Laser measurements of the carbon isotope ratio variations in human breath at atmospheric pressure

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Abstract.

A special technique is developed to measure the $^{13}CO_2/^{12}CO_2$ isotope ratio in human breath at atmospheric pressure with a 2-μm tunable diode laser. The procedure used to process the pressure-broadened spectra obtained and calculate the isotope ratio is based on the multidimensional linear regression of the spectra using stimulated $^{13}CO_2$, $^{12}CO_2$, and H_2O spectra. This technique allows measuring the ⇦-value for isotopic ratios with a precision of 0.007 % in a record time of 3 min at a long-term reproducibility of 0.02 %

Лазерные измерения вариаций углеродного изотопного отношения в выдохе человека при атмосферном давлении

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Реферат

Разработан метод измерений изотопического отношения $^{13}CO_2/^{12}CO_2$ в выдыхаемом воздухе при атмосферном давлении с поможью диодного лазера с перестройкой вблизи 2 мкм. Развита процедура обработки спектра и нахождения изотопного отношения с помощью многомерной линейной регрессии и моделирования спектров $^{13}CO_2$, $^{12}CO_2$, и H_2O, уширенных давлением. Метод позволяет определять ⇦-вариации изотопного отношения с точностью 0.007% за время регистрации 3 мин при долговременной воспроизводимости 0.02%

1 Introduction

The analysis of the ^{13,12}C concentrations is being widely used in geology, volcanology, criminology, medicine, and many other fields. The constant extension of the range of its applications stimulates the development of novel experimental techniques capable of high measurement precision and accuracy along with short test times. As far as the measurement precision and accuracy are concerned, the best results can be obtained with isotope ratio mass analyzers used in conjunction with chromatographic gas separation. This means can provide as high a precision as 0.01‰ [1], but it is very costly and can only be used at specialized laboratories, and so alternative methods are of great interest.

On the other hand, there are many practical applications that have no need for such precision. For example, the breath test precision actually required to detect the *Helicobacter Pylori* bacteria, the cause of gastric ulcers, is but 0.5 ‰; and the test procedure should take no more than a few minutes. Various optical and spectral methods meeting these requirements have been discussed in the literature in recent years (see, e.g. [2]).

The most common spectroscopic techniques rely on the absorption of infrared laser radiation in vibrational-rotational transitions in CO_2. The pertinent measurements can be taken with frequency tunable lasers using the methods of cavity ring-down spectroscopy (CRDS) [3], integrated cavity output spectroscopy (ICOS) and cavity enhanced absorption spectroscopy (ICOS) [4], wavelength modulation absorption spectroscopy (WMS) [5], and absorption with a difference frequency generation source [6].

CO_2 absorption measurements are usually taken in one of the following three spectral regions: near 4.3, 2.0, and 1.6 microns. Comparison between the CO_2 absorption features in the mid- and near-IR regions shows a significant reduction in the absorption coefficient. Specifically, the strongest lines at 1.6 μm are approximately

100 times weaker than those at around 2 μm. Similarly a 500-fold reduction is observed when going over from 4.3 to 2 μm.

The fundamental 4.3-μm band is preferred because of the high coefficient of absorption obtaining here. By using a quantum-cascade laser (QCL) in highly stabilized experimental conditions (sample cell temperature and pressure), Nelson and co-authors [7] managed to attain a CO_2 isotope ratio measurement precision of 0.02 ‰, with the test time as long as 400 seconds, which is the highest precision figure achieved by spectroscopic techniques by now. Unfortunately this approach requires that the photodetectors used should be cryogenically cooled; and the cost of QCLs is high.

Lasers and detectors operative in the 1.6-μm region have good characteristics. They can work at room temperature and are readily available. But the low absorption coefficient of the CO_2 molecule in this spectral region makes it necessary to use a sample cell around 1 kilometer in optical path length. Kasyutich and co-authors [8] used a high-precision cavity for the purpose and managed to achieve a measurement precision of 1.8 ‰.

The 2- μm region seems to be the most reasonable alternative. The diode lasers and detectors operative in this region can work at room temperature, and the absorption coefficient of the CO_2 molecule here is high enough, so that the CO_2 isotope ratio can be measured over an optical path a few meters long. Such an optical path can be provided by relatively simple and stable multipass systems using reflecting cavity optics that are not required to have very high performance characteristics. Another fortunate factor is that the absorption lines of water in this region are weak. Several papers have been published to date on the carbon isotope ratio measurements in the 2-μm region. Castrillo and co-workers [9] reported a long-term reproducibility of 0.5 ‰ in $^{13}CO_2/^{12}CO_2$ isotope ratio measurements with a DFB diode laser, the test time being 80 minutes long.

The measurement schemes used in all the above-mentioned works are based on comparison between the absorption data obtained for individual molecular vibrational-rotational lines at gas pressures reduced to 10--100 Torr to prevent spectral overlapping.

Our preceding work [11] stands out in this respect, for here we handled spectra containing overlapped pressure-broadened lines. We demonstrated that this approach could also provide for sufficiently high precision in spectral isotopic measurements comparable with the low pressure measurements. In principle it is not too much surprising because in both cases the precision and accuracy are limited by the comparison of the measured absorption spectral components intensities with the simulated ones thus the same way depending on the reliability of spectral database and the possibilities of measuring technique.

The aim of present work is to demonstrate the technique of measuring the $^{13}CO_2/^{12}CO_2$ isotope ratio at atmospheric pressure to meet the needs of medical practice. Note also that the parameters most important for the above objective are the relative measurement precision and reproducibility rather than the accuracy of the absolute isotope ratio value, this being essentially individual even for a group of healthy people. Bacterial diagnostics requires making reliable comparison between carbon isotope concentrations in a succession of samples of air exhaled by a selected person before and after (ten minute to hour) taking the special isotope enriched substance. That is why we did not try to highly improve the accuracy of the absolute isotope ratio values, these being determined in some cases with the help of most simple secondary standards.

According to these objectives in sec.2 we describe the experimental technique and its main parameters control. Sec.3 is devoted to the spectral interval choice, the necessary accuracy of frequency scale calibration, spectra processing technique and simulations of spectral line profile. The results are presented in sec.4. The analysis of the precision with the help of Allan variance performed and the long term stability studied. The advantages and shortages of atmospheric pressure measurements compare to low pressure ones are discussed, the examples of measurements presented.

2 Experiment

The experimental setup is shown schematically in Fig. 1. The spectrometer uses a three-channel optical scheme. The probe laser beam passes through the sample channel PhD1 including a Herriot multipass cell containing the sample gas. The cell is 900 mm in length and 22 mm in inside diameter. The total optical path length in the cell is around 24 m. Inside of the sample cell there are a temperature and a pressure sensor, their accuracies being 0.1 °C and 0.2 mbar, respectively. The beams for the remaining two channels (PhD2 and PhD3) are formed by a beam-splitting wedge. The baseline channel PhD2 is needed to normalize the beam intensity in the sample channel. The channel PhD3 includes a reference gas cell and is used to calibrate the laser radiation frequency. The cell is filled with the CO_2 gas at a pressure of 70 Torr. As shown elsewhere in the text, the laser frequency accuracy must be no worse than 10^{-5} cm^{-1}. The necessary accuracy was achieved by comparing the absorption spectra obtained in the reference gas cell with those tabulated for the line centers and their pressure shifts in the work by Rothman

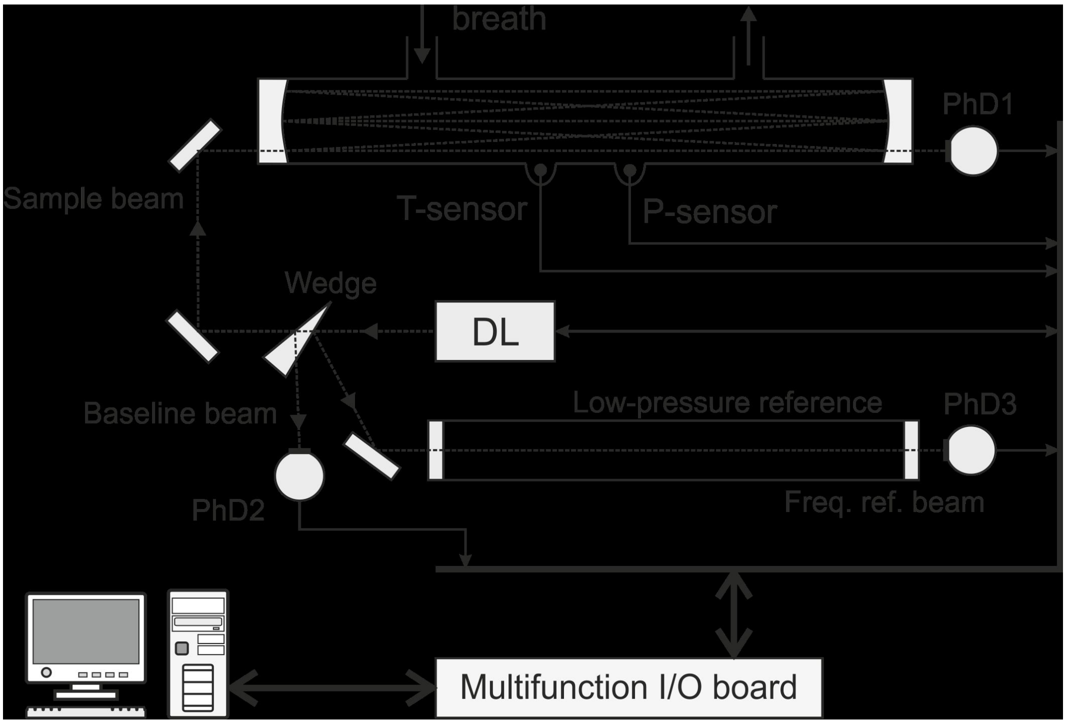

Figure 1. Sketch of the experimental setup

and co-workers [12]. The frequencies along the entire laser tuning curve were found by interpolation between the reference line center positions. The interpolation procedure is discussed in Sect. 3.

The light source used is a VCSEL laser diode (*Vertilas*) emitting some 0.5 mW of power near 2.007 μm. The laser is tuned by applying to it a periodic sawtooth injection current up to 7 mA in amplitude and 200 Hz in repetition rate.

The stabilization of laser radiation frequency is an important parameter and needs in stabilization of the temperature of laser cooler because the typical sensitivity is about 0.1cm^{-1}/K for diode lasers. It is known that the effective stability can be achieved up to the level $3\ 10^{-6}$K[13]. As in [13] we additionally use the feedback to tight the frequency grid to the center of absorption line in the reference cell so the long-term (1-h) temperature stability of the Peltier element is ensured at 10^{-4} K.

The use of the VCSEL laser offers a number of advantages over, say, its DFB counterpart. One is its close-to-linear current-frequency tuning curve that is typically wider than that of the DFB diode laser. This makes for improved accuracy of the above-mentioned interpolation procedure. Another is the high quality of the VCSEL laser beam, which helps obtain closely similar behavior of the laser basic lines in the split beams.

The photodetectors (PhD1 through 3) used in the setup are InGaAs photodiodes (Type G8372-01, *Hamamatsu*) placed in series with home-made trans-impedance amplifiers. The signals from the detectors are fed to a multifunction I/O board (Type NI-PCI-6120, 800 kHz, 16 bits, *National Instruments*) and computer processed using the LabView 9.0 software.

The absolute isotope ratio values were measured using commercially available CO_2 gas as a secondary standard. Its isotope ratio was determined with a Model MS200 quadrupole mass analyzer.

