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Precise $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotopic ratio measurements for breath diagnosis with a 2 μm diode laser

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ABSTRACT

A laser spectrometer based on a distributed-feedback semiconductor diode laser at 2 μm is developed to measure the changes of $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotope ratio in exhaled breath sample with the CO_2 concentration of $\sim 4\%$. It is characterized by a simplified optical layout, in which a single detector and associated electronics are used to probe CO_2 spectrum. The cell has 10 cm long base length with 26.4 m optical path length in total and 330 cm^3 volume. The cell pressure and temperature are controlled at 50 Torr and 28°C, respectively. The best $^{13}\delta$ precision of 0.06‰ was achieved by using wavelet denoising and Kalman filter.

Keywords: Stable isotopes, Laser spectroscopy, Human breath, Wavelet denoising, Kalman filter.

1. INTRODUCTION

Exhaled breath testing, as a non-invasion and safe diagnostic method, is an ideal approach to evaluate health status and disease types. The bacterium *Helicobacter Pylori*, being able to dissociated the urea molecule by the urease producing ammonia and carbon dioxide, is believed to cause peptic ulcer in humans while infecting the gastric mucous layer. The carbon dioxide produced by the bacterium is expelled through the expired breath. It means that an infected people will expire more ^{13}C labeled carbon dioxide than a healthy person when they ingest a small amount of ^{13}C labeled urea. Therefore, through the measurement of CO_2 isotopic ratio in human breath, gastrointestinal diseases related to *Helicobacter Pylori* infection are able to be identified.

Usually, isotope ratio measurements are achieved by means of isotope ratio mass spectrometry (IRMS), which is extremely sensitive and have rather satisfactory accuracy and stability. However, there are many limitations for IRMS. The samples to be measured must be pre-processed to be separated from other mass-overlapping species. In addition, isotopomers having nearly the same molecular weight can not be distinguished. Finally, The IRMS are cumbersome, very expensive and difficult to implement on-line measurements.

A tunable diode laser instrument operating in the near infrared region (NIR) using readily available distributed-feedback (DFB) lasers which have excellent quality and small volume and do not require additional cooling

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equipment, can be implemented in a relatively inexpensive portable configuration suitable for a real time and online diseases diagnosis device. However, in the 1.6 μm region, due to the low line strength, an optical path length of more than 1 kilometer is needed for achieving ideal spectral signal-to-noise ratio. Eric. R. Crosson et al. [1] adopted cavity ring-down spectroscopy (CRDS) technique to measure carbon dioxide isotope ratio in 5% CO_2 exhaled breath and achieved a precision of 0.22‰. ED H. Wahl et al. [2] obtained 0.15 ~ 0.25‰ precision by using the same method and CO_2 concentration. By using an off-axis cavity-enhanced absorption spectrometer (OA-CEAS), V. L. Kasyutich et al. [3] reached a precision of 1.8‰.

The 2.0 μm spectral region offers a practical and ideal alternative for the measurement of $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotope ratio in exhaled breath. Besides the advantages mentioned above in near infrared region, the CO_2 line strength is sufficiently large at 2.0 μm , so that the CO_2 isotope ratio can be measured using an effective path length of only a few meters, which can be provided by many available multipass cell. In addition, the water absorption lines are weak and even could be avoided. By using a 2.0 μm tunable diode laser, S. N. Andreev et al. [4] obtained 0.07‰ measurement precision of $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotope ratio in human breath.

In the present study, we demonstrate a carbon dioxide isotope ratio spectrometer based on a tunable DFB diode laser operating at 2.0 μm . The precision and stability of the system are discussion by measuring $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotope ratio in simulating human breath CO_2 concentration of ~4%.

