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New express method for melatonin determination in the human body

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Original fundamental properties of Yanson point contacts allow their application to research and technology development at a wide range of surrounding conditions. At low temperature these nanoobjects can be used as a main instrument of Yanson point-contact spectroscopy. At room temperature they can serve as a sensitive element of advanced nanosensors with excellent performance. The most important advantage of point-contact sensors in investigating complex gas media is the spectral nature of the response signal. The discovery of the spectral capabilities of point-contact sensors in the analysis of complex gas media allows us to speak in terms of spectral multifunctionality of Yanson point contacts and the expansion of the possibilities of their spectral application from the spectroscopy of electron-phonon interaction at low temperatures to spectroscopy of gaseous media at room temperatures. Using the spectral response of point-contact sensors, in this work we propose a new non-invasive method for the determination of melatonin, one of the important hormones characterizing the state of the human body. A series of procedures was proposed to find melatonin concentration in the human body as function of the response of a point-contact sensor to the action of the exhaled breath. It has been shown that the proposed method is accurate enough to be used for medical purposes in real time. The results of the study suggest that the problem of noninvasive determination of melatonin concentration in the human body can be successfully solved by using breath tests based on Yanson point contacts.

Keywords: Yanson point contact, breath profile, melatonin, quantum sensor, selective detection.

To I. K. Yanson on his anniversary

Introduction

The second half of the last century was marked by a row of serious achievements in the study of the electronic properties of metals, which entailed the evolution and active development of various methods for studying the electronic system of conductors [1]. At the same time, the technical possibilities for studying the phonon system of solids and the interaction of systems of electrons and phonons remained limited for a long time. Since the systems of electrons and phonons and the result of their interaction largely determine the fundamental properties of conductors, the importance of creating highly efficient instruments for their study was beyond doubt. In the 60–70s of the 20th century, the main methods used to determine the phonon density of states function and the electron-phonon interaction function were the method of scattering of thermal neutrons [2] and the study of the tunnelling effect in superconducting tunnel contacts [3]. However, it is obvious that the tunnelling method is applicable only in the study of superconducting materials, while the other is associated with great complexity of the study, since it is possible only with the use of nuclear reactors and the method of neutrons scattering has a relatively low resolution.

Therefore, the discovery of the fundamental possibilities of point contacts [4], which provide the simplicity and high efficiency of detecting the processes of interaction of electronic and phonon systems, led to the rapid evolution of Yanson point-contact spectroscopy [5, 6] and the development of an innovative technology for creating Yanson point contacts as a new class of nanostructures [7].

Yanson point-contact spectroscopy is based on the study of the deviation from linearity of the current-voltage characteristics of a point contact. These deviations are due to the inelastic interaction of charge carriers with scattering centres. The role of scattering centres can be played by various quasiparticles, such as phonons or magnons, and crystal structure defects. At low temperatures, the second derivative of the current-voltage characteristics of the Yanson point contact is directly proportional to the electron-phonon interaction function [8]. Due to this, it received the name of the point-contact spectrum. It is this property that determined the importance of Yanson point contacts, since the direct experimental determination of the electron-phonon interaction function, proposed by Yanson point-contact spectroscopy, is impossible by other known methods.

Yanson point-contact spectroscopy is a low-temperature instrument, since with an increase in temperature, due to blurring of the Fermi level, the spectral features in the point-contact spectrum broaden, which prevents the obtaining of quantitative information about the processes of electron-phonon interaction in the temperature range above the temperature of liquid helium. However, as it turned out, the application of Yanson point contacts is not limited to the study of inelastic interactions of charge carriers at low temperatures. In 2006, the discovery of the point-contact gas-sensitive effect was first announced [9]. This was the first push for the use of classical quantum objects in new applications [7, 10] at the room temperatures.

Gas nanosensorics is a promising direction of modern research. Gas nanosensors have significantly higher sensitivity, speed of response and selectivity than bulk chemical gas sensors. The place of traditional sensing elements in modern sensor applications is taken by sensors based on carbon nanotubes, porous silicon sensors, sensors based on metal oxides and metal nanowires [11]. However, these nanosensors are not devoid of tangible disadvantages. Among them are low reproducibility of the structure of complex nanostructures in the manufacture of sensors, a long relaxation time after exposure to a gas agent, and unstable baselines [12].

A separate direction of research in gas sensing is sensors for studying biological media, such as human breath [13]. The peculiarity of the breath lies in the very high amount of its specific components, as well as in their ability to react with each other in short periods of time [14]. Different components have a different nature and localization source: the gastrointestinal tract, lungs, oral cavity, or various exogenous factors such as smoking products and other atmospheric pollution [15]. The amount of unique compounds in breath is up to 2000 or more [16]. Many of these components carry direct information about the metabolic state of the human body [17]. The ability to detect and differentiate between these components and connect their presence to various pathogens and pathologic conditions is key to the successful implementation of sensors in modern medical technology. Breath sensors have already shown their effectiveness in solving such urgent problems as early detection of lung cancer [18], or diagnostics of carcinogenic strains of the bacterium *Helicobacter pylori* [19].