3 Measurement procedure details

3.1. Analytical spectral region

Subject to our analysis was a spectral fragment optimally chosen with due regard for the dynamic range of the absorption line intensities. The wavenumber of the radiation emitted by the diode laser at a fixed temperature of 24 ˉC was tuned in the range 4977.4—4978.9 cm^{-1}. Observed in this spectral region are three relatively strong absorption lines of the $^{12}CO_2$ vibrational bands $\leftrightarrow_! + 2\leftrightarrow_2 + \leftrightarrow_3$ and $\leftrightarrow_1 + 3\leftrightarrow_2 + \leftrightarrow_3 - \leftrightarrow_2$ (R0 $12^01 \leftarrow 00^00$, $\nu = 4978.607720\ cm^{-1}$, R17 $13^11 \leftarrow 01^10$, $\nu = 4978.204746\ cm^{-1}$, and R16 $13^11 \leftarrow 01^10$, $\nu = 4977.724534\ cm^{-1}$) and one line of the $^{13}CO_2$ vibrational band $\leftrightarrow_1 + 2\leftrightarrow_2 + \leftrightarrow_3$ (P16 $12^01 \leftarrow 00^00$, $\nu = 4978.022037\ cm^{-1}$). H_2O absorption lines are also observed in this spectral fragment at 4978.384300 cm^{-1} (000-110 line) and 4978.499960 cm^{-1} (000-011 line). All frequencies are for vacuum [12]. In our earlier work [11], we analyzed a much broader spectral range (4974.5—4980.7 cm^{-1}), but the present experiment showed that the choice of a wider spectral fragment worsened the precision of the $^{13}CO_2/^{12}CO_2$ isotope ratio measurements, because the dynamic range of the absorption line intensities in this case was too great. In [11] the ratio of absorption intensities was up to 70, presently it is not more 15 thus improves the signal/noise ratio about 2 times.

A typical example of sample and reference spectrum records is presented in Fig. 2.

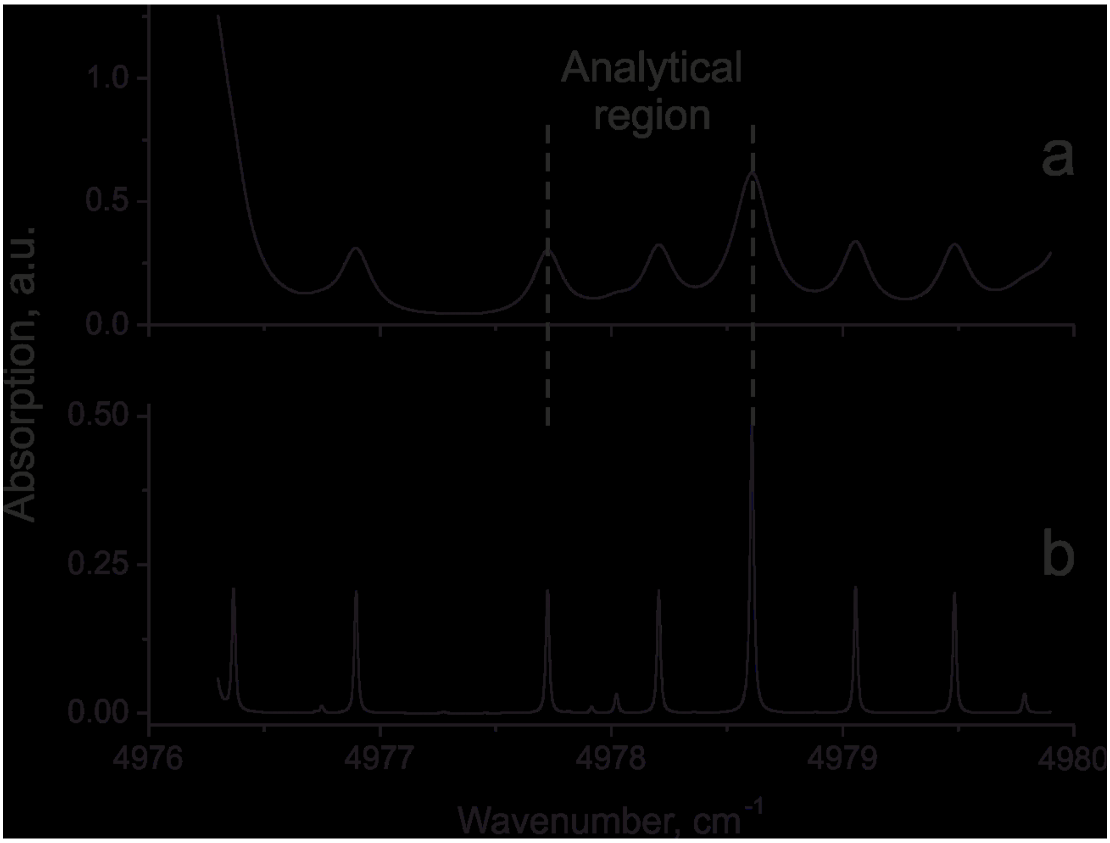

Figure 2. Spectra recorded in (a) the sample channel and (b) the reference channel.

3.2. Spectra processing

The procedure used to analyze the spectra recorded in the sample channel is based on multidimensional linear regression from simulated spectra of the $^{13}CO_2$, $^{12}CO_2$, and H_2O molecules:

$$\ln I_a(\nu) = \ln I_b(\nu) - \sum_i k_i \alpha_i(\nu) L + P^{(3)}(\nu), \qquad (1)$$

where $I_a(\nu)$ is the signal measured in the sample channel, $I_b(\nu)$ is that measured in the baseline channel, $\alpha_i(\nu)$ is the simulated absorption coefficient of the ith molecule, L is the optical path length in the sample cell, k_i is the linear regression coefficient, and $P^{(3)}(\nu)$ is a cubic polynomial allowing for the residual differences between the diode laser baselines in the optical channels PhD1 and PhD2. The result of the linear regression operation is a set of the coefficients k_i that are proportional to the concentrations of the respective molecules [11], the ratio $k(^{13}CO_2)/k(^{12}CO_2)$ giving the desired isotope concentration ratio. The results are presented in terms of the δ-values commonly used to characterize isotope ratios:

$$^{13}\delta = \left(\frac{([^{13}CO_2]/[^{12}CO_2])_m}{([^{13}CO_2]/[^{12}CO_2])_{std}} - 1 \right) \times 1000‰ , \qquad (2)$$

where $[^{13}CO_2]/[^{12}CO_2]_m$ is the isotope ratio measured and $[^{13}CO_2]/[^{12}CO_2]_{std} = 0.011237$ is the standard (PDB) isotope ratio.

3.3. Frequency scale

Our numerical analysis and experiment show that one of the most important factors that limit precision in determining the $^{13}CO_2/^{12}CO_2$ concentration ratio through linear regression to simulated absorption spectra is the calibration accuracy of the diode laser frequency tuning scale. We estimate that an error of a mere 10^{-5} cm^{-1} in the frequency scale of the spectra recorded leads to an error of 0.1 ‰ in the $^{13}CO_2/^{12}CO_2$ ratio measurement results. So, to achieve the δ measurement precision necessary for medical tests (0.5 ‰) the frequencies of the experimental absorption spectra should be locked to those of the simulated spectra with an accuracy no worse than a few 10^{-5} cm^{-1} units. This requirement primarily concerns the absorption line centers, it being less stringent for the line wings. The accuracy above can not be provided by means the commercially available wavelength meters. Therefore the line center positions are primarily determined in the low-pressure (70 Torr) reference gas cell, with proper corrections made for their pressure-induced shifts [12] with guaranteed accuracy of 10^{-6}cm^{-1}. Next the entire laser frequency tuning characteristic is restored by interpolation. A variety of interpolation techniques were tested; for example, linear, cubic spline, and Hermite polynomial. As far as the δ-value precision is concerned, the best results were obtained with the linear interpolation method. Table 1 presents the results of comparison between the above interpolation methods, with the other spectrum processing parameters in the regression scheme remaining the same.

Table 1. Comparison between various interpolation techniques

Type of interpolation	δ-value precision
Linear	0.07‰
Cubic spline	0.5‰
Hermite polynomial	0.1‰

As compared with the higher-order approximations, *the simple* linear approximation yields better results. This can probably be attributed to the limited number of reference line centers within the laser frequency tuning range. During the course of measurements the laser frequency was repeatedly calibrated every 160 milliseconds.

3.4. Sample spectra simulations

The spectral absorption coefficients $⇨_i(↔)$ for the $^{12}CO_2$, $^{13}CO_2$, and H_2O lines of interest were calculated for the comparison in the Lorentz or the Voigt approximation using the HITRAN-2008 database [12]. The data from the pressure and temperature sensors installed in the multipass cell were included in the spectrum simulations. The approximation quality was estimated from the difference between the measured and simulated spectra, following the approximation procedure with the given laser frequency tuning characteristic reconstruction method (linear interpolation).

In addition to the lines whose centers fall within the analytical spectral region (1.5 cm^{-1}), there are around ten lines in the vicinity of the region (±6 cm^{-1}) that were also included in simulations in order to allow for the contribution from their wings to the analytical line profile.

Figure 3 presents an example of application of the regression procedure to a CO_2 spectrum from human breath. Presented are the results for both the Lorentz and the Voigt approximation of the simulated spectra. One can see that the Voigt approximation (curves *e,d*) gives a much smaller residual difference between the spectrum recorded and the sum $⅄k_i☙_i(⑤)L$ for the $^{12}CO_2$, $^{13}CO_2$, and H_2O molecules than that in the case of Lorentz approximation (curve *f*). In the case of Voigt approximation, the residual difference for the total absorption coefficient is less than 10^{-3} of the measured spectrum intensity. There are two graphs of the residual for Voight profile. Graph (e) refers to averaging for 160 ms (a single laser frequency tuning cycle, 64 pulses) and graph (g) refers to 100 s averaging. One can see that curves (e) and (d) are not spectrally correlated. It means that the regular residual between the calculated Voight profile and

