# M8_on-line. The phenomenon of absorption and emission of light in analysis. Determination of concentrations of substances in solutions using fluorescence.

The aim of the exercise is to determine fluorescence spectra of water solutions of riboflavin. The fluorescence intensity will be registered for different known concentrations of riboflavin. And further, the unknown concentration of riboflavin will be determined.

**Problems to be prepared**:

- Quantum Theory of Light. Molecular *electronic transition*
- Absorption. Transmittance. The Beer-Lambert Law.
- Fluorescence. *Jablonski diagram.*
- Absorption and fluorescence spectra.
- Absorption and fluorescence spectroscopy – methodology, apparatuses.

## Manual

1. Determine excitation spectrum for riboflavin (Figure 1). By using arrows set an excitation wavelength $\lambda_{exc}$ on monochromator in range of 220 – 515 nm with 5 nm step and read corresponding photo-current $I$ (proportional to light intensity) on the ammeter. Fill the table below and plot a graph $I = f(\lambda_{exc})$.

| $\lambda_{exc}$ (nm) | $I$ (μA) |
|---|---|
| | |

2. Determine emission spectrum for riboflavin (Figure 2). By using arrows set an emission wavelength $\lambda_{em}$ on monochromator in range of 420 – 690 nm with 5 nm step and read corresponding photo-current $I$ (proportional to light intensity) on the ammeter. Fill the table below and plot a graph $I = f(\lambda_{em})$.

| $\lambda_{em}$ (nm) | $I$ (μA) |
|---|---|
| | |

3. Determine quantitative analysis for riboflavin (Figure 3). By using arrows set concentration $c$ in the sample in range of $10^{-6}$ – 9 x $10^{-6}$ mol/l with $10^{-6}$ mol/l step and read corresponding photo-current $I$ (proportional to light intensity) on the ammeter. Fill the table below and plot a graph $I = f(c)$.

| $c$ (mol/l) | $I$ (μA) |
|---|---|
| | |

4. Determine unknown concentration $c_x$ (Figure 4). Read photo-current $I$ (proportional to light intensity) on the ammeter corresponding to $c_x$. Knowing the parameters obtained from linear fit calculate $c_x$. Mark $c_x$ on the printed graph. Fill the table below.

| $c_x$ (mol/l) |
|---|
| |

5. Make the conclusions.

**Literature**


1. **For example**: http://nathan.instras.com/MyDocsDB/doc-800.pdf

Under this address one can find free version of Principles of Fluorescence Spectroscopy Third Edition Joseph R. Lakowicz

2. Other books on absorption, fluorescence spectroscopy.