Currently, the so-called electronic noses are widely used as sensors for human breath [20]. Usually, they are a set of several conductive objects, mainly nanostructured, with different electrophysical properties and, therefore, different response parameters to different gas components. However, the sensitive elements of such a sensor matrix have a significant disadvantage of low information content in the response signal, which greatly complicates the procedure for its processing in real time. As a result, the development of electronic nose sensors is limited by the capabilities of modern computing technology. Currently, most of these sensors are capable of detecting about 20–30 components of a gas medium. This limitation significantly affects the possibilities of using electronic nose sensors for the effective study of breath, which consists of more than 2000 components.

An alternative approach to sensory analysis of human breath is the use of Yanson point contacts [10, 13]. Application of Yanson point contacts in sensory research has become possible due to the fundamental properties of such objects [7]. The geometrical dimensions of the contact can reach the scale of a single atom, which provides an extremely high surface to volume ratio of the conductor and this, in turn, is one of the key parameters for highly sensitive nanosensors [21]. The potential drop at Yanson point contacts occurs directly in the area of constriction, which suggests that the change in the electric resistance of this area makes a dominant contribution to the resistance properties of the entire system “electrode–point contact–electrode” [9]. Another important feature of Yanson point contacts is the separation of thermal and nonequilibrium processes in space [22]. In the contact itself, there occurs a process of transferring the energy of electrons to various quasiparticles, while the release of thermal energy occurs on massive banks-electrodes. Due to this, in point contacts it is possible to achieve extremely high current densities up to 10^7 A/cm^2 at room temperature [9]. In homogeneous bulk objects, the distribution of thermal and nonequilibrium processes is uniform over the entire volume of the conductor, as a result of which, when the current density reaches 10^2–10^3 A/cm^2, such an object begins to melt. The high current density flowing through the Yanson point contact is another key feature that provides its sensor properties. Due to the high-density current, the probability of interaction of nonequilibrium electrons, which received excess energy in the electric field of a point contact, with gas molecules adsorbed on its surface increases. As a result of the interaction, gas molecules desorb from the contact, taking with them the excess energy of electrons. This process prevents heating of the constricted area, which could lead to the subsequent destruction of the nanoobject. A consequence of this mechanism is that the Yanson point contacts quickly recover their properties after the end of interaction with the gas agent [14, 19].

It should be noted that long relaxation times are a noticeable disadvantage of classical nanostructured sensors. Without external influence, conventional nanosensors, after the end of their interaction with gas, restore their initial state during a long period of time, which, for example, in the case of carbon nanotubes, can reach several hours [23]. Annealing of nanostructures in many cases is not applicable due to the low resistance of such objects to heating. The response time of Yanson point contacts to the action of the analyte is fractions of a second and is determined only by the diffusion rate of the analyte near the contact surface, while for conventional nanostructured sensors it ranges from several seconds to tens of minutes. This property is important to perform real-time measurements. In the case of breath, this feature becomes one of the key ones, since the reliability of the results directly depends on the efficiency of control. This is caused by the fact that the composition of the breath changes over time due to the spontaneous interaction of its components with each other. All of the above-mentioned features of Yanson point contacts predetermined the prospects of their use as a basic element of the sensors for human breath.

The most important advantage of point-contact sensors in investigating complex gas media is the spectral nature of the response signal [14, 19]. This property fundamentally distinguishes point-contact sensors from known sensors operating on the principle of changing electrical conductivity [7]. The discovery of the spectral capabilities of point-contact sensors in the analysis of complex gas media [19, 24] allows us to speak in terms of spectral multifunctionality of Yanson point contacts and the expansion of the possibilities of their spectral application — from spectroscopy of electron-phonon interaction at low temperatures to spectroscopy of gaseous media at room temperatures. We recently proposed a new method for the selective detection of complex gas media using the spectral response of point-contact sensors [14]. Developing the proposed approach, in this work we propose a new non-invasive method for the determination of melatonin, one of the important hormones characterizing the state of the human body.

Melatonin (N-acetyl-5-methoxy tryptamine, chemical formula $C_{13}H_{16}N_2O_2$) is one of the key hormones in the human body [25]. It is a product of pineal gland secretion that affects many physiological functions of the body. Melatonin acts as a photoregulator of circadian biorhythms, and all endogenous rhythms of the body are subordinated to its effect. Such biological rhythms are a universal tool for adaptation of living organisms to the environment. Melatonin also possesses unique anti and prooxidant properties that determine its protective capabilities against free radical damage to DNA, proteins and lipids. Due to the small size of the melatonin molecule, which is able to penetrate the plasma membrane, as well as the fact that the antioxidant effects of melatonin are not mediated by membrane receptors, it can affect free radical processes in any cell of the body.