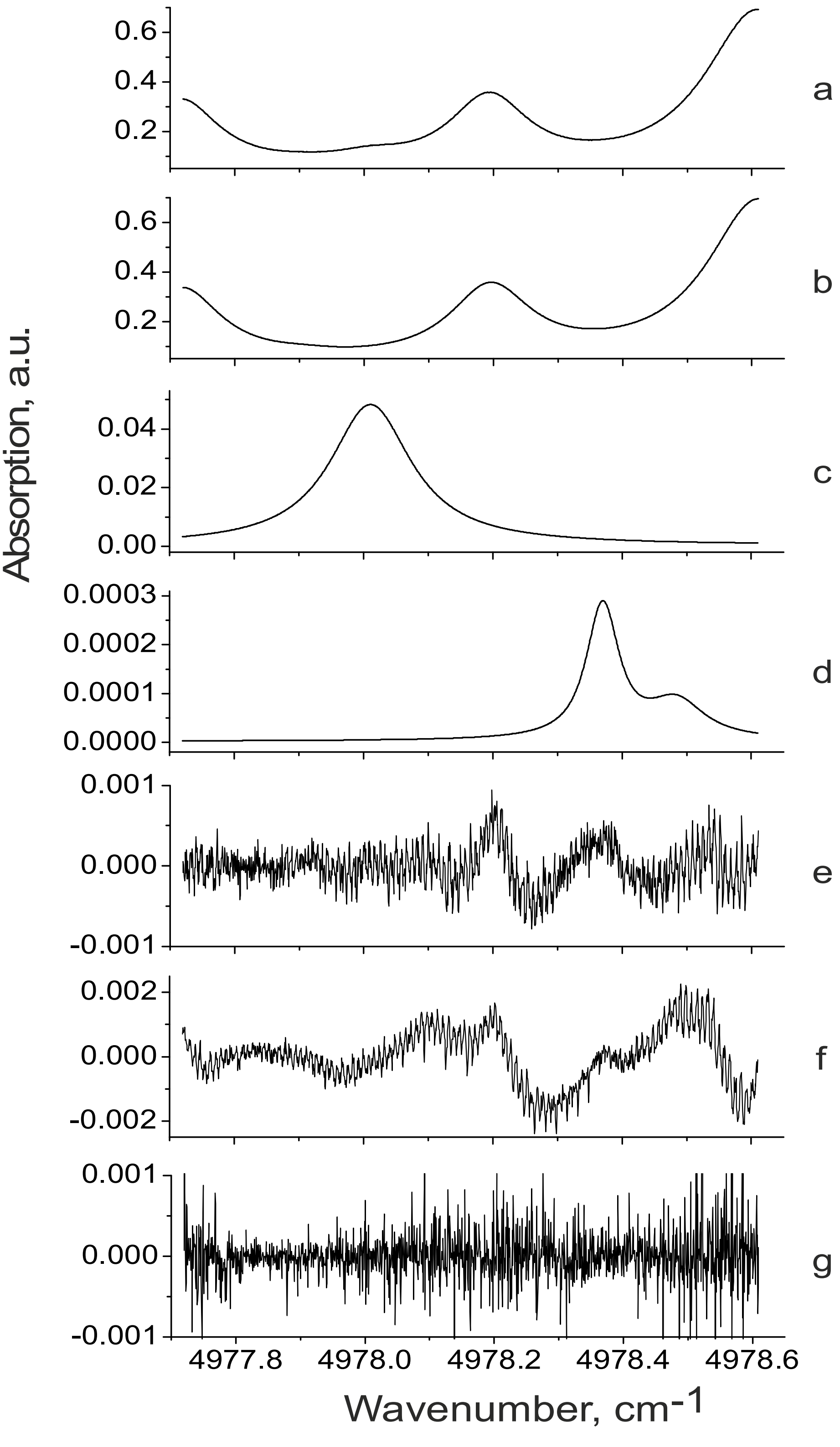

Figure 3. Spectrum of CO_2 in human exhalation and the results of multidimensional linear regression. a - recorded spectrum; b, c, d - simulated spectra (b - $^{12}CO_2$, c - $^{13}CO_2$, d - H_2O); e,g - residual of the regression for the Voigt approximation; f - residual of the regression for the Lorentz approximation. e,f – signal storage time 160ms, g – 100s. The absorption intensity and residual scales are in arbitrary but the same units for all graphs.

experimental record is not observed at the level of measurements precision. The same time the fine structure of residual with the spacing about 0.008 cm^{-1} reveals. We attribute it to interference fringes of optical system.

Some attempts were made to further improve the simulation scheme and to take into account all known broadening mechanisms in linear optics (apart the molecular response mechanism which can play some significance when the line broadening is comparable with transition frequency and not is our case so other authors also don't consider it). Specifically the possible effect of the Dike narrowing was taken into consideration. Since this narrowing affects only the Gaussian component of the Voigt profile, we purposefully varied (not in accordance with the temperature sensor data) the width of the Gaussian profile component down to 50 % of its real Doppler value, but the residual difference between the measured and simulated spectra only worsened. The physical reasons behind the small residual differences observed are difficult to discuss at the moment. Most likely there are several factors at work simultaneously, specifically the finite accuracies of the HITRAN spectral database [12] and our frequency scale calibration.

For rough estimate the above-mentioned residual difference value of 10^{-3} cm^{-1} can be approximately corresponded to the precision of the δ-value measurements. To find the real precision, one should resort to more detailed statistical processing. This matter will be considered in the next section.

4 Results and discussion

4.1. Measurements precision

The δ-value precision was estimated using the Allan variance [14]. Figure 4 presents the pertinent Allan deviation plot and

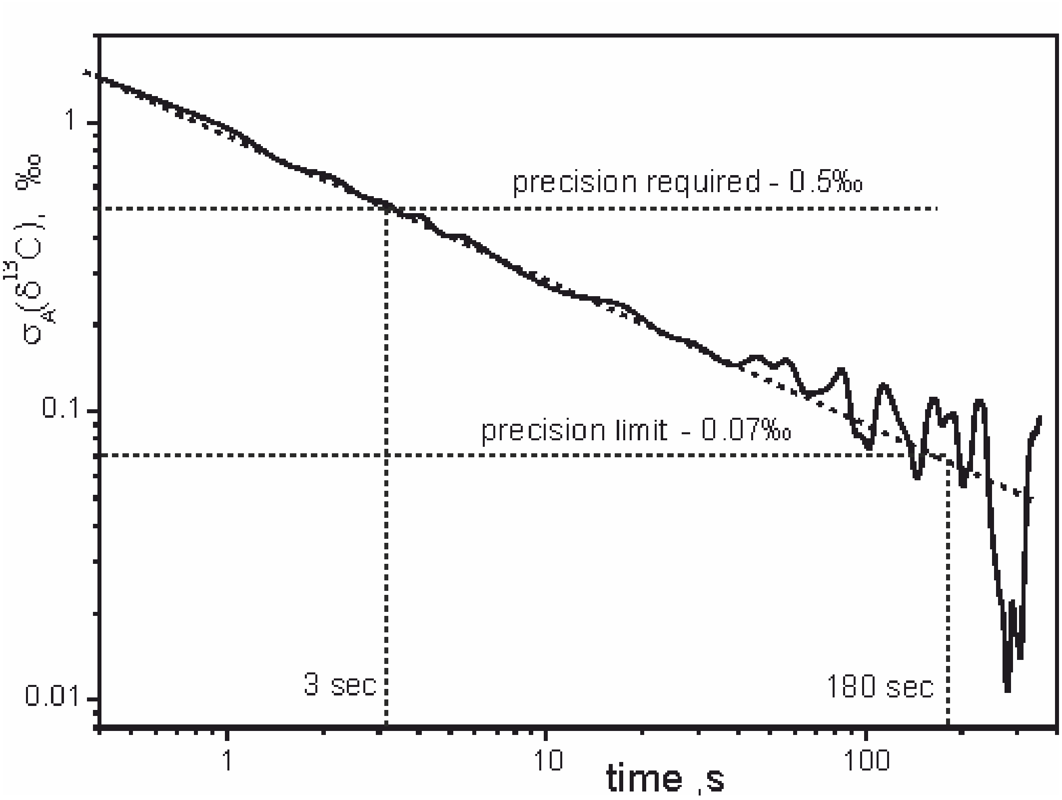

Figure 4. Allan deviation plot for the $\delta^{13}C$ isotope ratio

demonstrates a 0.07-‰ δ-value precision attained in taking human breath measurements with an averaging time of 3 minutes. As can be seen in fig.4 the precision 0.07 ‰ for δ-value was defined for a time at which the Allan plot fluctuations are of the factor 2 so before the minima of the curve. Such precision level is commensurable with that provided by isotope mass analyzers. The precision level necessary for medical breath tests (0.5 ‰) can be achieved with an averaging time of 3 seconds.

The precision level attained is higher than that reported in the majority of the previous works available in the literature, which also used optical methods in the infrared region of the spectrum [2]. Measurements in all of the above-mentioned works were taken at low sample gas pressures in order that the spectral lines of interest could be separated, and so the δ-values were obtained from a limited number of narrow line centers. Also, two gas cells were used in these works to measure the difference in δ, one of them being used as a secondary isotope standard.

