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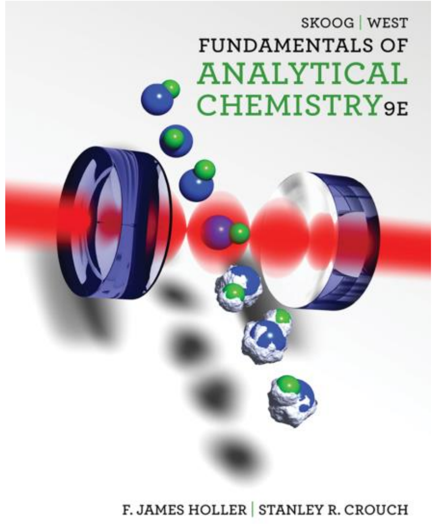
1.



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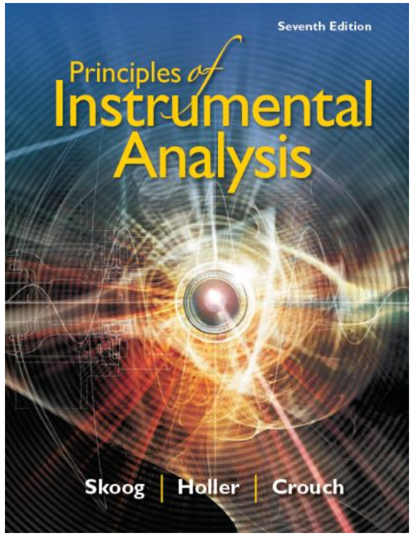


3.

ATAKOL's
lecture notes

1. Skoog, D.A., West, D.M., Holler, J.F., Crouch, S.R. 2013. Fundamentals of Analytical Chemistry (9E). Cengage Learning, Belmont, USA.
2. Skoog, D.A., Holler, J.F., Crouch, S.R. 2016. Principles of Instrumental Analysis (7E). Cengage Learning, Boston, USA.
3. Prof. Dr. Orhan ATAKOL's lecture notes.

