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Application of tunable diode lasers for a highly sensitive analysis of gaseous biomarkers in exhaled air

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***Abstract*. The application of tunable diode lasers for a highly sensitive analysis of gaseous biomarkers in exhaled air in biomedical diagnostics is discussed. The principle of operation and the design of a laser analyser for studying the composition of exhaled air are described. The results of detection of gaseous biomarkers in exhaled air, including clinical studies, which demonstrate the diagnostic possibilities of the method, are presented.**

Keywords: *tunable diode lasers, high-resolution molecular spectroscopy, biomarkers, analysis of the composition of exhaled air, medical diagnostics.*

1. Introduction

One of the promising applications of tunable diode lasers (TDLs) is a highly sensitive gaseous analysis of biologically active endogenous molecular metabolites formed in living organisms and taking part in a gas exchange, namely, in the process of external breathing. It is known that, aside from molecules O_2, N_2, H_2O, and CO_2, the exhaled air also contains the traces of more than 600 volatile compounds produced during metabolism in biochemical reactions and removed from organism during breathing [1]. The concentration of these compounds in exhaled air is, as a rule, substantially lower than 1 mg m^{-3}. The mechanisms of formation and transport of some of these compounds are highly specific, which allows their use as natural biomarkers. The continuity of breathing and a high efficiency of the gas exchange between blood and air in lungs permit one to monitor these processes. A distinctive feature of this diagnostics is that it is noninvasive, which provides a number of advantages during fundamental and applied biomedical studies.

TDLs provide good analytic characteristics required for microanalysis of the composition of exhaled air. They allow one to measure the volume concentrations of some gaseous substances in the range from 10^{-4} % to 10^{-8} % with the time constant of 1–10 s [2, 3], featuring a high selectivity and insensitivity to basic atmospheric components (O_2, N_2, H_2O, and CO_2) contained in a sample being analysed. Substances contained in a sample of exhaled air can be detected without any preliminary concentrating or enrichment. The analysis can be applied for detecting various gaseous compounds.

These possibilities are provided by a low level of the amplitude noise of TDLs, a narrow emission line (less than 0.001 cm^{-1}), and a broad tuning range (up to $\sim$ 200 cm^{-1}) achieved by varying temperature. TDLs made of different chemical compounds provide in combination a very broad tuning range, which permits the tuning to the wavelength that is optimal for analysis, an accurate detection of the fine structure of vibration–rotation absorption bands, and an exact measurement of the parameters of individual absorption lines.

The above analytic characteristics of TDLs can be achieved if the vibration–rotation molecular spectra are well resolved, which determines the scope of molecules that can be analysed in exhaled air by the method of diode laser spectroscopy for the purpose of medical diagnostics. First, this is the detection of traces of light gaseous molecular metabolites such as CO, NO, NO_2, N_2O, NH_3, H_2O_2, C_2H_4, C_2H_4, C_2H_6, CH_2O, CH_4, CH_3OH, C_2H_5OH, CS_2, H_2S, C_5H_{12}, C_2H_6, CH_2OHS in the concentration range from 10^{-7} % to 10^{-4} %. Second, this is an accurate detection of a number of molecular isotopes enriched with D such as ^{13}C, ^{15}N, ^{18}O, and ^{35}S. Third, this a long-term monitoring of the composition of exhaled air and its analysis during one breathing cycle, when a gaseous mixture being analysed cannot be accumulated or enriched. And, fourth, this is laboratory studies of a gas exchange for small animals and plants.

We consider in this paper some applications of TDLs in biomedical and clinical diagnostics and present the relevant results.

2. Spectroscopy of molecules under study and the choice of an analytic line

A proper choice of an analytic absorption line is very important in the spectral analysis of the composition of exhaled air. The concentrations of H_2O and CO_2 in exhaled air are higher than in atmospheric air (more than 3 %), and the spectra of these molecules are overlapped with the spectra of molecules under study. This is demonstrated in Fig. 1, where the absorption spectra of NO, CO_2, and H_2O in exhaled air are presented in the range 5–6 μm (1820–1920 cm^{-1}). One can see that the resonance absorption by CO_2 and H_2O can be several orders of magnitude greater

