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Laser analysis of the $^{13}C/^{12}C$ isotope ratio in CO_2 in exhaled air

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Abstract. **Tunable diode lasers (TDLs) are applied to the diagnostics of gastroenterological diseases using respiratory tests and preparations enriched with the stable ^{13}C isotope. This method of the analysis of the $^{13}C/^{12}C$ isotope ratio in CO_2 in exhaled air is based on the selective measurement of the resonance absorption at the vibrational – rotational structure of $^{12}CO_2$ and $^{13}CO_2$. The CO_2 transmission spectra in the region of 4.35 μm were measured with a PbEuSe double-heterostructure TDL. The accuracy of carbon isotope ratio measurements in CO_2 of exhaled air performed with the TDL was ∼ 0.5 ‰. The data of clinical tests of the developed laser-based analyser are presented.**

Keywords: *tunable diode lasers, $^{13}C/^{12}C$ isotope ratio, high-resolution molecular spectroscopy, medical diagnostics, respiratory tests.*

The analysis of the isotope composition of CO_2 in exhaled air, in particular, the relative content of the stable ^{13}C isotope is one of the new efficient methods of noninvasive medical diagnostics. Isotopic respiratory tests, which are based on preparations labelled by the ^{13}C isotope and taken by a patient and on the analysis of changes in the $[^{13}CO_2]/[^{12}CO_2]$ concentration ratio in exhaled air, find an ever-widening application in gastroenterology and hepatology for diagnosing the stomach, duodenum, liver, pancreas, and large and small intestine [1]. This is an alternative approach compared to the use of radioactive isotopes, such as ^{14}C, in medical practice.

In relative measurements, which are widely used in the carbon isotopic analysis, the carbon isotope ratio $^{13}C/^{12}C$ of the sample under study is compared to a standard ratio. The carbon isotope ratio in the limestone in the Pee Dee Formation in South Caroline is considered to be standard. It is equal to ∼ 1.1237 % and is abbreviated as PDB [2 – 4]. As a rule, the variations of the isotope ratio $^{13}C/^{12}C$ significant for diagnostics amount to a few percent and less. Therefore, the quantity $\delta(^{13}C)$ measured in *pro mille* (‰) is commonly used in practice. It is defined as [2, 4, 5]

$$\delta(^{13}C) = \left(\frac{R_a}{R_{std}} - 1\right)1000, \quad (1)$$

where R_a and R_{std} are the carbon isotope ratios for the studied and standard samples.

During respiratory tests with isotopes, the change in $\delta(^{13}C)$ is estimated in a certain time interval after a labelled preparation is taken. Consequently, the difference between the results for the tested (studied) and so-called basal samples of the exhaled air is measured [4]:

$$\Delta\delta(^{13}C) = \delta_{contr}(^{13}C) - \delta_{bas}(^{13}C). \quad (2)$$

The error in determining $\delta(^{13}C)$ and $\Delta\delta(^{13}C)$ must be below 0.5 ‰. This can be achieved if a high sensitivity, high accuracy, and a wide dynamic range (∼ 6 orders of magnitude) of concentration measurements are ensured. This is possible when using either mass spectroscopy combined with gas chromatography, or spectrophotometry methods. The former is currently the main technique for the practical analysis of the carbon isotope ratio in CO_2 [6, 7]. However, the instruments based on this technique are rather complex and expensive, thus impeding the wide application of the diagnostics using stable isotopes.