The currently known methods for the determination of melatonin, including nuclear magnetic resonance, enzyme immunoassay, radioimmunoassay, liquid chromatography, mass spectrometry are laborious and require the application of expensive equipment [25]. The most widely used methods are the enzyme immunoassay method and the radioimmunoassay method. However, these methods do not make it possible to view a large number of samples at a sufficient speed. The concentration of melatonin in the human body is determined by analyzing biological fluids such as blood, urine and saliva. To reliably measure the concentration of melatonin in the blood [25], the patient is given an intravenous catheter 2 h before the measurement, which, along with the invasiveness and long duration of the procedure, complicates the introduction of this analysis into widespread medical practice. The use of urine as a biological material is also associated with certain discomfort at the stage of sampling. At the same time, determining the concentration of melatonin in the human body is a very important analysis that should be included in the regular examination of all patients. Due to the effect of melatonin on the processes occurring in the human body, as well as due to a row of the difficulties and limitations peculiar to the listed methods, the development of a simple and quick-to-implement method for determining melatonin using modern advances in science and technology is a rather promising and demanded task.

For regular examination of patients, the most convenient biological material can be human breath, which directly includes gaseous products of saliva. In combination with the express mode for sensory analysis of breath, the undeniable advantage of this approach is obvious. Currently, there is no method for determining melatonin in the human body based on the analysis of breath. In this work, we propose for the first time to fill this gap and use this biological media for the non-invasive determination of such an important hormone. In addition to the breath, to implement the new method we employ an innovative point-contact sensor technology, which has no analogues and was tested earlier in our works (see, for example, Refs. 7, 10, 19), and an original method for analyzing the results based on the spectral nature of the response of sensors whose sensitive elements are Yanson point contacts (see Ref. 14).

Materials and methods

To develop a new method for the determination of melatonin in the human body, we researched the breath of patients using point-contact sensory matrices. To obtain them, the anion-radical salt of 7,7,8,8-tetracyanoquinodimethane (TCNQ) was used as a sensitive substance. The reason for choosing an organic conductor for creating point-contact sensors is associated with the features of the crystal structure of such materials and their electrophysical properties. Crystals of organic conductors grow predominantly in the form of needles, tubes, or plates in which molecules are packed in stacks. Due to the peculiarities of the crystal structure, such materials form layered crystals with high conductivity anisotropy. As a result, the conductivity of TCNQ salt crystals along and across the stack axis differs by three orders of magnitude.

Point-contact sensors were obtained by the method of combined electrochemical deposition in the constant voltage mode of salt $[\text{N-C}_4\text{H}_9\text{-iso-}Q_n](\text{TCNQ})_2$ from a saturated solution of acetonitrile on a substrate according to the method described in [26]. The substrate consisted of copper foil with the thickness of 50 μm, placed on a polymer composite.

In our case, the synthesis of the CuTCNQ compound occurs in the process of the sensor element's copper plate anodic polarization during the creation of an autonomous energy source integrated into the gas-sensitive point-contact array. Cu^+ ions travel from the copper crystal lattice to the intermediate layer and interact with the dissociation products of the $[\text{N-C}_4\text{H}_9\text{-iso-}Q_n](\text{TCNQ})_2$ salt. This process results in the formation of the CuTCNQ salt. As the compound deposits on the copper plate of the sensor element, it forms an active substance that has a more positive potential with respect to the second copper plate. This potential difference serves as an energy source in the formation of the output signal.

Since the formed CuTCNQ salt is not soluble in acetonitrile, in contrast to the initial substance, it becomes possible to grow microcrystals directly on the copper electrode. Thus, a film is formed which consists of layered crystals of the first phase of the CuTCNQ compound [27]. The surface morphology of the sensing element obtained in this way is shown in Fig. 1(a), while the layered nature of the formed crystals, typical for tetracyanoquinodimethane compounds, can clearly be seen in Fig. 1(b).

Point contacts were formed at the points of contact of the lateral faces of such crystals in accordance with the mechanism employed in the Chubov displacement technique [28] used in Yanson point-contact spectroscopy. For every square millimetre of the surface, there were more than $5 \cdot 10^5$ such contacts. As a result, a mesoscopic multistructure of Yanson point contacts was formed, which ensured a high level of the sensor output signal under the action of the gas media. A galvanic cell was integrated into this structure in the process of electrochemical synthesis using a special technology [26], which provided the functioning of the sensors as stand-alone objects for a long time without using external power sources.

