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LUMINESCENCE AND ITS APPLICATION IN MEDICINE

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Abstract

The article examines the physical foundations of the luminescence phenomenon, types of luminescence, its classification based on excitation methods and temporal characteristics (fluorescence and phosphorescence) fundamental laws of luminescence (Stokes' law and Vavilov's law), which determine the spectral and energetic features of this process, application of luminescence analysis in medical practice.

Keyword

luminescence and its laws, fluorescence, phosphorescence, photodynamic therapy, luminescence analysis, photosensitizers, medical diagnostics, quantum yield.

Luminescence is radiation that exceeds thermal radiation at a given temperature and in a given spectral range, and which has a duration of afterglow significantly exceeding the period of light waves. The first part of this definition was proposed by E. Wiedemann and distinguishes luminescence from equilibrium thermal radiation. The second part—the duration criterion—was introduced by S. I. Vavilov to distinguish luminescence from other types of secondary emission (such as bremsstrahlung of charged particles or Vavilov-Cherenkov radiation).

Types of luminescence, its characteristics, and laws.

Typically, when referring to luminescence, we mean radiation from complex molecules. For a substance to luminesce, its molecules must be brought into an excited state. Based on the method of particle excitation, the following main types of luminescence are distinguished:

1. Photoluminescence – excitation occurs as a result of the absorption of electromagnetic energy (usually in the visible or ultraviolet range).

2. Cathodoluminescence – excitation occurs due to the interaction of the substance's molecules with high-energy electrons (observed in kinescopes, cathode-ray tubes, etc.).

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3. X-ray luminescence– excitation is produced by X-rays (observed on X-ray luminescent screens).

4. Chemiluminescence– excitation occurs due to energy released as a result of a chemical reaction.

5. Bioluminescence – excitation of molecules occurs as a result of biochemical reactions taking place within a living organism (a specific type of chemiluminescence).

If the excitation of a molecule occurs continuously, the luminescence will also be continuous with a certain intensity I0, and in this case, it is called steady-state luminescence. If the excitation of the molecule is suddenly stopped, the emission does not disappear immediately; the intensity of the luminescence begins to decrease (decay according to an exponential law):

$$I_{люм} = I_0 e^{-\frac{t}{\tau}}.$$

Here, the parameter I is called the luminescence duration (afterglow duration or the lifetime of the excited state) and is the most important characteristic of a luminescent substance. It determines the time during which the luminescence intensity decreases by a factor of e ≈ 2.7. This time is usually determined from a graph obtained experimentally (Fig. 1).

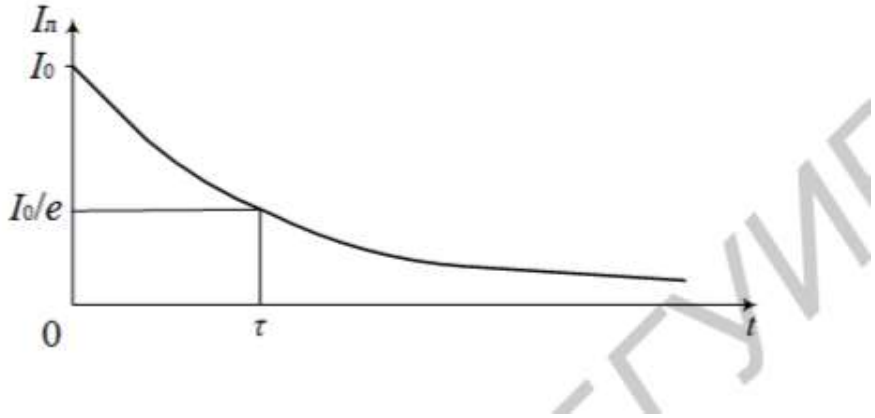

Fig. 1. Decay of luminescence intensity over time.

Based on the afterglow duration, luminescence is divided into two types:

Fluorescence, if 10^{-7} s, i.e., the luminescence decay occurs very rapidly (instantaneously to the human eye);

Phosphorescence, if 10^{-4} s; in this case, the decay is relatively slow and is often clearly visible to the naked eye.

The main characteristics of luminescence are:

1. Luminescence spectrum – the dependence of luminescence intensity on the wavelength of the emitted light.

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2. Luminescence duration – as previously noted, this is the time during which the luminescence intensity decreases by a factor of e.

3. Quantum yield of luminescence γ – the ratio of the number of luminescence quanta to the number of quanta absorbed during the excitation of a molecule: $\gamma = n_{lum}/ n_{abs}$. The quantum yield is always less than unity ($\gamma < 1$) due to the presence of non-radiative (non-optical) transitions. A substance is considered a good luminescent material if its quantum yield $\gamma > 0.01$, i.e., if $\gamma > 1$. **4. Excitation spectrum** – the dependence of the intensity of the radiation exciting the luminescence on its wavelength. It usually coincides with the absorption spectrum of the luminescing molecules.

Two fundamental laws of luminescence:

1. Stokes' Law. The luminescence spectrum of a substance is always shifted towards longer wavelengths relative to the spectrum of the absorbed radiation (Fig. 2).