The isotopic analysis can be significantly simplified and made much cheaper, if spectrophotometry principles based on the recording of the absorption spectra of the analysed substance are used. In this case, according to the Bouguer – Lambert – Beer law, relation (1) takes the form

$$\delta(^{13}C) = \left[\frac{([^{13}C]/[^{12}C])_a}{([^{13}C]/[^{12}C])_{std}} - 1\right]1000$$

$$= \left[\frac{(\varkappa_{13}/\varkappa_{12})_{std}(A_{13}/A_{12})_a}{(\varkappa_{13}/\varkappa_{12})_a(A_{13}/A_{12})_{std}} - 1\right]1000, \quad (3)$$

where $\varkappa_i$ and A_i are, respectively, the absorption coefficient and absorption value for the laser radiation at a certain wavelength for isotope modifications of CO_2 (isotopomers), and $i = 12$, 13 is the isotope number. One can see that, if the absorption coefficients are constant, the determination of the $\delta(^{13}C)$ value is reduced to the selective measurement of the absorption by the isotopomers, which can be accomplished, for example, by detecting individual lines of the fine vibrational – rotational structure of the IR spectra for CO_2. This is possible using high-resolution spectroscopy methods, such as laser spectroscopy methods.

One of such approaches is based on the use of tunable diode lasers (TDLs) [5, 8, 9], which are characterised by a unique combination of a narrow lasing line, a rather high radiation power, and the possibility of frequency tuning in a

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wide range. This ensures the high-resolution recording of vibrational–rotational absorption spectra of various molecules with a high sensitivity and high accuracy. In the case of CO_2, such recording makes it possible to distinguish the IR absorption spectra for its different isotopomers, namely, $^{12}CO_2$ and $^{13}CO_2$, to precisely measure the ratio of their concentrations in the gaseous medium under study. This paper describes a laser analyser of the carbon isotope ratio in CO_2, in which a TDL based on lead salts (PbEuSe) is used [10]. It operates in the middle IR region. This system is chiefly designed for the medical diagnostics using respiratory tests.

The wavelength region near $\lambda = 4.3$ μm, where the 00011–00001 fundamental band of CO_2 is located, is the most favourable region for the spectrum analysis of the carbon isotope ratio (Fig. 1). This band has the maximum integrated intensity in the middle IR and consists of fairly well isolated lines. The centres of this band for $^{12}CO_2$ and $^{13}CO_2$ are mutually shifted by ~ 65.5 cm^{-1}, and the lines with close intensities of these isotopomers are observed in the zone of the overlap of the *P*-branch of $^{12}CO_2$ and the *R*-branch of $^{13}CO_2$ in a range of 2290–2305 cm^{-1}. The lines with comparable intensities belonging to the 01111–01101 composite vibration band of $^{12}CO_2$ also lie in this region. The centre of this band is shifted relative to the centre of the 00011–00001 band by ~ 12 cm^{-1}.

An emulation of the CO_2 transmission spectrum in a range of 2280–2305 cm^{-1} for the conditions realised in practice (Fig. 2) makes it possible to select several pairs of neighbouring $^{12}CO_2$ and $^{13}CO_2$ lines suitable for the isotope spectrum analysis. They lie near the frequencies $\nu = 2293$, 2297, and 2301 cm^{-1}. The lines in these pairs have almost identical intensities at the natural isotope content, are at optimal distances from each other, and are sufficiently far from other lines. Their main parameters taken from the HITRAN 96 data base [11] are listed in Table 1.

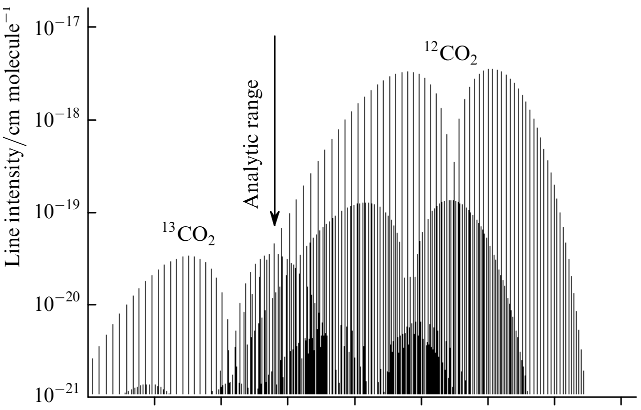

Figure 1. Absorption bands of a natural CO_2 isotope mixture in the range of 4.3 μm (the data from the HITRAN 96 atlas [11]).

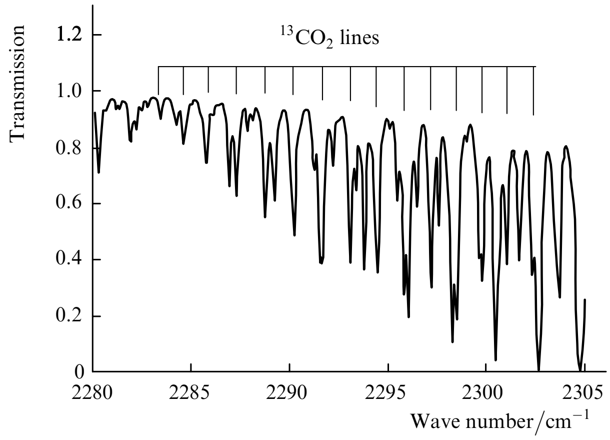

Figure 2. Emulated CO_2 transmission spectrum in the range of 4.35 μm in atmospheric air with the natural content of isotopes at $p_{atm} = 760$ Torr, $p_{CO_2} = 22$ Torr, $T = 293$ K, and an optical path length of 10 cm.

The use of TDLs as sources of tunable IR radiation simplifies the spectrum analysis of the carbon isotope ratio in CO_2 due to a number of specific features. Lasers of this type operate in almost the entire middle IR range, allowing one to select a spectral region most suitable for the analysis. The TDL radiation frequency is easily tuned to the required spectral region. The range within which a single-mode frequency tuning can be performed is sufficiently wide for the almost simultaneous recording of the required spectral features. The narrow emission line of these lasers ensures the completely resolved spectrum structure recorded, and low amplitude and frequency noises guarantee the necessary sensitivity to a change in the optical density. The high reproducibility of the TDL tuning curve allows for an increase in the sensitivity and detection accuracy due to the storage of the recorded signals. The isotope analysis of CO_2 near $\lambda = 4.35$ μm was performed using TDLs based on the $Pb_{1-x}Eu_xSe$ compound, which have a double heterostructure and a strip geometry of the active region [10]. Depending on their chemical composition, such lasers

Table 1. Parameters of the used analytic CO_2 lines.

Isotopomer	Band	Assignment	Frequency of the line centre ν_0/cm^{-1}	Line intensity*/10^{-20} cm molecule^{-1}	Air-caused broadening**/cm^{-1} atm^{-1}	Energy of the lower state/cm^{-1}
$^{13}CO_2$	00011–00001	*R*(12)	2293.093	3.41	0.076	60.874
$^{12}CO_2$	00011–00001	*P*(58)	2293.811	3.06	0.064	1333.768
$^{13}CO_2$	00011–00001	*R*(18)	2297.186	3.5	0.070	133.446
$^{12}CO_2$	01111–01101	*R*(43)	2297.580	1.59	0.067	1405.985
$^{13}CO_2$	00011–00001	*R*(24)	2301.064	2.82	0.070	234.095
$^{12}CO_2$	01111–01101	*P*(39)	2301.681	2.71	0.067	1276.448

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*With allowance for the isotopomer abundance;

**Half-width at half-maximum

operate in a wavelength range of 4.2 – 12 μm at temperatures of 80 – 140 K. When the temperature changes within this range, the spectral range of the operation of a single laser diode is 200 – 250 cm^{-1}. The range of continuous single-mode tuning reaches a value of 12 cm^{-1}. The width of the TDL lasing line is $\sim 10^{-4}$ cm^{-1}. If a pulsed pumping current is used, the frequency tuning rate is $5 \times 10^2 - 10^3$ cm^{-1} s^{-1} and depends on the shape and duration of the pumping pulse. The TDL output power in the single-mode regime can reach 0.5 mW. The threshold currents at $T = 80$ K are 0.1 – 0.6 A.

The optical layout and components of the laser analyser of the carbon isotope ratio in CO_2 are shown in Fig. 3. Liquid nitrogen provides low operating temperatures of the TDL. The laser temperature is maintained to an accuracy of $\sim 10^{-3}$ K. The diverging radiation from the TDL is focused at a nitrogen-cooled InSb photodetector with a CaF_2 objective lens. An optical cell ~ 10 cm long with a total volume of ~ 50 mL is placed between the lens and photodetector.

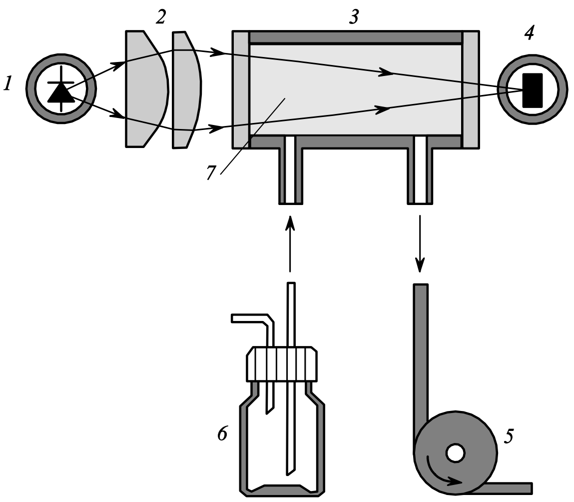

Figure 3. Schematic of the laser analyser of the carbon isotope ratio in CO_2 in exhaled air: (*1*) diode laser in a cryostat; (*2*) CaF_2 lens; (*3*) analytic cell; (*4*) IR InSb photodetector; (*5*) micropump; (*6*) container with a sample of the exhaled air; and (*7*) analysed gas mixture.

The laser is pumped by rectangular current pulses with an amplitude of 0.5 – 2 A, a duration of ~ 3 ms, and a repetition rate of ~ 100 Hz. During each pulse, a nonlinear change in the TDL lasing frequency occurs due to the nonstationary heating of the laser crystal and is periodically repeated from pulse to pulse to a high accuracy. In order to linearise the tuning curve, the TDL can be pumped by current pulses with a small slope of the linearly increasing amplitude [12].

After being amplified within a band of 0 – 3 MHz, the detected signal is digitised using a fast 8-bit ADC with a response time of 50 ns. The spectra are stored during their digital recording. The storage procedure is performed together with the controllable 4-bit sweeping of the electric zero of the recording system, which makes it possible to increase not only the signal-to-noise ratio but also the effective resolution of the recording. When the number of stored signals is 256, the relative discreteness of the amplitude recording decreases to $\sim 1.8 \times 10^{-6}$ [13], and the standard deviation for the white-noise component in the transmission spectra decreases to $\sim 1.2 \times 10^{-5}$. An additional increase in the measurement accuracy by an order of magnitude can be achieved using a programmed filtering of the digitised spectra based on the Fourier transform [13].

The operating conditions of the analyser were maintained with a PC-controlled special device, which ensured the selection of the TDL operating temperature, the parameters of the pump pulses, and the parameters of the system for transmission spectra recording. This device also assisted the digital recording and visualisation of signals and the data storing and archiving in the PC memory.

It is important that, when selecting optimal conditions for the analysis of the carbon isotope ratio, TDLs allow for a rather fine control of their spectral and power characteristics. In this case, only two variable parameters are used: the temperature and pump current of the laser crystal. Fig. 4 presents the main regularities in changes of the TDL tuning characteristics. Fig. 4a shows the transmission spectra of CO_2 in the exhaled air measured at a fixed temperature (87.2 K) of the heat sink and various initial pump-current amplitudes i_0 and rise rates s. Fig. 4b presents the tuning curves for each regime. One can see that during the entire pulse with a duration of 2855 μs, the laser actually operates at a single mode continuously tuned in a range of > 10 cm^{-1}. When rectangular ($s = 0$) pump pulses are used, the optical laser frequency increases nonlinearly during the pulse. As s grows, the tuning curve approaches a linear function. Depending on the current regime, the laser-frequency scanning rate changes within a fairly wide range. For example, for the doublet at a frequency of 2297 cm^{-1}, it is between $\sim 10^3$ and $\sim 4 \times 10^3$ cm^{-1} s^{-1}, which is taken into account when selecting the parameters of signal detection and digital spectra recording.

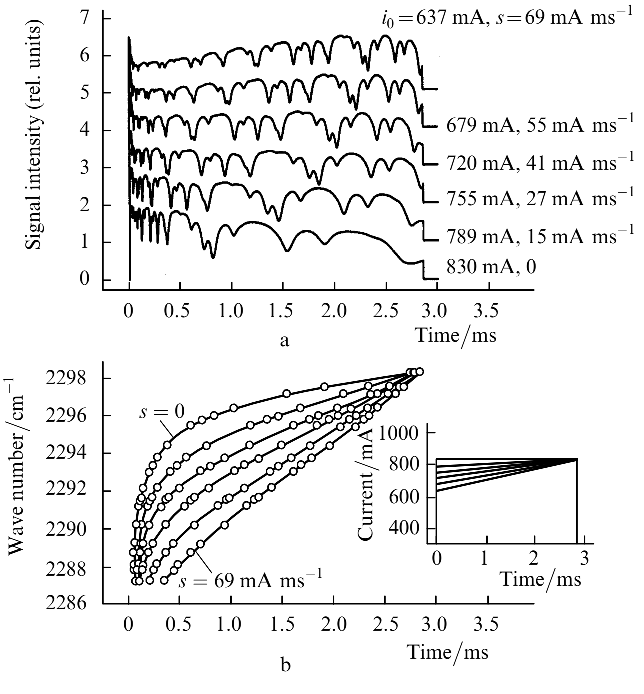

Figure 4. (a) CO_2 transmission spectra and (b) tuning curves at various initial amplitudes i_0 and TDL pumping current rise rates s. The temperature of the heat sink is 87.2 K, and the pulse duration is 2855 μs. The inset shows the current pulse shape.

Below, we present typical parameters of the pump pulses, recorded spectra, recording system, and the recorded spectral data arrays, which are used in the analysis of the carbon isotope ratio in CO$_2$:

Parameters of the TDL pump-current pulse:	
Initial amplitude/mA	500 – 1200
Current rise rate/mA ms^{-1}	20 – 40
Duration/μs	1500 – 3000
Repetition rate/Hz	100 – 200
Parameters of the recorded spectra	
Spectral region/cm^{-1}	2292 – 2305
Spectrum length/cm^{-1}	5 – 15
Analytic interval/cm^{-1}	3 – 4
Reproducibility error/cm^{-1}	< 0.001
Tuning rate/cm^{-1} s^{-1}	$3 \times 10^3 - 10^4$
Duration of recording the analytic absorption lines/μs	30 – 100
Radio frequency band/MHz	0 – 3
Signal-to-noise ratio	> 100
Parameters of the recording system	
ADC capacity/bit	8
Response speed/ns	> 50
ADC zero sweeping/bit	4
Dynamic range/V	0 – 3
Controlled bias voltage/V	±3
Number of stored signals	1 – 256
Characteristics of spectra after recording	
Discreteness of recording/ns	100 – 800
Number of points per spectrum	4000 – 6000
Number of points per absorption line	200 – 300
Relative discreteness of amplitude recording	$\sim 2 \times 10^{-6}$
Signal-to-noise ratio	$\sim 8 \times 10^4$
Time of recording a spectrum with a size of 4000 points and a number of stored signals of 256/s	10 – 30

Typical CO$_2$ transmission spectra recorded with a TDL and used in the analysis of the carbon isotope ratio are shown in Fig. 5. The concentrations of the isotopomers is determined on their basis using the Bouguer – Lambert – Beer law, which establishes a relation between the laser signal intensities before (I_0) and after (I) the passage through the cell:

$$I(\nu) = I_0(\nu)\exp\{-(\varkappa_{12}(\nu)[^{12}\mathrm{C}] + \varkappa_{13}(\nu)[^{13}\mathrm{C}])L\}, \tag{4}$$