To register the sensor response under the action of breath in real time, a portable measuring complex created in our group was used [19]. The complex was connected to a modern personal computer with the original SensorResponse 2 software, developed at B. Verkin Institute for Low Temperature Physics and Engineering of the National Academy of Sciences of Ukraine. The change in the conductivity of gas-sensitive sensors under the influence of a gas medium (response signal) was recorded by measuring the voltage drop on a precision resistor connected in series with the sensor itself in an electrical circuit. The measurement technique is detailed in Ref. 19. The sensor output was 10 times amplified. The control of the data obtained using a personal measuring complex was carried out with a Keithley 2100 multimeter. The gas sensitivity and selective reaction of point-contact sensor arrays to the action of human breath and its components, as well as the approbation and advancement of a portable measuring complex, were scrutinized in the works [19, 24, 26, 29, 30]. Detailed information

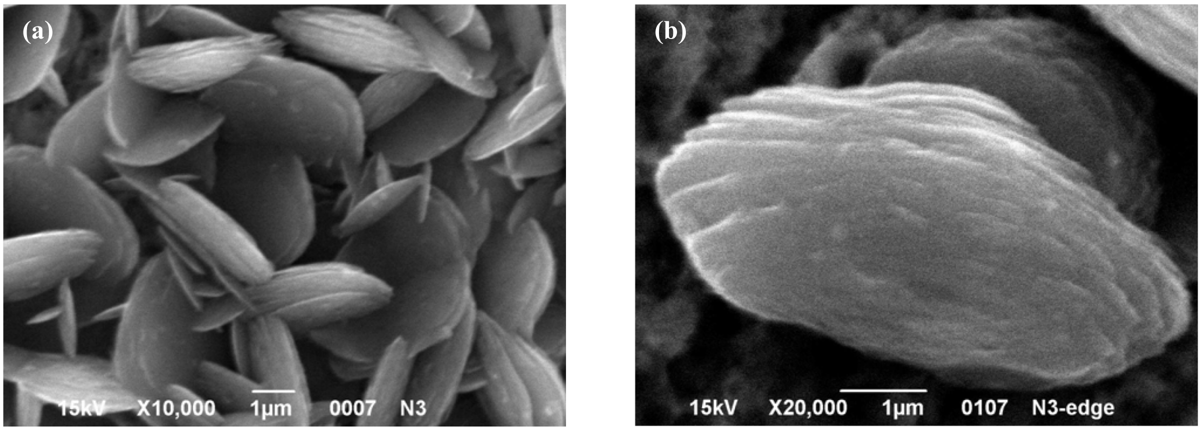

Fig. 1. (a) General view of the surface of a point-contact sensing element based on the TCNQ compound, (b) the layered crystal structure of the CuTCNQ compound.

about the manufacturing technology of Yanson point contacts and sensors based on them is presented in Ref. 7.

The study was made with the breath of patients treated at SI "Institute for Children and Adolescents Health Care" of NAMS of Ukraine. There were 20 patients in total aged from 12 to 18. The average age was 15. All measurements were performed in the framework of a year-long research on the fundamental mechanisms of the development of diseases of the upper gastrointestinal tract.

For the breath test, the sensor was placed between the copper clamps of a specially designed fluoroplastic holder and reliably fixed. A disposable plastic tapered tube was then fitted onto the holder to act as a test chamber. For measurements, such a cell was placed in the oral cavity and exposed to the patient's breath for a minute. In our previous studies [24], it was found that a time interval of 1 min is a sufficient time for all components of the breath to leave their "trace" on the recorded sensory profile of breath. The measurements were carried out in accordance with the method developed by us earlier for the diagnosis of the bacterium *Helicobacter pylori* [19]. After the end of the exposure of the sensor to the breath, the test chamber was slowly removed from the patient's mouth. Further phase of measuring was relaxation of the sensor which took place under environmental conditions. During exposure and relaxation, the sensor response was recorded in real time. To increase the reliability of the results obtained, 4 parallel tests were carried out using the breath of the same patient. All tests were performed in the morning after an overnight fast. The day before the study, the patients did not take any medication.

The clinical diagnosis was established by a thorough analysis of complaints, anamnesis, the results of objective and instrumental examinations. When making a diagnosis, attention was paid to the detection and detailing of clinical symptoms according to the history, the presence of an aggravating heredity for diseases of the digestive system, the duration of the disease, *etc.* in accordance with the Rome IV criteria.

To adjust the point-contact sensor matrix and verify the breath test, the content of melatonin in the urine of patients was determined by the fluorometric method [31].

The processing of the results, analysis of the response curves of point-contact sensors to the action of breath, and the search for correlations between the sensor response signal and the melatonin level were performed using the WEKA data mining software [32]. Additional control and verification of processing results was carried out using the SPSS statistical package [33]. Statistical analysis of the data was carried out using standard parametric and nonparametric methods of statistics [34].

Ethics. The research protocol was approved by the Institutional Review Board and the Ethical Committee of the SI "Institute for Children and Adolescents Health Care" of NAMS of Ukraine. All patients gave their written consent to participate in the study.

