Task I. Basics optical laboratory methods

Required knowledge: Optical laboratory methods

1. Spectrophotometry – Absorption curve and determination of concentration of eosin

Main tasks:
Determination of absorption maximum from the absorption curve of eosin.
Determination of concentration of eosin in the unknown sample.

Task 1
Measuring of absorption curve of eosin and determination of its maximum in visible part of electromagnetic spectrum.

Measurement aids and implements:
Spectrophotometer Specol, cuvettes, automatic pipette, eosin solution, distilled water

Procedure:
1) Switch on the spectrophotometer and set the wavelength of light at 450 nm by means of wavelength selector.
2) Fill one cuvette by distilled water and the second by studied solution. Cuvette has two different sides. Always grip the cuvette at opaque surfaces. Cuvettes must be filled almost up to the edge.
3) Put the cuvette with distilled water into the first movable holder of the spectrophotometer. Move the holder to the other side and place a cuvette with studied solution (eosin) there.
4) Press button "E" on the spectrophotometer to set absorbance measurement mode when the cuvette with distilled water in the measurement position. Than press button "R" to do the calibration for distilled water. The value 0.000 (± 0.005) must appear on the display. That means that the absorbance value was automatically set to 0.000 for distilled water. Now shift the holder with cuvettes to measure the eosin solution and read its absorbance A_1. This is the value of absorbance for a specific wavelength (for the starting 450 nm).
5) Slide again the holder with cuvette with distilled water into the measuring area and increase the value of the wavelength by 5 nm. Press again "R" to do the re-calibration of the absorbance for distilled water and slide the holder to the cuvette with studied liquid to read the absorbance A_2.
6) Repeat this procedure until you reach the wavelength of 550 nm (step is always 5 nm) and read the specific absorbance values of the studied liquid (A_3…..A_N).
7) Plot a graph how the absorbance depends on the wavelength of light and highlight the maximum value of wavelength.

Task 2
Spectrophotometric determination of concentrations of solutions.

Measurement aids and implements:

Spectrophotometer Boeco, automatic pipette, stand with test tubes, studied solutions of known concentration of eosin, sample of unknown concentration, distilled water, cellulose wadding.

Procedure:

1. Start the procedure by the preparation of the solution (eosin) with specific concentrations. Use pipettes to dilute the stock solution of known eosin concentration (5μg/L) and prepare the others with lower concentrations of 4, 3, 2 and 1μg/L. All of the new solutions dilute into test tube. To know the right ratio of dilution use the so called dilution rule. (e.g. aspirate by pipette 4 parts of the stock solution and add 1 part of distilled water to have the concentration of 4μg/L. By means of the same dilution rule and different ratios prepare solutions of the other concentrations into test tubes).
2. Switch on the spectrophotometer and set the wavelength of light at absorption maximum of eosin, determined in the Task 1 (515 nm).
3. Fill the first cuvette with distilled water, the second cuvette with studied solution (start with the stock solution concentration (5μg/L). Always grip the cuvette at opaque surfaces!
4. Open the cover of spectrophotometer and insert the cuvette with distilled water first. Close the cover and calibrate by pressing CAL. Be aware of the right placing of cuvette into the holder – clear sides have to be on right and left!)
5. The value 0.000 must appear on the display, that means the absorbance value was automatically set to 0.000 for distilled water. Now insert cuvette with studied solution of highest concentration and read its absorbance A_1.
6. Repeat this procedure with all the prepared solutions of known concentrations (A_2…..A_5). Always calibrate the spectrophotometer with distilled water before measuring each of the samples.
7. Do the last measurement with the **solution of unknown concentration (x)**. Prepare a table and record there concentrations and their adequate absorbances. Plot a graph how absorbance depends on concentration. Find out from the plotted graph the real **concentration** of the unknown sample and highlight its value in graph.

2. Refractometry – Determination of NaCl concentration

Main tasks:
Determination of concentration of NaCl in an unknown sample.

Measurement aids and implements:
Refractometer (when using the Abbe refractometer a lamp is required for illumination), crystalline NaCl, stand with test tubes, automatic pipettes, distilled water, cellulose wadding, laboratory scales, weighing vessel.

Procedure:

1. Suppose that the concentration of the unknown NaCl solution is not higher than 200g/L. Therefore prepare 10ml of NaCl solution with concentration of 200g/L (dissolve 2g of NaCl in distilled water to have 10 ml of solution). From the solution of concentration 200g/L prepare the other **by diluting with distilled water into test tubes**: 150g/L, 100g/L, 50g/L. Use pipette to keeping the ratio (**do not use crystalline NaCl anymore)**.
2. Swing away the illuminating (with matted surface) and measuring prisms of the refractometer and make sure that the instrument is clean. If you find remains of some solution or traces of dry NaCl on any of the surfaces of the prisms or in their surrounding, rinse them with distilled water and dry gently with cellulose wading.
3. Turn ON the lamp placed close to the refractometer and start the measurement with distilled water. Proceed from the less concentrated solution to the more concentrated ones. Put a drop of solution on surface of the prism and close the prisms (lock the prism by turning the switch).
4. Find the line inside the eyepiece by turning of the bottom switch on refractometer and set it into the center of the cross hairs to determine the refraction index of studied liquid. This interface can be coloured and not sharp (with respect to the phenomenon of optical dispersion). To remove this aberration turn the upper switch on the refractometer which controls the Amici prisms that compensate dispersion of the measured substance. Set the compensated interface to the center of cross hairs.
5. Read the value of optical dispersion of the solution by the eyepiece (bottom scale). (with accuracy at least of three decimal places)
6. Repeat the measurement for all of the solutions with specific concentrations.
7. Write down all measured results into a table and then plot a graph of the dependence of the refraction indices on the concentration. Lastly measure the refraction index of **unknown solution** and highlight it in graph. Find out the real **concentration** of unknown sample from the plotted graph.
8. After finishing of measurement clean the surfaces of both prisms with distilled water and dry them carefully with cellulose wadding.