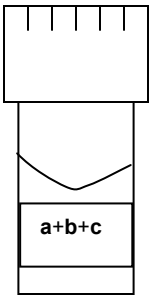
I. Introduction: In this experiment, you will be given an unknown mixture of acetone, benzene and chloroform you will determine the %v/v[1] composition of the solution by analyzing the UV absorbance spectrum of a 1:100 dilution of your sample in acetonitrile. The spectrum that you record will reveal that all three of these analytes are present because you will see the spectral fingerprints of all three. In your spectrum the absorbance at any given wavelength will be the sum of the absorbances of the individual components.



The infrared spectra that you record will be for qualitative analysis.

Time permitting; we will also perform a gas chromatography (GCMS) analysis, using the method of standard additions, to confirm the quantitative analysis that we performed with the UV spectrum.

II. Theory: The reference standards that you prepare will be measured on the UV spectrometer and will give you the following spectra:

- Acetone (A): $A_A(\lambda) = \varepsilon_A(\lambda)bC_{A,S}$
- Benzene (B): $A_B(\lambda) = \varepsilon_B(\lambda)bC_{B,S}$
- Chloroform (C): $A_C(\lambda) = \varepsilon_C(\lambda)bC_{C,S}$

(See figure 1 on the next page.)

- Mixture (M) (diluted): $A_M(\lambda) = \varepsilon_A(\lambda)bC_{A,M} + \varepsilon_B(\lambda)bC_{B,M} + \varepsilon_C(\lambda)bC_{C,M}$

(See figure 2 on the next page.)

  - A = absorbance (unitless)
  - λ = light wavelength (nm)
  - ε = extinction coefficient ($\%^{-1}cm^{-1}$)
  - b = light pathlength (1 cm in all cases here)
  - C = concentration (%)
  - Subscripts A,B,C,S,M refer to acetone, benzene, chloroform, standard and mixture respectively

a. The diluted sample that you measure will give you a spectrum of the *mixture*: (In the mixture, the concentrations are not the same as in the standards.)

b. You must use the reference spectra to determine ε_A, ε_B and ε_C at strategically chosen wavelengths. (See **IV.h** below)

c. Your job is to use the spectra to determine $C_{A,M}$, $C_{B,M}$ and $C_{C,M}$ – the concentrations of the components in the mixture.

[1] Note that %v/v is not generally a good concentration unit to use when doing exact computations because mixing of solutions can cause volume changes – e.g. 10 mL of acetone and 10 mL of water do not equal 20 mL of solution. In this case the solutions are dilute enough and the molar volume change on mixing small enough that they can be ignored.

d. The problem is one of three equations (II.d at three λ's) and three unknowns $C_{A,M}$, $C_{B,M}$ and $C_{C,M}$. If you choose your wavelengths properly, this can be somewhat simplified. (See **IV.h** below)

e. The three equations are taken from absorbances at three different wavelengths: λ_1, λ_2, λ_3

$$A_M(\lambda_1) = \varepsilon_A(\lambda_1)bC_{A,M} + \varepsilon_B(\lambda_1)bC_{B,M} + \varepsilon_C(\lambda_1)bC_{C,M}$$
$$A_M(\lambda_2) = \varepsilon_A(\lambda_2)bC_{A,M} + \varepsilon_B(\lambda_2)bC_{B,M} + \varepsilon_C(\lambda_2)bC_{C,M}$$
$$A_M(\lambda_3) = \varepsilon_A(\lambda_3)bC_{A,M} + \varepsilon_B(\lambda_3)bC_{B,M} + \varepsilon_C(\lambda_3)bC_{C,M}$$

Simplifying the notation, dropping 'b' since it is the same in all cases and =1.00 cm:

$$A_1 = \varepsilon_{A1} C_A + \varepsilon_{B1} C_B + \varepsilon_{C1} C_C$$
$$A_2 = \varepsilon_{A2} C_A + \varepsilon_{B2} C_B + \varepsilon_{C2} C_C$$
$$A_3 = \varepsilon_{A3} C_A + \varepsilon_{B3} C_B + \varepsilon_{C3} C_C$$