Results and discussion

To understand the principle of operation of a point-contact sensor, let us compare the typical point-contact sensory profile of human breath and the point-contact spectrum of the electron-phonon interaction recorded at low temperatures in conductors using Yanson point-contact spectroscopy (Fig. 2). It should be noted that the response curve of the point-contact sensor is the dependence of the electrical conductivity of the Yanson point contact on time as a result of interaction with the gas medium [Fig. 2(a)]. It is fundamentally different from similar dependences of

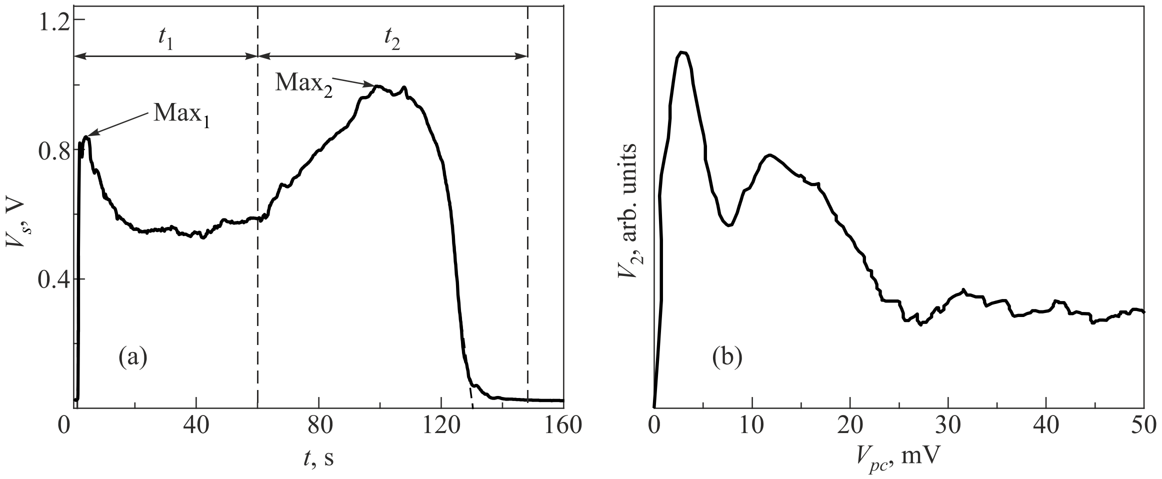

Fig. 2. (a) Typical response curve of point-contact sensor based on TCNQ compound under the action of breath of a healthy person. V_s is the voltage drop, which occurs in the sensor, t is the time, t_1 is the exposure time, t_2 is the relaxation time. (b) The point-contact spectrum of an organic conductor β-(BEDT-TTF)$_2$I$_3$ obtained using Yanson point contacts in the direction of current flow that is perpendicular to the layers of organic molecules [35]. V_2 is the second derivative of the current-voltage characteristics of the point contact, V_{pc} is the contact voltage drop, R_0 is the point-contact resistance at $V_{pc} = 0$. $R_0 = 130\ \Omega$, $T = 4.2$ K.

various sensors operating on the principle of electrical conductivity changing. For example, in contrast to traditional semiconductor sensors, which demonstrate a monotonic response in the form of a δ-function under the influence of a gas medium, point-contact sensors based on TCNQ compounds show a complex non-monotonic curve of the response on time [Fig. 2(a)] [7, 10], which includes a series of features similar to those in the spectra of electron-phonon interaction in metals [Fig. 2(b)].

The point-contact spectrum includes the characteristics of all interactions in the quantum system of solids. Shown as an example in Fig. 2(b), the spectral dependence of electron-phonon interaction processes in point contacts of an organic conductor β-$(BEDT\text{-}TTF)_2I_3$ [35] contains information on the energies of phonons, their density of states, the parameter and anisotropy of the electron-phonon interaction in the compound under study, *etc*. The parameters of the point-contact spectrum make it possible to determine the general characteristics of the phonon system of solids and the interaction of the electron system with the phonon one. Employment of Yanson point-contact spectroscopy technique to record and analyze the profile of human breath allows us to obtain an effective tool for studying this complex gas medium using point-contact sensors.

Consideration of the features of the response curve of sensors under the influence of human breath using correct mathematical processing of the results provides the possibility of converting them into characteristic parameters, which can later be used to analyze the profile of breath with the separation of individual areas corresponding to different metabolic processes that occur in the human body.

Before we present and discuss the obtained results, let us briefly describe the process of experimental study of human breath using point-contact sensors based on TCNQ compounds. Before measurements, gas-sensitive elements of the sensor matrix are in equilibrium with the atmosphere. Each sensing element is a Yanson point contact between two layered crystals of Cu-TCNQ. As already said above, every square millimetre of the sensor matrix contains more than $5\cdot10^5$ such contacts, which taken together make up a complex mesoscopic structure responsible for the high level of the sensor output signal. In atmosphere, each sensing element of the active point-contact sensor is surrounded by atmospheric nitrogen, which preserves them from interacting with other atmospheric gases such as oxygen.

The measurements start with the sensor matrix being placed in the oral cavity of a patient. Under the action of the exhaled breath, the thermodynamic parameters of the medium the sensing elements are in contact with change drastically. The temperature and humidity of the medium in the patient's mouth activate the galvanic element which is integral part of the sensor structure. Electric current starts flowing through the sensor elements. Nitrogen molecules at the active centres on the surface of the conduction channel of Yanson point contacts recombine with the breath components which are human metabolic products. Electric field triggers a series of processes related to inelastic interaction of electrons with molecules of the adsorbed gas, in analogy to the electron-phonon interaction in Yanson point-contact spectroscopy. A cascade of such interactions occurring during a minute-long period of the sensor being under action the breath leads to a new quasi-equilibrium state.

