

## Measurements of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ content ratio with the use of near IR diode lasers

A.I. Nadezhdinskii,<sup>1</sup> G.Yu. Grigoriev,<sup>2</sup> S.L. Malyugin,<sup>2</sup>  
A.V. Makkaveiskii,<sup>1</sup> Sh.Sh. Nabiev,<sup>2</sup> D.B. Stavrovskii,<sup>1</sup>  
Ya.Ya. Ponurovskii,<sup>1</sup> Yu.P. Shapovalov,<sup>1</sup> and A.P. Babichev<sup>2</sup>

<sup>1</sup> Center for the Natural Science Research at the A.M. Prokhorov Institute of General Physics,  
Russian Academy of Sciences, Moscow

<sup>2</sup> Institute of Molecular Physics at the "Kurchatov Institute" Russian Scientific Center, Moscow

Received June 10, 2005

The paper reports on the development of instrumentation for express-measurements of  $^{13}\text{CO}_2$  concentration using the  $\text{CO}_2$  absorption spectrum in the near-infrared range. The relative error in the  $d$ -factor determination in the breath-test obtained from 100 single measurements summed was within 0.7%. To determine the absolute error of the isotopic ratio measurements, we have conducted independent calibration measurements with five  $\text{CO}_2$  samples of different isotope composition using both the diode laser spectroscopy (DLS) and mass-spectrometers.

The  $^{13}\text{C}$  isotope is of interest mostly due to development of respiratory diagnosis aimed at identification of people infected with the *Helicobacter pylori* bacterium (the so called Urea Breath Test). The *H.pylori* causes gastroenteropathy, gastrointestinal diseases, primarily, stomach ulcer. In brief, the idea of the method is as follows. A patient takes a solution of carbamide  $\text{CO}(\text{NH}_2)_2$  marked with the carbon  $^{13}\text{C}$ . For *Helicobacter pylori*, the carbamide is a food, consumption of which results in emission of carbon dioxide. Then the  $\text{CO}_2$  finds its way through the stomach's walls and blood vessels into lungs and is breathed out. In measuring the isotopic ratio  $^{13}\text{CO}_2/^{12}\text{CO}_2$  in the expired air, it is possible to judge on whether the patient is infected with *H.pylori* or not and estimate the clinical stage. The discover of this bacterium was a significant step towards perfection of treatment methods for gastrointestinal diseases. An important contribution to this field came from the isotopic diagnosis, which, in addition to the above possibilities, made it possible to follow the curing dynamics and to choose the most efficient medicines.

At present, the breath-test method has gained a wide application abroad, particularly, in the USA. By having overgrown the research stage, it has reached the stage of practical application. Determination of the degree of the *H.pylori* contamination from the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio is preferable compared to measuring the radioactive  $^{14}\text{C}$  concentration, because it can be applied to all patients without exception, including pregnant women and babies.

Besides *H.pylori* infestation, the isotope breath-test can be used in diagnosis of a series of other diseases.<sup>1</sup> Therefore, in addition to carbamide, several

other specimens marked by  $^{13}\text{C}$  can be used as active reagents. Nevertheless, in all cases the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio in the expired air is a failure warning indicator.

Ideas of using measurements of isotope ratios in the expired air were formulated in early 1950s. Different possibilities of applying the measured results to diagnosing functioning of different organs, first of all, lungs, have been considered.

In 1969, a review of the Australian researcher K.T. Fowler was published,<sup>2</sup> in which application of mass-spectrometers to breath-tests was analyzed. The idea of *H.pylori* diagnosis with the help of the isotopic breath-test was proposed in two years after discovery of the bacteria.<sup>3</sup> Immediately, the development of diagnostic methods has begun, and soon a great number of publications appeared in medical, chemical, and physical literature.<sup>4-9</sup> Leading manufacturers of mass-spectrometric instruments have designed and commercialized mass-spectrometers of IRMS isotope ratio for isotopic breath diagnosis.<sup>10,11</sup>

Such instruments provide a high measurement accuracy, but are rather expensive. Therefore, different groups of researchers began designing new instruments based on optical measurement methods. Yet, in 1975 C.S. Irving has proposed<sup>10</sup> a nondispersive approach to measuring the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  isotopic ratio. Based on this principle, while using photo-acoustic detectors, a commercial instrument produced by "Wagner" company was designed.<sup>11,12</sup> Another commercial instrument based on opto-galvanic effect is produced by Alimenterics company.<sup>13,14</sup> These instruments are a bit cheaper than mass-spectrometers, but they remain sufficiently expensive for the Russian market.

A series of works on the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio laser measurements were published,<sup>15-19</sup> reporting on

results of isotopic breath diagnosis implementation, in cooperation with physicians, into practice of Russian clinics.

Since the invention of the method of isotopic diagnostics of the breath, the problem of its accuracy and reliability has arisen.<sup>20–25</sup> Comparison between the optical and mass-spectrometric measurements was conducted, which demonstrated all the commercial instruments to provide a required accuracy.

The  $^{13}\text{CO}_2/^{12}\text{CO}_2$  isotopic ratio is measured in the air breathed out by a patient before taking in the tracer and in some time after its acting

$$\delta = \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{final}}}{^{13}\text{C}/^{12}\text{C}_{\text{initial}}} - 1 \right) \cdot 1000 \text{ ‰ (pro mille)}.$$

The problem of the so-called “cut-off point,” i.e., on the  $\delta$  value, at which the patient is considered infected, is of a great importance. Most often, 5‰ was taken as the cut-off point. This value was found in the very beginning of the practice on isotope breath-test application and then it was introduced in the European protocol of standards. However, later, it was shown in a series of studies<sup>20</sup> that at the urea dose from 75 to 100 mg this value must be decreased to 3.5‰. At present, this value is universally accepted, although the range of 3.5 to 5‰ is called the gray zone, where an unambiguous conclusion on the infection with *H. pylori* is impossible.

As to other diseases and diagnostic means, there is no a universally accepted value of  $\delta$  because of unfinished laboratory tests of the method. The cut-off point determines requirements to the instrumental measurement accuracy. To reliably determine  $\delta$  value, the measurement accuracy must be at least  $\delta/3$  from the point of view of physical measurements. In practice, it must be at the level of 0.5 to 0.8‰. The accuracy of specialized mass-spectrometers is 0.1–0.2‰. Manufacturers of optical IR-instruments declare the same accuracy, but actually it is somewhat lower, i.e., 0.5–0.8‰.

In Russia, apparently, the main obstacle for wide use of the breath test is high price (about 50–80 thousand USD) of the equipment needed. The price of the laser opto-galvanic instruments is at the same level. An infrared device with photo-acoustic recording is a bit cheaper.

Therefore, at the “Kurchatov Institute” Russian Scientific Center, new methods for measuring the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio have been developed in parallel with the creation of “Kolonna” dividing complex for production of the  $^{13}\text{C}$  isotope.<sup>28</sup> The goal of the work is to find the method enabling one to develop a device acceptable for the Russian market. To organize interaction with physicians and to conduct comparative experiments, two chromato-mass-spectrometers produced by Medichems company (Republic of Korea) were bought with the help of the Ministry of Nuclear Industry, which were specially intended for the breath

diagnostics. Using these devices, the breath test measurements are now conducted in cooperation with a series of Moscow clinics and medical centers.<sup>29–32</sup>

As an alternative, the method of diode laser spectroscopy (DLS) is under study. A tunable diode laser ( $\lambda = 1.6 \mu\text{m}$ ) with distributed feedback and fiber-coupled output of radiation, as well as a multi-path optical cell with a total length of optical path of 42 m are used. The choice of the radiation range (1.6  $\mu\text{m}$ ) is primarily determined by the above goal: to develop a low-cost instrument for the isotope breath diagnostics.

A  $\text{Pb}_{1-x}\text{Eu}_x\text{Se}$ -laser emitting at 4.3  $\mu\text{m}$  center wavelength has been used.<sup>17–19</sup> This range coincides with an intense absorption band of the  $\text{CO}_2$  molecule, but both the laser and photodetector for this range require cooling down to 80–100 K temperatures. This increases the price of the system and limits its applicability, because the liquid nitrogen is not available in any clinic.