You will solve this problem in two ways:

1. Selective Elimination:
This method requires a wavelength where A does not overlap B or C, and one where B does not overlap C.
Select wavelength 1 such that $\varepsilon_{A1} \neq 0$ and $\varepsilon_{B1} \approx 0$ and $\varepsilon_{C1} \approx 0$, solve for C_A
Select wavelength 2 such that $\varepsilon_{B2} \neq 0$ and $\varepsilon_{C2} \approx 0$, solve for C_B
Select wavelength 3 such that $\varepsilon_{C3} \neq 0$, solve for C_C

2. Matrix Inversion Approach (does not require $\varepsilon \approx 0$):
This method can be applied to 3 or more mutually overlapping spectra.

The set of three equations above can be expressed in matrix notation as:

$$\begin{vmatrix} A_1 \\ A_2 \\ A_3 \end{vmatrix} = \begin{vmatrix} \varepsilon_{A1} & \varepsilon_{B1} & \varepsilon_{C1} \\ \varepsilon_{A2} & \varepsilon_{B2} & \varepsilon_{C2} \\ \varepsilon_{A3} & \varepsilon_{B3} & \varepsilon_{C3} \end{vmatrix} \times \begin{vmatrix} C_A \\ C_B \\ C_C \end{vmatrix}$$

Or simply:
$\mathbf{A} = \varepsilon \times \mathbf{C}$

If there exists a matrix ε^{-1} such that $\varepsilon \times \varepsilon^{-1} = \mathbf{I}$ (the identity matrix)
Then:
$\varepsilon^{-1} \times \mathbf{A} = \varepsilon^{-1} \times \varepsilon \times \mathbf{C} = \mathbf{C}$

So:

$\mathbf{C} = \varepsilon^{-1} \times \mathbf{A}$

The trick, of course, is finding ε^{-1}…

Small matrices such as the 3x3 matrix of ε's above, can be inverted and multiplied in Excel.

For example, I generated a table of epsilon values below from some students' calibration data.

This means that I looked at the calibration spectra at λ=300nm, then I took the absorbance from the calibration standard for, e.g. acetone, and divided by the standard concentration and 1 cm:

$$A_{300} = \varepsilon_{300,ACETONE} b C_{ACETONE}$$

so

$$\varepsilon_{300,ACETONE} = A_{300} / b C_{ACETONE}$$

The ε-values are computed and put into a 3x3 matrix of cells (Eps below), and then the Excel function 'minverse' is used to compute ε^{-1}.

Eps	acetone	benzene	chloroform
λ=300	0.861494	0.083347	0.007792
261	1.615213	13.47864	0.019722
221	0.117172	2.138121	0.68208

eps-1	acetone	benzene	chloroform
λ=300	1.172227	-0.00515	-0.01324
261	-0.14083	0.075152	-0.00056
221	0.240073	-0.23469	1.470147

To make the inverse matrix do the following:

i. highlight at 3x3 area of cells
ii. type '=minverse(T7:V9)' – but don't hit the **Enter** key…
iii. oops – you hit **Enter**, didn't you? Go back to i. above …
iv. type **Ctrl-Shift-Enter**

Everyone hits Enter – but this messes things up – you have to use **Ctrl-Shift-Enter!**

To get the concentration matrix back, simply multiply the ε^{-1} matrix by the absorbance matrix (**A**=ε**C** so **C**=ε^{-1}**A**).

