyclohexane; your sample is a solution of the two PAH isomers in cyclohexane. You will also be supplied with stock solutions of your analytes. Prepare four calibration standards for each PAH in the range of 3 – 50 μg/mL. Use 25 mL volumetrics for the standards.

Your first task is to choose the wavelengths that you will use for your analysis. Using your most concentrated standard, collect the absorption spectra of both analytes from 400 – 290 nm, at a scan rate of 100 nm/min. You will also want to check the absorption spectrum of your sample mixture. Can you tell from your spectrum that your sample contains a mixture of the PAHs? Based on the spectra you have taken, choose two wavelengths for your multicomponent analysis.

Once the wavelengths are chosen, obtain accurate measurements of the absorbance of the blank (cyclohexane) and all the standards at *each* of the chosen wavelengths. Also determine the absorbance of the sample solution at the appropriate wavelengths. You might want to check that the measurements of your sample solution are within the calibration range, but check with your instructor before making any dilutions of the sample.

MULTICOMPONENT ANALYSIS : *DATA SHEET*

Name: _____ unknown #: _____

Preparation of calibration standards

	1-methylanthracene	9-methylanthracene
conc of stock solutions		
standard 1 (pipet vol)	mL	mL
standard 2 (pipet vol)	mL	mL
standard 3 (pipet vol)	mL	mL
standard 4 (pipet vol)	mL	mL

Absorbance Measurements

	λ_1 = _____ nm	**λ_2 = _____ nm**
blank		
std 1 (1-methylanthracene)		
std 2 (1-methylanthracene)		
std 3 (1-methylanthracene)		
std 4 (1-methylanthracene)		
std 1 (9-methylanthracene)		
std 2 (9-methylanthracene)		
std 3 (9-methylanthracene)		
std 4 (9-methylanthracene)		
sample solution		

Results (as 95% confidence intervals)

	1-methylanthracene	**9-methylanthracene**
ε @ λ_1 ($cm^{-1}M^{-1}$)		
ε @ λ_2 ($cm^{-1}M^{-1}$)		
concentration (μg/mL):		

MULTICOMPONENT ANALYSIS: *DATA TREATMENT*

You will need to report confidence intervals for (a) ***molar*** absorptivities (in $cm^{-1}M^{-1}$) for both PAHs at both wavelengths (4 intervals total); and (b) concentrations (in μg/mL) for both PAHs in the sample solution. While the standard error of the molar absorptivity can be calculated from the standard error of the slope of the appropriate Beer's Law plot, determining the standard error of the concentration estimate is a little more complicated. Note that the pathlength, *b*, of a standard cuvette is 1 cm.

Multivariate Error Analysis

You will measure the absorbance of the "unknown" sample at two wavelengths; the concentration of the two analytes, *a* and *b*, are determined by solving the following simultaneous equations:

$$A_1 = k_1^a[a] + k_1^b[b]$$
$$A_2 = k_2^a[a] + k_2^b[b]$$

where A_1 and A_2 are the two absorbance measurements at λ_1 and λ_2, respectively. The *k* values, the products of absorptivity and pathlength, must be determined by separate calibration (i.e., Beer's Law plots). By Kramer's rule, the solution to the above two equations is

$$[a] = \frac{1}{D}(k_2^b A_1 - k_1^b A_2)$$

$$[b] = \frac{1}{D}(k_1^a A_2 - k_2^a A_1)$$

where
$$D = k_1^a k_2^b - k_2^a k_1^b$$

Measurement error in A_1 and A_2 will result in errors in the concentration $[a]$ and $[b]$ calculated from the above equations. You might be able to see that, by simple error propagation of the solution equations (ignoring the calibration error in the values of *k* obtained from the Beer's Law plots),

$$\sigma_a^2 = \frac{1}{D^2}\left[(k_2^b)^2\sigma^2(A_1) + (k_1^b)^2\sigma^2(A_2)\right]$$

$$\sigma_b^2 = \frac{1}{D^2}\left[(k_1^a)^2\sigma^2(A_2) + (k_2^a)^2\sigma^2(A_1)\right]$$

where σ_a and σ_b are the standard deviations of the calculated concentration values, and $\sigma^2(A_1)$ and $\sigma^2(A_2)$ are the measurement variances of A_1 and A_2, respectively. If we assume homogeneous variance for the measurements of A_1 and A_2, so that $\sigma(A_1) = \sigma(A_2) = \sigma$, then we may simplify the above equations to

$$\sigma_a = \frac{\sigma}{|D|}\sqrt{(k_1^b)^2 + (k_2^b)^2}$$

$$\sigma_b = \frac{\sigma}{|D|}\sqrt{(k_1^a)^2 + (k_2^a)^2}$$

In order to use these equations, you will need an estimate of σ, the shared measurement noise in A_1 and A_2. This estimate can be obtained from your data (actually, *four* such estimates are available).