The next phase is relaxation of the sensor in atmosphere, which usually lasts for 1–3 min. The relaxation process is related to desorption of the breath molecules and their subsequent substitution with atmospheric nitrogen. The main role in the fast desorption of the breath molecules from the surface of Yanson point contacts is played by the active transfer of the excess energy of nonequilibrium electrons in the contact to the adsorbed substances. The high probability of these processes is a result of the superhigh current density in Yanson point contacts (10^7 A/cm^2 [9]). At this stage, in contrast to the phase of sensor exposure to the breath, the temperature and humidity of the medium around the sensor decrease. Breath molecules desorb, electric resistance of the sensor matrix increases, while the electric field generated by the sensor galvanic element decreases. In 1–3 min, depending on the exhaled breath composition, the sensor returns to its initial state without the need for any additional action.

The point-contact sensor considered in this work is fundamentally different from the presently available analytical devices. Due to the high surface density of single sensor elements in the gas-sensitive point-contact array (more than $5\cdot10^5$ Yanson point contacts per 1 mm^2 of visible surface [27]) the output signal of a point-contact nanosensor is a high-intensity integral curve that reflects the energy spectrum of the interaction of Yanson point contacts with the entire ensemble of molecules of the analyzed medium [7, 10, 19]. This is possible thanks to the unique properties of the Yanson point contact, which allows the energy interactions in the contact and on its surface to be clearly manifested [5–7]. They also include the threshold of resistive sensitivity at the level of individual molecules in contact with the surface of the conduction channel of the nanostructure [36]. Such a gas-sensitive array allows obtaining a high-resolution energy portrait of the analyte. The fine visualization of the portrait is limited by the resolution of the device that records the output signal of the sensor, since a huge number of interactions "analyte molecule–conduction channel" occur per time unit, and numerous elementary responses form a complex energy spectrum.

It is known from statistical physics that the ergodic hypothesis is applicable to systems consisting of a large number of structural elements [37]. This means that the average value of the nanosensor output signal at each moment of time is a correct averaging of the response of a single point contact placed in the analyzed medium over a long testing period, i.e., it is obtained by averaging the results of many parallel experiments. This is evidence that the response of the point-contact sensor adequately reflects the features of the nature and state of the analyte.

The complexity of the analyzed systems and their dynamism which is caused by numerous interactions within the system do not allow one to directly calculate the level of contribution of each component to the output signal of the analytical device. In this case, methods of comparative analysis combined with the techniques of correlation analysis and mathematical statistics successfully used in sensor technology for the development of methods of medical diagnostics are effective [38]. The key factor that complicates the process of identification of the individual contributions of all components of the analyzed environment is that during the entire analytical act, i.e., in the process of measurement, the role of each component in the formation of the output signal changes significantly. Therefore, one of the fundamental tasks of the proposed method of analysis is to establish a time range, which is characterized by the maximum contribution of the target component to the output signal. The knowledge of the time range allows us to determine the signal level of the sensor placed in a medium with a known analyte concentration and obtain a point on the plane of the calibration characteristic with the coordinate axes "concentration of the target component–the level of the output signal". An averaged straight line can be drawn through all such points, which allows us to estimate the concentration of a component in the control sample of a gaseous medium of similar nature.

Regarding the determination of melatonin in breath, the procedure is as follows. For a number of patients with known results of medical analyses of melatonin, breath profiles are obtained using a point-contact sensor. Using the entire array of these profiles, we can find the correlation coefficients of melatonin concentrations and the level of the breath profile in the form of voltage drop on the sensor at the appropriate time. The maximum in the dependence of the correlation coefficient on time corresponds to the time of the maximum effect of melatonin on the output characteristic of the sensor, i.e., on the breath profile. Then, in the coordinates "melatonin concentration–the level of the sensor output signal at the selected time", we fix the point for each patient. By drawing an averaged straight line through the whole set of these points, we obtain a calibration dependence for determination of melatonin in the body of any newly arrived patient (patient of the control group). The scatter of points on the calibration characteristic is estimated as standard deviation from the averaging straight line. In accordance with the theory of mathematical statistics, the double value of the standard deviation of the sample corresponds to the confidence interval into which any result of a homogeneous measurement will fall with a probability of 95 % [39]. This means that a doctor using a point-contact sensor, can obtain a breath profile within 3–5 min and, using the calibration characteristics, estimate with a 95 % reliability the level of melatonin in the patient's body within the double mean square deviations without sending the patient for a long expensive procedure. In most cases, this is sufficient for prompt medical diagnosis. Naturally, the larger the sample size of patients with known medical test results, the higher the reliability of the estimates made using the sensor. However, this is a technical aspect which can easily be solved in the clinical practice of a medical institution.

