Lab.7. Spectrophotometry

Key words:
Spectroscopy; light (a particle, a wave or both), wave properties, how radiation and matter interacts, electromagnetic spectrum, what do spectroscopists measure, UV-VIS spectrophotometry, absorbance, chromophores, Beer’s Law, energy level diagrams for molecules, instruments for measuring absorption.

Literature:
D. A. Skoog, D. M. West, F. J. Holler, S. R. Crouch; Analytical Chemistry. An Introduction, 7th ed., Section 1, Section IV

Theoretical background

Absorption Spectroscopy and Beer's Law
Light brings us news of the universe. Our perception of light is mostly of the visible range of radiant energies, the slot between roughly 400 and 800 nm within the electromagnetic spectrum. But because most light is invisible, it must be detected by other means. Through the transmission, absorption, and scattering of light, we can gain information about the hidden world beyond what usual observations reveal.

Principles
Spectroscopy is the study of the interaction of electromagnetic radiation with matter. In spectroscopy, two terms are inescapable: transmittance and absorbance. Transmittance (T) is defined as the ratio of the intensity of light after it passes through the medium being studied (/) to the intensity of light before it encountered the medium (/o), as shown in Equation below.

\[ T = \frac{I}{I_0} \]  (1)
Spectroscopists more commonly refer to percent transmittance (\%T), which is simply: 
\[ T \times 100\% \]. Often the same spectroscopic information that is reported as the percent 
transmittance is more conveniently expressed as absorbance (A):

\[
A = - \log \left( \frac{I}{I_0} \right)
\]  

(2)

Note that

\[
A = 2 - \log (\% \, T)
\]

(3)

If one knows the percent transmittance, one can calculate absorbance and vice versa. Some
non-digital spectrophotometers have both a \%T and an absorbance scale displayed on a meter. 
Because the \%T scale is lines', it can be read with good precision over the entire range of 
transmittances. However, the absorbance scale is a logarithmic scale and cannot be read with
precision at high absorbance values. Therefore, if the absorbance is larger than 0.7, it is 
preferable to calculate the absorbance, using the \% T, rather than to read the absorbance 
directly. Modern digital spectrophotometers may be programmed to display either absorbance 
or percent transmittance by simply touching a mode selection button. An operational statement 
of the Beer-Lambert Law can be represented as

\[
A = \varepsilon \cdot c \cdot l
\]

(4)

where \( c \) is the concentration in Mole L\(^{-1} \) of some absorbing substance in solution,
\( l \) is the optical path length, and \( \varepsilon \) is the molar absorptivity.
The molar absorptivity is a constant that depends on the nature of the absorbing system (the
solute-solvent combination) and the wavelength of the light passing through it. A plot that
shows the dependence of \( A \) (or \( e \)) on wavelength is called a spectrum.
When absorbance measurements are made at a fixed wavelength in a cell of constant path 
length, \( \varepsilon \) and \( l \) are constant. Therefore, the absorbance, \( A \), should be directly proportional to \( c \), 
the concentration of the solute. A solution that shows such a linear relation between \( A \) and \( c \) is 
said to obey the Beer-Lambert Law, which is the optimum situation for a spectrophotometric
method of analysis.
It is also possible, although less desirable, to perform spectrophotometric analyses in systems 
that deviate from the Beer-Lambert Law, as the Cu\(^{2+} \)/NH\(_3\) system is shown to do in Figure 1.
Figure 1. Cu²⁺ / NH₃ solutions do not obey Beer's Law.
A proper calibration curve would be a smooth line drawn through the data points. The deviation results from the equilibrium distribution of Cu²⁺ between the absorbing species Cu(NH₃)₄²⁺ and several nonabsorbing complexes. In such cases, the absorbance versus concentration measurements results in a calibration curve, rather than the linear plot predicted by the Beer-Lambert Law.

The simplest equipment for spectrophotometric measurement is presented in Fig.2. It consists of light source, monochromator system, glass absorption cell (cuvette) and detector.

Fig.2. The block diagram of spectrophotometer UV-VIS
(from http://faculty.sdmiramar.edu/fgarces/LabMatters?instruments/UV_VIS/Cary_50.htm)
The spectrophotometers are divided into two systems: single beam spectrophotometers and double-beam spectrophotometers.

**EXPERIMENTAL PART**

**Procedure**

1. **Preparation of standard solutions.**
   
   **Step 1**
   Use a burette to dosage 0.1, 0.25, 0.5, 1.0, 1.5; 2.0, 2.5 mL of the Ni(NO$_3$)$_2$ [concentration 0.05 mg mL$^{-1}$] into the seven flasks (with maximal volume 50 mL).

   **Step 2**
   In the same way add into all flasks 30 mL of distilled water, 3 mL of 3% solution of (NH$_4$)$_2$S$_2$O$_8$ and 5 mL of solution dimethylglyoxime (remember to keep this order).

