

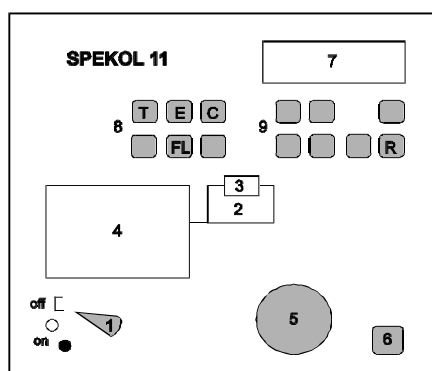
FLUORESCENCE ANALYSIS

Aim of the practical

The aim of the practical is to become familiar with the phenomenon of luminescence and its use for quantitative analysis in laboratory practice. To this end it is necessary to record the fluorescence excitation spectrum of an aqueous fluorescein solution in the visible range. The fluorescence intensity is measured at different excitation wavelengths, and the results are plotted. The wavelength corresponding to the maximum fluorescence intensity is determined from the plot. This wavelength is then used to measure the fluorescence intensity of solutions with different fluorescein concentrations. A calibration curve is prepared and used to determine the concentration of the studied solutions.

Equipment and solutions

1. SPEKOL 11 spectrophotometer.
2. Fluorescein solution.
3. KI solution.



1. Light-shutter switch.
2. Cuvette holder.
3. Cuvette.
4. Photocell housing.
5. Wavelength-setting drum.
6. Mains switch.
7. Display window.
8. "Measurement mode" keypad.
9. Data-entry keypad for measurement parameters.

Pic 1. Front panel of the SPEKOL 11

Course of measurements:

ATTENTION! On entering the laboratory, plug in the SPEKOL-11 and press the mains switch (6).
After about 20 min the instrument is ready for measurements.

I. Measurement of fluorescence intensity as a function of excitation wavelength.

1. Fill a cuvette with distilled water up to $\frac{3}{4}$ of its height; place it in the cuvette holder (2).
2. Insert the cuvette into the light path using the cuvette-changer holder.
Gently move the holder **downwards**, insert the cuvette, rotate the holder by 180° (as far as it goes) and **gently raise** it back to the previous position — this brings the cuvette into the excitation-light path.
3. Turn the light-shutter switch (1) to the middle position (between "ON" and "OFF", marked [o]).

4. Set the wavelength on the drum (5) to **490 nm**.
5. Press the **FL** key — the instrument is programmed for fluorescence measurement.
The LEDs above the keys Z-FL MIN and FAKT flash.
6. Press **Z-FL MIN** — zero is set for the distilled-water sample.
The LEDs above FAKT and R flash.
7. Press **FAKT** — window (7) displays the stored factor value 1.000.
8. Insert into the monochromator light path the second cuvette filled to $\frac{3}{4}$ height with the stock solution of fluorescein. Do this as in step 2.
9. Press **FAKT**, and after a moment **R**.
10. Rotate the drum (5) to change the wavelength from **470** to **540 nm**, reading the fluorescence intensity in the window (7) at **10 nm** intervals.
Take readings approximately 10 seconds after inserting the sample into the light path.
11. Enter the results into Table 1.
12. Using the data from Table 1, plot the fluorescence intensity as a function of the excitation wavelength $f = f(\lambda)$ next to the emission spectrum of fluorescein (second page of the report), and find the wavelength at which the fluorescence intensity is maximal.

II. Measurement of the fluorescence intensity as a function of fluorescein concentration.

1. Prepare **7 samples** of different concentrations, each **10 ml** in volume, from the stock solution of fluorescein.
2. Fit a tip from box **3** onto pipette **3** and set the appropriate volume using the upper knob.
3. Using the pipette, transfer 1, 2, 3, 4, 6, 8 and 10 ml of the stock solution into seven clean test tubes; then top up each solution with distilled water to a total volume of 10 ml. Mix the prepared solutions with a glass rod.
4. Using the drum (5), set the monochromator to the wavelength corresponding to the maximum fluorescence intensity determined from the plot obtained in part I.
5. Pour the prepared solutions successively into the measuring cuvette (up to $\frac{3}{4}$ of its height), starting with the **lowest** concentration, and read the values shown in the display window (7).
Pour the measured samples back into their respective test tubes, as they will be needed later in the experiment.
6. Enter the measurement results into Table 2.
7. Using the obtained results, plot the fluorescence intensity as a function of solution concentration on the plotting paper — this yields the calibration curve $f = f(c)$.
8. Determine the unknown concentrations of the fluorescein solutions **C₁** and **C₂**.
9. Pour the solutions from bottles **C₁** and **C₂** successively into the measuring cuvette and record the readings in window (7).
10. Using the calibration curve (step 7) and the measured fluorescence-intensity values, find the unknown concentrations.

III. Effect of a fluorescence quencher on the emission intensity.

1. Fit a tip from box **2** onto pipette **2** and set the appropriate volume using the upper knob.
2. Add **200 µl** of potassium iodide (KI) solution with a pipette to each solution prepared in step II.3. Mix with a glass rod and wait 5 minutes.
3. Pour the prepared solutions successively into the measuring cuvette (up to $\frac{3}{4}$ of its volume), starting with the **lowest** concentration, and read the values shown in the display window.

4. Enter the data into Table 3.
The dilution effect caused by the increase in sample volume is negligible and can be ignored when calculating the solution concentration.
5. Using the results, plot the dependence of the fluorescence intensity on concentration (on the same graph as in step II.7) and compare it with the plot obtained without quenching.

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 20 Fluorescence analysis	
..... Student names	Faculty: Group number: Date:	
Grade:	Tutor's signature:	

I. Fluorescence intensity of fluorescein as a function of excitation wavelength (excitation spectrum).

Table 1.

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

II. Fluorescence intensity of a fluorescein solution as a function of its concentration.

The concentration of the initial solution is $1,5 \cdot 10^{-6} \text{ mol/dm}^3$.

Table 2.

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									

III. Fluorescence intensity of a fluorescein solution with KI quencher, as a function of fluorescein concentration.

Table 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]
Concentration [mol/dm ³]							
Fluorescence intensity							

Emission spectrum of fluorescein