In this study, the instrumentation for express-measurements of  $^{13}\text{CO}_2$  concentration by the  $\text{CO}_2$  absorption spectrum in the near IR range has been developed for the first time.<sup>33,34</sup>

The absorption lines *R4*  $^{12}\text{CO}_2$  and *P12*  $^{13}\text{CO}_2$  located within 6232.7–6232.5  $\text{cm}^{-1}$  frequency range were recorded. Further, the ratio of  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$  concentrations was calculated from the ratio between the absorption coefficients measured at these lines.

The diode module (DL-module) incorporated a diode laser (Anritsu company) with the distributed feedback and a fiber-coupled output of radiation ( $\lambda = 1.6 \mu\text{m}$ ), a thermo-resistor, and a Peltier thermo-element.

The multi-path cell with a 25 cm base and the total optical path of 42 m had a fiber input of radiation. The cell’s construction allowed its evacuation and filling with the studied gas to be done at a reduced pressure.

A photodetector (PD) with a preamplifier (PA), set on the cell, were used to record the output radiation. The interface module, intended for control of the DL-module and recording system, incorporated a source of the DL pumping current, a DL thermo-stabilizing system, and a preamplifier for the power supply.

To control the DL pumping, thermo-stabilizing systems, signal recording and processing, NI DAQ PCI-6052E board by National Instrument company was used. A computerized control of the measurement process was performed within the NI LabView 7.0 medium.

The DL was pumped by trapezoidal current pulses of the pulse amplitude about 80 mA ( $I_{\text{work}}$ ), a pulse length of 0.4–1 ms at the repetition frequency of 1–2 kHz. The DL generation frequency scanning ( $\Delta\nu \sim 2 \text{ cm}^{-1}$ ) was conducted by varying the efficient index of refraction of the laser active range during the pump pulse.

The thermo-stabilizing system allowed a smooth tuning of generation frequency within  $60 \text{ cm}^{-1}$  due to variation of the crystal temperature with the help of the Peltier thermo-element.

Figure 1a presents a model spectrum of  $\text{CO}_2$  transmission near  $1.6 \mu\text{m}$  (according to spectra from HITRAN database). Top curves show the contribution from  $^{13}\text{CO}_2$  absorption at a natural isotope concentration. The chosen spectral range and analytical spectral lines of the two  $\text{CO}_2$  isotopes are presented as well.

The analytical spectral lines were chosen in such a way that the gas temperature in the cell almost did not affect the measurement results, i.e., the lines with similar temperature dependence of the integral absorption cross section (Fig. 1b): R4 for  $^{12}\text{CO}_2$  and P12 for  $^{13}\text{CO}_2$  were used.

Figure 1c demonstrates the  $\text{CO}_2$  absorption spectrum in the analytical spectral range obtained in this work in measuring the  $\text{CO}_2$  isotope composition in the expired air. The air sample pressure in the cell was 30 Torr.

Under the experimental conditions chosen, the absorption coefficient amplitude in the strong line, corresponding to the main isotope, makes about 0.024, while in the weak line – about 0.0006.

Taking into account the required measurement accuracy (about 0.5‰), we arrive at a conclusion that the necessary accuracy of the absorption measurements must be no worse than  $3 \cdot 10^{-6}$ . In the spectral range chosen, the  $^{13}\text{CO}_2$  absorption cross section is 46 times weaker than that of the main one. Taking into account this fact, the procedure for measuring isotope composition in the expired air includes five principal steps. Schematically, those are shown in Fig. 2.

1–2. Spectrum recording and normalization. The absorption coefficient is calculated and the frequency scale is linearized. In the case of the DL with a distributed feedback, the frequency scale can be described sufficiently accurately by a linear function with the shape of the pumping current pulse as the argument.

3. Spectral filtration. The filtration is performed with the use of cross-correlation functions, as well as the Butterworth filter of the second order.

4. Spectrum approximation. This is performed by the Doppler contour's second derivative and a linear function.

5. Computation of  $\delta$  value. The ratio  $R$  of the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  integral absorption coefficients and  $\delta = (R/R_0 - 1) \cdot 1000\text{‰}$  are found.