Quantum theory provides the following explanation for this law: after absorbing a radiation quantum hv and transitioning to an excited state, a molecule may lose part of its energy by transferring it to other molecules through thermal motion, and the remaining energy is emitted as a luminescence quantum h_{lum}. If we denote the energy lost by the molecule as A, then $hv_{lum} = hv-A$. Consequently, the frequency of the luminescence radiation is lower than the frequency of the absorbed radiation, and the wavelength, accordingly, is longer.

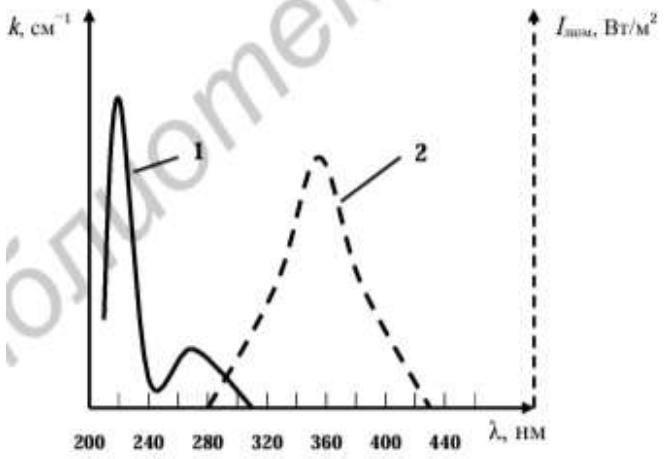

Fig. 2. Absorption (curve 1) and luminescence (curve 2) spectra.

2. Vavilov's Law. The quantum yield and the luminescence spectrum of complex molecules do not depend on the excitation wavelength. It can be any wavelength within the absorption band of the substance. Like the absorption spectrum, the luminescence spectrum is unique to each substance. This is utilized in the study of substance structures through luminescence analysis. Both the type

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and concentration of a luminescent substance can be determined from its luminescence spectrum. It should be noted that luminescence analysis is more than 1,000 times more sensitive than absorption spectral analysis. For instance, it allows for the detection of a luminescent substance in a mixture at extremely low concentrations: down to 10-12 g/L with a quantum yield of only about one percent.

Luminescence analysis in medicine: Many important biological objects are characterized by autofluorescence or contain fluorescent components called fluorophores. Protein fluorescence is due to the presence of three aromatic amino acids: tryptophan, tyrosine, and phenylalanine, which have absorption maxima at 280, 275, and 257 nm, respectively. Their fluorescence maxima in aqueous solutions occur at wavelengths of 348, 303, and 282 nm, with quantum yields of 20%, 21%, and 4%, respectively. The primary contribution to protein luminescence is provided by tryptophan; in its absence, tyrosine contributes, and only when both are absent does the weak fluorescence of phenylalanine manifest. For example, the ultraviolet fluorescence of collagen and elastin, which do not contain tryptophan, is associated with the tyrosine and phenylalanine within their composition. The fluorescence of collagen and elastin can be used to distinguish between different types of tissues, such as epithelium and connective tissue containing these proteins. In the red region of the spectrum, porphyrins are the primary fluorescing compounds.

In certain cases, the nature of the luminescence allows for the differentiation of pathologically altered tissues from healthy ones. For instance, the use of a fiber-optic catheter to excite and measure the fluorescence of the inner wall of arteries enables the diagnosis of atherosclerosis. Since the tissue begins to fluoresce with a different spectrum in cases of atherosclerosis compared to normal tissue, the presence of the disease can be determined based on these spectral differences (Fig. 3).

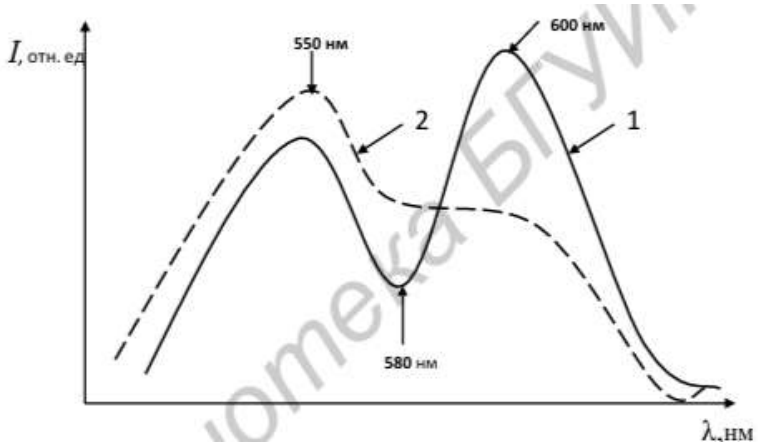

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Fig. 3. Fluorescence spectra of the artery wall: normal (curve 1) and pathological (curve 2).

Another example: when using radiation from a 440 nm LED laser to excite luminescence, a broad band in the red wavelength range with a maximum at 680 nm is observed in the fluorescence spectrum of a carious tooth (Fig. 4). Its appearance is associated with the luminescence of bacteria present in the carious cavity, and its intensity depends on the degree of tooth decay.