ε-1				abs_mix		conc.	
1.172227	-0.00515	-0.01324		0.271475		0.30281	[a]
-0.14083	0.075152	-0.00056	x	1.582426	=	0.080382	[b]
0.240073	-0.23469	1.470147		0.549284		0.501314	[c]

To do the matrix multiplication:

i. highlight a 1x3 area of cells
ii. type 'mmult(
iii. type the range of cells for the 3x3 ε^{-1} matrix
iv. type a comma ','
v. type the range of cells for the 1x3 **A** matrix
vi. type the right bracket ')'
vii. oops – you hit **Enter**, didn't you? Go back to i. above …
viii. type **Ctrl**-**Shift**-**Enter**
ix. the concentrations are in the resulting matrix!

[acetone] = 0.30%
[benzene] = 0.080%
[chloroform] = 0.50%

CAUTION: ***Absorbance values greater than 2 are unreliable in most cases.*** This is because they are based on very small transmittances, i.e. very low light levels.

CAUTION: ***The reference sets for benzene and chloroform have absorbances greater than 2 below 220 nm*** – therefore the reference spectra are unreliable below 220 nm.

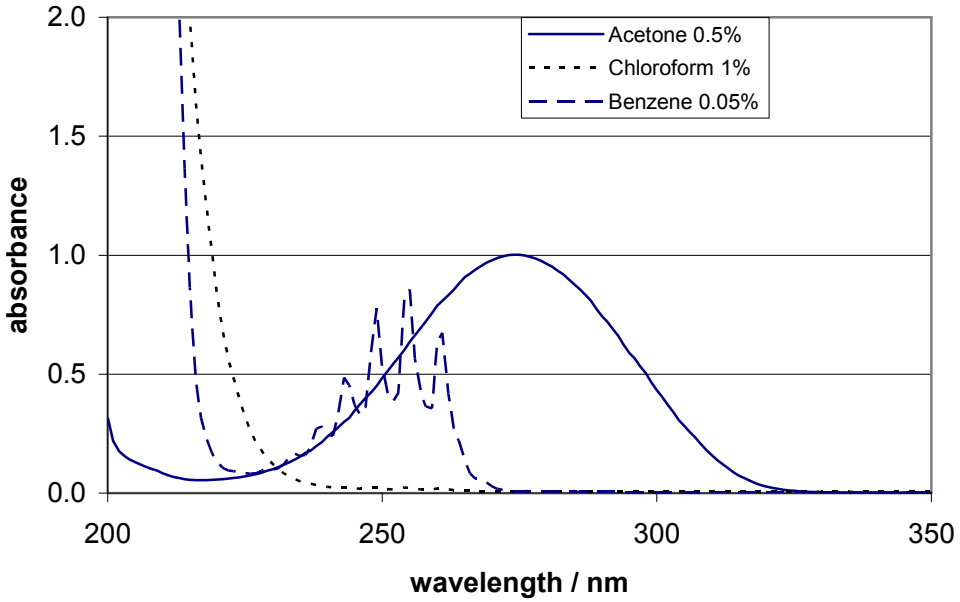
CAUTION: ***Do not use wavelengths for which the ε values for two different species are identical.*** This will give you a situation where there is no solution to the problem – the matrix inversion will fail.

Examples of the calibration spectra and a solved system are below.