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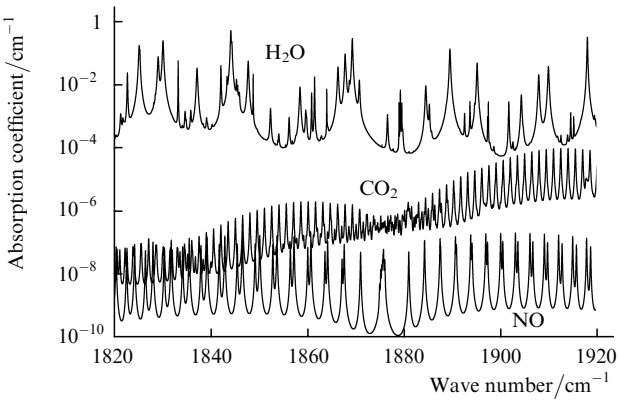

Figure 1. Absorption spectra of H_2O, CO_2, and NO in the 5–6-μm region at concentrations corresponding to the composition of exhaled air (6 %, 3 %, and 10^{-6} % of H_2O, CO_2, and NO, respectively).

than absorption by detected NO molecules. In addition, many intense absorption lines of NO, which can be used for analysis, coincide with the absorption lines of H_2O and CO_2. A similar situation is observed in the 9–11-μm region, which is used for detecting $^{14}NH_3$, $^{15}NH_3$, and C_2H_4 molecules, and in the range from 3 to 3.5 μm, which is used to analyse hydrocarbons, as well as in other spectral regions. In the case of such strongly overlapped vibration–rotation spectra, the choice of the optimal analytic absorption line is performed with the help of the algorithm, which is based on the determination of conditions required for the preservation of an extremum in the absorption spectrum, which is caused by the overlap of the wings of the analytic and interfering lines. This approach was described in detail in paper [4], where it was shown that, if a weak analytic line of a Lorentzian shape with the intensity S_a and the coefficient γ_a of broadening by a buffer gas is located at a distance δ from a much more intense absorption line of the interfering gas with parameters S_i and γ_i, then the extremum will disappear when the concentration of a substance being analysed is

$$c_a \propto \frac{S_i}{S_a}\left(\frac{\gamma_i}{\delta}p\right)^2\left(\frac{\gamma_a}{\delta}p\right)^{n+1}c_i\,,$$

where c_i is the concentration of the interfering gas; p is the total pressure of the gas mixture under study; and n is the spectrum differentiation degree ($n = 0, 1, 2$). This expression allows one to compare the analytic characteristics of different absorption lines and estimate an increase in the detection sensitivity in passing from the direct detection of the spectrum to the detection of its derivatives, as well as upon reducing the pressure of a gas mixture under study. In particular, it was found, by using this expression, that the absorption lines, whose parameters are presented in Table 1, are most suitable for analysis of the content of CO, NO, $^{14}NH_3$, $^{15}NH_3$, CH_4, and C_2H_4 in exhaled air.

3. Sensitivity of the TDL gas analysis

While the limiting sensitivity of detection of resonance absorption with the help of TDLs is determined by their noise parameters (quantum noise, relaxation oscillations, fluctuations of the carrier mobility and mode competition [5–7]), in practice, as a rule, the sensitivity is restricted by the noise of a detection system, the time response and the dynamic range of the spectrum digitisation procedure, and by optical interference noise [2, 3]. In the case of repetitively pulsed TDLs and digital recording of molecular transmission spectra used in this paper, a number of instrumental and program methods are used to reduce the influence of these factors [8, 9]. The instrumental differentiation and integration of the transmission spectra allow one to narrow down a radio-frequency detection band. In addition, the differentiation enhances the contrast of the spectrum and shifts the dynamic range of the signal detection to a small-signal region. The computer differentiation is also used for this purpose [9].

Program methods for enhancing the detection sensitivity are based on the separation of the useful component of a digitally detected signal from noises with the help of a digital filtration, for example, using the Fourier transformation. The Fourier transformation can improve the signal-to-noise ratio upon detection of a resonance absorption line against a white-noise background and reduce the relative amplitude of interference modulations [9]. Fig. 2 illustrates the efficiency of this approach by the example of filtration of the preliminary digitised transmission spectrum of CO_2 detected with the help of a TDL in the region of 2280 cm^{-1}. The transmission at the intense line of CO_2 and at its left satellite is ~ 1.4 % and ~ 0.075 %, respectively, the width of both lines being ~ 230 points. The intensity of the interference noise with the half-period ~ 50 points before filtration was ~ 0.03 %, and the peak amplitude of white noise was ~ 0.02 %. The spectrum was subjected to the

Table 1. Parameters of the absorption lines optimal for detecting of some gaseous biomarkers in exhaled air.

Molecule	Concentration range in exhaled air	Required sensitivity/ppb	Spectral range/μm	Spectral band	Optimal analytic line			
					Assignment	Frequency of the line centre/cm^{-1}	Line intensity/10^{-19} cm molecule^{-1}	Broadening coefficient/cm^{-1} atm^{-1}
CO	0.1–10 ppm	5	4.7	1–0	$P(5)-P(10)$ $R(5)-R(10)$	2100–2180	3–4	0.06
NO	3–1000 ppb	0.5	5.5	1–0	$P(7.5)$	1850.17	1.03	0.0598
$^{14}NH_3$	1–200 ppb	1	10.3 9.3	$v_2(0100a-0000s)$ $v_2(0100a-0000s)$	$sO(3.3)$ Multiplet $aR(4)$	967.346 1065.6	5.45 6.34	0.075 0.075
$^{15}NH_3$	1–1000 ppb	1	9.2	$v_2(0100a-0000s)$	$sR(3.3)$	1079.3	5.20	0.075
C_2H_4	1–100 ppb	1	9.8	v_7	$P(9.7)$	1023.688	0.314	0.087
CH_4	1–100 ppm	50	3.3	$v_3(00011001-00000000)$	Triplet $R(3)$	3057.7	1.2–2.1	0.06

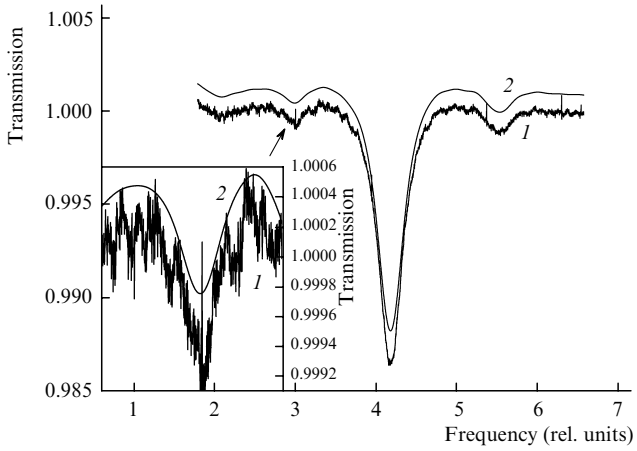

Figure 2. Application of the Fourier transformation to the transmission spectrum of CO_2 recorded with the help of a TDL. (*1*) measured spectrum; (*2*) spectrum after filtration.

Fourier transformation with the filter width equal to 100 points. The inset in Fig. 2 shows the left satellite. One can see that the Fourier transformation resulted in a substantial reduction of white noise and periodic interference noise. While the signal-to-noise ration before filtration was ~ 3, it increased after filtration, according to our estimate, by more than a factor of 30.

4. TDL analyser of the composition of exhaled air

A laser analyser of the content of the gaseous micro-components of exhaled air represents a highly sensitive, high-resolution IR spectrometer. The typical scheme of such an analyser is shown in Fig. 3. Depending on the spectral range, lasers based on the $A^{III}B^{V}$ or $A^{IV}B^{VI}$ compounds of different structures can be used as IR radiation sources. We used a repetitively pulsed TDL for recording absorption spectra. The typical duration of current pump pulses for double-heterostructure lasers was 3–10 ms, and the pulse repetition rate was varied from 50 to 200 Hz [9]. In this regime, the lasing frequency was scanned within the entire spectral region under study (of width 10–30 cm^{-1}) during each laser pulse, which was caused by a periodic nonstationary heating of the laser crystal during the current pulse. The high reproducibility of the laser tuning from pulse to pulse was provided by a precise (with an error of 0.001 K) stabilisation of the temperature of a heat sink on which the TDL was mounted and by the high reproducibility of the parameters of current pulses. This allowed us to improve the signal-to-noise ratio by accumulating the signal. The spectral resolution of the analyser, which was determined by the width of the lasing line and the tuning stability, was $\sim 3 \times 10^{-4}$ cm^{-1}.