The program written within the LabView medium allows a real-time monitoring of the measurement process and computation of  $\delta$ . The time of a single  $\delta$  measurement did not exceed 0.2 s. With averaging over 100 measurements, relative error in  $\delta$  did not exceed 0.7‰, which is quite acceptable for the clinical practice.

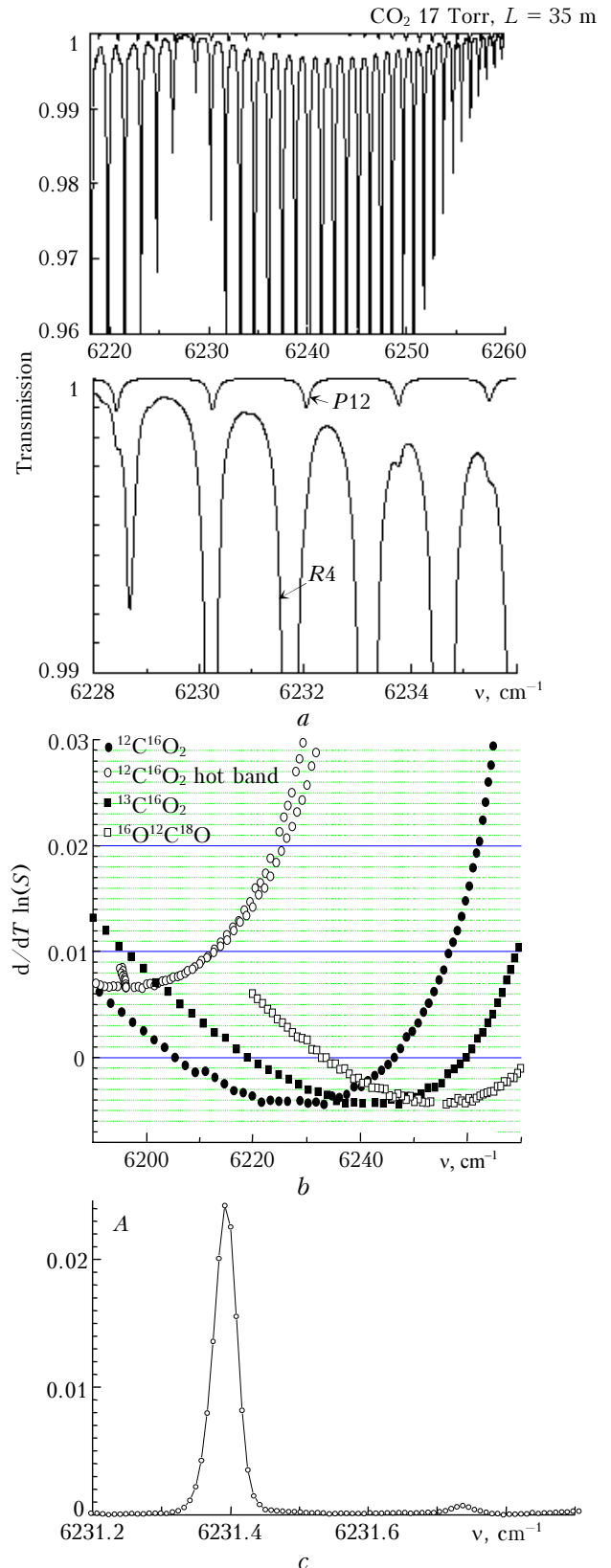


Fig. 1. Model absorption spectrum for  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$  (HITRAN) (a); the coefficient of relative temperature dependence of the spectral line integral absorption coefficients (b); and  $\text{CO}_2$  absorption spectrum in the analytical spectral range (c).