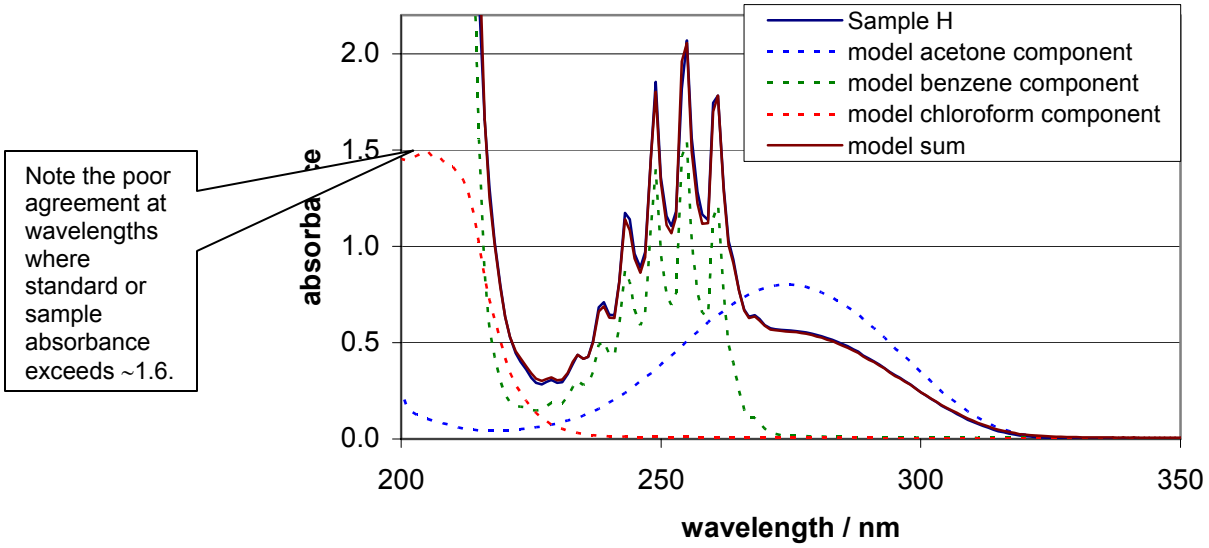
In the first figure, the calibration spectra are shown.

In the second figure, the sample spectrum along with the calibration spectra scaled so that they sum to equal the sample spectrum, and the sum (i.e. the model) is shown as well.

Calibration data



Sample and Model together



Note the poor agreement at wavelengths where standard or sample absorbance exceeds ~1.6.

III. Instruments and Materials:

- **a.** Cary 30 Bio UV-Vis absorption spectrometer.
- **b.** One pair of 1 cm quartz cuvettes.
- **c.** Mattson Fourier Transform Infrared (FTIR) Spectrometer with Attenuated Total Reflection (ATR) accessory.
- **d.** Several Pasteur pipettes and bulb.
- **e.** One 200μL autopipettor and several tips.

IV. UV spectroscopy with the CARY-30:

- **a.** Note: Acetonitrile (CH_3CN) is your solvent and is nearly transparent above 200nm. Any small CH_3CN absorbance is effectively subtracted because you will be using CH_3CN as a blank (or baseline as described in the Cary 30 Scan software).
- **b.** First dilute your sample 1:100 (v/v) with CH_3CN for UV absorbance. This will require that you pipette 0.100 mL unknown into a vial and dilute it with CH_3CN to a total volume of 10.0 mL. You will need a 200 μL autopipettor for this. This can be checked out from the chemistry service center, or alternatively I can supply you with one if mine is not in use. We have only three of these so you may need to arrange to share one with another student.
- **c.** Measure the absorbance spectrum of your sample. Use baseline correction and dual-beam mode. This will require a pure CH_3CN blank.
- **d.** Save the files – use your name in the file name so that you can recognize them – record the file names in your notebook.
- **e.** Measure the absorbance spectrum of the supplied reference solutions of acetone, benzene and chloroform - 1% $CHCl_3$, 0.5% $(CH_3)_2CO$, 0.05% C_6H_6 (all V/V percents) diluted in CH3CN.
- **f.** Using the software cursor, record the absorbance values (x.xxxx digits) for the sample and calibration standards at three wavelengths. This will be a total of 12 absorbance values. Record the wavelengths (yyy.y) that you used as well.
- **g.** Cautions: Depending on noise levels, as absorbance levels exceed about 2, they become unreliable and may sometimes jump erratically to '10' a fictitious value. Remember large absorbance means small signal, and therefore poor signal to noise ratio. If your sample has an absorbance value greater than 2.2, it will need to be diluted again.
- **h.** Use the software to determine the precise absorbance and wavelength values at:
  - i. A wavelength where only acetone absorbs
  - ii. A wavelength where only acetone and benzene absorb
  - iii. A wavelength where acetone, benzene and chloroform absorb but that is greater than 220 nm.
- **i.** Record the above absorbance and wavelength data.
- **j.** Print out the absorbance spectra (overlapping on one plot).

