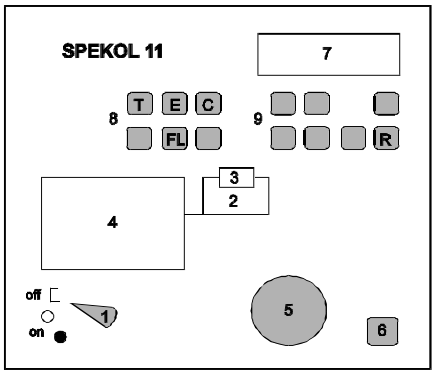
FLUORESCENCE ANALYSIS

ATTENTION! Plug in the spectrometer "SPEKOL -11"
and press the button of the power-switch (6).
After ca 20 minutes the device is ready to work.

Equipment:

1. Spektrometer "SPEKOL - 11"
2. Stock solution of fluorescein.
3. Solution of fluorescence quencher (KI).
4. Set of test-tubes- 7 tubes.
5. Measuring cuvettes - 2 pcs.
6. Pipettes: 0.5 – 5 ml volume range regulated; 0,2 ml for the fluorescence quenching solution.



Description of symbols:
1 – light diaphragm switch. 2 – cuvette holder. 3 - cuvette. 4 – photodetector case. 5 – wavelength setting-up knob. 6 – power-switch. 7 – display window. 8 – key buttons for setting the operation mode. 9 – key buttons for entering the data to the memory.

Your first task is to obtain the fluorescent spectrum of the stock solution of fluorescein in the visible light range. For this purpose, you have to measure the intensity of fluorescence as a function of excitation wavelength and plot it on a graph. The wavelength of the maximal fluorescence intensity should be read off from the spectrum. You are going to use this wavelength for measurements of fluorescence intensity as a function of fluorescein concentration. If you plot the obtained results you will obtain a calibration curve (fluorescence intensity vs. fluorescence concentration). The calibration curve will be then used for determination of fluorescein concentration of analyzed solutions.

Course of measurements:

1. Measure fluorescence intensity as a function of the excitation wavelength in the range 400 - 560 nm. First pour distilled water into one cuvette (fill it to about 3/4 of its height) and place the cuvette into the holder (2); lift up the holder with the cuvette to place the cuvette in the spectrometer. Set the light diaphragm switch (1) in the middle position (sign o). Using the screw (5) set the excitation wavelength to 490 nm. Press the key button FL to set the fluorescence operation mode. Press the key Z-FL to set the zero value of fluorescence (see the display window). Press the key FAKT (you should now see 1.000 in the display window). Then pour the stock solution of fluorescein to another cuvette and place this cuvette into spectrometer. Press the key FAKT again, and then the key R. After a while you see the value of fluorescence intensity in arbitrary units displayed in the window (7). Now, by turning the knob (5) change the wavelength in the range from 460 to 560 nm every 10 nm, and read off the values of the intensity from the display window. Write down the data to the Table 1. Draw a spectrum of the fluorescence intensity as a function of the excitation wavelength on a graph paper. Read off the wavelength at which the fluorescence intensity reaches the maximum.

2. Measurements of the fluorescence intensity as a function of fluorescein concentration.
Dilute the stock solution of fluorescein to obtain 7 solutions of different concentrations of fluorescein. Using a pipette with the regulated volume pour 1; 2; 3; 4; 6; 8 and 10 cm^3 of the fluorescein stock solution to the test-tubes and add distilled water to obtain the volume of 10 cm^3. Using the knob (5) set the excitation wavelength to the value for maximal fluorescence intensity obtained previously. Pour the first fluorescein solution into the cuvette and read off window the measured intensity of fluorescence from the display. Start the measurements with the solution with the lowest concentration of fluorescein. Write down the data to the Table No 2.

ATTENTION: After each measurement the measured sample of solution must be poured back to the same test-tube it was taken from. The solutions will be used in the last part of the practical.

On the graph paper draw the calibration curve of the fluorescence intensity (N) as a function of fluorescein concentration N = f (c).

3. Determine the fluorescein concentration in solutions of unknown concentrations. Pour the samples one after another to the cuvette and read off the measured fluorescence intensity from the display window. Using the calibration curve find the concentration of fluorescein in the analyzed solution.

4. Examine the effect of fluorescence quencher. Add 0.2 ml of potassium iodide (KI) to the fluorescein solutions in the test tubes. Mix the tubes' content and wait c.a. 5 minutes. Measure the fluorescence intensity of the solutions with KI. The dilution effect caused by the addition of KI to the solutions is minimal and can be neglected. Plot the measured intensities as a function of fluorescein concentration. Use the same graph as you used to plot the calibration curve. Compare the curves obtained in the absence and presence of the quencher.

Required theoretical knowledge:

1. Luminescence.
2. Mechanisms of fluorescence and phosphorescence.
3. The Stokes shift.
4. Quantum yield of fluorescence.
5. Fluorescence quenching.
6. Application of fluorescent measurements for qualitative and quantitative analysis.
7. Application of fluorescence markers in examination of membrane transport processes and intracellular processes.
8. Application of fluorescence in cancer diagnostics
9. Bioluminescence.

Wrocław Medical University Department of Biophysics and Neuroscience	**Practical No 4** **Fluorescence analysis**
……..…… ……..…….. ……..…… Student names	Faculty: …….. Group number: …… Date: ……
Grade:	Tutor's signature:

1. Measure the intensity of fluorescence (N) of the fluorescein stock solution as a function of excitation wavelength.

λ [nm]	**460**	**470**	**480**	**490**	**500**	**510**	**520**	**530**	**540**	**550**	**560**
Fluorescence intensity											

2. Draw the spectrum of fluorescence intensity as a function of excitation wavelength: $N = f(\lambda)$, next to the fluorescein emission spectrum. Find out the wavelength at which the intensity reaches its maximum - λ_{max}.

3. For this wavelength (λ_{max}) measure the fluorescence intensity as a function of fluorescein concentration.

The fluorescein stock solution concentration is **$1{,}5 \cdot 10^{-6}$ mol/dm^3**

Amount of fluorescein added	1 [cm^3]	2 [cm^3]	3 [cm^3]	4 [cm^3]	6 [cm^3]	8 [cm^3]	10 [cm^3]	C_x [mol/dm^3]	C_y [mol/dm^3]
Concentration [mol/dm^3]									
Fluorescence intensity									

4. On the graph paper draw the fluorescence intensity as a function of fluorescein concentration (calibration curve). Measure the fluorescence intensity for solutions of unknown fluorescein concentrations (c_x and c_y) and determine their the concentration using the calibration curve.

5. For the wavelength λ_{max} measure the fluorescence intensity as a function of fluorescein concentration in the presence of potassium iodide added to quench the fluorescence.

Amount of fluorescein added	1 [cm^3]	2 [cm^3]	3 [cm^3]	4 [cm^3]	6 [cm^3]	8 [cm^3]	10 [cm^3]
Concentration [mol/dm^3]							
Fluorescence intensity							

6. Plot fluorescence intensities obtained in the presence of KI as a function of fluorescein concentration.

Emission spectrum of fluorescein