Task I. Basics optical laboratory methods

Required knowledge: Optical laboratory methods

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

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

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

Needs for measurement:
Spectrophotometer Specol, automatic pipette, solution, distilled water

Procedure:
1) Switch On spectrophotometer and by means of wavelength selector turn to set the wavelength of light at 450nm.
2) Always grip the cuvette at eggshell surface.
3) Into the first movable holder of spectrophotometer Specol grip a cuvette with distilled water. In the other grip a cuvette with studied solution. Cuvettes must be filled almost up to the edge.
4) Perform absorbance measurement for distilled water. For water press button "E" to set absorbance and button "R" to make reset. The value 0.000 must appear on the display. That means that for distilled water the absorbance value was automatically set to 0.000. Now shift into the measuring site of the module the cuvette with studied solution and read its absorbance A_1.
5) Into the measuring site shift again the cuvette with distilled water and increase the value of the light wavelength by 5nm. After zero setting the display (The distilled water absorbance is 0.000 again) insert into the measuring site the cuvette with studied solution and read his absorbance A_2.
6) Repeat this procedure again and again until you obtain the absorbance value 550 nm (A_3…..A_N)
7) Plot a graph how the absorbance depend on the wavelength of light and mark its maximum, that means the wavelength at which the light is absorbed most.

Task 2
Spectrophotometrical determination of concentrations of solutions.

Needs for measurement:

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

Procedure:

1. Dilute the stock solution of known concentration (5µg/L) so as to obtain some other solutions of known lower concentration than the original. It`ll be the best, if you use the dilution rule.(e.g. From solution of eosin of concentration of 5µg/L by means of the dilution rule we obtain solutions of concentrations of 4, 3, 2 and 1µg/L by diluting with distilled water).
2. Switch on spectrophotometer and by means of wavelength selector set the wavelength of light at maximum of eosin, known from previous task (515 nm).
3. Always grip the cuvette at eggshell surface
4. Cuvettes must be filled almost up to the edge. Into the single grip of the moveable holder of spectrophotometer Boeco first insert the cuvette with distilled water and after insert cuvette with studied solution of lowest concentration.
5. Perform absorbance measurement for distilled water. The value 0.000 must appear on the display by pressing CAL. That means that for distilled water the absorbance value was automatically set to 0.000. Now shift into the measuring site of the module the cuvette with studied solution and read its absorbance A_1.
6. Into the measuring site shift again the cuvette with distilled water. After zero setting the display (The distilled water absorbance is 0.000 again) insert into the measuring site the cuvette with diluted solution of known concentration and read his absorbance A_2
7. Repeat this procedure again and again with all prepared solutions of known concentrations (A_3…..A_5). Always calibrate the spectrophotometer before each new concentration with distilled water. (absorbance of distilled water - 0.000). Don`t change the wavelength. Take down used concentrations and measurement results into a table and made a graph of depend absorbance on concentration.
8. Last measuring do with the **solution of unknown concentration (x)**. Find out from plotted graph the real **concentration** of unknown sample.

2. Refractometry – Determination of NaCl concentration

Main tasks:
Determination of concentration of NaCl in unkown sample.

Needs for measurement:
Refractometer (For using the Abbe refractometer a lighting lamp is required.), crystalline NaCl, stand with test tubes, automatic pipettes, distilled water, cellulose wadding, laboratory balance, weighing vessel.

Procedure:

1. Suppose that the concentration of the unknown NaCl solution is not higher than 200g/L. Therefore prepare 10ml NaCl solution of concentration 200g/L (10ml of distilled water and 2g of NaCl). From prepared concentration 200g/L prepare the others by diluting with distilled water: 150g/L, 100g/L, 50g/L. Use pipette to keeping the ratio.
2. Swing away the lighting and measuring prisms of the refractometer and make sure that the instrument is clear. If you find on any of the surfaces of the prisms or in their surroudings

remains of some solution or traces of dry NaCl, wash them with distilled water and dry gently with cellulose wading.

3. Put a trace of studied liquid on surface of the prism and close the prisms again.

4. By means of the bottom switch of the refractometer set the instrument so you can observe the interface of light and shade by the eyepiece. This interface can be coloured and not sharp (with respect to the phenomenon of optical dispersion). For removing this aberration there is upper switch on the instrument which moves with compensation prisms that compensate dispersion of the measured substance. Set the compensate interface to the center of reticle.
Note: We find illuminated field by light of which optical dispersion is at most similar to optical dispersion of used liquid, by setting the compensation prisms.

5. Read the value of optical dispersion of liquid by the eyepiece (bottom part). (with accuracy at least of three decimal places)

6. Gradually measure refraction indexes of calibration solutions, including original solution. Firstly measure distilled water then proceed from the less concentrated solution to the more concentrated one. Take down all measured results into a table and then plot graph of the dependence of the refraction indexes on the concentration.

7. Lastly measure the refraction index of **unknown solution** and plot it into graph, find his concentration from the graph.

8. After finalization of measuring clean the surfaces of both prisms with distilled water and dry them again with cellulose wadding.