2. EXPERIMENTAL DETAILS

2.1 Spectrometer set-up

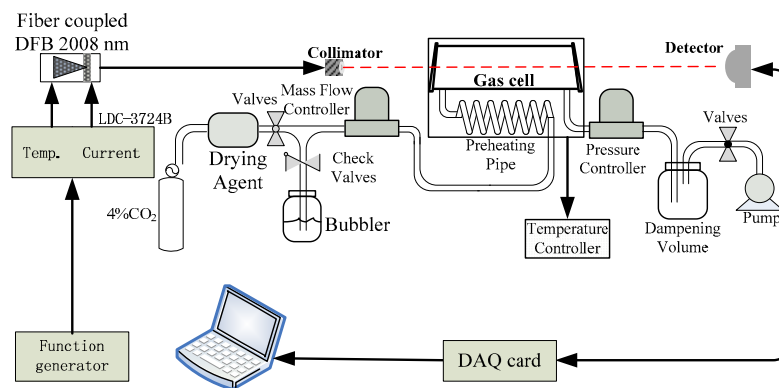


Fig. 1 Experimental set-up

The experimental sketch was shown in Fig. 1. The light source is a tunable room temperature operating DFB diode laser radiating a single mode at 2.0088 μm with an output power of 1 mW at 100 mA. The diode laser is driven by a controller (ILX-Lightwave, model LDC-3724C), capable of providing both the injection current and the temperature stabilization within 0.01 mA and 0.01 K, respectively. The wavelength tuning characteristics are 0.27 cm^{-1}/K and 0.13

cm⁻¹/mA rates. The laser beam, after collimated, is directly lead into a multipass cell (mirror diameter: 65mm, basal length: 10 cm, volume: 0.33 liter, effective optical path length: 26.4 m) filled with 4% CO₂ sample, and then detected by a thermoelectrically cooled photodiode InGaAs detector (THORLABS PDA10DT). The laser frequency was periodically scanned by means of a sawtooth wave at 9 Hz. The data was acquired through a 16-bit DAQ card (NI: USB-6353) which connected with a computer and analyzed by a LabVIEW program. A drying pipe was applied to dry sample as much as possible. The temperature in absorption cell was controlled by a two-order heating instrument: pre-heating and direct heating. A high precision pressure controller (MKS: 640B) was used to stabilize the pressure in cell. Gas flow was controlled at 40 SCCM (cubic centimeters per minute at standard temperature and pressure) by a mass flow controller (MFC).

2.2 Spectral acquisition and analysis method

The absorption spectrum of sample and reference gases was acquired successively. A cubic polynomial function was used to fit the spectroscopy for obtaining a baseline. Both sample and reference signal divide the baseline to normalize the spectrum. The selected line pairs for the measurement of CO₂ isotope ratio were ¹²CO₂ R(17) at 4978.2047 cm⁻¹ and ¹³CO₂ P(16) at 4978.0220 cm⁻¹[5].

The stable carbon dioxide isotope ratio is usually expressed in parts per thousand as a delta value which is given by:

$$^{13}\delta = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \quad (1)$$

Where

$$R = ^{13}C / ^{12}C \quad (2)$$

is the molecular ratio of heavy to light isotope of the sample. $R_{standard}$ is the stable isotope ratio in standard sample, which is the international PDB-standard (Belemnite of the Pee Dee Formation in South Carolina, [¹³C]/[¹²C]=0.011237).

Following the approach published by Erdelyi et al. [6], each scan window of the reference and sample gases were divided into two parts relate to the absorbance of ¹²CO₂ and ¹³CO₂ respectively, and then the absorbance for both isotopes were plotted for the sample and reference gases. The plot was fitted to a linear fit function, and the slopes of the fitted curves offer the concentration ratio as follows:

$$^{12}Slope = ^{12}C_{sample} / ^{12}C_{reference} \quad (3)$$

$$^{13}Slope = ^{13}C_{sample} / ^{13}C_{reference} \quad (4)$$

The ¹²C_{sample or reference} and ¹³C_{sample or reference} is the isotope gas concentration.

If the isotope ratio of reference gas is known as $R_{reference}$, and thus that of sample gas can be calculated as:

$$R_{sample} = \frac{^{13}C}{^{12}C} = \frac{^{13}Slope}{^{12}Slope} \times C_{reference} \quad (5)$$

The ratio of the fit slopes gave the $\delta^{13}C$ value formula expressed as :

$$^{13}\delta = \left(\frac{^{13}Slope}{^{12}Slope} - 1 \right) \times 1000 \quad (6)$$

The 12 slope and 13 slope represent the fit slope of $^{12}CO_2$ and $^{13}CO_2$ respectively. The standard deviation of the fitted line slope gives the minimum detectable concentration.