Laser radiation passes through an analytic multi-pass cell, containing a gas mixture under study, and is focused on a fast IR detector (CdHgTe or InSb). Transmission spectra were recorded using schemes with 8-bit ADCs, which digitised the spectrum with the time resolution to 50 ns. The dynamic range of the digital recording of the spectrum could be enlarged up to 12–16 bit by accumulating a signal simultaneously with a controlled sweep of the electric zero [9].

The concentration of a substance under study was measured from the amplitude of the resonance absorption

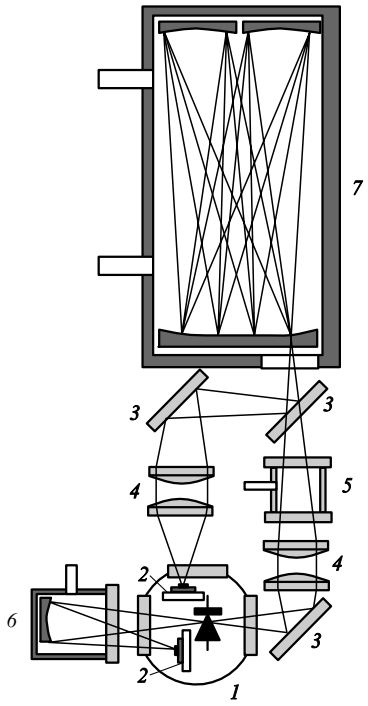

Figure 3. Optical scheme of a highly sensitive TDL gas analyser: (*1*) TDL in cryostat; (*2*) IR detectors in cryostat; (*3*) deflecting mirrors; (*4*) two-lens IR objectives; (*5*) calibration cell; (*6*) reference cell; (*7*) multi-pass analytic cell.

line using the Bouguer–Lambert–Beer law, taking into account the parameters of its shape determined by collision and Doppler broadening.

5. Applications of TDLs in biomedical diagnostics

We tested the TDL methods and systems by analysing the content of endogenous molecules CO, No, NH_3, CH_4, and C_2H_4 in exhaled air. Consider the detection of such biomarkers as CO and NO in exhaled air, which is most interesting for biomedical diagnostics [10–12].

Endogenous CO in exhaled air. CO is formed in the organism due to the enzymatic decomposition of heme-containing structures [13]. It is buffered and transported from the places of its formation to lungs due to the reversible binding with myoglobin and hemoglobin, competing with O_2. The rate of CO release with exhaled air depends on the rate of its formation and the efficiency of its binding with buffer systems, which in turn depends on pH of a medium, the oxygenation of tissues, and the content of some substances in the organism. Our studies revealed the basic properties of the exhalation of endogenous CO from the human organism during breathing.

The typical transmission spectrum of CO detected with a TDL and used for measuring the concentration of this molecule in exhaled air is shown in Fig. 4, where the assignment of the absorption lines of ^{12}CO is presented. This figure also shows the transmission spectrum of the ^{13}CO isotopic molecule, which is used for the assignment of the absorption lines of the main isotope and the frequency calibration of the detected spectrum with an error of $\sim 10^{-3}$ cm^{-1}. The sensitivity of detection of the volume concen-

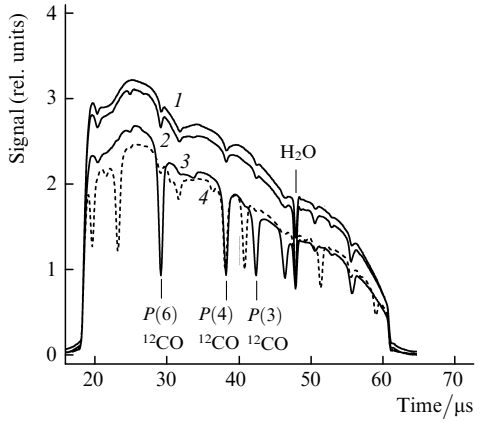

Figure 4. Transmission spectra of the atmospheric air (*1*), exhaled air (*2*), calibration CO-air mixture (*3*) and ^{13}CO-air mixture (*4*) recorded with a TDL in the region of 2120 cm^{-1}.

tration of CO in exhaled air for 5 s was $\sim 5 \times 10^{-7}$ %.