where L is the length of the cell. Since, as is shown in Fig. 5, the CO$_2$ absorption lines belonging to different isotopomers are not fully isolated, it is difficult to determine the absolute absorption value in separate lines and, consequently, to directly measure the concentrations of the detected compounds. However, relative concentration measurements used for the analysis of the carbon isotope ratio can be simplified. In particular, when measuring the absorption in the detected resonant structure, instead of the exact value of the intensity $I_0(\nu)$ at the centre frequency ν_0 of each of the analytic lines, one can use a value obtained by a linear interpolation of the values of the nearest peaks $I(\nu)$. Fig. 6 shows a resolved analytic region of the CO$_2$ transmission spectrum near $\nu = 2297$ cm^{-1}, and the quantities utilised in the spectrum processing are presented.

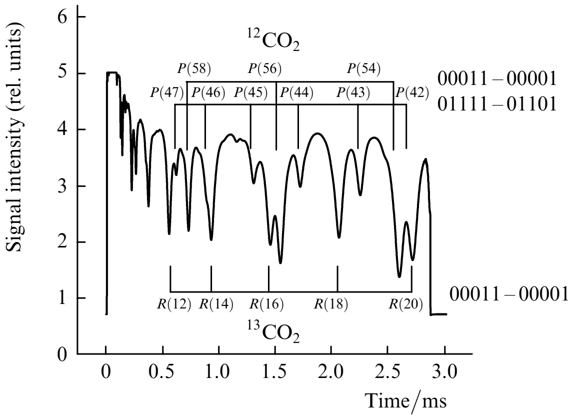

Figure 5. CO$_2$ transmission spectrum used for calculating the carbon isotope ratio and identifying the recorded lines.

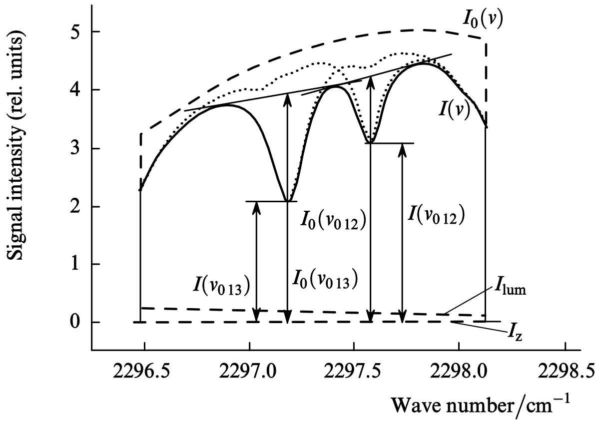

Figure 6. Analytic CO$_2$ doublet near $\nu = 2297$ cm^{-1}; I_{lum} is the luminescent signal component, I_z is the optical zero level, and dotted lines are the signals in the absence of absorption in the analytic lines.

As a result of the spectrum processing for each analytic line, a certain optical density is measured:

$$\chi_{\mathrm{meas}\,i} = \varkappa_{\mathrm{eff}\,i}(\nu_{0i})\left[^{(i)}\mathrm{C}\right]L = -\ln\left[\frac{I(\nu_{0i})}{I_0(\nu_{0i})}\right], \tag{5}$$

where $i = 12$, 13 is the isotope number. Note that the effective absorption coefficient $\varkappa_{\mathrm{eff}\,i}(\nu_{0i})$ differs from the absorption coefficient in the line determined by its spectral parameters. The ratio of the optical densities for the ^{12}CO$_2$ and ^{13}CO$_2$ lines is defined as

$$R_\chi = \frac{\chi_{\mathrm{meas}\,13}}{\chi_{\mathrm{meas}\,12}}. \tag{6}$$

If the ratio of the measured absorption coefficients $\varkappa_{\mathrm{eff}\,12}(\nu_{0\,12})$ and $\varkappa_{\mathrm{eff}\,13}(\nu_{0\,13})$ is maintained constant for the analysed and reference gas mixtures, then expression (1) can be written in the form

$$\delta(^{13}\mathrm{C}) \approx \left(\frac{R_{\chi\mathrm{a}}}{R_{\chi\,\mathrm{std}}} - 1\right)1000. \tag{7}$$

Hence, the absolute value of R_χ is insignificant in determining $\delta(^{13}\mathrm{C})$.