   **Step 3**
   Add another portion of distilled water into the flasks to bring the solution up to the calibration mark on the neck of the flask. Mix the solution thoroughly.

   **Step 4**
   Take the flask labelled “x” for unknown mixture. Ask TA how many mL of nickel nitrate solution should be added. The remaining components of mixture add in the same order like for solution with known nickel nitrate concentration.

   **Step 5**
   Prepare blank test (analytical blank). Add all components from step 2 to 3 (without nickel nitrate solution) into 50 mL volume flask.

2. **Determination of the Spectrum of the complex solution and selection of the analytical wave [the absorbance maxima].**

   **Step 6**
   Fill one of the cells in 2/3 with blank test solution to use as a reference.

   **Step 7**
   Set the wavelength of the spectrophotometer at 400 nm; then insert the cell in the cell compartment. Close the compartment door and set the instrument to exactly zero absorbance.

   **Step 8**
Next time fill the sample cell with a small portion of the solution (from flask No 4) and discard the rinsing. Fill the cell about two-thirds full with solution, insert it in the sample compartment. Close the sample door and read absorbance as accurately as possible.

Step 9
Change the wavelength to 410 nm and again adjust the zero (absorbance) settings using the reference cell as described in previous steps.

Step 10
Repeat this procedure at 10 nm intervals over 400 – 560 nm range.

Step 11
Record your data on the summary deport Sheet (Table 1). Prepare a plot of absorbance versus wavelength. Determine the wavelength of maximum absorbance for your solution of the complex.

Table 1. Absorbance versus wavelength for nickel-dimethylglyoxime complex solution.

<table>
<thead>
<tr>
<th>$\lambda$ [nm]</th>
<th>A</th>
<th>$\lambda$ [nm]</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>490</td>
<td>410</td>
<td>500</td>
</tr>
<tr>
<td>420</td>
<td>510</td>
<td>430</td>
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<td>460</td>
<td>550</td>
<td>470</td>
<td>560</td>
</tr>
<tr>
<td>480</td>
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</tbody>
</table>


Step 12
Set the spectrophotometer wavelength to the absorbance maximum and adjust the zero (zero absorbance) setting, using the reference cell as usual. Rinse and then fill the sample cell with one of the diluted nickel dimethylglyoxime complex (solution 1) and measure absorbance of the solution. Record your data on the Table 2.
Table 2. Absorbance vs. nickel concentration dependence.

<table>
<thead>
<tr>
<th>Flask</th>
<th>Concentration of nickel [mol L⁻¹]</th>
<th>Absorbance</th>
<th>Molar absorptivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
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<td>7</td>
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<tr>
<td>X</td>
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</tbody>
</table>

Remember that the concentrations of nickel nitrate presented in the Table 2 are in mol/L whereas the initial concentrations are given in mg/mL so it should be converted into mol/L. (Molecular mass of nickel nitrate is 182.7 g/mol).

Step 13
Repeat procedure from the step 1 for solution labelled number 2 to 7. Record your data on the Table 2.

Draw plot absorbance versus the concentration of your salt (nickel nitrate) for the seven solutions. If the data display a linear relationship, draw the best straight line through the experimental points, including the origin.

Step 14
Repeat procedure from the step 1 for solution label “x”. Determine the concentration of nickel nitrate from the calibration plot obtained in steps 1 to 2.

* Use the spreadsheet for calculation and calibration curve.
Step 15

Get from the TA the volume of nickel nitrate added to the flask “x”; determine the absolute and relative error (%). Record these data in Table 3.

Table 3. Sample data.

<table>
<thead>
<tr>
<th>Flask</th>
<th>Absorbance</th>
<th>Concentration of nickel determined from calibration plot [mol L(^{-1})]</th>
<th>Real concentration [mol L(^{-1})]</th>
<th>Absolute error</th>
<th>Relative error</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
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</tbody>
</table>

Absolute error = concentration determined from plot – real concentration

Relative error = \(\frac{\text{concentration determined from plot} - \text{real concentration}}{\text{real concentration}}\) \times 100\%
Step 16

Calculate the molar absorptivity of the nickel nitrate solution according to the formula below.

\[ \varepsilon = \frac{A}{b \cdot c}, \quad c \, \text{[mole} \cdot \text{L}^{-1}], \quad b \, \text{[cm]} \]

Put these data to Table No 2.

**Example of calibration curve (straight line) obtained with help of an Excel is presented below.**

Questions to answer:

1. Does nickel-dimethylglyoxime solution obey Beer’s Law?

2. What is the wavelength of maximum absorbance for nickel-dimethylglyoxime solution?

3. What was the concentration of nickel-dimethylglyoxime in sample X?

4. What is the absolute and relative error of the experiment?

5. Does the molar absorptivity of nickel-dimethylglyoxime depend on the solution concentration?