Near-IR laser-based spectrophotometer for comparative analysis of isotope content of CO_2 in exhale air samples

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Abstract. **We present a laser spectrophotometer aimed at high-accuracy comparative analysis of content of $^{12}CO_2$ and $^{13}CO_2$ isotope modifications in the exhale air samples and based on a tunable near-IR diode laser (2.05 μm). The two-channel optical scheme of the spectrophotometer and the special digital system for its control are described. An algorithm of spectral data processing aimed at determining the difference in the isotope composition of gas mixtures is proposed. A few spectral regions (near 4880 cm^{-1}) are determined to be optimal for analysis of relative content of $^{12}CO_2$ and $^{13}CO_2$ in the exhale air. The use of the proposed spectrophotometer scheme and the developed algorithm makes the results of the analysis less susceptible to the influence of the interference in optical elements, to the absorption in the open atmosphere, to the slow drift of the laser pulse envelope, and to the offset of optical channels. The sensitivity of the comparative analysis of the isotope content of CO_2 in exhale air samples, achieved using the proposed scheme, is estimated to be nearly 0.1‰.**

Keywords: tunable diode lasers, molecular absorption spectra, CO_2 isotopic modifications, analysis of isotope composition of exhale air.

1. Introduction

The possibility of using the methods of diode laser spectroscopy (DLS) for high-sensitivity analysis of CO_2 isotope composition has been repeatedly discussed [1–6]. In particular, laboratory systems based on tunable diode lasers (TDLs) aimed at measuring the relative content of $^{12}CO_2$ and $^{13}CO_2$ were reported. The urgency of these products is due to their use in medical diagnostics based on the isotope testing of exhale air [7]. It is essential that these tests do not require measurement of the absolute concentrations of $^{12}CO_2$ and $^{13}CO_2$. It is sufficient to determine the change in the isotope content of the exhale air, caused by administering the ^{13}C-labelled preparations. It is always supposed that there are two or more samples of the gas mixture whose isotope composition may slightly differ. The analysis of publications [4–6, 8, 9] shows that this specific feature is not taken into account by the researchers even when double-beam or differential optical schemes are used, although this could provide the required analytic characteristics using more simple and reliable methods.

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The use of TDLs for the analysis of isotope composition of CO_2 in the exhale air is quite adequate to the complexity of the considered problem. The wide spectral range covered by lasers of this type allows the choice of the spectral region most suitable for the analysis. The tuning characteristics of TDLs allow simultaneous recording of both $^{12}CO_2$ and $^{13}CO_2$ absorption lines. The DLS methods can provide recording of relative changes in the optical density in the absorption lines at the level of 10^{-6}–10^{-7} [10, 11] and solving the problems of optical diagnostics with the use of weak absorption bands. Finally, the all-electronic control of the TDL radiation parameters allows application of novel digital methods for recording and processing of the spectral data and, thus, significant reduction of the noise level.

Below we describe the spectrophotometer aimed at high-sensitivity (with the accuracy of ~0.01%) quantitative analysis of differences in the isotope composition of CO_2 in exhale air samples and based on the use of a TDL operating in the near-IR range (2.05 μm). Original schemes and constructions of the laser analytic unit, electronics and software, as well as algorithms for spectral data processing, are used in the presented system. The system can serve as a prototype analyser for clinical applications, associated with isotopic diagnostics of digestive apparatus diseases.

2. Specific features of the analytic problem to be solved

The main goal in solving the present analytic problem is to determine, with maximal sensitivity, the difference in the CO_2 isotope composition between the so-called basal and control samples. The basal sample is taken from the tested subject before the ^{13}C-labelled preparation is administered and the control sample is taken some time after it. It is agreed that the acceptable reliability of the exhale air testing is provided by distinguishing the isotope composition of the exhale air samples within the accuracy of ‰ (1% = 0.1‰). Provided that the standard abundance of $^{13}CO_2$ in nature is ~1.1237% [12], the content of CO_2 in the exhale air is ~3% and the pressure is equal to the atmospheric one, such sensitivity of the isotope analysis is possible if the concentration sensitivity to CO_2 molecules amounts to 0.03 ppm.