Laser analysis allows both static and dynamic studies of the CO content in exhaled air. Fig. 5 demonstrates a continuous and prolonged monitoring of the CO content and the blood acidity with the time response close to the real-time response. We studied the CO exhalation during a physical load and hyperventilation. The air exhaled by a patient was pumped through a laser analyser cell, free breathing being provided with the help of a small buffer reservoir.

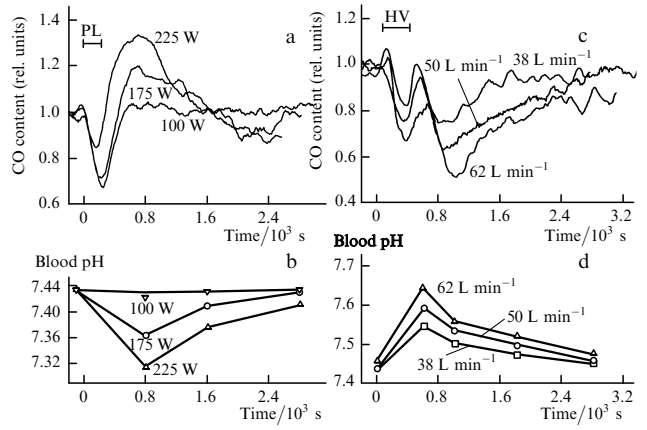

Figure 5. Dynamics of the CO content (a, c) and the blood acidity (b, d) under different physical loads (PL) (a, b) and hyperventilation (HV) at different depths (c, d).

Fig. 5a shows the dynamics of the CO concentration in exhaled air during physical loads of different powers (veloergometry). One can see that the CO concentration decreases during five-minute physical load, which is caused by the enhanced ventilation of lungs, and then increases during the relaxation period after exercises under a large load (175 and 225 W). On the contrary, after two-minute hyperventilation (Fig. 5c), the CO concentration in exhaled air decreases. Both these effects, observed in two different physiological tests, have the same nature – a change in the concentration of CO_2 and other metabolic products in blood, which affect its acid-alkali state. The concentration of these species in blood increases under physical load and decreases during hyperventilation. The value of pH in blood changes correspondingly (Figs 5b, d). The exhalation of CO during breathing is sensitive to these variations in the chemical composition of blood because they determine the strength of CO binding with blood cells. Therefore, the laser analysis of the CO concentration in exhaled air can be used for noninvasive monitoring of variations in the chemical composition of blood and its gas-transport properties. This analysis can be used in studies of normal and hyperbaric physiology, sport medicine, and for studying the functional state of the human organism in a changed gas medium.

We also investigated variations in the exhalation of endogenous CO caused by some diseases (Fig. 6). For this purpose, the CO concentration in the air exhaled by a patient was measured during a natural breathing at the state of rest. We performed a statistical analysis by sampling exhaled air to an intermediate reservoir of volume 2–3 L. The data averaged over the groups of pathologic states show that the exhalation of CO decreases in the cases of chronic obstructive lung disease, lung fibrosis, anaemia, and bronchial asthma. The CO exhalation increases substantially compared to norm in the case of liver disease, which is caused by the enhanced metabolism of bilirubin resulting in the destruction of heme-containing structures [13]. These results show that exhaled CO can be used as a marker of some diseases in clinical diagnostics.

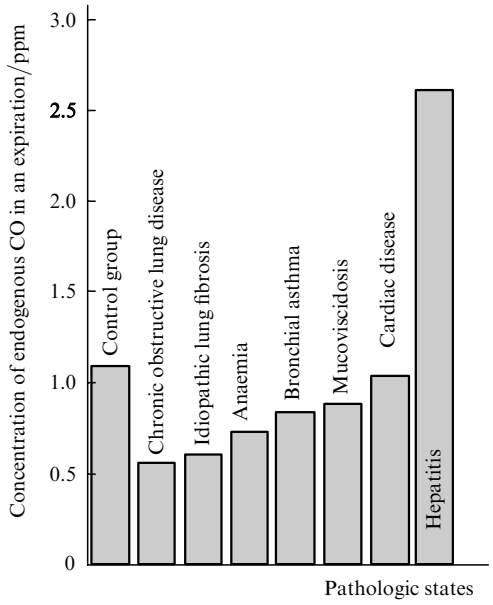

Figure 6. Average CO concentrations in exhaled air for a control group and for different pathologic states.