The proposed method of analysis of the breath profile to determine the content of melatonin in the human body is based on finding the section of the sensor response curve which corresponds to the maximum correlation between the response signal and the substance under study [14]. Since time position of any section of the response curve is related to the energy which needs to be transferred to the adsorbed gas to make it desorb, finding the time interval which displays the relevant peculiarities in the response curve allows one to find the binding energy between the analyzed breath component and the point-contact surface. Therefore, similarly to how a point-contact spectrum in Yanson point-contact spectroscopy makes it possible to find many characteristics of the studied material, including the function of electron-phonon interaction, the point-contact profile of breath can provide us with rich information about the adsorption and conductive properties of gas molecules on the surface of active centres.

The process of realization of the new express method for detection of melatonin can be divided in several phases. At first, at the basic group of patients, there were determined melatonin concentrations in urine and sensor response curves to the action of breath for each patient. Then for this group of patients the temporal dependence of the correlation coefficient for the studied substance (melatonin) and the sensor signal was found. After that, we used temporal sections of the response curve corresponding to a correlation level not lower than 2 % of the maximum value to find the dependence of voltage drop on melatonin concentration in the patient's urine in accordance with the methodology described in detail in Ref. 14. Finally, the last dependence allowed us to obtain a linear regression equation, which served as a calibration characteristic for sensory determination of melatonin concentration.

The linear correlation coefficient (Pearson correlation coefficient) r between the response signal voltage V and the melatonin concentration C in the human body can be calculated using the following equation:

$$r=\frac{\mathrm{cov}_{VC}}{\sigma_V\sigma_C}=\frac{\sum_{j=1}^{n}(V_j-\overline{V})(C_j-\overline{C})}{\sqrt{\sum_{j=1}^{n}(V_j-\overline{V})^2\sum_{j=1}^{n}(C_j-\overline{C})^2}}. \qquad (1)$$

Here cov_{VC} is the covariance of the values V and C, σ_V is the root-mean-square deviation of the value V, σ_C is the root-mean-square deviation of the value C, V_j is the response voltage at a given time for the jth patient (out of n patients), C_j is the melatonin concentration of the jth patient, $\overline{V}$ and $\overline{C}$ are the mean values of the respective samplings:

$$\overline{V} = \frac{1}{n}\sum_{j=1}^{n} V_j, \quad \overline{C} = \frac{1}{n}\sum_{j=1}^{n} C_j. \tag{2}$$

Applying the strategy of using sensors to detect concentration of hormones in the human body [14] and using the experimental data obtained in this study, we have shown the possibility of finding singular sections of the complex curve of point-contact nanosensor response to the action of the exhaled gas which correlate with the melatonin content in the human body.

The initial data were the results of the breath tests of 16 patients with a known concentration of melatonin in the urine. Using the sensor response as a time series with a step of 0.5 s, we obtained the temporal dependences of the correlation coefficient between the response voltage and the melatonin concentration (Fig. 3).

The maximum negative correlation between the melatonin level in the urine and the voltage drop in the sensor is observed for the time interval 63.5–67.5 s. This means that this interval corresponds to the energies in the response curve which characterize with a maximum probability the contribution of melatonin and products of its interaction to the breath profile. The values of average voltage $\overline{V}_s$ of the breath profile of a patient for this period of time (Fig. 3) and of melatonin concentration C_{mel} in the urine determine the position of points in the coordinates “response voltage–melatonin concentration” (Fig. 4). Since there is no *a priori* information about the nature of the interrelation between melatonin concentration and sensor voltage, it would be logical to use a linear approximation and draw a straight line through the points using the method of least squares:

$$C_{\mathrm{mel}}[\mathrm{nmol/l}] = 78.5 - 134\overline{V}_s. \tag{3}$$

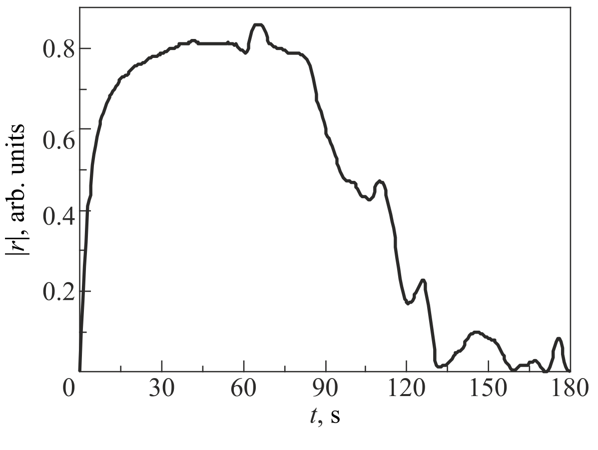

Fig. 3. Temporal dependence of the absolute values of correlation coefficient $|r|$ between the response voltage from the patients’ breath tests and the melatonin concentration from the patients’ medical analyses.