For the isotope analysis using a TDL operating at room temperature, we have chosen the spectral region near 2.05 μm. In this region the R-branch of the 20013–00001 band of $^{12}CO_2$ overlaps with the P-branch of the 20012–0001 band of $^{13}CO_2$ (Fig. 1) [13], which makes it possible to choose a pair of closely spaced lines of $^{12}CO_2$ and $^{13}CO_2$ convenient for the analysis. For the gas mixture corresponding to the composition of the exhale air, the absorption coefficient in the peaks
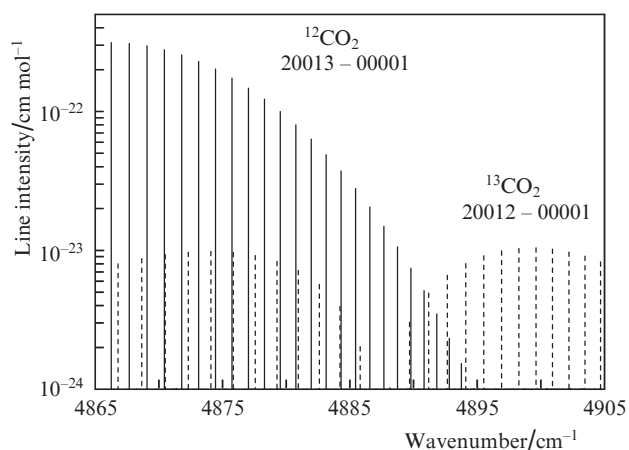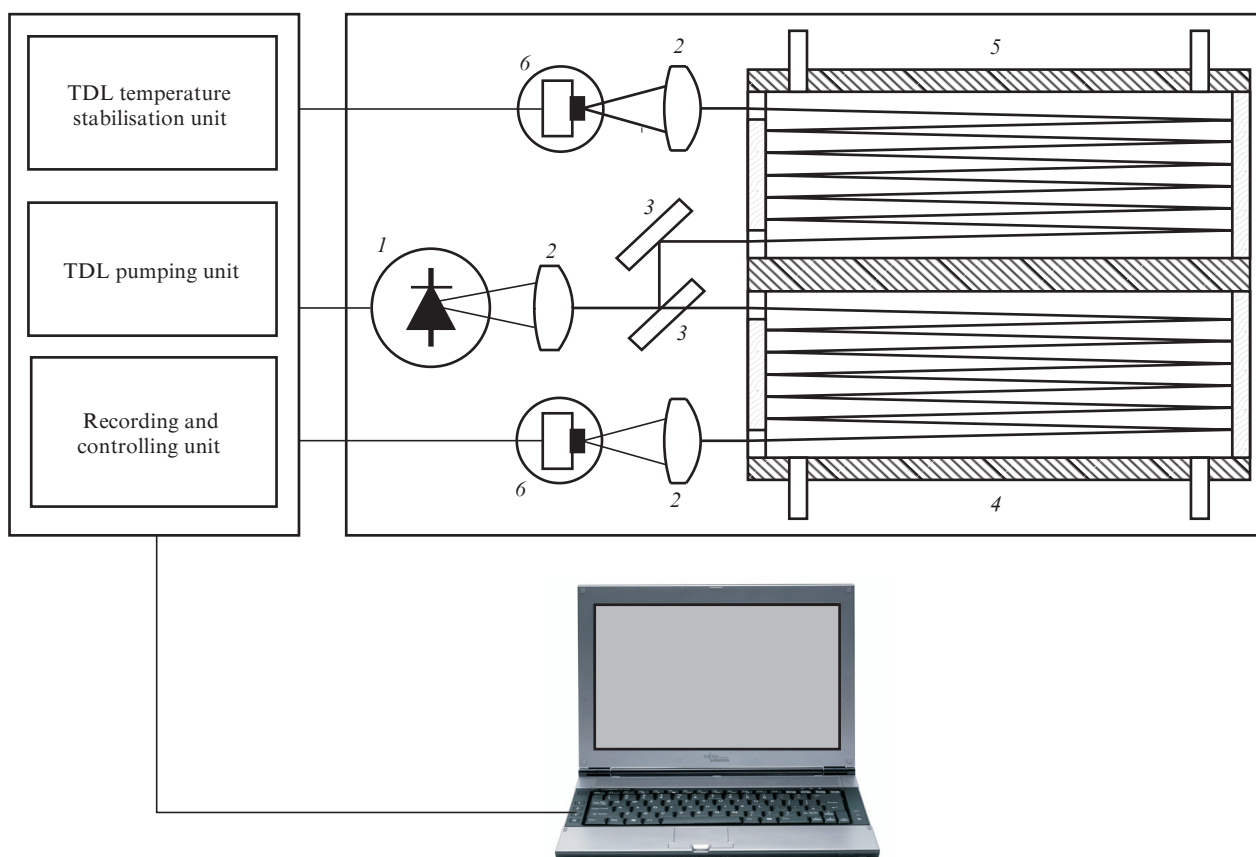
Figure 1. Relative position and intensity of $^{12}CO_2$ and $^{13}CO_2$ absorption lines in the spectral region near 2.05 μm, convenient for the isotope analysis of CO_2 [11].