Endogenous NO in exhaled air. NO plays an important role in a number of biochemical processes proceeding in organism. This molecule takes part in the transmission of nervous pulses and the regulation of the tonus of blood vessels, and, being an efficient oxidant, exerts a bacteriostatic action during inflammatory processes [11, 14]. The laser analysis of NO can be used for diagnostics and therapy of some diseases accompanied by inflammatory processes. For example, the measurement of the NO content in exhaled air is important in the study of diseases of airways

(bronchial asthma and obstructive lung disease), while the measurement of the NO concentration in stomach is important in the medical treatment of diseases of digestive organs (gastritis). The NO concentration in these cases varies in range from 1 to 1000 μg m^{-3} and can be measured with a TDL analyser in the presence of great amounts of water and CO_2.

The traces of NO in exhaled air were detected with a sensitivity of ~ 1 μg m^{-3} by differentiating the experimental transmission spectra. Fig. 7a shows the transmission spectrum of the air exhaled by a smoker at 1850 cm^{-1}, which was recorded directly. The NO concentration greatly exceeds the norm, amounting to 50 mg m^{-3}. The absorption lines of CO_2 and H_2O preventing the measurement of NO traces are observed. Fig. 7b shows the second derivative of the transmission spectrum of the nasal air sampled from nasal airways, where the NO concentration is ~ 1 mg m^{-3}. One can see that the contrast of this spectrum is substantially higher, which allows the analysis sensitivity to be increased to several μg m^{-3}.

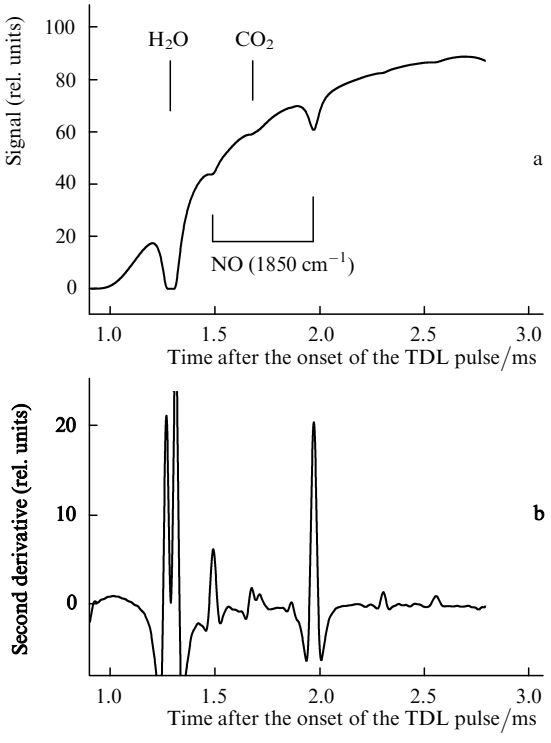

Figure 7. Transmission spectrum of air exhaled by a smoker recorded with a TDL in the region of 1850 cm^{-1} at the NO concentration equal to ~ 50 mg m^{-3} (a) and the second derivative of the transmission spectrum of the nasal air at the NO concentration equal to ~ 1 mg m^{-3}.

When the NO biomarker is used for diagnostics of diseases of respiratory organs [10, 11, 13], it is necessary to distinguish its content in the initial and final portions of each expiration [15]. We used for this purpose the so-called alveolar interceptor, which separates mechanically the air exhaled in each expiration into fractions and gradually accumulates in a cell the air from a part of airways under study. Fig. 8 demonstrates the use of the interceptor for measuring the NO concentration. Before its switching, the NO concentration averaged over an expiration was measured (the initial plateau). After the interceptor switching and sampling of the initial portion of the expiration, the NO concentration increased, which was caused by a higher level of NO producing in the upper airways, including nasal airways. The NO concentration in the final (alveolar) portion of the expiration was lower than the average value.