The algorithm for processing the transmission spectra involves the following steps. The zero level of the optical signal is determined, and an analytic spectrum region is selected. The Fourier filtering of the spectrum is performed, which is aimed at a decrease in the high-frequency component of amplitude noises. The positions of the peaks nearest to the both analytic lines are determined in transmission spectra. For each analytic line, straight lines are plotted through these peaks (Fig. 6) and are used instead of the pulse envelope for normalising and calculating the optical density $\chi_{\text{meas}\,i}$ at the centre of each isotopomer line. The ratio of the found absorption coefficients for $^{13}CO_2$ and $^{12}CO_2$ (i.e., the value of R_χ), the parameter $\delta(^{13}C)$ for the tested and basal gas mixtures, and the sought-for difference $\Delta\delta(^{13}C)$ are then calculated. The spectra are processed according to the algorithm described above using a special program. The execution time of a complete processing cycle in the interactive mode is ~ 60 s.

Errors in determining the carbon isotope ratio using TDLs may be caused by various factors: variations in the spectral properties of the studied medium under changes of the ambient conditions, unstable characteristics of laser radiation, features of the radiation passage through the analysing system, limitations of the recording system, effects of the discreteness of signal digitisation and spectral-data processing, errors in gas sampling, etc. The effects of the factors that lead to systematic errors decrease in relative measurements or can be taken into account by introducing corrections.

Random errors are the most significant. They are determined by uncontrollable temperature variations in the analysed gas mixture in the cell and by signal intensity modulations due to the interference of laser radiation reflected by optical elements of the analyser, in particular, by a time instability of such modulations. These errors are reduced by stabilising the gas temperature in the cell, observing a unified protocol of spectral measurements, and also due to a thorough adjustment of the optical system and the use of special design solutions that reduce the probability of the interference of repeatedly reflected laser rays.

Samples of the exhaled air for the analysis of the carbon isotope ratio are collected in airtight glass containers with a volume of 70 ml. In this case, one holds its breath in order to ensure an increased content of CO_2 of $4\%-5\%$ with respect to its normal fraction. Using probes, the mixture from the container is transported to the analyser cell. The data reproducibility is achieved by the fulfilment of a certain protocol of the cell filling and evacuation. Three transmission spectra are recorded and processed for each sample. The total time of the analysis of a single gaseous sample is ~ 6 min.

The laser analyser described was used in the diagnostics of Helicobacter pylori (Hp) infectious bacteria in organisms. The presence of these bacteria in the digestive tract provokes gastrities and stomach and duodenum ulser [14, 15]. In order to detect Hp in the digestive tract, a special so-called urea breath test (^{13}C-UBT) has been developed. It utilises a specific capability of Hp bacteria of producing the ferment urease, which decomposes urea [14, 16]. The ^{13}C-UBT test is based on the peroral dosing of an aqueous solution of urea labelled with ^{13}C and the subsequent analysis of the changes in the carbon isotope ratio in exhaled CO_2. The presence of Hp in the digestive tract leads to a decomposition of the labelled urea and an increase in the $^{13}CO_2$ content in the exhaled air. Fig. 7 shows a typical time dependence of $\delta(^{13}C)$ in exhaled CO_2 for infected and noninfected persons after they took the labelled urea. The dose of the taken urea solution was selected in accordance with the ^{13}C-UBT protocol [16], so that the peak of the obtained curve corresponded to the size of the colony of the bacteria in question. For practical purposes, one tested sample of the exhaled air taken 30 min after the basal sample is sufficient. If $\Delta\delta(^{13}C) > 3$ ‰, this value is considered positive and testifies to the presence of Hp infection.

