Group Projects

Projects
- Identification of flavor compounds with GC/MS
- Quantification of Cr and Mn in different types of steel with UV/VIS
- Quantification of Zn and Cu in pennies with AAS
- Detection of caffeine in coffee/tee with HPLC and UV/VIS

Logistics
- Your TA splits you in four groups of 3-4 students
- Work responsibilities are distributed uniformly amongst the group members
- Each student keeps his/her own notes and submit his/her own report
- ONE set of printouts with UV/vis spectra, chromatograms, etc. per group
- The printouts must be approved by your TA at the end of the lab, taken with you, and submitted with your reports (one set per group)
- Blaming your group members for the failure to deliver the final result is unacceptable
1. GC/MS of Flavor Compounds

1. Ethyl Butyrate
2. Isoamyl Acetate
3. Benzaldehyde
4. Cinnamaldehyde
5. Unknown mixture containing 2-5 of these compounds
6. Composite mixture containing all 5 compounds to be used for the method optimization

Goal 1: identify compounds in your unknown mixture:

1. By their retention time in gas chromatography (GC) column
2. By their electron impact mass spectrum (MS)
3. By their smell

Goal 2: study the effect of GC/MS column temperature on chromatographic separation
GC/MS Instrument

- Ultrahigh purity helium in a pressurized tank
- Gas lines, valves, and flow regulators
- Manual injector for injection of analyte into the He flow ($T_{\text{injector}} = \text{fixed}$)
- Long, temperature programmed column
- Electron impact ion source ionizes molecules that come out from the column ($T_{\text{detector}} = \text{fixed}$)
- Quadrupole mass spectrometer (QMS) sorts the resulting ions by their m/z values
- Ion detector counts the ions that make it through the QMS
- Computer controls all the instrument components, processes signal from the ion detector, displays data, etc.

**Important Note:**
- Your syringe must withdraw only headspace AIR from the vial, NOT the liquid!!!
- The syringe and the needle must be dry. Do not allow the needle come in contact with the liquid, touch the walls of the vial.
Inside the GCMS Tube (Not to Scale)

Fused Silica Open Tubular (FSOT)

He Gas Mobile Phase (Eluent)  Capillary Tube  0.25 mm ID

Non-polar liquid stationary phase  ~ 1 µm

CH₃
CH₃ – Si – O
CH₃

CH₃ – Si – O
CH₃

CH₃
CH₃

CH₃ – Si – O
CH₃

CH₃
CH₃

n

(polydimethylsiloxane)

~ 5% of methyl groups substituted as –C₆H₅
Inside a GC Column

Partition ratios: \( K_A = \frac{3}{7} \) \( K_B = \frac{6}{4} \)

Selectivity Factor, \( \alpha = \frac{K_B}{K_A} \) (measure of column resolving power)

B is retained stronger and it will come out of the column later.

Temperature of the column will affect the partition ratios and the rate with which molecules move through the column VERY significantly. In this lab, you will change the temperature to study its effect on the retention time.
Each peak corresponds to a separate compound, which can be identified by a unique retention time.
Sampling and Injection

Withdraw headspace air without needle touching any of the liquid.

When the GC/MS instrument is ready align the syringe with the injection port vertically.

Insert the needle fully trying not to bend it. Do not force it if you feel obstacles!

Inject the gas and immediately press the "Start" button. Be gentle with the syringe – it does not take much to break it!
2. UVVIS Analysis of Steel

**Goals:**
1. Quantify Mn and Cr in your steel sample
2. Learn how to quantify multiple absorbing compounds in situations when their absorption spectra overlap with each other

- Weigh out a solid steel sample
- Dissolve this steel sample in boiling acids (HNO₃, H₂SO₄, H₃PO₄)
- Oxidize Cr and Mn to Cr₂O₇²⁻ and MnO₄⁻
- Prepare calibrated solutions of Cr₂O₇²⁻ and MnO₄⁻
- Measure absorbance of your solution at the two selected wavelengths
- Measure absorption spectra of Cr₂O₇²⁻ and MnO₄⁻ Pick two wavelengths corresponding to the peaks in the absorption spectra
- Verify that Beer's law is valid for your concentration range
- Analyze your data
- Pick two wavelengths corresponding to the peaks in the absorption spectra
Basics of Absorption Spectroscopy