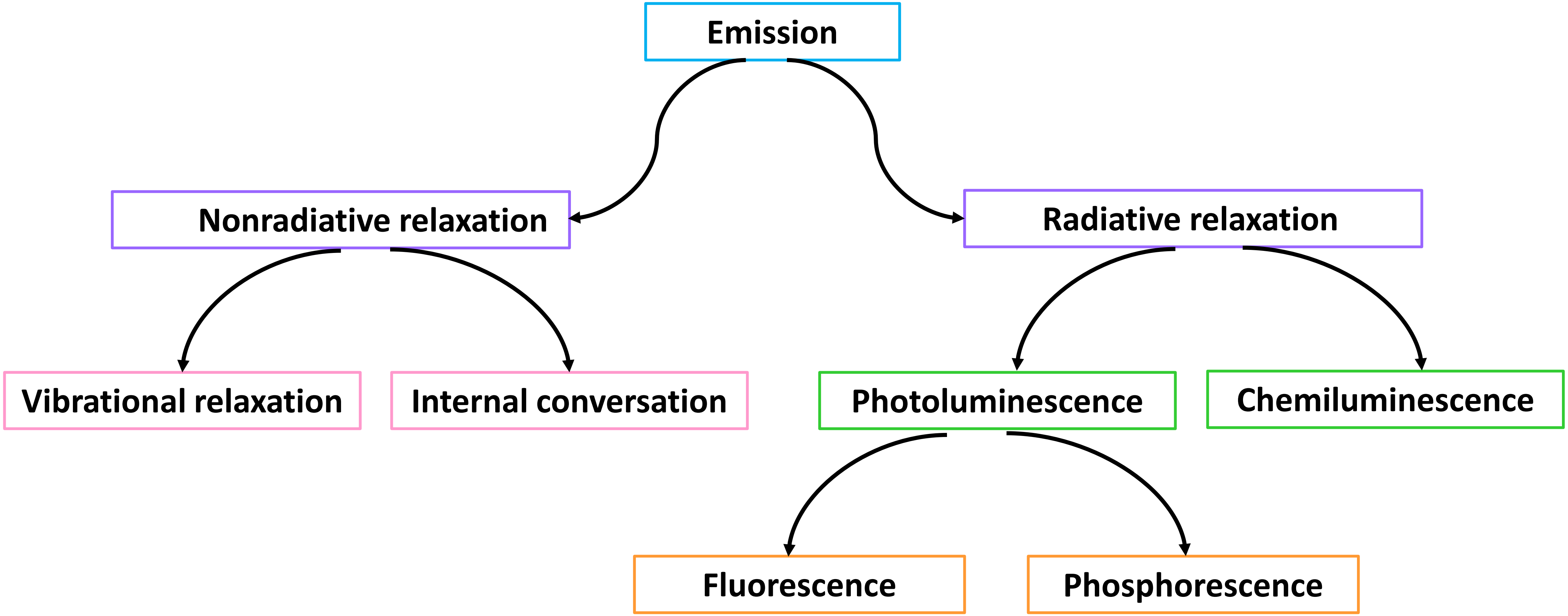
LUMINESCENCE METHODS-FLORIMETERS

The excited species relax to the ground state, giving up their excess energy as photons (emission). **Emission** is examined under two headings: radiative and non-radiative relaxation.

In **photoluminescence spectroscopy** the emission of photons is measured following absorption. The most important forms of photoluminescence for analytical purposes are **fluorescence** and **phosphorescence spectroscopy**.

Fluorescence is a photoluminescence process (emission with radiative relaxation) in which atoms or molecules are excited by absorption of electromagnetic radiation.

Chemiluminescence is produced when a chemical reaction yields an electronically excited molecule, which emits light as it returns to the ground state.


Emission
Nonradiative relaxation
Radiative relaxation
Vibrational relaxation
Internal conversation
Photoluminescence
Chemiluminescence
Fluorescence
Phosphorescence

Molecular Fluorescence

- Molecular fluorescence is measured by exciting the sample at an absorption wavelength, also called the excitation wavelength, and measuring the emission at a longer wavelength called the emission or fluorescence wavelength.
- Usually photoluminescence emission is measured at right angles to the incident beam to avoid measuring the incident radiation.
- The short-lived emission that occurs is called fluorescence, while luminescence that is much longer lasting is called phosphorescence.
- Fluorescence emission occurs in 10^{-5} s or less.
- In contrast, phosphorescence may last for several minutes or even hours
- Fluorescence is much more widely used for chemical analysis than phosphorescence.

Advantages of Fluorescence

- One of the most attractive features of molecular fluorescence is its inherent sensitivity, which is often one to three orders of magnitude better than absorption spectroscopy.
- Another advantage is the large linear concentration ranges of fluorescence methods, which are significantly broader than linear concentration ranges in absorption spectroscopy.
- Fluorescence methods are, however, less widely applicable than absorption methods because of the smaller number of chemical systems that show appreciable fluorescence.
- Fluorescence is also subject to many more environmental interference effects than absorption methods.

Fluorescence measurements

For this measurement, two wavelength selectors are needed to select the excitation and the emission wavelengths. The selected source radiation is incident on the sample and the radiation emitted is measured, usually at right angles to avoid detecting the source radiation and to minimize scattering.

In the measurement of the radiant power emitted as the analyte returns to the ground state can give information about its identity and concentration. The results of such a measurement are often expressed graphically by a spectrum, which is a plot of the emitted radiation as a function of frequency or wavelength.

Fluorescent Species and The Quantum Yield

- The **quantum yield** of molecular fluorescence is simply the ratio of the number of molecules that fluoresce to the total number of excited molecules, or the ratio of photons emitted to photons absorbed.

$$\Phi = \frac{R_f}{R_f + R_{nr}}$$

Φ = Quantum Yield
R_f = Fluorescence relaxation
R_{nr} = Nonradiative relaxation

- Highly fluorescent molecules, such as fluorescein, have quantum efficiencies that approach unity under some conditions.
- Species that do not fluoresce or that show very weak fluorescence have quantum efficiencies that are essentially zero.