In addition to its capability of being used at atmospheric pressure, i.e., with no need for vacuum equipment, the approach suggested offers the following advantages compare the reduced pressure case which are connected with the analysis of absorption:

(a) The regression scheme involves a vast array of spectral fragments, including not only the central parts of the lines of interest, but also overlapped parts of the spectrum and line wings, which improves statistics.

(b) There is no need for the gas temperatures and pressures in the sample and the reference cell to be identical, for the latter is only used for laser frequency calibration purposes. It is well known that fluctuations of the Boltzmann factor due to temperature variations have a strong influence on the δ-value measurement results. Castrillo and co-workers [9] and Gagliardi and co-workers [10] reported an 11 ‰ K^{-1} measurement accuracy attained in the 2-μm spectral region (8 ‰ K^{-1} accuracy in this work, thanks to the optimization of the analytical spectral region). In the approach suggested, use can be made of but relatively low-level passive temperature stabilization, for possible temperature (hence gas density) variations are recorded by the sensors and are taken into account in spectrum simulations within the framework of the regression scheme. In other words, with our approach it is necessary to measure the temperature in the sample cell, rather than stabilize it. Figure 5 presents as an example a sample gas temperature record plotted with a time resolution of 5×10^{-3} s. One can

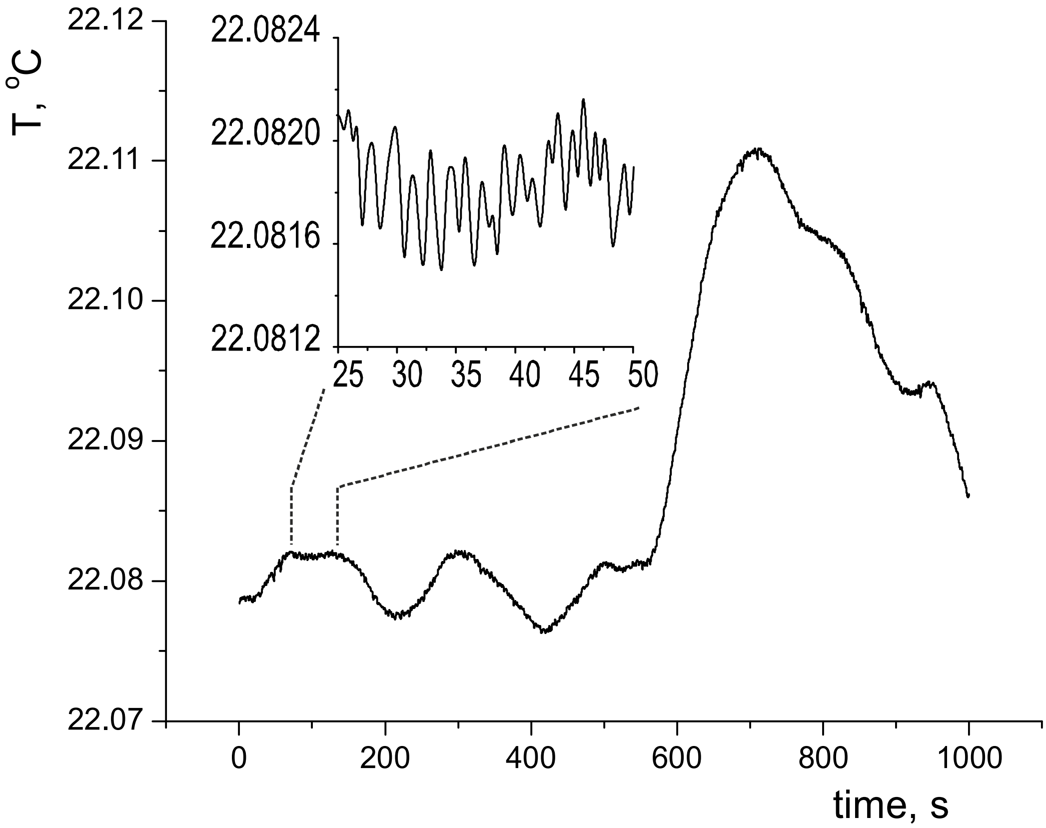

Figure 5. Temperature fluctuations in the sample cell

see that temperature fluctuations in the figure are much below the minimum that the absolute accuracy of the sensors (see Sect. 2) allows them to record. Typical slow temperature fluctuations are on the order of 0.03 K. They occur on a time scale of 10—100 s and can be taken into account (the necessary characteristic regression cycle time is around 0.2 s). Noise-like fluctuations are on the order of 0.001 K, and so their influence should not be over 0.01 ‰. The temperature factor is thus not the cause of measurement precision limitation. The possible effect of slow temperature fluctuations on the isotope ratio measurement precision in our experimental conditions is no more than 0.3 ‰. The typical parameters of the record plotted in Fig. 5 for a 6-min interval remain the same for much longer times, say, on the order of tens of hours.

(c) Data obtained from smoothed spectrum structures should be less sensitive to the effect of experimental uncertainties and external factors.

(d) The Dicke narrowing (DN) effect appears when the absorbing particle mean free pass became comparable or less than the absorption wavelength. For CO_2-N_2

collisions and 2mkm wavelength it correspond the pressure about 30Torr. DN influence the Doppler component and convert it from Gaussian to dispersive one [15] thus disturb the Voight profile. The influence is more pronounced when the input of Doppler component to the Voight convolution is essential. For the lines under consideration the Doppler to Lorentz widths ratio ☚⑤$_D$/☚⑤$_L$ at 300K is about (0.15-0.07) at atmospheric pressure and about (2.5-1) at 50 Torr. In such situation the results of simulations at elevated pressure is less sensitive to details of line profile models like Galatry or Rautian-Sobelman models compare to Voight one (see the results in sec.3.4). For these more sophisticated models the new fitting parameters for DN are needed [16, 17]. For collision broadening and shift the precise data adapted to standard atmosphere can be taken from Hitran [12] include the dependence on the rotational state for the standard air with its oxygen, argon, water… for both isotopomers. The Hitran database contains the information on CO_2 self broadening parameters so it is easy to correct the broadening factors for exhaled air compare the standard air. Even the negligibly small changes in oxygen density in the breath compare to air are compensated by the nitrogen at fixed pressure and the broadening factors for them are practically equal. The small influence of tails of the water lines was also accounted (sec.3.4). From this point of view the line profile modeling at atmospheric pressure is not more complicated and less reliable than those in case of reduced pressure for the purposes of applications under discussion.

