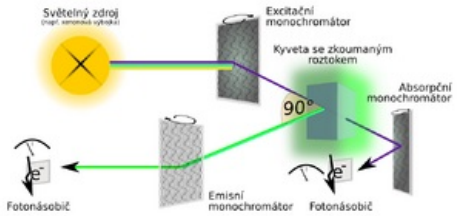
Luminescence methods

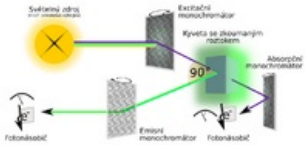
Among the more technically demanding, but very sensitive optical methods are procedures that use '*light emitted by the determined substance*. Strictly speaking, this would also include flame emission photometry, in which an electron is excited to a higher energy state by a flame and emits light when it returns to a lower energy level. Mostly, however, the electron is excited by radiation (photoluminescence, used in fluorimetry, and phosphorescence), or a substance with an excited electron is created in a chemical reaction (chemiluminescence, used in luminimetry).

Fluorimetry and spectrofluorometry

Fluorimetry uses the photoluminescence phenomenon. The fluorescent substance is excited by monochromatic light, which excites one of the valence electrons to a higher energy level. When returning to its original energy state, part of the energy is wasted as heat, part is emitted in the form of a photon. '*The energy of the emitted radiation is therefore always lower than the energy of the excitation radiation*, i.e. the emitted light has a longer wavelength. The emitted light is usually scanned in a direction perpendicular to the excitation beam, and after passing through the emission monochromator, its intensity is measured by a photomultiplier.



Most fluorimeters use interference filters as excitation and emission monochromators. More expensive devices are equipped with optical gratings, so that the excitation and emission wavelengths can be continuously adjusted. In that case it is called **spectrofluorimetry**'.



Compared to photometric methods, spectrofluorimetry has higher specificity (in addition to the absorption, i.e. excitation spectrum, the emission spectrum of the substance is also taken into account) and sensitivity (thanks to the photomultiplier, very small light intensities can be measured and since it is measured in the direction perpendicular to the excitation and at a different wavelength than the one at which it is excited, the emitted light is minimally "polluted" by the excitation radiation). Unfortunately, the method hides a number of technical pitfalls: fluorescent reagents are often very sensitive to minimal changes in pH, ionic strength or polarity, to the presence of oxidizing agents or so-called quenchers (substances that enable the descent of an excited electron to the basic energy level without would emit a photon – often these are e.g. trace amounts of some transition metals), in addition, relatively expensive and complex equipment is required.

The analytical possibilities of spectrofluorimetry can be further expanded by including polarization filters in the excitation and emission parts of the device - so-called *polarization fluorimetry*. This technique takes advantage of the fact that an excited electron returns to the ground state with a certain delay. If this delay were completely negligible or the fluorescent molecules were completely immobile, the emitted radiation would retain the same polarization as the excitation radiation. However, if the time between excitation and photon emission is sufficient to rotate the excited molecule, the plane of polarization of the emission radiation can be rotated with respect to the plane of polarization of the excitation radiation. Under laboratory conditions, the rotational mobility of fluorophores in solutions is sufficient to partially depolarize the emission radiation. At the same time, small molecules can rotate faster than large ones, so the size of the fluorescent molecule can be deduced using polarization fluorimetry. This is used, for example, in immunochemistry, where in this way it is possible to distinguish between a small free fluorescently labeled antibody and a large complex of this antibody with antigen.

Especially in research, a number of other variants of spectrofluorimetry are used: interesting data can be obtained, for example, if the sample is illuminated with very short flashes of light and the time course of fluorescence is measured, the spectrofluorimeter can be connected to a fluorescence microscope, chromatograph, etc. as in the case of photometry, it can be measured in special microtitre plates, etc.

Luminimetry

In chemiluminescence, electrons are excited by a chemical reaction. It must be a significantly exoergic event, almost exclusively oxidation. However, the released energy must not be released as heat. Some substances can emit part of the energy in the form of a photon directly, which is manifested by a short flash of light. In other cases, it is necessary to add another substance to the system, to which the energy is transferred and which then (usually for a number of seconds to minutes) **emits light*. It can be synthetic luminophores or the natural enzyme luciferase of fireflies - then we talk about* bioluminescence*. Finally, it is possible to use the oxidation of a suitable substance electrically at the anode, which is referred to as** *electrochemiluminescence*''.

Luminometric methods tend to be quite sensitive. Luminimeters are similar in principle to the emission part of a fluorimeter. They often use filters as a monochromator and a photomultiplier is usually connected as a detector.

Links

- ws:Luminiscenční metody

References

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