Attenuated Total Reflection ATR-FTIR on the Mattson Instrument:

I. If you are the first customer at the FTIR, run a blank (or reference as described by Mattson) on the clean, dry ATR crystal. Use F9 to bring up the reference/scan control panel. Click on Method Setup and set the resolution to 4 cm^{-1}, and the frequency range to 700 to 4000 cm^{-1}. Set the instrument to display 'Absorbance' as opposed to 'Transmittance'.

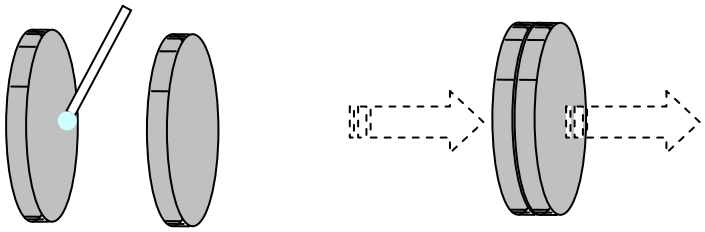
II. Dispense enough of your **undiluted** mixture from the small sample vial (**NOT** the 1:100 dilution) onto the ATR crystal to completely cover the crystal. Gently spread the liquid onto the crystal surface with the plastic pipette tip if necessary. Close the ATR crystal with the sealing top. Clear any vapors out of the sample chamber by fanning it briefly.

III. Run the scan and then print it out by pressing F7, or file, plot.

IV. Dry the crystal when you are done.

V. Repeat the above (2-4) for pure samples of acetone, benzene and chloroform.

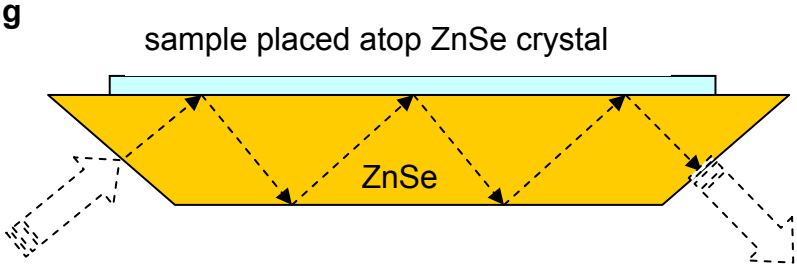
1. "salt plate" sampling



KBr disks

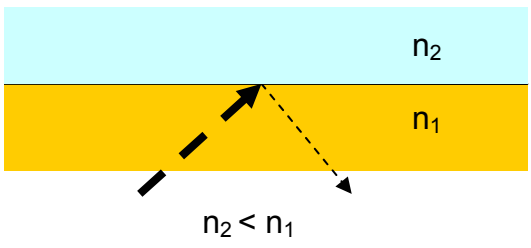
thin layer of sample sandwiched "sandwiched"

2. ATR sampling method



"Total" internal reflection occurs at ZnSe | sample interface.

If sample absorbs IR radiation, then the IR beam is **attenuated** as though it had passed through ca. 1μm of sample.



Report:

I. Cover page should have your name, the date and your unknown letter.
II. Attach your UV spectra.
III. Calculate the concentrations of acetone, benzene and chloroform in the mixture using the UV absorption data.
  a. Use the elimination method - show your calculations.
  b. Use the matrix inversion method – print out your spreadsheet.
IV. Attach your ATR-FTIR spectra: mixture and three standards.
V. Identify two different group frequencies for each standard spectrum.
VI. Identify the corresponding peaks in the sample spectrum.