FACTORS AFFECTING THE FLUORESCENT FEATURE

* Structural Rigidity

* Conjugate double-bonded structures

* pH

* Temperature and Solvent Effects

* Electronic transitions

* Number of rings

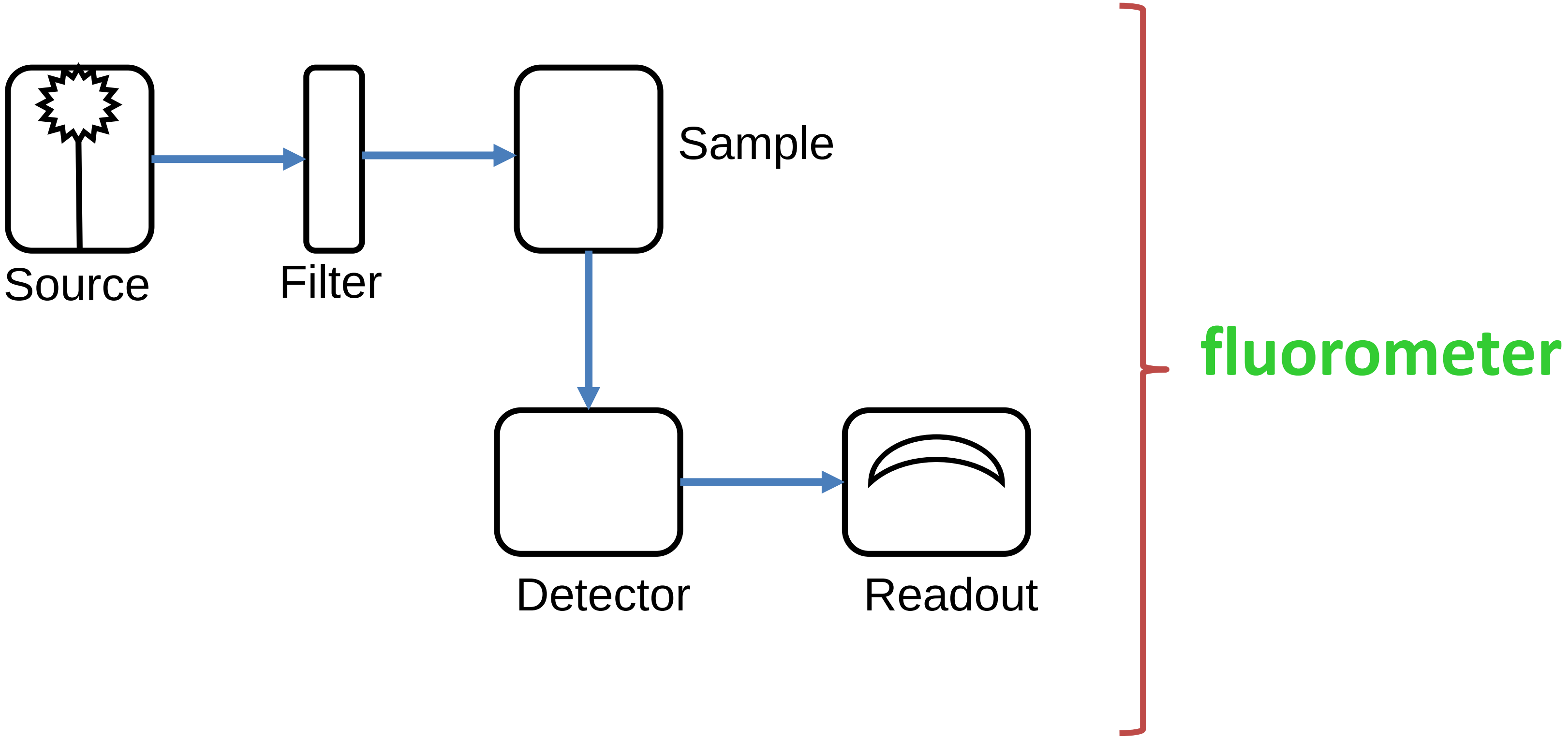
* Presence of heteroatoms

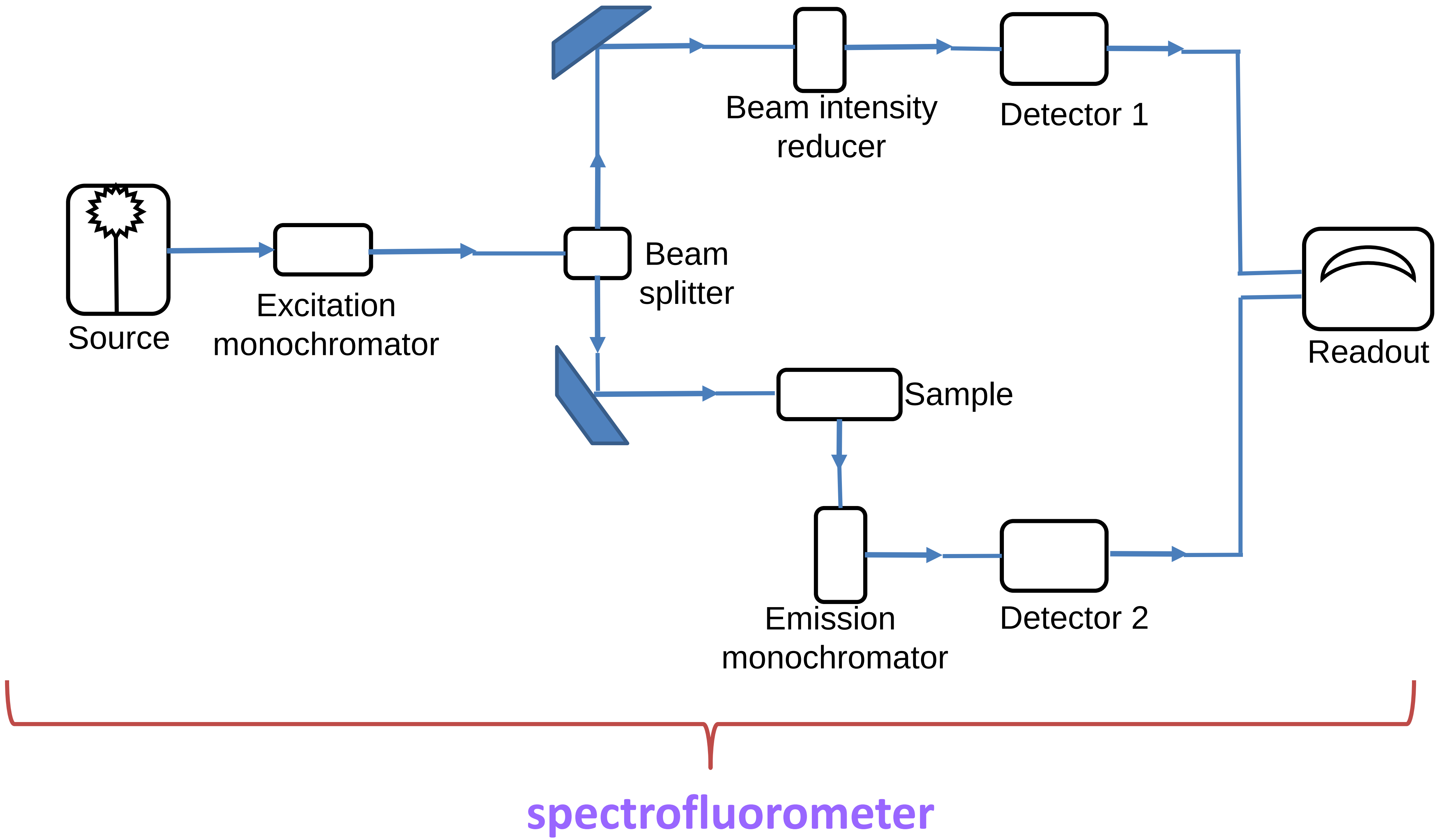
* Formation of chelates

Fluorescence Instrumentation

There are several different types of fluorescence instruments:

- If the two wavelength selectors are both filters, the instrument is called a fluorometer.
- If both wavelength selectors are monochromators, the instrument is a spectrofluorometer.




Beam intensity reducer
Detector 1
Source
Excitation monochromator
Beam splitter
Readout
Sample
Emission monochromator
Detector 2
spectrofluorometer

Applications of Fluorescence Methods

- Quantitative fluorescence methods have been developed for inorganic, organic, and biochemical species
- Inorganic fluorescence methods can be divided into two classes:
  - direct methods and
  - indirect methods
- **Direct methods** are based on the reaction of the analyte with a complexing agent to form a fluorescent complex.
- **Indirect methods** depend on the decrease in fluorescence, also called quenching, as a result of interaction of the analyte with a fluorescent reagent Quenching methods are primarily used for the determination of anions and dissolved oxygen.