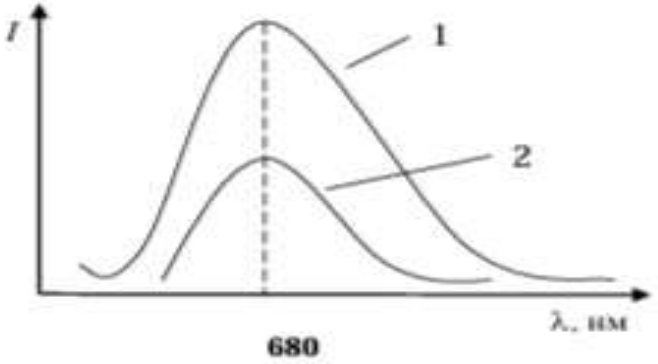

Fig. 4. Fluorescence spectra of a dental carious cavity before (curve 1) and after (curve 2) its mechanical treatment.

This fact can also be utilized for diagnostic purposes. Thus, after mechanical treatment of the carious cavity, the fluorescence intensity decreases due to the removal of bacterial microflora. Consequently, the fluorescence intensity can serve as an indicator of how well the carious cavity has been prepared for filling. The lower the fluorescence intensity, the higher the quality of the preparation. A number of biological objects do not possess intrinsic luminescence, or its intensity is too low. In such cases, special substances (fluorescent tags or probes) are used, which bind to the biological object to form a luminescent complex. Fluorescein is the most common fluorescent tag. A few seconds after injecting 1–2 mL of a 10–15% aqueous solution of fluorescein, bright green fluorescence can be observed in the eye, on the teeth, on the oral mucosa, etc. Fluorescein is harmless and is excreted from a healthy organism within 50–70 hours.

The fluorescein tag is used, for example, to delineate tumor boundaries during oncological surgeries. The latter is based on the ability of malignant tumors to accumulate fluorescein. If a patient who has been injected with fluorescein has a malignant tumor, for instance, of the gastrointestinal tract, this tumor glows lime green against the dark blue background of the intact mucosa, which allows its boundaries to be determined and makes the surgical intervention more conservative. Some fluorescent probes are used in photodynamic therapy of

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tumors. Such substances are called photosensitizers. Like fluorescein, they possess the property of accumulating in malignant neoplasms while being poorly retained in normal tissues. Their molecules are readily excited by visible light. When illuminated by a laser, photosensitizers produce an active form of oxygen, known as singlet oxygen, which is capable of damaging the tumor without disrupting healthy tissue.

In medical practice, hematoporphyrin-based drugs ("Photogem" in Russia) are most widely used as sensitizers. After the administration of the sensitizer, its fluorescence is used to assess the size and location of the tumor (Fig. 5).

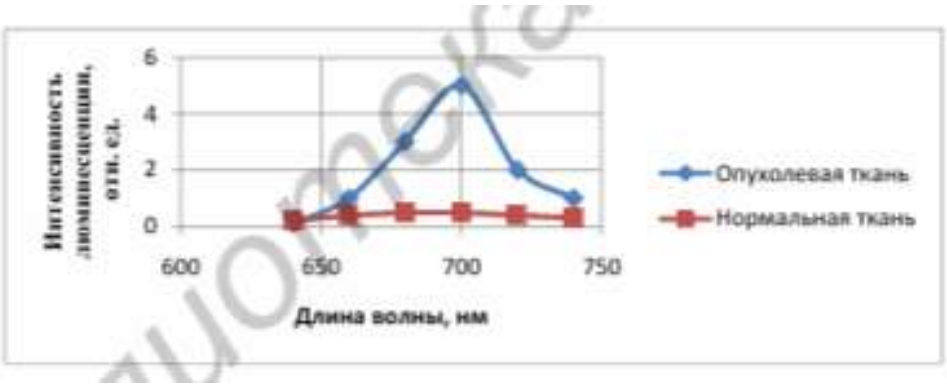

Fig. 5. Fluorescence spectra of tumor and normal tissues with the "Photogem" photosensitizer (2 mg/kg, 24 hours post-injection). Subsequently, the affected area is irradiated with a laser for 15–20 minutes. This process utilizes a light guide system that allows the laser light to be delivered to internal organs. The destruction of the malignant tumor and the partial or complete restoration of the affected areas are determined by the decrease in tumor fluorescence intensity, measured after a specific period of time (from 2 to 4 weeks) following the irradiation of the affected tissue. **Conclusion**

Observations and experiments demonstrate that luminescence is a highly sensitive and informative physical method widely applied in modern medicine. Due to the capability of performing non-invasive diagnostics at the molecular level, luminescent analysis methods enable the detection of pathological processes (such as atherosclerosis and carious lesions) at early stages. The use of fluorescent tags and photosensitizers opens new prospects in oncology, ensuring precise delineation of tumor boundaries and the implementation of selective photodynamic therapy. The high sensitivity of the method, which exceeds absorption analysis thousands of times over, makes it an indispensable tool in clinical laboratory diagnostics and surgical practice, significantly improving the efficacy and organ-preserving nature of medical interventions.

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