EXPERIMENT 2.4.
UV-VISIBLE SPECTROPHOTOMETRY

OBJECTIVE
The aim of the experiment is to learn the basic principles of UV-visible spectrophotometry and how to measure concentration by a UV-visible spectrophotometer.

PRELIMINARY QUESTIONS
1. Define spectrophotometry, spectrophotometer and analyte.
2. What is absorption and transmission of electromagnetic waves? What about absorptivity and transmittivity? Do they always sum up to give unity?
3. Does a light beam possess a frequency value?
4. How can a light spectrum be produced?
5. What is the function of a monochromator? Define monochromatic light.
6. What is the simultaneous effect of UV-Visible light on the molecules after absorption?
7. Express absorbance by formula. Discuss why it is expressed so?
8. Define blank solution and list the possible errors that can be ameliorated by using a blank rather than inserting source intensity $I_o$ in the absorbance equation.
9. Answer the questions written in text in parenthesis.

INTRODUCTION
Ultraviolet spectrometry is used for measuring concentration of a specific molecule in a solution. This technique is appropriate for transition metal ions, highly conjugated organic compounds and biological macromolecules as analytes. It is based on absorption of light in visible or ultraviolet region. Absorption of ultraviolet or visible light occurs simultaneously with an electron jumping into an excited state from ground state and this explains why transition metal ions and conjugated organic compounds can be analysed by this method. The energy gaps between ground and excited states depend on the chemical nature of the molecule of interest and therefore the wavelength of the light is chosen accordingly for absorption by that molecule.

In UV-visible spectrophotometry, amount of absorbed light increases with but is not proportional to concentration (Think about why) and hence another parameter proportional to concentration is defined as below and named as absorbance, $A$:

$$A = \log\left(\frac{I_o}{I}\right)$$

where $I_o$ and $I$ are the intensities of light coming from the source and transmitted light, respectively. Defined as above, absorbance is proportional to concentration according to Beer’s Law:

$$A = \varepsilon BC$$
In this equation; $B$ is the length of the cell parallel to light direction, $\varepsilon$ is a constant depending on temperature, pressure and molecular nature of the analyte and $C$ is the analyte concentration. The values of $B$ and $\varepsilon$ are not important most of the time since plotting a calibration line using standard samples of known concentration is a much more accurate way. (Do you agree? why or why not?).

A UV-visible spectrophotometer consists of a light source, a monochromator, sample cell and a detector. Among the light sources, most common are tungsten filament (300-2500nm), deuterium arc lamp (190-400nm) or xenon arc lamp (160-2000nm) or LED for visible wavelengths. Monochromator provides a continuous spectrum using the light coming from the source so that light beam at a specific wavelength can be selected and sent to the solution inside the sample cell. Detector measures the intensities of the light coming from both the blank sample and analyte solution. Hence the absorbance value is calculated for each solution.

**Figure 1.** A representative sketch for a UV-Visible Spectrophotometer

**EXPERIMENTAL**

**Equipment and Material:**
- UV-Visible Spectrophotometer
- Methylene-blue
Procedure:
- Prepare 7 different aqueous methylene-blue solutions in the range between 50ppm-1000ppm.
- Find the wavelength of the UV-Visible light specific to methylene-blue by continuous scanning.
- Read the absorbance values for each solution.
- Draw absorbance vs. concentration plot and fit a line on this plot.
- Read the absorbance values for the unknown solutions.
- Predict the concentration values for the unknown solutions.

Reference Readings: