
CONDENSED-MATTER
SPECTROSCOPY

A Method for Determining Iron-Containing Components of Liquor in Neurosurgical Problems¹

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Abstract—Results of spectral studies of human liquor aimed at determining the specific features of its composition arising as a result of neurosurgical diseases and during the recovery period are presented. A method for detecting characteristic bands attributed to pathology, which is based on precision measurements of the UV–VIS absorption spectra of liquor and on their subsequent computer analysis is presented. With this method, iron-containing components of liquor can be qualitatively and quantitatively determined, which is important in solving neurosurgical problems.

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INTRODUCTION

Cerebrospinal fluid (liquor) is an internal medium of central nervous system, which contains various proteins, mineral compounds, a small amount of cells (leukocytes, lymphocytes). Due to the occurrence of the hematoencephalic barrier, the liquor most completely characterizes the functional activity of different mediator systems of cerebrum and medulla. Traumatic injuries and cerebrovascular disorders affect the permeability of the hematoencephalic barrier, as a result of which iron-containing blood proteins, in particular, hemoglobin, appear in the liquor.

Methods that are capable of detecting and differentiating small damages to the central nervous system are of considerable interest. First of all, this refers to spectral analytical methods. Earlier, we performed spectral studies of the liquor by different methods in addition to UV spectroscopy [1]. This additional spectral information on the liquor reflects neuroprocesses and cannot be obtained with the help of standard biochemical techniques. We developed a method based on precision measurements with subsequent mathematical analysis. The spectra were measured and mathematically processed using a previously developed spectral–computing complex. This method makes it possible to qualitatively and quantitatively characterize small damages to the central nervous system. It should be emphasized that standard methods of separation of spectra into component bands (e.g., the Alentsev–Fock method, etc.) cannot be applied here.

EXPERIMENTAL

The spectral–computing complex makes it possible to record spectra of absorption, reflection, luminescence, and photoexcitation in the range 0.19–4.0 μm . The measurement accuracy is up to four decimal points, and, in the case of the measurement of the absorption coefficient k , the accuracy in various spectral intervals was varied from 0.1 to 0.01%. Mathematical processing was performed using a program with which the complex was supplied. In most cases, the absorption of the liquor was measured in the range 190–600 nm. Since the absorption coefficient of the liquor in the UV range varies from 0.1 cm^{-1} to higher than 100 cm^{-1} , to determine this parameter in the short-wavelength spectral range, we had to dilute the liquor up to 100 times. Figure 1 shows typical absorption spectra of the liquor of a patient.

SPECTRUM PROCESSING METHODS

Analysis of the most intense part of the absorption spectrum of the liquor (192–240 nm) showed that, in this range, the absorption continuously and exponentially decreases toward longer wavelengths. Here, the absorption is a superposition of the wings of absorption bands whose maxima lie in the range of the vacuum ultraviolet. An intense absorption of water in this range prevents the detection of these bands. The sum of decreasing spectral bands was modeled by a corresponding exponential function. Such a procedure was physically substantiated and experimentally confirmed in [2]. Then, this dependence was subtracted from the initial absorption spectrum of the liquor. As a result, we succeeded in observing bands whose intensity was

[†] Deceased.

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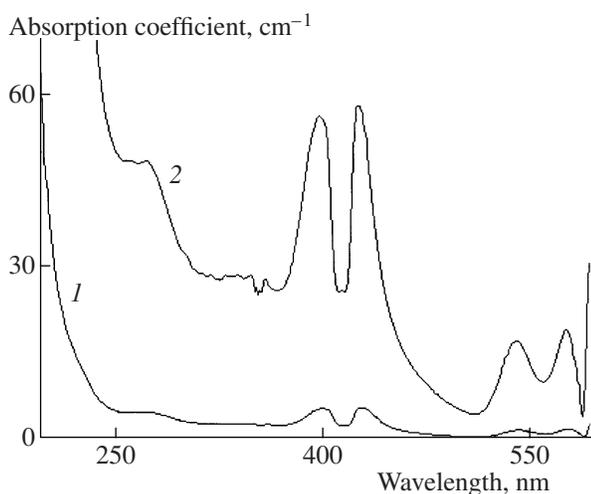


Fig. 1. (1) Absorption spectrum of liquor of a patient and (2) the long-wavelength part of the spectrum expanded tenfold along the vertical axis.

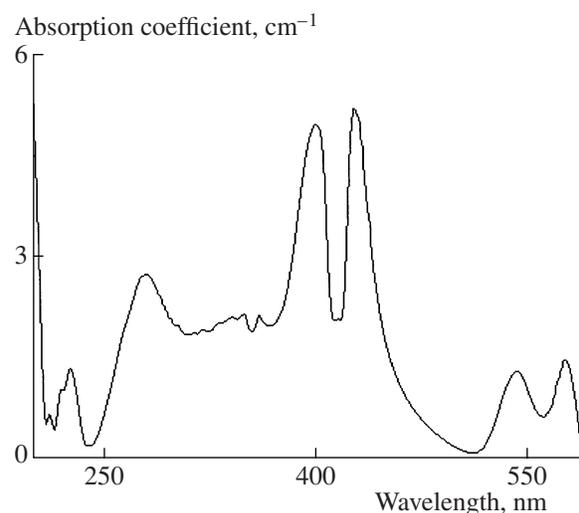


Fig. 2. Absorption spectrum of liquor of a patient after the subtraction of the short-wavelength band.

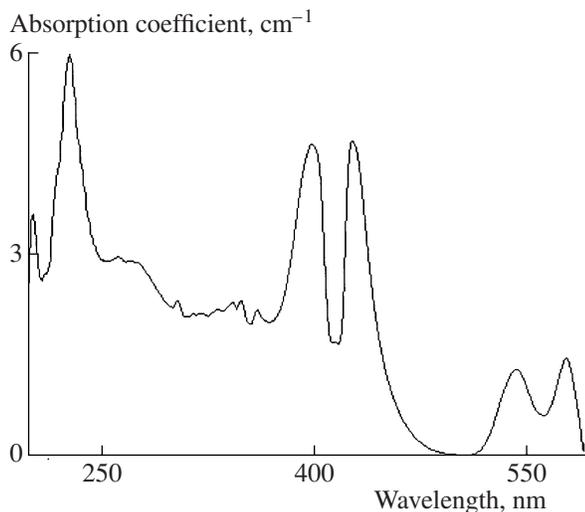


Fig. 3. Difference between the absorption spectrum of liquor of a patient and absorption spectrum of liquor averaged for a group of healthy patients.

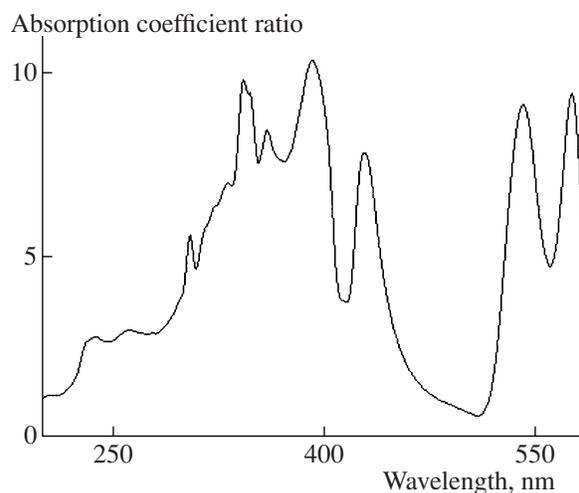


Fig. 4. Ratio between the absorption spectrum of liquor of a patient and absorption spectrum of liquor averaged for a group of healthy patients.

100 times lower than the intensity of the decreasing continuum (Fig. 2).

We studied the shape of the pronounced band located near 280 nm. We observed that, in general, this base has a symmetric Gaussian profile in the energy coordinate system, which allowed us to model this function and to subtract it from the initial spectrum. As a result, we were able to determine weak absorbing components.

In another approach, we measured the absorption spectra of the liquor for a group of conventionally healthy patients and used the averaged spectrum for determining spectral features of the liquor of sick

patients. To do this, we subtracted the averaged spectrum from the absorption spectrum of the liquor of a sick patient (Fig. 3). This method allowed us to determine additional absorption bands characteristic of the liquor of sick patients using the procedure described above.

Measuring the ratio of the absorption spectrum of the liquor of a sick patient to the averaged spectrum (Fig. 4) allows one to determine a change in the absorption in the state of pathology compared to the normal state.

For practical use, it is important to know the relative change in the concentration of a compound, which is

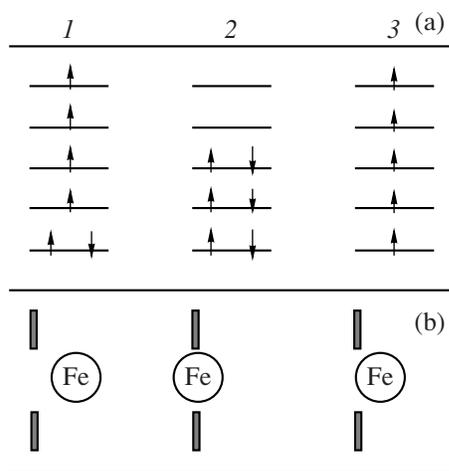


Fig. 5. Characteristics of the hemoglobin molecule in different states: (a) hem electronic structure and (b) position of the iron atom with respect to the plane of the porphyrin ring in (1) deoxyhemoglobin, (2) oxyhemoglobin, and (3) methemoglobin.

determined by the area of the absorption band. For this purpose, we integrated the spectra over several characteristic ranges; namely, the spectra were integrated over the short-wavelength (200–250 nm) and the long-wavelength (250–360 nm) UV ranges and over the short-wavelength (380–450 nm) and the long-wavelength (500–580 nm) visible ranges.

RESULTS

By analyzing the UV absorption spectra of the liquor with the help of computer-assisted mathematical processing, we revealed the following particular features. The absorption spectra of the liquor of patients with different neurosurgical diseases have characteristic bands whose maxima are frequently located at the following wavelengths: 208, 214, 219, 222, 226, 274, 276, 278, and 280 nm. The averaged absorption spectrum of the liquor of healthy patients has a smaller number of maxima, which are observed at 213 ($\Delta k = 0.2 \text{ cm}^{-1}$), 219 and 224 ($\Delta k = 2.2 \text{ cm}^{-1}$), 265 ($\Delta k = 0.1 \text{ cm}^{-1}$), and 277 nm ($\Delta k = 1.8 \text{ cm}^{-1}$). Comparison between the absorption spectra of the liquor in the normal state and in the state of pathology showed that, in the case of an inflammatory process, k increases ~ 4.5 times near 220 nm and ~ 2 times near 280 nm. In this case, the concentrations of the components of the liquor change about fourfold.

Analysis of the visible absorption spectra of the liquor containing blood allowed us to single out bands corresponding to iron-containing blood proteins. These are hemoglobin and its derivatives deoxyhemoglobin and oxyhemoglobin. Hemoglobin is the protein of erythrocytes, which carries molecular oxygen. By its chemical structure, hemoglobin consists of protein,

termed globin, and hem, a complex of Fe(II) with protoporphyrin.

Figure 5 schematically shows the electronic structure of the hem and the position of the iron atom with respect to the plane of the porphyrin ring of the hem [3].

In the deoxyhemoglobin molecule, the iron atom is displaced out of the plane of the porphyrin ring by 0.5–0.6 Å. Among the six 3d electrons of Fe(II), two electrons are located on one of the lowest d orbitals, while the remaining four electrons occupy the other d orbitals, and the spin is $S = 2$. The magnetic moment of the hem in this state is ~ 5.5 Bohr magnetons, and the absorption spectrum in the visible range exhibits a characteristic band peaked at 556 nm [3].

In oxyhemoglobin, the iron atom lies practically in the plane of the porphyrin ring, all the six d electrons are paired in the three lowest d orbitals, and the spin is $S = 0$; i.e., oxyhemoglobin is a diamagnetic [3]. In the visible range, there are two characteristic absorption bands, which are located at 543 and 576 nm [4].

In methemoglobin, oxygen is replaced by water, and the iron atom is located closer to the plane of the hem than in deoxyhemoglobin. All the five d electrons are unpaired and occupy the five d orbitals. The spin is $S = 5/2$, and the magnetic moment is ~ 5.91 Bohr magnetons [3]. The characteristic bands in the visible range are located at 420, 542, and 578 nm [4].

Analysis of the visible absorption spectra of the liquor of patients with neurosurgical diseases made it possible to locate the characteristic bands at the following wavelengths: 398, 428, 542, and 578 nm. The absorption bands at 428, 542, and 578 nm belong to the hemoglobin derivatives, oxyhemoglobin and methemoglobin. The occurrence of blood proteins in the liquor indicates that, due to neurological diseases or injuries, the permeability of the hematoencephalic barrier is disturbed or the barrier is destroyed. The method for investigating the absorption spectra of liquor that we proposed above can be used for diagnostic and prognosis for diseases. Comparison between the absorption spectra of the liquor in the normal and pathological states showed that, in the presence of blood in the liquor, k increases ~ 10 times in the range at 400 nm, ~ 8 times at 420 nm, and ~ 9 times in the range 540–580 nm.

Collation of the spectra with the clinical presentation of diseases showed the following. In the case of osteochondrosis of spine, the absorption coefficient at 200 nm, $k = 40\text{--}100 \text{ cm}^{-1}$, was close to the normal value, and the integral increase in the concentration of liquor components exceeded the normal value by a factor of ~ 1.6 . In the case of disk herniation, k increased to $\sim 230 \text{ cm}^{-1}$, and, in the presence of a pain syndrome with a duration of up to half a year, k was greater than 300 cm^{-1} . In this case, an increase in the concentration of components increased from 1.1 to 2.4 times. In the case of a complicated spinal injury with a duration from 0.5 to 5 years, $k = 70\text{--}260 \text{ cm}^{-1}$, if the duration of the

disease is 30 years, $k = 69\text{--}142\text{ cm}^{-1}$; an increase in the concentration of components increased by a factor of 1.3–1.7. For the patient with a noncomplicated injure of spinal motor segment (the injure duration was 15 years), $k = 80\text{ cm}^{-1}$, and the concentration of components was close to the normal value or increased ~1.5 times. The observation of the normal concentration of components in the liquor indicates that the clinical course was marked by compensation.

CONCLUSIONS

In our investigation, we observed marked changes in the absorption spectra of the liquor in the UV and visible ranges depending on the type of a disease and severity of injury. Thus, in the case of traumatic injures and cerebrovascular accidents, iron-containing blood proteins (hemoglobin and its derivatives) were observed in the liquor. We proposed a method for detecting iron-containing components of liquor and determining their concentration. The method is based on precision measurements of spectra and their computer-assisted processing using a spectral–computing complex. The high sensitivity of the method allows one to use it in detect-

ing small damages to the central nervous system. Summarizing, we should note that new methods that were developed in the intersection area between physics and clinical medicine and that involve computing make it possible to estimate in more detail and interpret states of qualitative properties of liquor of neurosurgical patients.

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