Light source (e.g., laser) \rightarrow \text{Cell filled with clean water} \rightarrow \text{Photodetector (e.g., photodiode)} \rightarrow I_0

The same light source \rightarrow \text{Cell filled with (diluted) sample} \rightarrow \text{The same photodetector} \rightarrow I < I_0

Quantitative measure: Transmittance (T)

\[ T = \frac{I}{I_0} \]
**Beer-Lambert Law**

**Qualitative**
- Different molecules absorb light to a different extent
- More concentrated sample $\Rightarrow$ less light transmitted
- Larger cell $\Rightarrow$ less light transmitted

**Quantitative**

$$T = \frac{I}{I_0} = 10^{-A}$$

$$A = \varepsilon \times l \times C$$

- $A =$ absorbance (sometimes called $\text{OD} =$ Optical Density)
- $l =$ length of the absorption cell
- $\varepsilon(\lambda) =$ molar extinction coefficient (depends on the specific analyte molecule and on the wavelength $\lambda$ of the light source)
- $C =$ molar concentration of the analyte molecule
Absorption Spectrometer

Xe-lamp: emits white light
D₂-lamp: emits UV light

Monochromator: transmits only one specific wavelength (Chem 152)

50:50 beam splitter

100% reflective mirror

Sample cell

Reference cell

Sample detector

Reference detector

Absorbance

Wavelength

Spectrum

Recorder (computer)

Voltage

Amplifier

$I$ $I_0$
## Transmittance vs Absorbance

<table>
<thead>
<tr>
<th>Situation</th>
<th>$T$</th>
<th>$A = -\log(T)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All light goes through (transparent sample)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>50% light goes through</td>
<td>0.50</td>
<td>0.30</td>
</tr>
<tr>
<td>10% light goes through</td>
<td>0.10</td>
<td>1</td>
</tr>
<tr>
<td>1% light goes through</td>
<td>0.01</td>
<td>2</td>
</tr>
<tr>
<td>0.1% light goes through</td>
<td>0.001</td>
<td>3</td>
</tr>
<tr>
<td>Nothing goes through (opaque sample)</td>
<td>0</td>
<td>Infinity</td>
</tr>
</tbody>
</table>

Useful range for analytical chemistry applications

Useless! Dilute your sample if your $A > 1$
Two Absorbers: Two Wavelengths Needed

Beer-Lambert law applied to a mixture of two absorbers: $X=\text{Cr}_2\text{O}_7^{-}$ and $Y=\text{MnO}_4^{-}$

$$A_{\text{total}}(\lambda_1) = A_X(\lambda_1) + A_Y(\lambda_1) = b \times \{\varepsilon_X(\lambda_1)C_X + \varepsilon_Y(\lambda_1)C_Y\}$$

$$A_{\text{total}}(\lambda_2) = A_X(\lambda_2) + A_Y(\lambda_2) = b \times \{\varepsilon_X(\lambda_2)C_X + \varepsilon_Y(\lambda_2)C_Y\}$$

- $A(\lambda)$ = absorbance at wavelength $\lambda$
- $b$ = absorption cell path length
- $\lambda_1$ and $\lambda_2$ = selected wavelengths where compounds $X$ and $Y$ both absorb
- $\varepsilon_X(\lambda)$ = extinction coefficient of compound $X$ at wavelength $\lambda$
- $C_X$ = unknown concentrations of compound $X$
- The four required values $\varepsilon_X(\lambda_1)$, $\varepsilon_X(\lambda_2)$, $\varepsilon_Y(\lambda_1)$, and $\varepsilon_Y(\lambda_2)$ are determined by measuring absorbance from your calibration solutions that contain only one compound
- For your unknown solution you are measuring $A_{\text{total}}$ at two wavelengths. This gives you two equations with two unknowns; they can be solved by routine linear algebra techniques

Four calibration plots are needed in this project (2 compounds $\times$ 2 wavelengths)
Solving for Concentrations

- Obtain all four extinction coefficients from linear fits (a sample Excel file is provided)
- Solve for the unknown concentrations algebraically. Here is an example of how it can be done using MathCAD (you can also do it in a standard way by following example 26-3)

First, we define the cell length in **centimeters** (it is 2 cm in this particular example)

\[ \text{cell} := 2.00 \]

Then we define the matrix of extinction coefficients, where columns correspond to compounds X and Y, and rows correspond to the two selected wavelengths. The units are \( \text{M}^{-1} \text{ cm}^{-1} \). In this particular example:

- Compound X has molar extinction coefficients of 3000 and 700 at wavelengths 1 and 2, respectively
- Compound Y has molar extinction coefficients of 800 and 2000 at wavelengths 1 and 2, respectively

\[
\text{ext} := \begin{pmatrix} 3000 & 700 \\ 800 & 2000 \end{pmatrix} \quad \text{product := ext \cdot cell}
\]

Define the vector of absorbances, wherein the vector index corresponds to the wavelength

\[
\text{Ab} := \begin{pmatrix} 0.500 \\ 0.900 \end{pmatrix}
\]

Total measured absorbance at wavelength 1

Total measured absorbance at wavelength 2

Now we are going to solve the linear equation for concentrations. Because of the units we used, the answer will be in \( \text{mol/L} \).

\[
\text{product}^{-1} \cdot \text{Ab} = \begin{pmatrix} 3.401 \times 10^{-5} \\ 2.114 \times 10^{-4} \end{pmatrix}
\]

Concentration of compound 1

Concentration of compound 2
Goals: quantify the amounts of Zn and Cu in
1. Old American pennies (mostly copper and some zinc)
2. New American pennies (mostly zinc and some copper)
The flame is hot enough to completely vaporize the sample down to the level of the individual atoms (Zn and Cu atoms in our example). The instrument then measures optical absorption through by the flame, which is proportional to the concentration of atoms in the flame, which is in turn proportional to the concentration of atoms in the original liquid sample.

\[ \text{Absorbance}_{Zn \text{ lamp}} = \text{constant}_{Zn} \times [Zn^{2+}] + \text{offset}_{Zn} \]

\[ \text{Absorbance}_{Cu \text{ lamp}} = \text{constant}_{Cu} \times [Cu^{2+}] + \text{offset}_{Cu} \]

The proportionally constants are determined using calibration against solutions with known [Zn$^{2+}$] and [Cu$^{2+}$].
4. Caffeine Project (HPLC & UVVIS)

**Goal 1:** estimate the amount of caffeine in:
1. Caffeinated coffee or tea, as opposed to
2. Decaffeinated coffee or tea

**Goal 2:** study the effect of the HPLC solvent composition on the chromatographic separation

- Prepare a stock solution of caffeine
- Weight and extract two tea or coffee samples (caff + decaff)
- Record several UVVIS absorption spectra for several samples of known dilution (absorbance should cover the range of 0-1)
- Dilute by a known amount until the UVVIS absorbance at 273 nm is in range of 0-1. Measure the absorption spectrum.
- Dilute to about the same level as the caffeine stock solution
- Record several HPLC chromatograms using different types of mobile phase
- Determine the extinction coefficient at 273 nm from Beer's law
- Estimate the amount of caffeine in your samples from the UVVIS spectra
- Use the mobile phase composition that gives the best results for your coffee/tea samples
- Compare the results
Chromatograms

A + B

A + B

A + B

A + B

A + B

A + B

A + B
Inside an HPLC Column

Mobile phase ($\text{H}_2\text{O}$, $\text{CH}_3\text{OH}$, $\text{CH}_3\text{CN}$, THF, …)

**Gradient elution**: mobile phase composition changes during the chromatogram (this is to temperature-programmed elution in GC)

**Isocratic elution**: fixed solvent composition (used in this lab). Your goal will be to find the optimal solvent to use for caffeine.
Injection

A manual injector with a 20 µL sample loop will be used to inject the solution of your analyte (caffeine in H₂O) into the flow.

The valve must be in the **LOAD** position while filling the sample loop with the solution of your analyte.

Shifting the valve into the **INJECT** position will start the chromatogram.

**IMPORTANT**: Do not leave the valve in between the **LOAD** and **INJECT** position. It will ruin the instrument.

**IMPORTANT**: Use a proper HPLC syringe (with a dull needle tip) for injections. It is different from the syringe used in the GCMS lab, which has a septum-piercing needle tip.
Diode Array Detection

As soon as the analytes leave the column they enter a miniature absorption cell where they are detected with a diode array absorption detector.

The detector can measure absorbances at 5 different wavelengths simultaneously. You will see 5 corresponding chromatograms on the screen.