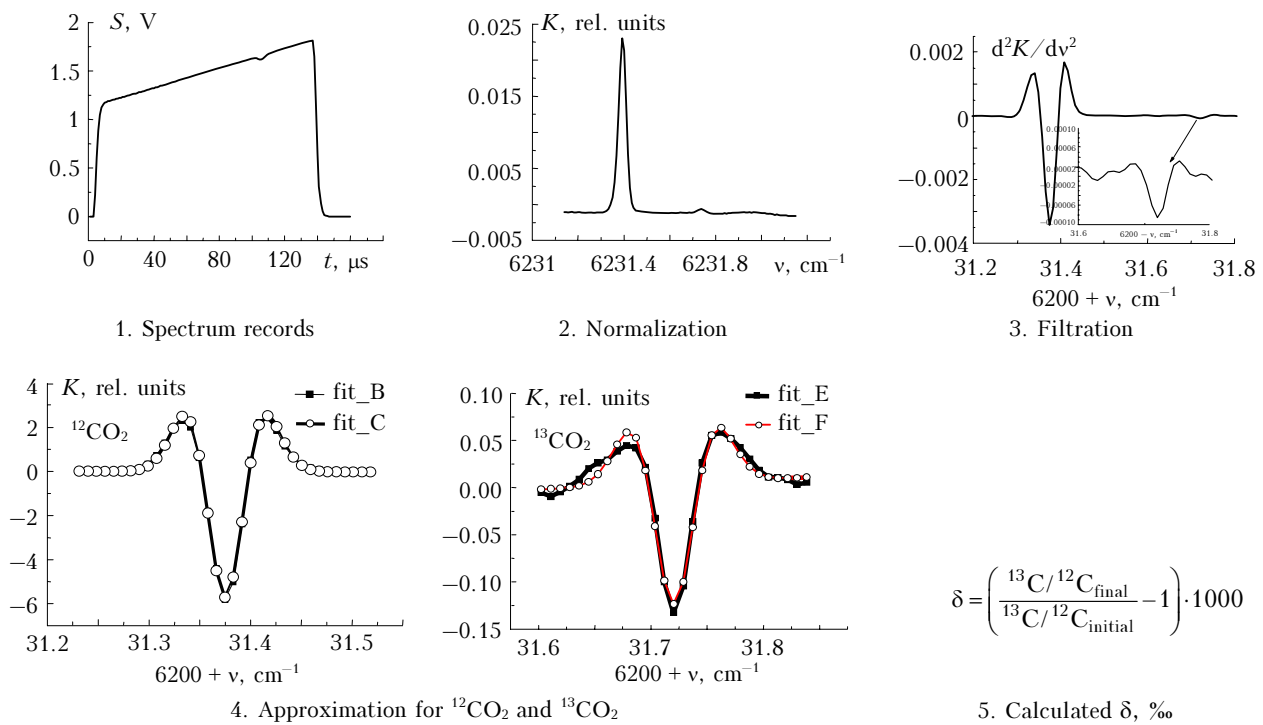


Fig. 2. Main steps of the procedure of  $\delta$  determination.

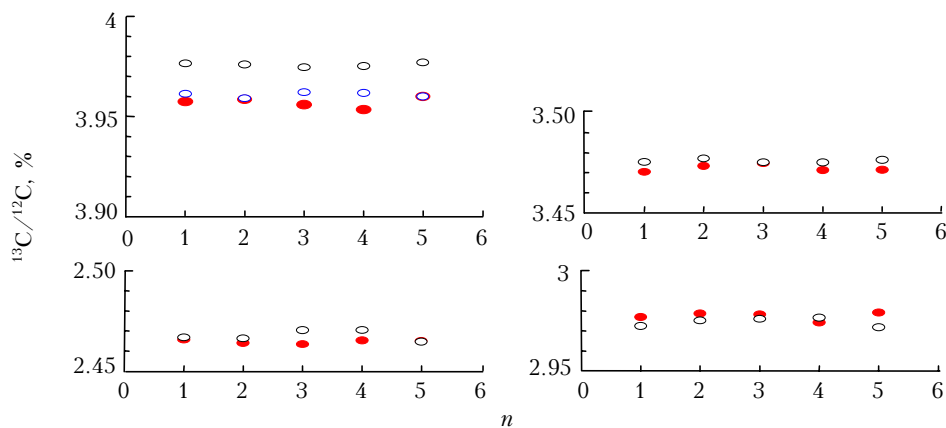


Fig. 3. Results of independent DL measurements of isotope composition for one and the same  $\text{CO}_2$  sample. Each point corresponds to realization of one measurement.

The device calibration in  $R$  measurements is based on values of integral cross sections taken from the HITRAN-2004 database. To estimate the systematic error, five  $\text{CO}_2$  samples with different isotope compositions were prepared. The  $\text{CO}_2$  isotope composition in the samples was preliminary measured with a mass-spectrometer. The DL measurement results are shown in Fig. 3, and data obtained with the help of DL gas-analyzer and mass-spectrometer (averaged values) are presented in the Table.

Sample number	15	5	25	3	1
$^{13}\text{C}/^{12}\text{C}$ , %, DLS	3.97	3.47	2.46	2.97	1.08
$^{13}\text{C}/^{12}\text{C}$ , %, mass-spectrometer	3.97	3.48	2.54	2.94	1.10

Statistical analysis of the data allowed estimation of the systematic error by different measurement cycles of one and the same sample, which did not exceed 1.2‰. For this measurement procedure, it is caused by the error in determination of the gas mixture pressure equal to 0.5 Torr. However, in measuring  $\delta$ , the systematic error is often compensated for, and then, as it was said above, relative error for  $\delta$  does not exceed 0.7‰. Thus, in this work, the instrumentation for express-measurements of  $^{13}\text{CO}_2$  concentration by the  $\text{CO}_2$  absorption spectrum in the near infrared has been developed. Comparative tests for patients were conducted using the DL gas-analyzer model and the HeliView device.

Figure 4 presents the time-dependent  $\delta$  values for infected and non-infected patients. The laboratory

prototype of the DL gas-analyzer can serve a basis for creating of a commercial analyzer for the isotope breath diagnosis.

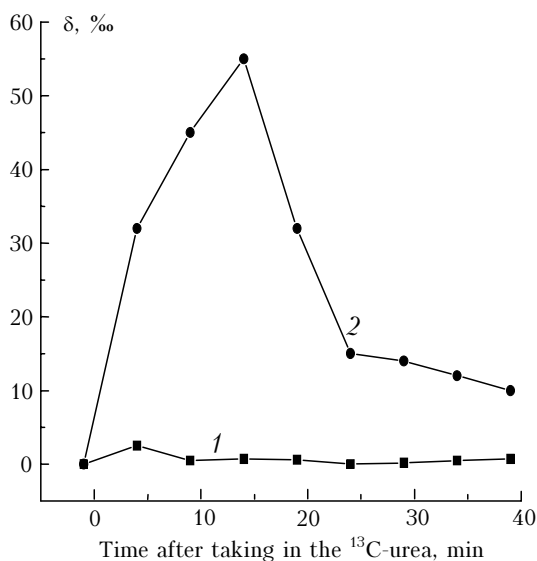


Fig. 4. Isotope breath test: non-infected patient (1), infected patient (2).

## References

- V.G. Grishina, V.I. Nevmerzhtskii, and E.B. Svirshchevskii, *Isotope Breath Test. Isotopes. Properties, Manufacturing, Application*, ed. by V.Yu. Baranov (Fizmatlit, Moscow, 2005), Vol. 2, 311 pp.
- K.T. Fowler, *Phys. Med. Biol.* **14**, No. 2, 185–199 (1969).
- D.Y. Graham, P.D. Klein, D.J. Evans, et al., *The Lancet* **343**, 1174–1177 (1987).
- R.P.H. Logan, S. Dill, F.E. Bauer, et al., *Eur. J. Gastroenterol. Hepatol.* **3**, 915–921 (1991).
- R. Guilluy, F. Billion-Rey, C. Pachiaudi, et al., *Anal. Chim. Acta* **259**, 193–202 (1992).
- B. Braden, M. Haisch, L.P. Duan, et al., *Z. Gastroenterol.* **32**, 675–678 (1994).
- S. Koletzko, M. Haisch, I. Seeboth, B. Braden, K. Hengels, B. Koletzko, and P. Hering, *The Lancet* **345**, 961–962 (1995).
- <http://www.europa-uk.com>
- <http://www.medicchems.com>
- C.S. Irving, *Anal. Chem.* **5S**, 2172–2175 (1975).
- B. Braden, F. Schäfer, W.F. Caspary, and B. Lembcke, *Scand. J. Gastroenterol.* **31**, 442–445 (1996).
- <http://www.wagner-bremen.de/>
- D.E. Murnick and B.J. Peer, *Science* **263**, 945–947 (1994).
- R. Schuman, B. Rigas, A. Prada, and G. Minoli, *Gastroenterol.* **108**, A961 (1995).
- E.R. Crosson, K.N. Ricci, B.A. Richman, F.C. Chilese, T.G. Owano, R.A. Provencal, M.W. Todd, J. Glasser, A.A. Kachanov, B.A. Paldus, T.G. Spence, and R.N. Zare, *Anal. Chem.* **74**, No. 9, 2003–2007 (2002).
- D.E. Cooper, R.U. Martinelli, C.B. Carlisle, H. Riris, D.B. Bour, and R.J. Menna, *Appl. Opt.* **32**, 6727–6731 (1993).
- E.V. Stepanov, *Kvant. Elektron.* **32**, No. 11, 981–986 (2002).
- E.V. Stepanov, “*Laser spectroscopy and analysis of microelements in expired air*,” Author’s Abstract of Doctor Phys.-Math. Sci. Dissert., Moscow (2003), 41 pp.
- E.V. Stepanov, V.A. Milyaev, and Yu.G. Selivanov, *Usp. Fiz. Nauk* **170**, No. 4, 458–462 (2000).
- B.J. Johnston, S. Levi, and P.G. Johnson, *Gut* **39**, (suppl. 2), A122 (1996).
- F. Stellaard and B. Geypens, *Gut* **43**, 2–6 (1998).
- F. Mion, G. Rosner, M. Rousseau, et al., *Clin. Sci.* **93**, 3–6 (1997).
- R.M. Zagari, P. Pozzato, F. Bazzoli, et al., *Digestion* **59** (suppl. 3), 461 (1998).
- C.D. Mansfield and H.N. Rutt, *Phys. Med. Biol.* **47**, 689–696 (2002).
- Working Party of the European Helicobacter Pylori Study Group. Guidelines for clinical trials in Helicobacter pylori infection*, *Gut* **41** (suppl. 2), 1–23 (1997).
- V. Savarino, D. Vaira, P. Dulbecco, et al., *Gut* **43** (suppl. 2), A50 (1998).
- S. Ohara, M. Kato, M. Asaka, and T. Toyota, *Helicobacter* **3**, 49–53 (1998).
- A.P. Babichev, I.P. Gnidoi, G.Yu. Grigoriev, K.A. Dyma, A.A. Dyatlov, D.A. Kersnovskii, N.V. Komissarov, V.V. Stepanov, A.L. Ustinov, and A.S. Polevoi, in: *Proc. of VII Intern. Conf. on Physical-Chemical Processes in Selection of Atoms and Molecules* (CNIIAtominform, Moscow, 2002), p. 290.
- P.L. Shcherbakov, I.I. Balabolkin, V.I. Nevmerzhtskii, E.A. Tsoi, V.Yu. Baranov, G.Yu. Grigoriev, V.G. Grishina, S.A. Senchenkov, and S.G. Levchenko, in: *Abstracts of VII Congress of Pediatricians of Russia*, Moscow (2002), p. 349.
- G.Yu. Grigoriev and A.A. Sysoev, in: *Collection of Scientific Papers of MIPHI*, Moscow (2000), Vol. 8, p. 44.
- G. Grigoriev and A. Sysoev, in: *Abstracts of 15th International Mass Spectrometry Conference*, Barcelona (2000), p. 73.
- G. Grigoriev and A. Sysoev, in: *Proc. of Fifth Intern. Conf. on Physical-Chemical Processes in Selection of Atoms and Molecules* (CNIIAtominform, Moscow, 2000), p. 205.
- A. Babichev, G. Grigoriev, S. Malyugin, Sh. Nabiev, A. Nadezhdinskii, Ya. Ponurovskii, and D. Stavrovskii, in: *Proc. of IX Intern. Conf. on Physical-Chemical Processes in Selection of Atoms and Molecules* (Zvenigorod, 2004), p. 64.
- A. Nadezhdinskii, Ya. Ponurovskii, D. Stavrovskii, G. Grigoriev, and Sh. Nabiev, in: *Abstr. of Int. Conf. on Analytical Chemistry “Analytics of Russia”*, Moscow (2004), p. 116.