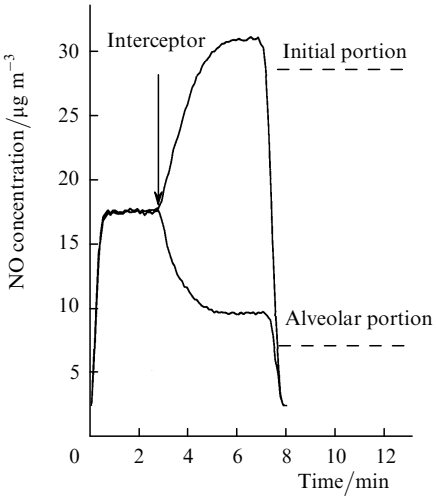

Figure 8. Dynamics of the NO concentration in exhaled air sampled with an alveolar interceptor.

Fig. 9 shows the NO concentrations in exhaled air measured for various diseases. One can see from Fig. 9a that the NO concentration in the final portion of exhaled air averaged over a group of patients increases in the cases of bronchial asthma, obstructive lung disease, and fibrosis. It follows from Fig. 9b that to distinguish bronchial asthma from obstructive lung disease, the ratio of NO concen-

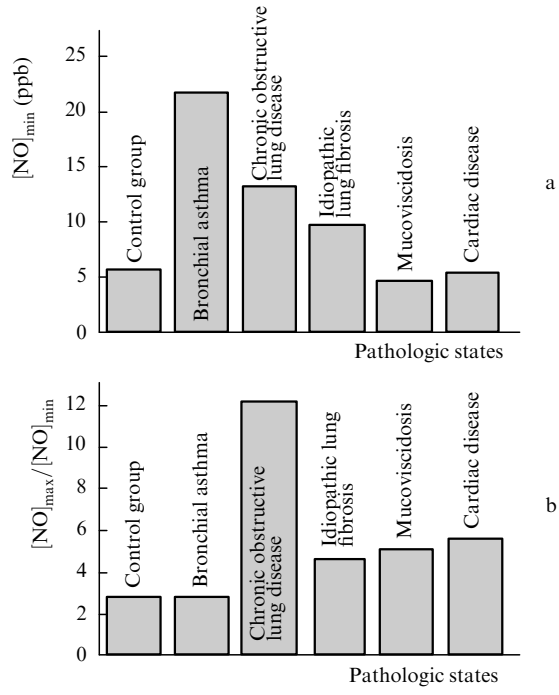

Figure 9. Average concentrations $[NO]_{min}$ in the final portion of an expiration (a) and average ratios of concentrations $[NO]_{max}/[NO]_{min}$ in the initial and final portions of the expiration (b) for some pathologic states.

trations in the initial and final portions of exhaled air can be used. This ratio substantially exceeds the norm in the case of obstructive lung disease, in contrast to bronchial asthma.

The use of NO as a marker can be promising for studying the action of various factors causing inflammatory reactions in the human organism. Thus, we have found an increase in the NO concentration in exhaled air for patients using mobile telephones [15].

This simple and noninvasive method is promising for rapid diagnostics of diseases and can be used during therapy for efficient medical treatment.

6. Conclusions

The highly sensitive laser spectral analysis of gaseous molecular biomarkers in exhaled air is promising for fundamental and applied (clinical) diagnostic studies. The use of TDLs for this analysis extends the scope of objects and problems that can be solved and opens up new approaches in medicobiological studies (real-time measurements, long-term continuous monitoring, noninvasive analysis, large-scale screening studies, use of new loading tests, etc.). The application of the method proposed in this paper for diagnostics of diseases along with conventional methods can provide noninvasive, rapid, and economical studies. After an appropriate development, this method can be used for the correlation diagnostics of diseases, diagnostics involving stable isotopes, the optimisation of various therapeutic treatments, diagnostics of extremal physical states (in sport, astronautics, etc.), in pharmacological studies, including laboratory animals, for the environmental pollution measurements (for example, for ecological monitoring) and for screening epidemiological tests.

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