One of these wavelengths will be 273 nm – the peak wavelength of the caffeine near-UV absorption spectrum. You will use the 273 nm chromatogram for your quantitative analysis.
Linear Calibration (AAS, UVVIS, HPLC Labs)

1. Chapter 8 (Sampling, Standardization, and Calibration)
2. Sample Excel file on the course website

<table>
<thead>
<tr>
<th>[Standard]</th>
<th>Signal</th>
<th>$x^2$</th>
<th>$y^2$</th>
<th>$x'y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.019</td>
<td>0.01</td>
<td>0.00361</td>
<td>0.0019</td>
</tr>
<tr>
<td>0.2</td>
<td>0.041</td>
<td>0.04</td>
<td>0.01681</td>
<td>0.0082</td>
</tr>
<tr>
<td>0.3</td>
<td>0.078</td>
<td>0.09</td>
<td>0.06084</td>
<td>0.0234</td>
</tr>
<tr>
<td>0.4</td>
<td>0.102</td>
<td>0.16</td>
<td>0.010404</td>
<td>0.0408</td>
</tr>
<tr>
<td>0.5</td>
<td>0.129</td>
<td>0.25</td>
<td>0.016641</td>
<td>0.0645</td>
</tr>
<tr>
<td>0.6</td>
<td>0.16</td>
<td>0.36</td>
<td>0.0256</td>
<td>0.096</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>6</td>
<td>number of calibration points</td>
</tr>
<tr>
<td>$\text{Sum of } x$</td>
<td>2.100E+00</td>
<td>sum of $x$ values</td>
</tr>
<tr>
<td>$\text{Sum of } y$</td>
<td>5.290E-01</td>
<td>sum of $y$ values</td>
</tr>
<tr>
<td>$\text{Sum of } x^2$</td>
<td>9.100E-01</td>
<td>sum of squares of $x$ values</td>
</tr>
<tr>
<td>$\text{Sum of } y^2$</td>
<td>6.077E-02</td>
<td>sum of squares of $y$ values</td>
</tr>
<tr>
<td>$\text{Sum of } xy$</td>
<td>2.348E-01</td>
<td>sum of $x'y$ values</td>
</tr>
<tr>
<td>$S_{xx}$</td>
<td>1.750E-01</td>
<td>see Section 8C</td>
</tr>
<tr>
<td>$S_{yy}$</td>
<td>1.413E-02</td>
<td>see Section 8C</td>
</tr>
<tr>
<td>$S_{xy}$</td>
<td>4.965E-02</td>
<td>see Section 8C</td>
</tr>
<tr>
<td>$&lt;x&gt;$</td>
<td>3.500E-01</td>
<td>average of $x$ values</td>
</tr>
<tr>
<td>$&lt;y&gt;$</td>
<td>8.817E-02</td>
<td>average of $y$ values</td>
</tr>
<tr>
<td>$m$</td>
<td>2.837E-01</td>
<td>slope of the line</td>
</tr>
<tr>
<td>$b$</td>
<td>-1.113E-02</td>
<td>intercept of the line</td>
</tr>
<tr>
<td>$S_r$</td>
<td>3.332E-03</td>
<td>standard deviation about the regression</td>
</tr>
<tr>
<td>$S_m$</td>
<td>7.966E-03</td>
<td>standard deviation of the slope</td>
</tr>
<tr>
<td>$S_b$</td>
<td>3.102E-03</td>
<td>standard deviation of the intercept</td>
</tr>
<tr>
<td>$SS_{resid}$</td>
<td>4.442E-05</td>
<td>sum of squares of fit residuals</td>
</tr>
<tr>
<td>$R^2$</td>
<td>9.969E-01</td>
<td>R-squared for this fit</td>
</tr>
<tr>
<td><strong>unknown</strong></td>
<td><strong>1.200E-01</strong></td>
<td>signal of the unknown ($y_u$)</td>
</tr>
</tbody>
</table>

Note: values in blue are entered manually; the rest of the values (including the number of measurements) are calculated automatically.

- UVVIS lab: concentrations in ppm $\rightarrow$ A; absorbances $\rightarrow$ B
- AAS lab: concentrations in ppm $\rightarrow$ A; absorbances $\rightarrow$ B
- HPLC lab: concentrations in $\mu g/mL$ go $\rightarrow$ A; peak areas $\rightarrow$ B

The unknown concentration will be in the same units as the units used in column A.