Spectroscopy Laboratory
SPECTROFLUORIMETRY

1. Determination of the effect of the concentration of a fluorescent compound on the fluorescence intensity.

- Having a solution of rhodamine G6 C=1 $mg \cdot mL^{-1}$, prepare a series of methanol solutions with the following concentrations: 0,1 $mg \cdot mL^{-1}$, 10 $\mu g \cdot mL^{-1}$, 5 $\mu g \cdot mL^{-1}$, 1 $\mu g \cdot mL^{-1}$, 0,5 $\mu g \cdot mL^{-1}$. Compare the glow intensity of individual solutions under UV light. Record the absorption spectrum in the range of 300-700 nm.
- Determine the excitation wavelength and plot the fluorescence emission spectra of all solutions in the range of 400-700 nm.

2. Determination of the concentration of a fluorescent substance in a sample using the standard curve method.

- For the samples from point 1, determine the standard curve by recording the emission spectra at the maximum excitation wavelength. For this purpose, use the appropriate device software (width of both slits: 2.5 nm).
- Using the software, record the emission spectrum for a sample with an unknown concentration of a fluorescent compound. Then determine the concentration of the fluorescent compound.

3. Comparison of the quenching of fluorescein fluorescence by halide ions.

- Prepare the fluorescein stock solution. For this purpose, take 0.25 mL of a solution of fluorescein with a concentration of 7×10^{-4} M in 0.1 M NaOH and fill it up to 25 mL with distilled water.
- Plot the absorption spectrum of fluorescein in the range of 250-700 nm, and then the fluorescence emission spectrum (400-700 nm) by exciting the sample with light with a wavelength of 301 nm (width of both slits: 2.5 nm).
- Prepare two series of fluorescein solutions (final volume = 1.5 mL) containing: 0.037 mL; 0.075mL; 0.15mL; 0.3 mL; 0.45 mL 0.5 M KI or 0.5 M KCl. Plot the emission spectra.

Przyrządy, materiały i odczynniki

- Spectrofluorimeter Jasco FP-8500
- rodamin G6 solution (1 $mg \cdot mL^{-1}$)
- methanol
- fluorescein solution (7×10^{-4} M w 0,1 M NaOH)
- 0,5 M KI
- 0,5 M KCl
- plastic cuvettes with lids
- automatic pipette with tips
- bottles for preparing solutions with lids
- distilled water
- UV lamp
- volumetric flask (25 mL) with stopper

Preparation of results

- Place the determined absorption and emission maximums in the table. Describe the recorded fluorescence absorption and emission spectra, present examples of rules characterizing fluorescence. Discuss the effect of concentration on fluorescence intensity.

- Draw a standard curve and calculate, using the appropriate formula, the concentration of the fluorescent agent in a sample of unknown concentration.

- Calculate the concentration of fluorescein, KI and KCl in the tested solutions and the I0/I ratio. Collect the results in a table. Based on the obtained results, prepare a Stern-Volmer chart (I0/I vs [Q]).

Sterna-Volmera equation:

$\frac{I_0}{I} = 1 + K_{sv}[Q]$, gdzie:

I_0 - fluorescence emission intensity without quencher;
I - fluorescence emission intensity with quencher;
K_{SV} - Sterna-Volmera constant [M^{-1}];
Q - quencher concentration [M]

Determine the Stern-Volmer constant from the graph. Discuss the results. Which quencher is more effective?

The scope of material:

- Jabłoński diagram;
- fluorophores;
- rules characterizing fluorescence: Stokes' rule, mirror symmetry rule, Kasha's rule, Vavilov's rule;
- fluorescence quantum yield;
- fluorescence lifetime;
- fluorescence quenching (dynamic and static), Stern-Volmer equation and diagram; the use of fluorescence quenching in protein structure studies.

Literature:

- W. Szczepaniak „Metody instrumentalne w analizie chemicznej”, Wydawnictwo Naukowe PWN;
- P. Suppan "Chemia i światło" Wydawnictwo Naukowe PWN, 1997;
- A. Kozik, M. Rąpała-Kozik, I. Guevara-Lora "Analiza instrumentalna w biochemii. Wybrane problemy i metody instrumentalnej biochemii analitycznej" seria wydawnicza IBM UJ, 2001;
- J. Lakowicz „Principles of fluorescence spectroscopy” Springer, New York, 2006