On the other hand, we want precise spectrum simulations and compliance with rather strict requirements on the accuracy of the experimental frequency scale. This is the very factor that limits precision and accuracy, and so the progress in precision demonstrated in this work seems to be the result of a trade-off of this factor and factors (a) through (d) above.

4.2. Accuracy and reproducibility

As far as medical breath tests are concerned, the long-term reproducibility of carbon isotope ratio measurements is a very important parameter, because the test procedure involves comparison between two exhaled air samples taken and measured at different times. To estimate the accuracy and long-term stability of the spectrometer, a sample of human air exhalation was repeatedly tested for 16 hours. The results of the pertinent δ-value measurements are presented in Fig. 6. It can be seen that during this time interval the

total variance of the δ-values reached 0.6 ‰, their standard deviation being 0.2 ‰. Fig. 6 shows that the spectrometer deviate the value of isotopic ratio not more than 0.015% after one hour of work. At 10 hours after taken the enriched substance the precision of measurements comes to the threshold of medical criteria so might be not satisfactory when using our laboratory device but it seems too long time for real practical needs.

The δ-values shown in Fig 6 represent absolute deviations from the PDB standard [2]. To get these data, we calibrated our optical spectrometer using commercially available CO_2 gas as a secondary standard. The isotope ratio in this gas was determined with a Model MS200 quadrupole mass analyzer. The δ-value deviation was

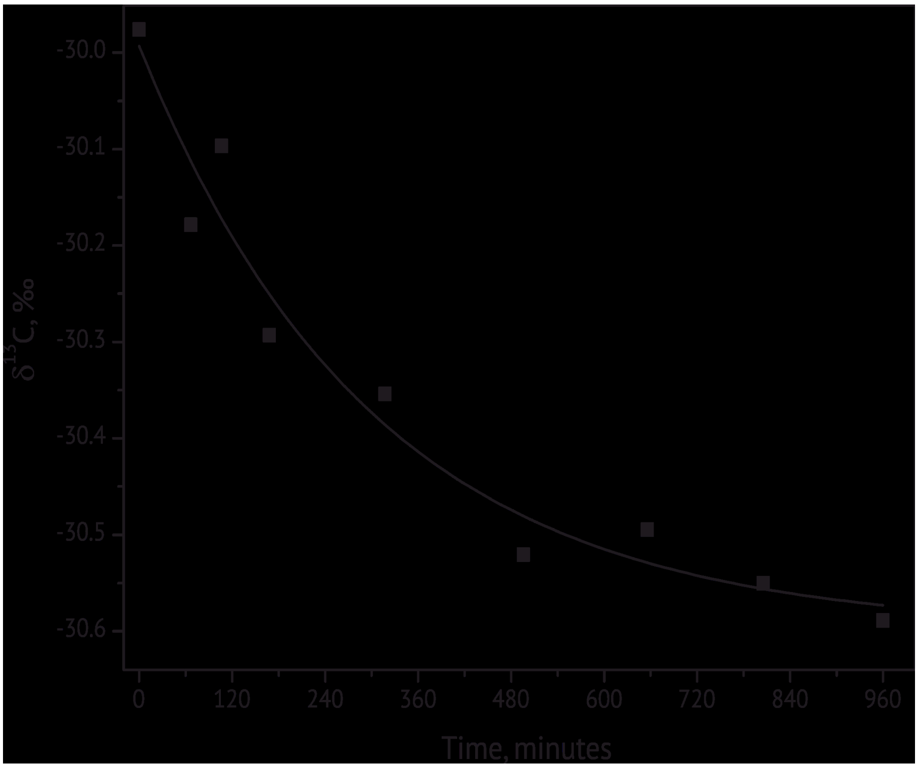

Figure 6. Long-term (16-hr) reproducibility for $\delta\,^{13}CO_2$

measured at ī(18⬅3) ‰ relative to the PDB value. The corresponding correction was made in absolute optical measurements.

Comparison of the techniques used and results attained in this work with those for our earlier work (precision 0.4 ‰) [11], as well as for pressure-broadened lines, shows that the precision improvement accomplished here owes primarily to the optimization of the analytical spectral interval and the use of temperature and pressure sensors in the sample cell. One can expect further improvement on account of optimization of the multipass Herriot cell geometry aimed at suppressing the present influence of residual interference fringes. It is not improbable that precision can furthermore be improved by reducing the mechanical instability of the spectrometer that at present seems to be the cause of the long-term drifts (Fig. 6).

Curve *a* in Fig 7 illustrates an example of isotope ⇦-value measurements in exhaled air samples taken from a group of 5 people.

