# LASER SPECTROSCOPY METHOD IN THE STUDING OF VIPERA LEBETINA OBTUSA VENOM

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PL spectra of vipera venom and its metabolits were studied exciting them by LGI-21 of nitrogen laser (337,1 nm, 10 ns). PL spectra cover the region of wavelength 360-630nm. Vipera venom and proteins have luminescence of different intensity and maxima at 490-530 nm. Half –width of PL spectra of vipera venom and proteins are at 0,62-0,83 eV.

Luminescent analysis finds a wide use in medicine and biology.

Laser diagnosis is the new prospective direction being effective means to study biological systems from biomolecules to cells, biotissues and the individual organs of animals and human [1,2].

In spite of the fact that laser medicine diagnosis is one of the effective directions, using of laser in biomedicine do not find the proper development [3].

Summarizing literary which is represented the various methods of laser diagnosis in biology and medicine is absent, what hampers their using in practice.

It is known that 90 % of whole fluorescence of proteins is conditioned by the presence of aromatic aminoacid – tryptophane in them. Proteins absorb time light near $\lambda$=280 nm, but strongly fluoresce in the region of $\lambda$=300-350 nm. Lifetime of fluorescence of tryptophane in proteins lies in 1-7 ns range, and depends on protein type and tertiary structure.

They also fluoresce in the amino-acids proteins as tyrosine, phenylalanine, cysteine and cystine [3].

The purpose of the investigation was studies on spectral – luminescent properties of proteins of the transcaucasian viper venom.

Proceeding from above spectral luminescent characteristics of the whole venom and its proteins divided by the method of gel – chromatography in the column with Sefadex –75 0.04 M phosphate sodium buffer were studied.

14 venom proteins with molecular mass from 20 to 149 kD were extracted from viper venom by the gel-filtration method.

Exciting spectrum and photoluminescence of samples were photographed in stationary and dynamical regimens in stationary regimens exciting – spectrum of venom covers the region of 440÷670 nm. It was used xenon lamps and mercurial lines 254, 313, 365 nm to excite of samples. Typical maxima at 470, 520 and 630 nm respectively are appeared in the exciting spectrum.

In dynamical regimen the samples were exciting by impulse nitrogen laser of LGI-21 type (337,1 nm, 10 ns). PL spectra are covered the region of wavelength 360÷650 nm. Maxima at 530 (fig.1). Were found out in the PL spectra.

Luminescence intensity of viper venom and its proteins were high during impulse nitrogen laser.

Venom protein luminescence is characterized by blue luminescence with the various intensity, that is proteins with the different molecular mass have luminescence maximum at unequal wavelength though the difference between them is not (within 10-40 nm).

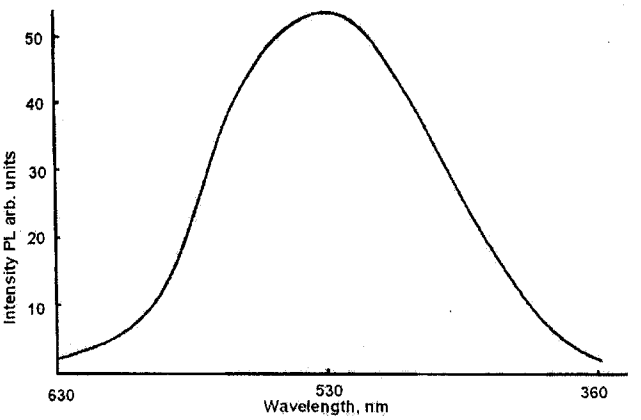


*Fig.1.* Photoluminescent spectrum of vipera venom for $\lambda_{max}$=530 nm.

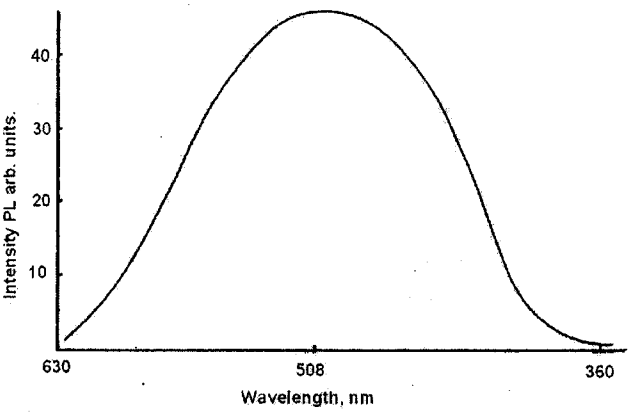


*Fig.2.* Photoluminescent spectrum of protein of vipera venom for maximum $\lambda_{max}$=508 nm.

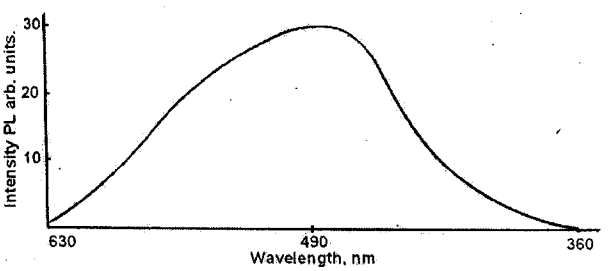


*Fig.3.* Photoluminescent spectrum of protein of vipera venom for maximum $\lambda_{max}$= 490 nm.

Spectral luminescent characteristics of venom and its proteins were present in table 1.

Table1. Spectral-luminescent characteristics of venom and its proteins.

| Mol. Mass of venom proteins, kD | Half-width of spectrum PL eV | $\lambda_{max}$ |
|---|---|---|
| 149 | 0.70 | 490 |
| 146.5 | 0.70 | 490 |
| 132.5 | 0.74 | 525 |
| 101 | 0.68 | 510 |
| 99 | 0.72 | 510 |
| 92.5 | 0.70 | 505 |
| 79 | 0.62 | 510 |
| 66 | 0.75 | 525 |
| 56.5 | 0.65 | 520 |
| 51.5 | 0.66 | 530 |
| 45 | 0.72 | 510 |
| 35 | 0.78 | 508 |
| 32 | 0.79 | 513 |
| 20 | 0.69 | 510 |
| snake | 0.83 | 530 |

PL spectra of venom and it's proteins (fig.2,3,4) have similar form, though one of them shine strongly others weakly.

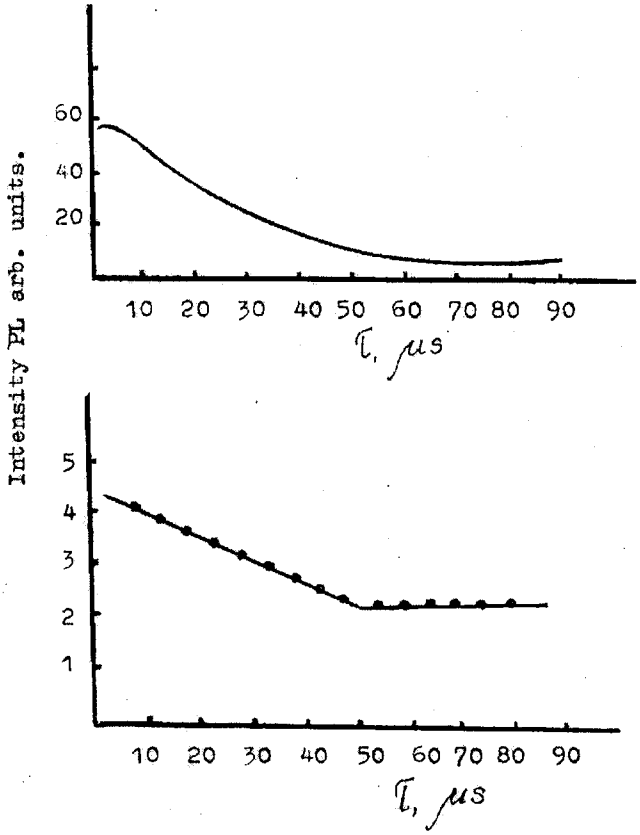


*Fig.4.* Time dependence of PL spectrum of vipera venom for maximum $\lambda$=530 nm at 300 °K.

Kinetics of luminescence of venom protein was studied (table 2).

Time dependence represented in semi logarithm scale for analyzing of experimental data

$$lg\ I = \int(t)$$

From formula $I(t)$ defined

$$\tau_D = \frac{0.43(t_2 - t_1)}{lg\ I_1 - lg\ I_2}$$

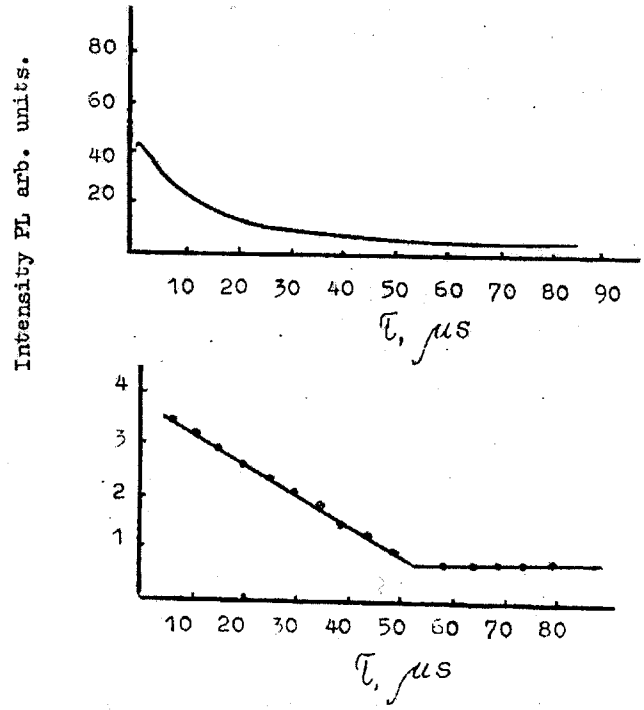


*Fig.5.* Time dependence of PL spectrum of protein of vipera venom for maximum $\lambda$=490 nm at 300 °K.

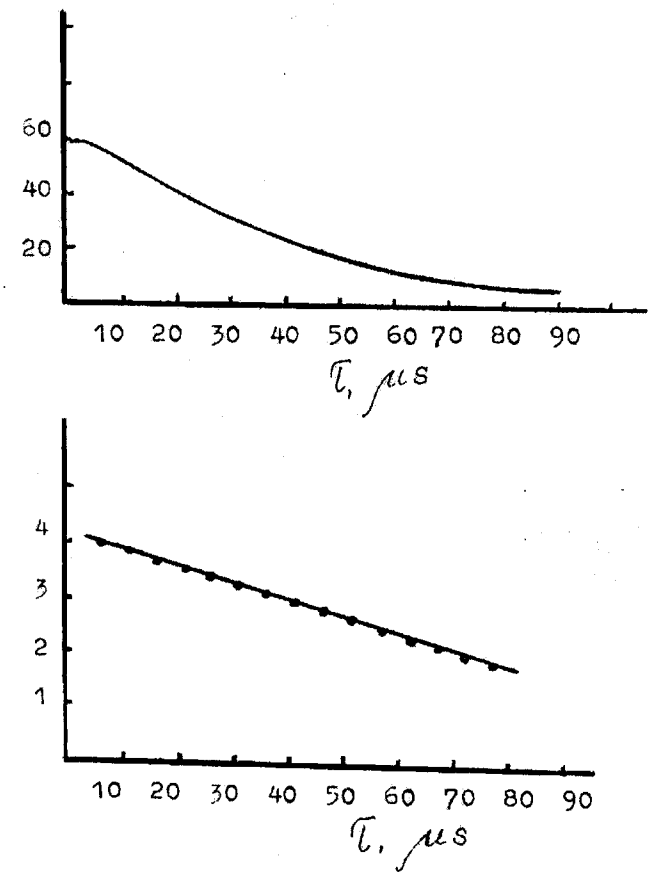


*Fig.6.* Time dependence of PL spectrum of protein of vipera venom for maximum $\lambda$=508 nm at 300 °K

Table 2. Temporal depend of luminescence of venom and it's proteins

| Intensity, $I$ in orbitraly units | | | Time $t$ in sec | | |
|---|---|---|---|---|---|
| Viper venom | Venom proteins M.m., kD | | Viper venom | Venom protein M.m., kD | |
| 52 | 35 | 149 | | 35 | 149 |
| 43 | 55 | 30 | 5 | 5 | 5 |
| 36 | 46 | 24 | 10 | 10 | 10 |
| 26 | 40 | 20 | 15 | 15 | 15 |
| 20 | 35 | 14 | 20 | 20 | 20 |
| 16 | 31 | 108 | 25 | 25 | 25 |
| 14 | 28 | 6 | 30 | 30 | 30 |
| 12 | 24 | 4 | 35 | 35 | 35 |
| 10 | 20 | 3 | 40 | 40 | 40 |
| 9 | 16 | 2.5 | 45 | 45 | 45 |
| 9 | 13 | 2.5 | 50 | 50 | 50 |
| 8 | 10 | 2 | 55 | 55 | 55 |
| 8 | 9 | 2 | 60 | 60 | 60 |
| 8 | 8 | 2 | 65 | 65 | 65 |
| 8 | 7 | 2 | 70 | 70 | 70 |
| 8 | 6 | 2 | | 75 | 75 |
| 8 | 4 | 2 | | 80 | 80 |
| 8 | 5 | | | 85 | 85 |
| | 4 | | | 90 | |

As figures showed 2 linear sections were revealed in the samples of venom protein patterns. As it was indicated incidences of these sections are differed. Presence of two linear sections in the dependence of $lg\ I = \int (t)$ is illustrated the occurrence of 2 luminescent centers in the studied samples of venom.

From time dependence of PL intensity of venom (fig.5) and its proteins (fig.6) the time of exciting state of luminescent centers was determined. It was equal to $3 \cdot 10^{-5}$ for venom and proteins.

Data on time dependence of maximum of luminescence for all proteins of venom were presented in table 2.

Metods of laser macro and micro diagnostics have high sensibility, considerable space solving allowing to analyse the trace concentrations of zootoxins.

Thus, luminescence analysis of biologic objects allowed to identify proteins of venom.

[1] *E.G. Oreshenkova.* Spectral analysis, M.: Bishaya shkola, 1982, p.375.

[2] *N.N. Barashkov.* Luminescent and lysis in heltn service, M.: Nauka, 1985, p.95.

[3] *A.N. Zoujdel.* Atomic phluorescent analysis, M. Nauka, 1980, p. 192.

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## LAZER SPEKTROSKOPİYA VASİTƏSİLƏ ZAQAFQAZİYA GÜRZƏSİ ZƏHƏRİNİN ZÜLALLARININ TƏDQİQİ

Gürzə zəhəri və onun zülallarının fotolüminessensiya spektrləri tədqiq edilmişdir. Nümunələr qabaqcadan LQİ-21 tipli impuls azot lazeri ilə (impulsun müddəti 10 ns, dalğa uzunluğu 337.1 nm) həyəcanlandırılmışlar. Fotolüminessensiya FL spektrləri 360÷630 nm intervalı əhatə edir.

Gürzə zəhəri və onun zülalları müxtəlif lüminessensiya intensivliyinə malikdir və işıqlanma maksimumu 490÷530 nm dalğa uzunluğunu əhatə edir. FL spektrinin yarım eni 0.62÷0.83 eV hüdudundadır.

**Ш.А. Топчиева**

## ЛАЗЕРНАЯ СПЕКТРОСКОПИЯ В ИЗУЧЕНИИ БЕЛКОВ ЯДА ЗАКАВКАЗСКОЙ ГЮРЗЫ

Исследованы спектры фотолюминесценции яда гюрзы и его белков при возбуждении их импульсным азотным лазером типа ЛГИ-21 (длительность импульса 10 нс, длина волны 337,1 нм). Спектры ФЛ охватывают область 360÷630 нм. Яду гюрзы и его белкам свойственна люминесценция разной интенсивности и максимумы свечения в пределах длин волн 490÷530 нм.

Полуширина спектра ФЛ яда гюрзы и его белков находится в пределах 0.62÷0.83 eV.

*Дата поступления: 18.10.99*

*Редактор: Б.Г. Тагиев*