3. The laser spectrophotometer

The mentioned specific features of the problem under solution were taken into account in the design of the spectrophotometer based on the distributed-feedback TDL operating at room temperature near the wavelength 2.05 μm (Laser Components, Germany). The precise tuning of the optical frequency of the laser radiation was performed by varying the laser temperature in the range from –10 to +50 °C by a thermoelectric cooler. The repetitively pulsed regime of the TDL pumping was used to provide the frequency scanning. The pulse repetition rate was 200 – 300 Hz, and the pulse duration was 3 – 4 ms. The laser was pumped using electric current pulses with a linearly increasing amplitude, which allowed linearisation of the frequency tuning rate during the pulse. In this regime the tuning rate of the laser radiation frequency was ~ 2 cm^{-1} ms^{-1}, and the frequency changed by 5 – 8 cm^{-1} during a single pulse. The laser radiation power amounted to ~ 0.5 mW. The TDL temperature was stabilised with the accuracy up to $\sim 10^{-3}$ grad. Together with the high reproducibility of the current pump parameters (the duration, the pulse period, and the amplitude), this provided the required reproducibility of the frequency tuning, the radiation amplitude and the transmission spectra recorded.

The specific features of the spectrophotometer scheme (Fig. 2) provide sufficient absorption in the analytic lines, simultaneous analysis of the reference (basal) and the analysed (control) gas mixtures, close laser radiation intensities and equality of temperatures in the two optical channels. The

of the most intense CO_2 lines of this band amounts to 3×10^{-5} cm^{-1}. With such an absorption coefficient and sensitivity to absorption at the level of 3×10^{-7}, achievable using TDLs [10, 11], the required concentration sensitivity can be implemented for the optical path length equal to ~ 100 cm. The additional technical requirement is that the volume of the gas sample should not exceed 100 mL, which is determined by the volume of the container for collecting the samples of exhale air.

Figure 2. Schematic diagram of the laser analyser of the CO_2 isotope composition in the exhale air:
(*1*) tunable diode laser; (*2*) aspherical microlenses; (*3*) mirrors; (*4*) reference cuvette; (*5*) analytic cuvette; (*6*) photodetectors.

optical beam, outgoing from the laser crystal, was collimated into a parallel one by an aspherical lens with the focal length ~8 mm. The beam width was limited using an aperture with the diameter ~5 mm. Using a beamsplitter, the beam was split into two beams with nearly equal intensities, each passed through one of practically identical multipass cuvettes 200 mm long. The multipass regime was implemented using two plane mirrors. The number of the ray passes in each cuvette was 14, so that the total optical path length in each cuvette was ~280 cm. After leaving the cuvettes, laser radiation was focused onto the InGaAs photodetectors (Hamamatsu) cooled to –50 °C. The cuvettes with inner volume ~50 cm^3 each were made of duralumin, and their construction, providing good thermal contact with the base of the optical unit, ensured practically equal temperatures of the gas samples in both cuvettes. Each cuvette was equipped with branch pipes to provide the gas circulation.

Operation of the spectrophotometer is based on using the original software–hardware complex that consists of three digital units providing the TDL temperature stabilisation, the TDL pumping, and the control of the transmission spectra recording. The scheme of the latter unit is based on the use of programmable logical matrices (Altera) and fast high-order ADCs (Texas Instruments). The sampling rate of the laser signal is 5 MHz, and the discreteness is 16 bit. This unit provides simultaneous and independent recording of laser spectra in two optical channels. The high sampling rate of the signal and the data exchange between the recording system and the controlling computer, corresponding to the protocol USB 2.0, allows recording all laser pulses in both channels simultaneously. Using the software of the controlling PC, operating under the WindowsXP/Vista OS, the following features are implemented and displayed: the system control terminal, the process of the complex control, the introduction of the system parameters, the choice of algorithms for spectral data processing, the real-time visualisation of the recorded spectra and the visualisation of the results of the spectral data processing.

4. Algorithm for recording and processing of spectral data

To provide the required accuracy of the isotope composition analysis of the exhale air samples, a special algorithm that allows detection of very small variations in the absorption spectrum of the analysed gas mixture with respect to the spectrum of the reference mixture was proposed and implemented. Due to this algorithm, the obtained results become insensitive to the influence of the general CO_2 concentration changes, to the variations in the environment temperature and to the parameters of laser radiation, which may cause random or systematic errors.

The proposed algorithm is organised as follows.

(i) Using the TDL the chosen spectral region is scanned within the width of 2–10 cm^{-1}, containing the absorption lines of both $^{12}CO_2$ and $^{13}CO_2$.

(ii) Two identical optical channels with multipass cuvettes providing nearly the same total optical path length are used. The first channel serves for recording the transmission spectra of the reference gas mixture, while the second channel is aimed at testing the analysed mixture. The design of the cuvettes provides virtually similar temperatures of the gas mixtures in both channels.

(iii) Both cuvettes are first blown with gaseous nitrogen. Then simultaneously the transmission spectra of 'empty' reference and analytic cuvette are recorded.

(iv) The reference gas mixture (basal exhale air sample) is let into the reference cuvette and the analysed gas mixture (control exhale air sample) is let into the analytic cuvette. The transmission spectra of both are simultaneously recorded.

(v) The recorded spectra are processed following the procedure described below.

Note, that the spectra, recorded in the reference and the analytic channel may differ in the total CO_2 concentration in the gas mixtures, relative content of $^{12}CO_2$ and $^{13}CO_2$, intensities of laser signal, laser pulse envelope spectra, reproducible noises, e. g., interference-like ones, random disturbances and noise.

5. Processing of the laser spectra

The transmission spectra, recorded in the reference and analytic channels with the cuvettes filled with appropriate gas samples, can be presented as follows:

$$I_r^m(v(t)) = I_{r0}(v(t))\exp[-\alpha_r^{12}(v(t),T_r)C_r^{12}l_r - \alpha_r^{13}(v(t),T_r)C_r^{13}l_r],$$

$$I_a^m(v(t)) = I_{a0}(v(t))\exp[-\alpha_a^{12}(v(t),T_a)C_a^{12}l_a - \alpha_a^{13}(v(t),T_a)C_a^{13}l_a]. \quad (1)$$

Here $I_{r0}(v(t))$ and $I_{a0}(v(t))$ are the laser signal envelopes in the reference and analytic channels, respectively, recorded after blowing of the cuvettes with gaseous nitrogen; $\alpha_r^{12}, \alpha_r^{13}, \alpha_a^{12}, \alpha_a^{13}$ are the absorption coefficient spectra of $^{12}CO_2$ and $^{13}CO_2$ in the reference and the analytic cuvette; T_r and T_a are the gas temperatures in the cuvettes; $C_r^{12}, C_r^{13}, C_a^{12}$, and C_a^{13} are the concentrations of $^{12}CO_2$ and $^{13}CO_2$ in the cuvettes; l_r and l_a are the optical path lengths in the cuvettes.

After recording the transmission spectra listed above, they are normalised to the appropriate envelope functions I^e of the laser signals in both channels, and the logarithm of the final result is calculated:

$$A_r(v, T_r) = -\ln\left(\frac{I_r^m(v)}{I_r^e(v)}\right) = \alpha_r^{12}(v, T_r)C_r^{12}l_r + \alpha_r^{13}(v, T_r)C_r^{13}l_r,$$

$$(2)$$

$$A_a(v, T_a) = -\ln\left(\frac{I_a^m(v)}{I_a^e(v)}\right) = \alpha_a^{12}(v, T_a)C_a^{12}l_a + \alpha_a^{13}(v, T_a)C_a^{13}l_a.$$

Each of the spectra A_a and A_r is a sum of $^{12}CO_2$ and $^{13}CO_2$ optical density spectra. Using normalisation, we eliminate the influence of the laser intensity variations due to misbalance of channels and variation of the laser pulse envelope function, as well as the reproducible interference noises. Then the ratio of the spectra is calculated:

$$\frac{A_a(v, T_a)}{A_r(v, T_r)} = \frac{\alpha_a^{12}(v, T_a)C_a^{12}l_a + \alpha_a^{13}(v, T_a)C_a^{13}l_a}{\alpha_r^{12}(v, T_r)C_r^{12}l_r + \alpha_r^{13}(v, T_r)C_r^{13}l_r}. \quad (3)$$

The concentrations of $^{12}CO_2$ and $^{13}CO_2$ are related with their partial fractions and the total CO_2 concentration via the expressions:

$$C_r^{12} = C_r\,^{12}P_r, \quad C_r^{13} = C_r\,^{13}P_r, \quad C_a^{12} = C_a\,^{12}P_a, \quad C_a^{13} = C_a\,^{13}P_a,$$

$$^{13}P_r + {}^{12}P_r = 1, \quad ^{13}P_a + {}^{12}P_a = 1.$$

Here, C_r and C_a are the total CO_2 concentrations in the reference and the analysed gas mixture; $^{12}P_r$, $^{13}P_r$, $^{12}P_a$, $^{13}P_a$ are the fractions of $^{12}CO_2$ and $^{13}CO_2$. Then, keeping in mind that $\Delta P = {}^{13}P_a - {}^{13}P_r = {}^{12}P_r - {}^{12}P_a$ and assuming the optical path lengths in the cuvettes to be equal, we obtain

$$\frac{A_a(v, T_a)}{A_r(v, T_r)} = \frac{C_a}{C_r}\left\{1 + \frac{\Delta^{13}P[\alpha_a^{13}(v, T_a) - \alpha_a^{12}(v, T_a)]}{\alpha_r^{12}(v, T_r) + {}^{13}P_r[\alpha_r^{13}(v, T_r) - \alpha_r^{12}(v, T_r)]}\right\}. \quad (4)$$