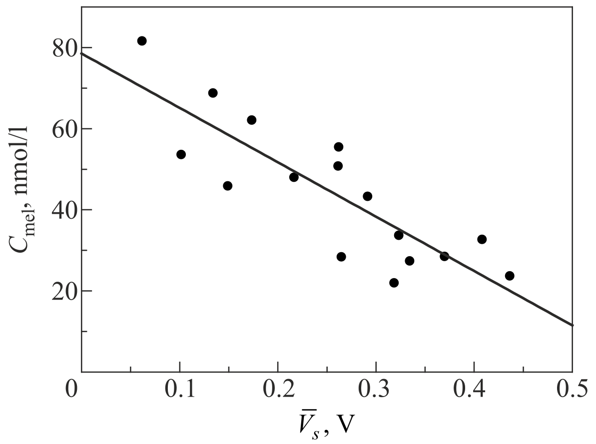

Fig. 4. Dependence of the analytical concentration C_{mel} of melatonin on the average response voltage $\overline{V}_s$ in the area of maximum correlation. Points are data of medical analyses, straight line is the result of the linear approximation (3).

This line is a calibration characteristic for estimation of melatonin concentration by means of sensor technique based on the breath test. Statistical analysis has shown that the root-mean square deviation of the medical analysis data from the approximation line does not exceed 7.6 nmol/l. Thus, with a probability of 95 %, the confidence interval is 15.2 nmol/l.

Model (3) was verified by calculation of melatonin concentration using breath test data of four new patients with a known melatonin concentration in their urine (Table 1).

Table 1. Verification of the sensor model for determination of melatonin

Patient’s number	Average response voltage $\overline{V}_s$ in the area of maximum correlation, V	Melatonin concentration C_{mel}, nmol/l		
		Analysis data	Estimation from Model (3)	Model error
1	0.29277	34.8	39.3	–4.5
2	0.25246	46.5	44.7	1.8
3	0.14417	53.3	59.2	–5.9
4	0.14318	64.5	59.3	5.2

Verification of the linear mathematical model demonstrates that all results of the sensor analysis fall into the corresponding confidence intervals. This proves that the statistical processing was made correctly and the proposed mathematical model can be used to estimate melatonin concentration in the human body using the point-contact breath test.

Conclusions

Determination of melatonin concentration in the human body is an important and nontrivial problem. Since the hormone impacts many functions of a living organism, knowledge of its rhythmics and concentration in the body makes it possible to predict pathological processes and choose the optimal therapy. Despite being quite numerous, the methods for determination of melatonin level in the human body are not without shortcomings. The nature of melatonin which manifests itself in melatonin's fast decay makes it difficult to use many of the existing methods for its determination. This is one of the reasons why the approach we propose in the present study may be of great value for practical applications.

The present study has resulted in the development of a new express method for determination of melatonin concentration in the human body using Yanson point contacts. A series of procedures was proposed to find melatonin concentration in the human body as function of the response of a point-contact sensor to the action of the exhaled breath. It has been shown that the proposed method is accurate enough to be used for medical purposes.

The results of the study suggest that the problem of noninvasive determination of melatonin concentration in the human body can be successfully solved by using breath tests based on Yanson point contacts. Employment of scientific and technological achievements of the past and of the present combined with mathematical tools makes it possible to successfully solve a number of problems which cannot be solved or present significant difficulties when approached with other methods. Many years ago, Yanson point-contact spectroscopy offered unique possibilities for solving scientific and technological problems of the time. Now, 40 years later, Yanson point contacts can be successfully applied in new cross-disciplinary areas of research.

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Новий експрес-метод для визначення мелатоніна в організмі людини

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Оригінальні фундаментальні властивості точкових контактів Янсона дозволяють використовувати їх для досліджень та розробки технологій у широкому діапазоні умов навколишнього середовища. При низькій температурі ці нанооб’єкти використовуються як основний інструмент мікроконтактної спектроскопії Янсона. При кімнатній температурі вони можуть служити чутливим елементом інноваційних наносенсорів з відмінними характеристиками. Найважливішою перевагою точково-контактних сенсорів при дослідженні складних газових середовищ є спектральний характер сигналу відгуку. Відкриття спектральних можливостей точково-контактних сенсорів при аналізі складних газових середовищ дозволяє говорити про спектральну багатофункціональність точкових контактів Янсона та про розширення можливостей їх спектрального застосування — від спектроскопії електрон-фононної взаємодії при низьких температурах до спектроскопії газоподібних середовищ при кімнатній температурі. Запропоновано новий неінвазійний метод визначення мелатоніна, одного з важливих гормонів, що характеризує стан людського організму, на основі використання спектрального відгуку точково-контактних сенсорів. Для його реалізації запропоновано низку процедур для визначення концентрації мелатоніна в організмі людини як функції реакції точково-контактного сенсора на дію газу, що видихається. Показано, що запропонований спосіб є достатньо точним для використання в медичних цілях в режимі реального часу. Результати дослідження свідчать, що проблему неінвазійного визначення концентрації мелатоніна в організмі людини можна успішно вирішити, використовуючи дихальні тести, які засновані на точкових контактах Янсона.

Ключові слова: точковий контакт Янсона, профіль газу, що видихається, мелатонін, квантовий сенсор, селективне виявлення.