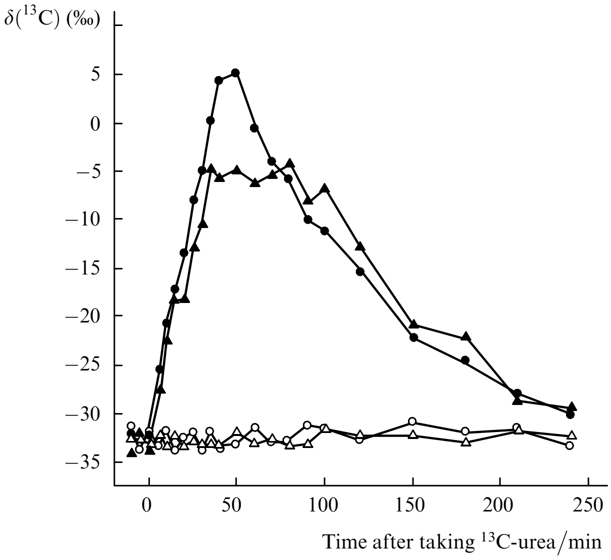

Figure 7. Time dependences of $\delta(^{13}C)$ in exhaled CO_2 obtained as a result of ^{13}C-UBT of two infected (dark dots) and two noninfected (light dots) persons.

Fig. 8 shows the results of a laser analysis of a run of ^{13}C-UBT tests for 13 persons. We see that six persons are infected. The $\Delta\delta(^{13}C)$ values are given for the positive tests. A slight trend of the basal level, which is caused by a change in the gas-sample temperature, should be noted. Taking this effect into account, the deviation of the basal data from their average value is no larger than ± 1.3 ‰ (the standard deviation is ~ 0.63 ‰). This difference between the R_χ values of the basal samples for different tested persons is partially associated with features of their diets. At the same time, our estimates show that, in this experimental run, the standard deviation determined by the instrumental error is ~ 0.22 ‰.

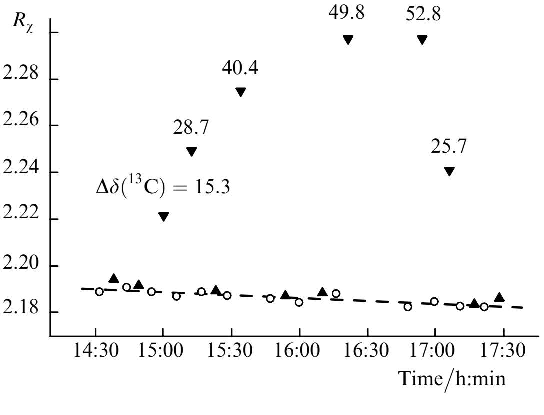

Figure 8. Results of R_χ measurements in (○) basal and (▲) reference samples of the exhaled air in a cycle of tests for 13 persons. The $\Delta\delta(^{13}C)$ values are given for positive tests.

More than 600 ^{13}C-UBT tests based on the laser analysis of the carbon isotope ratio have been performed jointly with specialists of the Vasilenko Clinic of Internal Disease Propedeutics (Sechenov Moscow Medical Academy). The results of the laser diagnostics were compared to the data of gastroscopic and morphological investigations, as well to ^{13}C-UBT tests carried out by the mass spectrometry technique combined with the gas chromotography. The data of our tests were used by clinic physicians for determining the infected patients, verifying the results of a therapy intended for eradicating Hp bacteria, and for estimating the efficiency of various treatment schemes.

Thus, this study has demonstrated the possibility of using TDLs for high-precision analysis of the isotope composition of CO_2 in exhaled air. The design and operating principles of the laser analyser of the carbon isotope ratio, the algorithms for processing the spectral data, and the sources of the main errors in this analysis are described. The results of clinical tests of this system, which were aimed at the diagnostics of stomach and duodenum diseases, are presented. The laser approach developed can be applied to the diagnostics of other digestive organs. This diagnostics is also based on the use of preparations labelled with ^{13}C. In addition, the offered principles can be basic in the analysis of stable isotopes of other elements, such as oxygen (^{18}O) or nitrogen (^{15}N).

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