In the spectral regions, where the $^{12}CO_2$ absorption coefficient is close to zero and the absorption of $^{13}CO_2$ dominates, provided that the temperatures of the gas mixtures in the analytic and reference cuvettes are equal, we obtain

$$\left.\frac{A_a}{A_r}\right|_{\alpha^{12}\to 0} \approx \frac{C_a}{C_r}\left(1 + \frac{\Delta^{13}P}{{}^{13}P_r}\right) = \frac{C_a}{C_r}\frac{{}^{13}P_a}{{}^{13}P_r}, \quad (5)$$

and in the regions where the absorption of $^{12}CO_2$ dominates, and the absorption coefficient of $^{13}CO_2$ is close to zero

$$\left.\frac{A_a}{A_r}\right|_{\alpha^{13}\to 0} \approx \frac{C_a}{C_r}\left(\frac{1 - {}^{13}P_a}{1 - {}^{13}P_r}\right) = \frac{C_a}{C_r}\frac{{}^{12}P_a}{{}^{12}P_r}. \quad (6)$$

Using relations (5) and (6), we obtain the quantity $\Delta\delta^{13}C$, traditionally measured in the isotope analysis and characterising the difference in isotope compositions between the reference and the analytic gas mixture:

$$\Delta\delta^{13}C = \left(\frac{R_a}{R_r} - 1\right) = \left(\frac{C_a^{13}/C_a^{12}}{C_r^{13}/C_r^{12}} - 1\right) = \left(\frac{C_a^{13}/C_r^{13}}{C_a^{12}/C_r^{12}} - 1\right)$$

$$\approx \left[\left(\frac{A_a}{A_r}\right)_{\alpha^{12}\to 0}\left(\frac{A_a}{A_r}\right)_{\alpha^{13}\to 0}^{-1} - 1\right]1000‰.$$

Since $^{13}P_r \approx 0.011$ and its significant relative variations do not exceed 10%, the quantity $(1 + \Delta^{13}P)$ is close to unity. In this case Eqn (6) yields the ratio of total CO_2 concentrations in the reference and analytic cuvettes with the accuracy $\sim 0.1\%$, and in the case when these concentrations are equal

$$\Delta\delta^{13}C \approx \left[\left(\frac{A_a}{A_r}\right)_{\alpha^{12}\to 0}\left(\frac{A_a}{A_r}\right)_{\alpha^{13}\to 0}^{-1} - 1\right]1000$$

$$\approx \left[\left(\frac{A_a}{A_r}\right)_{\alpha^{12}\to 0} - 1\right]1000 = \frac{\Delta^{13}P}{{}^{13}P_r}1000‰,$$

i.e., the measured quantity in this case corresponds to the change of the partial fraction of $^{13}CO_2$ in the analysed gas mixture with respect to the reference one.

Figure 3 shows the spectrum of the quantity A_a/A_r, calculated in the interval $4870-4905$ cm^{-1} using the data from the bank of molecular high-resolution spectral data HITRAN96 [14] and the spectra of absorption coefficient of $^{12}CO_2$ and $^{13}CO_2$. In the calculation two model transmission spectra of the exhale air were used, one for the standard concentration ratio of CO_2 isotope modifications ($^{12}CO_2$:$^{13}CO_2$ = 98.8763:1.1237) and the other for the fraction of $^{13}CO_2$ increased by 0.09% against the standard content. The total CO_2 concentration was assumed to be 3%, the temperature was 296 K and the optical path length was 200 cm.

One can see that in the presented fragment of the spectrum the ratio A_a/A_r demonstrates a sufficiently contrast reso-
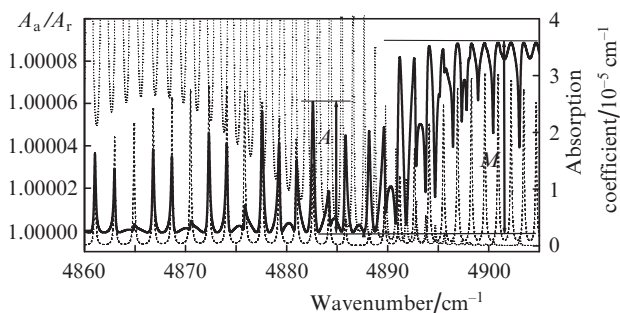
Figure 3. Model spectrum of relative absorption A_a/A_r (solid curve) for two gas mixtures, in which the content of $^{12}CO_2$ and $^{13}CO_2$ isotope modifications differs by 0.09%, and the absorption spectra (dashed curve) in the spectral range $4880-4900$ cm^{-1}. The total concentrations of CO_2 in the reference and the analysed gas mixtures are equal.

nance structure with the maximal modulation depth $M \sim 0.00009$, corresponding to the difference of isotope compositions of the reference and analysed gas mixtures. In correspondence with Eqn (6) the ratio A_a/A_r takes the values, close to the ratio of concentrations of $^{12}CO_2$ in the reference and analysed gas mixture (~ 1) at frequencies, corresponding to maxima of absorption by $^{12}CO_2$, provided that the absorption by $^{13}CO_2$ is small. On the contrary, at frequencies, corresponding to maximal absorption by $^{13}CO_2$ the ratio of spectra A_a/A_r is close to the ratio of concentrations of $^{13}CO_2$ in the reference and analysed gas mixture (~ 1.00009), provided that the absorption by $^{12}CO_2$ is small.