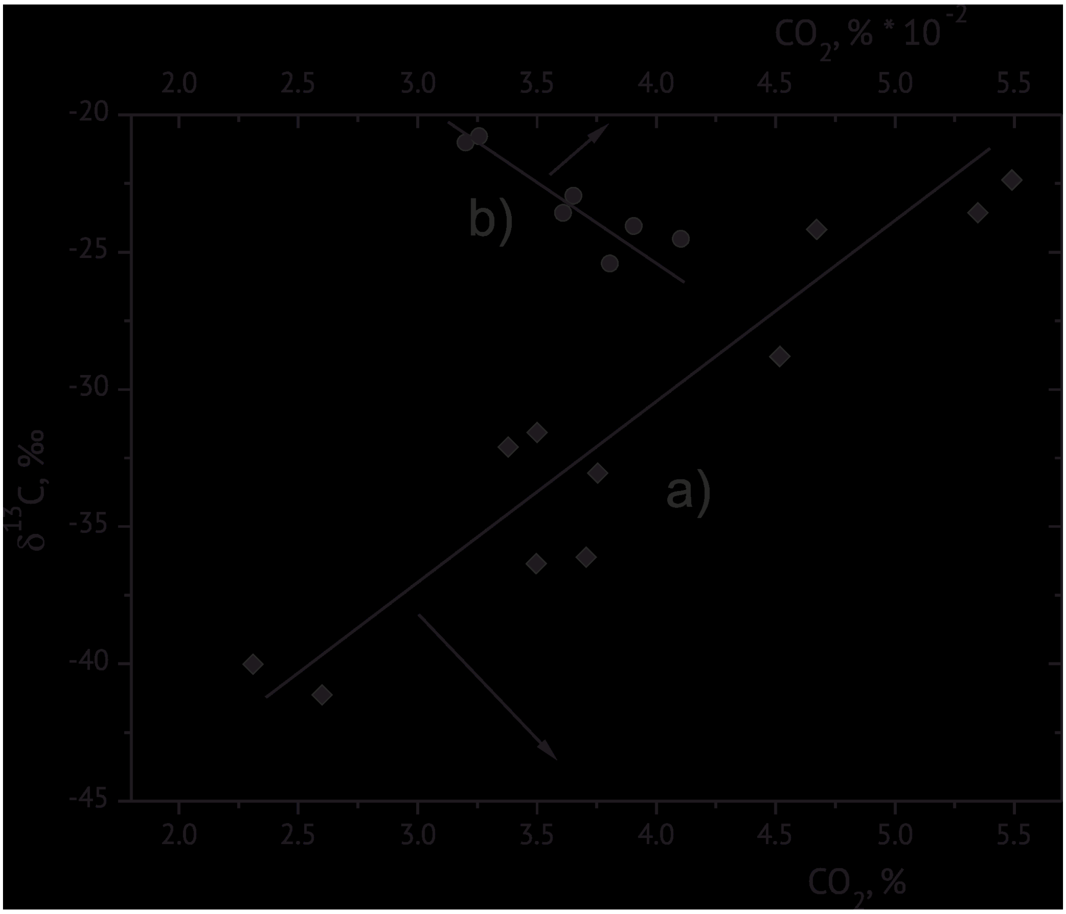

Figure 7. Example of exhaled air measurements. (a) - $\delta^{13}CO_2$ as a function of the total CO_2 concentration in the exhaled air for 5 people; (b) - the same for plants [18]

The measurements were taken at different instants of time during the course of a day. In that case, no measures were taken to control the depth of inhalation, breath holding, and the other sampling details. Also measured at the same instants of time was the total carbon dioxide concentration (↘, %) in the exhaled air. Although the ⇦- and ↘-values for each subject are individual, their correlation can be seen in the figure. The deviation of the isotope ratio ⇦ from the standard value decreases with increasing carbon dioxide concentration in the exhaled air. The only objective of these tentative experiments it was to show the existence of correlation between the absolute carbon dioxide concentration and isotope ratio. This matter probably deserves a more comprehensive investigation. Note only that the existence of correlation between these values was also reported in the early works on the geochemical analyses of air near living plants [17]. The relationship between ⇦ and ↘ for plants is illustrated by curve *b* in Fig. 7. The type of correlation in this case is the opposite of that for humans, but one should take into consideration the fact that plants consume carbon dioxide, whereas humans produce it.

5 Conclusions

The results presented in this work as to the precision of optical measurements of carbon isotope ratio in exhaled air at atmospheric pressure show that the diode laser operative in the 2-↘m spectral region reliably provides the necessary conditions for taking stable and precise measurements of relative changes in the carbon isotope ratio ⇦ as required by medical bacterial diagnostics. The ⇦-value precision determined by means of the Allan deviation plot with an averaging time of 3 minutes amounted to 0.07 ‰. Such precision is comparable with that provided by modern isotope mass analyzers. The standard deviation of the ⇦-value measured for 16 hours was no more that 0.2 ‰, no special measures being taken to thermally and mechanically isolate and stabilize the experimental setup. The existence of correlation was observed between the ⇦-value and the total CO_2 concentration in the exhaled air. In our opinion, the further improvement of the optical spectroscopic approach suggested by us should be aimed at enhancing the absolute accuracy of the ⇦-value. This may prove important in many applications, not to mention medical breath tests.

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References

1. P. Ghosh, W.A. Brand, Int. J. of Mass Spectr. **228**, 1 (2003)
2. E. Kerstel, L.Gianfrani, Appl. Phys. B **92**, 439 (2008)
3. E.H. Wahl, B. Fidric, Ch.W. Rella, S. Koulikov, B. Kharlamov, S. Tan, A.A. Kachanov, B.A. Richman, E.R. Crosson, B.A. Paldus, Sh. Kalaskar, D.R. Bowling, Isotopes in Env. Health Stud. **42**, 21 (2006)
4. R. Wehr, S. Kassi, D. Romanini, L. Gianfrani, Appl. Phys. B **92**, 459 (2008)
5. G.B. Rieker, J.B. Jeffries, R.K. Hanson, Appl. Phys. B **94**, 51 (2009)
6. D. Richter, B.P. Wert, A. Fried, P. Weibring, J.G. Walega, J.W.C. White, B.H. Vaughn, F.K. Tittel, Optics Lett. **34**, 172 (2009)
7. D.D. Nelson, J.B. McManus, S.C. Herndon, M.S. Zahniser, B. Tuzson, L. Emmenegger, Appl. Phys. B **90**, 301 (2008)
8. V.L. Kasyutich, P.A. Martin, R.J. Holdsworth, Appl. Phys. B **85**, 413 (2006)
9. A. Castrillo, G. Casa, E. Kerstel, L. Gianfrani, Appl.Phys. B **81**, 863 (2005)
10. G. Gagliardi, R. Restrieri, G. Gasa, L. Gianfrani, Opt.Laser Eng. **37**, 131 (2002)
11. E.S. Mironchuk, I.V. Nikolaev, V.N. Ochkin, S.S. Rodionova, M.V. Spiridonov, S.N. Tskhai, Quant. Electr. **39**, 388 (2009)
12. L.S. Rothman, et al, J. of Quant. Spectr. Radiat. Trans. **110**, 533 (2009)
13. T.M.Shay, Y.C.Chung.Opt. Eng. v.29, p.p.681-683, (1990)
14. P. Werle, R. Muecke, F. Slemr, Appl. Phys. B **57**, 131 (1993)
15. Vladimir N.Ochkin. *Spectroscopy of Low Temperature Plasma.* WILLEY-VCH, 609p., (2009)
16. J.W.Brault e.a. J. Molec. Spectr. **222**, 220, (2003)
17. E.R.Crosson e.a. Anal. Chem.**74**,2003, (2002)
18. E.M. Galimov, *Geochemistry of stable carbon isotopes* (Nauka, Moscow, 1968, in Russian)