3. RESULTS AND DISCUSSION

3.1 Temperature and pressure stability

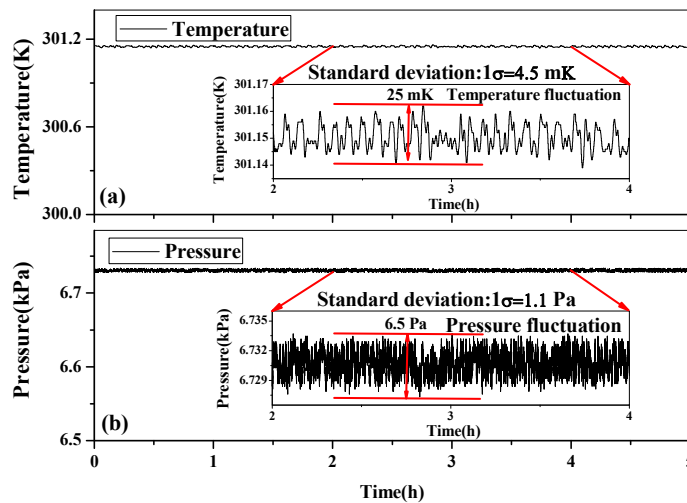


Fig. 2 The stability of (a) temperature and (b) pressure in absorption cell for 5 hours.

The insets shows their fluctuation amplitudes

The stability of temperature and pressure is very important for the measurement of carbon dioxide isotope ratio by using absorption spectroscopy technique. Fig. 2 shows the results of the long-term measurements of temperature and pressure in the cell. The temperature and pressure were found to be well controlled at 301.15 K and 6.66 kPa with fluctuation amplitude of 25 mK and 6.5 Pa illustrated in Fig. 2(a) and (b) respectively during 5 hours of continuous measurements.

The variation $\Delta\delta$ in the isotope ratio caused by a temperature shift ΔT is evaluated to a good approximation by [7]:

$$\frac{\Delta\delta}{\Delta T} = \frac{\Delta E}{k_B T^2} \quad (7)$$

Where k_B is the Boltzman constant, T is the temperature, and ΔE is the lower energy levels of the transitions associated with the carbon dioxide isotope molecular absorption lines. For the line pair used in this work, the ΔE is 681 cm^{-1} and so (7) yields a $\Delta\delta$ 11.2‰/K. The 1σ standard deviation of temperature was 4.5 mK during this measurement for 5 hours, so the temperature fluctuations contributed a precision of 0.049‰ to the precision of this instrument. But the effect of pressure on instrument precision could be neglected considering its measurement precision of 1.1 Pa.

3.2 Wavelet denoising

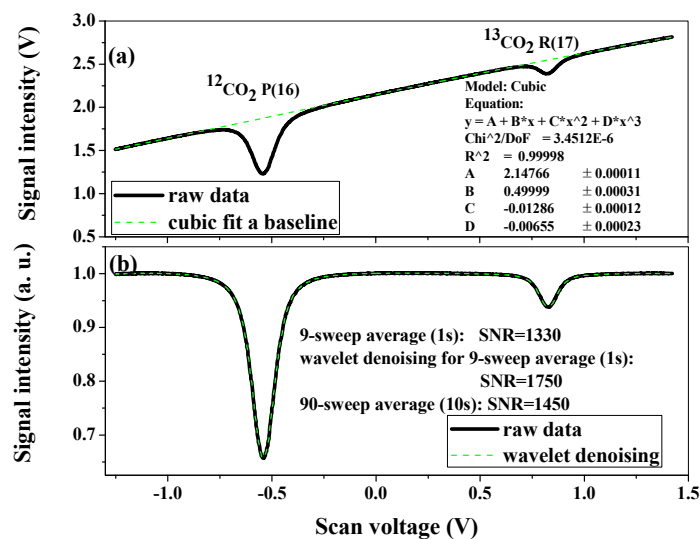


Fig. 3(a) A typical 9-sweep (1s) average absorption spectrum (solid line) and its three order polynomial fitted baseline (dashed line).
 (b) A normalized 9-sweep (1s) average absorption spectrum (solid line) and the spectroscopy after wavelet denoising (dashed line).