If the resonance absorption peaks of one isomer are located against the background of significant nonresonance absorption of the other one, like, e. g., near 4878 or 4883 cm^{-1}, the resonance singularities are observed with the amplitude A smaller than the maximal modulation depth M. From Eqns (4) and (5) it is easy to conclude that in the case of equal temperatures in the cuvettes the ratio of these quantities is expressed as

$$\frac{A}{M} = \frac{\Delta P^{13}[\alpha^{13}(v) - \alpha^{12}(v)]}{\alpha^{12}(v) + {}^{13}P_r[\alpha^{13}(v) - \alpha^{12}(v)]}\left(\frac{\Delta P^{13}}{{}^{13}P_r}\right)^{-1}$$

$$= \frac{{}^{13}P_r}{[\alpha^{13}(v)/\alpha^{12}(v) - 1]^{-1} + {}^{13}P_r}. \quad (7)$$

It follows from Eqn (7) that the amplitude of the resonance singularity in the spectrum of A_a/A_r in the case of finite $\alpha^{13}(v)$ and $\alpha^{12}(v)$ is proportional to the maximal amplitude $M = \Delta^{13}P/{}^{13}P_r$ of the spectrum modulation, the coefficient of proportionality being dependent on the ratio of $^{12}CO_2$ and $^{13}CO_2$ absorption coefficients at the given frequency and the quantity $^{13}P_r$. It may be found by calibration measurements.

6. Effect of noise

Taking the random noise component of the transmission spectrum (1) and the envelope functions into account results in the appearance of additional terms in Eqn (3).

$$\frac{A_a(v)}{A_r(v)} = \frac{\kappa_a(v) + i_{na}/I_{a0}(v)}{\kappa_r(v) + i_{nr}/I_{r0}(v)} \approx \frac{\kappa_a(v)}{\kappa_r(v)}\times$$

$$\times \left(1 + \frac{i_n}{I_0(\nu)\kappa(\nu)} + \frac{i_{nr}^2}{(I_{r0}(\nu)\kappa_r(\nu))^2}\right). \tag{8}$$

Here κ_a and κ_r are the optical densities A_a and A_r, defined by relation (3), in the absence of random noises, and the noise is represented by a random variation in the amplitude of the recorded signal with the maximal peak-to-peak value i_n.

At sufficiently large values of the optical density κ_r, the major contribution to the noise component will come from the second term. According to (8), the sensitivity level D of determining the difference in the isotope composition of gas mixtures in the reference and analytic cuvettes will be related to the amplitude of spectra recording noise amplitude as

$$D = \frac{\Delta^{13}P}{^{13}P_r} = \frac{i_n}{I_0(\nu_j)^{13}\kappa_{max}(\nu_j)},$$

where κ_{max} is the optical density of the reference gas mixture in the absorption maximum of the most intense absorption line of CO_2 centred at the frequency ν_j. Then the required signal-to-noise ratio, characterising the process of spectrum recording, is expressed as

$$\frac{I_0(\nu_j)}{i_n} = \frac{1}{^{13}\kappa_{max}(\nu_j)D}. \tag{9}$$

It follows from Eqn (9) that to achieve the isotope analysis sensitivity $D = 0.1\permil = 10^{-4}$ using the $^{13}CO_2$ absorption lines from the spectral region shown in Fig. 3, provided that the optical density in the absorption maximum is $\sim 5 \times 10^{-3}$ and the optical path length is 200 cm, the signal-to-noise ratio of spectrum recording should be $\sim 1.7 \times 10^6$.

One can reduce this noise component by accumulating spectra in the course of recording, or by filtering, e. g., using the Fourier transform. However, at small optical densities the third (quadratic) term in Eqn (8) becomes dominant. Since it takes only positive values, the filtering of spectra will not reduce the noise amplitude.

Based on the analysis of the noise effect, one should prefer the $^{13}CO_2$ lines within the spectral range $4870-4885$ cm^{-1} when choosing the optimal spectral region for isotope diagnostics. In this range the amplitudes of resonance singularities of the A_a/A_r spectrum are somewhat smaller than the maximal modulation depth M, and the noise component is more than three orders of magnitude smaller than in the R-branch region, which is caused by higher non-resonance absorption between the CO_2 lines.

7. Estimation of sensitivity to temperature

As mentioned above, in the designed analytic system two thermally coupled cuvettes are used, which provides equal temperatures of the reference and analysed gas samples with good accuracy (~ 0.01 deg) and, therefore, the results are independent of random variations in the gas medium temperature, at which the analysis is performed. Nevertheless, in the computer simulation of the algorithm we studied the possible dependence of the obtained results on the temperature difference between the reference and the analysed gas samples. In this case, additionally to the spectra mentioned above three absorption spectra were calculated at the temperatures 276, 286, and 306 K for the $^{12}CO_2$ and $^{13}CO_2$ mixture, differing from the standard $^{13}CO_2$ content by 0.09‰. The spectra of κ_a/κ_r for these temperatures are shown in Fig. 4. One can see that

the maximal modulation depth of the obtained resonance pattern is rather stable with respect to the difference between the temperatures of the reference of the analysed gas mixtures – the observed deviations are only a few per cent of the maximal peak-to-peak value. Meanwhile, the amplitudes of the resonance peaks, corresponding to the $^{12}CO_2$ lines that fall within the region of significant nonresonance absorption in the R-branch of $^{13}CO_2$ (the spectral range $4890-4900$ cm^{-1}) change by several times at the temperature difference of $20-30$ deg. Using such resonance singularities for isotope analysis, one should ensure the closeness of temperatures in the reference and analysed gas mixtures or, alternatively, measure the temperature difference and introduce appropriate corrections in the course of data processing. One can see that in the spectral range, optimal for isotope analysis ($4870-4885$ cm^{-1}), the effect of temperature is very small, and the temperature connection of cuvettes deliberately provides the results which are independent of the temperature difference between the reference and analysed gas samples.
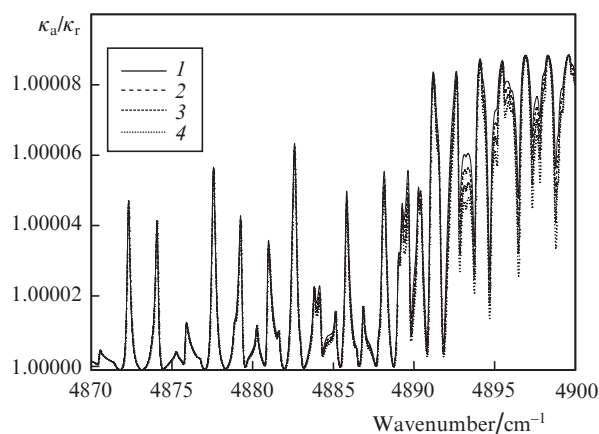
Figure 4. Spectra of relative absorption κ_a/κ_r at a fixed temperature of the reference gas mixture (296 K) and the temperature of the analysed gas mixture equal to 276 (*1*), 286 (*2*) 296 (*3*) and 306 K (*4*). The difference of content of $^{12}CO_2$ and $^{13}CO_2$ isotope modifications in the reference and the analysed gas mixtures is 0.09‰.

8. Experimental results

Figure 5 presents the experimental transmission spectra, measured using the spectrophotometer described above, and the result of their processing using the developed algorithm. Figure 5a shows fragments of the transmission spectrum of the 'empty' reference cuvette and the basal sample of exhale air, taken from the subject before administering the ^{13}C-urea. One can see that during the ~ 4-ms pulse the spectrum nearly 8 cm^{-1} wide was recorded ($4875-4883$ cm^{-1}). This region contains several lines of $^{12}CO_2$ and two lines of $^{13}CO_2$ convenient for performing the isotope analysis. The absorption in the $^{13}CO_2$ lines is $\sim 0.5\%$. The presented spectra were recorded with accumulation over 10 realisations. In the analytic channel similar transmission spectra were recorded for the control (analysed) exhale air sample, taken from the patient 20 minutes later than the isotope-labelled urea was administered. These spectra differ insignificantly from those obtained in the reference channel and, therefore, are not shown in the figure.

In the isotope analysis the number of accumulations in the process of transmission spectrum recording was increased up
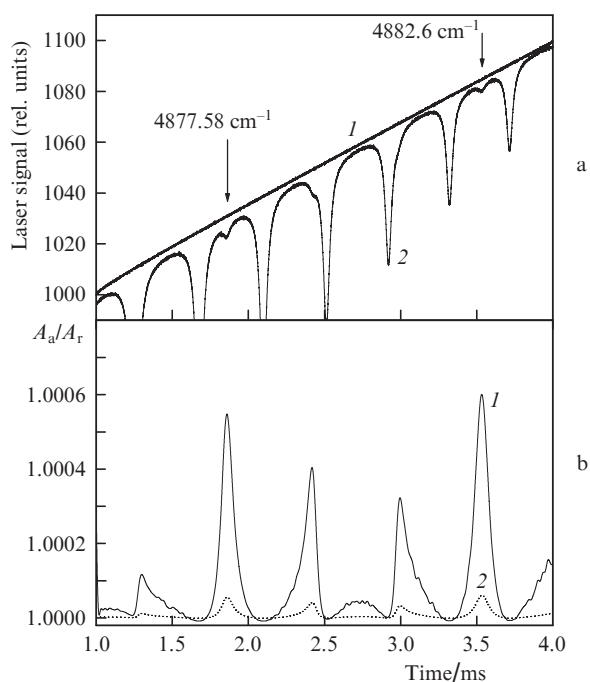
Figure 5. Experimental laser spectra of the 'empty' analytic cuvette (*1*) and analytic cuvette filled with the exhale air sample (*2*) in the spectral range 4873–4885 cm^{-1} (a) as well as experimental spectrum A_a/A_r for two samples of exhale air with measured difference in the content of $^{12}CO_2$ and $^{13}CO_2$ equal to 0.9‰ (*1*) and calculated spectrum of the ratio A_a/A_r for $\Delta\delta^{13}C$ equal to 0.1‰ (*2*).