Fig. 3(a) shows that the three order polynomial curve is suitable to simulate spectral baseline. Significantly, this method removes the laser power fluctuation efficiently. At the mean time, wavelet denoising was used to improve SNR, which is clearly demonstrated in Fig. 3(b). From the figure, we could see that, wavelet denoising is better than averaging, and one more advantage is that, it saves time, which is very important in the real time measurement.

3.3 Allan variance

The instrument performance and stability was tested using the Allan variance method. The $^{13}\delta$ value was measured

with 1 Hz sampling rate during a 800 s period. Time series of this data is shown in Fig. 4. An optimum averaging time of 20s can be derived according to the plot. This corresponds to a short term precision of 0.06‰. A precision of 0.25‰ was obtained for 1 s average.

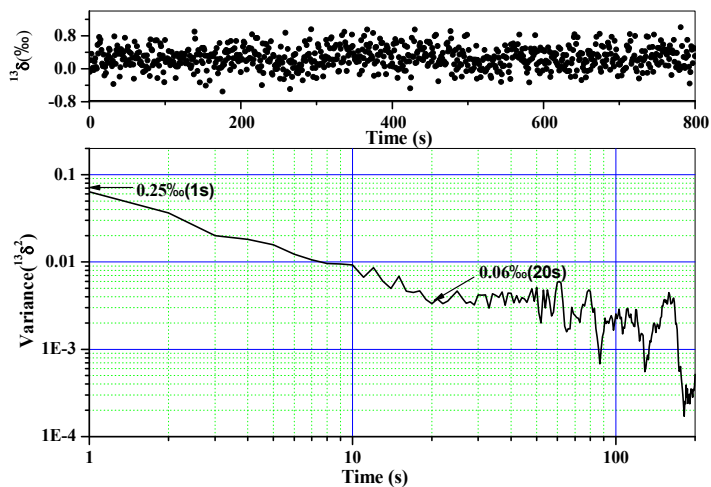


Fig. 4 Time series of $^{13}\delta$ value and Allan variance plot. An optimum averaging time of 20 s is shown.

3.4 Kalman filter

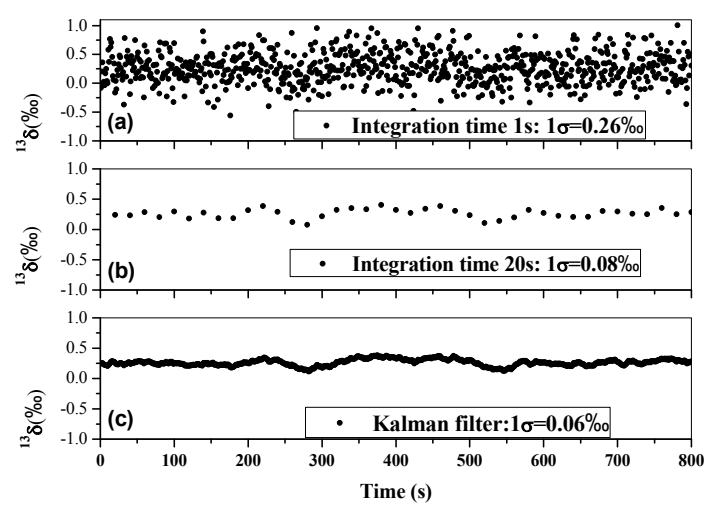


Fig. 5(a) 1s-raw data of the δ - value and its corresponding Kalman filter output 5(c), 5(b) the average of 30 δ - value.

Kalman filter was firstly applied for the isotope measurement by T Wu et al. [8]. In the present work, the Kalman filter was also used to process the $^{13}\delta$ - values, which is illustrated in Fig. 5(a) acquired with a 1Hz sampling rate in 4% sample. Fig. 5(c) shows the filtering results for a period of 800 s. A precision of 0.06‰ was achieved. The precision is better than that of 0.08‰ shown in Fig. 5(b) which was derived with an integration time of 20 s.

4. CONCLUSIONS

In summary, base on a tunable DFB diode laser, an instrument for $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotope ratio in human breath was developed. A precision of 0.06‰ was derived by combining different approaches, including hardware and The performance and precision of this device satisfy the requirements of real-time measurement of carbon dioxide ratio in human breath.

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