to 1000 (the time of spectrum measurement ~10 s), and the signal-to-noise ratio, which is determined mainly by the level of shot noise of the pre-amplifier and the photodetector, amounted to ~10^4. The contribution of the used 16-bit ADC digit capacity was negligibly small (~1.3×10^{-8}). Additional digital filtering of these random noises based on the Fourier transform allows increasing the signal-to-noise ratio of recording the resonance absorption against the background of random noises up to the required ~2×10^6. In this case an important role is played by a decrease in the level of reproducible disturbances (interference modulation of the spectrum, absorption in the open atmosphere), which was provided by normalising the transmission spectra in each channel to the spectrum of an 'empty' cuvette. Note, that the scheme of the optical unit was designed to provide the maximal reduction of the effect of interference noises. For this aim, the antireflection coatings and the special mutual alignment of optical elements were used. As a result, after the spectra recording the level of interference noises was less than 10^{-5} of the incident power, and after the normalisation of spectra it became less than ~10^{-6}. The latter became possible due to good reproducibility of the interference noise pattern and its actual insensibility to manipulations, related to refilling the reference and analytic cuvettes with gas mixtures. The pressure and temperature of all used gas mixtures were the same and corresponded to the room conditions of the lab where the measurements were performed. Therefore, the change in gas media in the cuvettes did not cause significant changes in the optical parameters of the system.

Figure 5b shows the spectra processed in correspondence with the described algorithm. After filtering the random noises off the spectrum of A_a/A_r using the Fourier transform (the filter width 100 points, the whole spectrum about 2×10^4

points), the spectrum was normalised following Eqn (6) to its value in the region of minimal absorption by $^{13}CO_2$. The amplitudes of resonance singularities, corresponding to the $^{13}CO_2$ lines near 4877.58 and 4882.6 cm^{-1}, agree with the difference of isotope composition ~0.9‰ between the basal and control samples of exhale air. The isotope ratio in the same samples analysed using the nonselective IR analyser UBiT 200 (Photal, Japan, potential accuracy 0.7‰) appeared to be ~1.2‰, which demonstrates satisfactory agreement of results obtained by devices, based on different physical principles. The reproducibility of the obtained value in five measurements is characterised by the standard deviation 0.037‰.

To estimate the sensitivity of the developed approach, we also show in Fig. 5 the spectrum of A_a/A_r calculated for the difference 0.1‰ of isotopic compositions. The comparison of the experimental and the calculated spectra allows estimation of attainable sensitivity with the value less than 0.1‰. This level is determined by the temperature instability of the spectrophotometer optical system geometry leading to slow and badly reproducible variations in the laser spectrum envelope function in both optical channels. We plan to confirm the attainability of this value experimentally and to determine the accuracy of the analysis in the future, since this process is complex both in methodology and in technical implementation and requires the use of expensive standardised isotope mixtures $^{12}CO_2$ and $^{13}CO_2$, or high-accuracy mass-spectrometry instrumentation.

9. Conclusions

Thus, we have developed the laser spectrophotometer aimed at high-accuracy comparative analysis of $^{12}CO_2$ and $^{13}CO_2$ isotope content in the exhale air samples and based on the use of tunable diode lasers in the near-IR region (2.05 μm). The spectrophotometer uses the two-channel optical scheme, small-volume multipass cuvettes and special all-digital control system.

The analysis has been implemented using a simple algorithm for spectral data processing, aimed at determining the difference in the isotope composition of gas mixtures. Several spectral regions (near 4878 and 4882 cm^{-1}) are proposed as optimal for isotope analysis of CO_2 in the exhale air.

The use of the proposed spectrophotometer scheme allows minimisation of the effects of interference in optical elements, absorption in open atmosphere, slow drift of the envelope function of the laser pulse, and offset of spectral channels. This is achieved at the expense of providing high reproducibility of the spectral characteristics of laser radiation, as well as simultaneous and independent high-speed recording of spectra in the reference and analytic channels. The use of two thermally coupled optical channels allows neglecting the temperature difference between the analytic and reference gas mixtures.

The influence of random noise on the result of the isotope analysis using the proposed algorithm is estimated. It is shown that if the sensitivity with respect to resonance absorption is ~10^{-7} and the optical density in the absorption peaks of the analytic lines is ~10^{-2} the sensitivity of the isotope analysis can be ~0.1‰.

The proposed approach may be applied for comparative analysis of isotope composition of any other gaseous molecules, possessing similar spectral properties.

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