

Simultaneous NO and CO₂ measurement in human breath with a single IV–VI mid-infrared laser

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A tunable diode laser absorption spectroscopy (TDLAS) system equipped with a IV–VI mid-IR laser operating near $5.2\ \mu\text{m}$ was used to measure exhaled nitric oxide (eNO) and carbon dioxide (CO₂) simultaneously in human breath over a single exhalation. Breath was sampled in real time, and eNO levels were measured from seven volunteers, two steroid-naïve asthmatics and five nonasthmatics. Measured CO₂ levels were used as an internal standard to verify correct breath collection and calculate eNO values. Calculated eNO concentrations agreed well with reported values for asthmatic and nonasthmatic individuals. © 2002 Optical Society of America

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Nitric oxide is an important physiological molecule. It plays a role in airway inflammatory diseases, respiratory tract infections, neurotransmission, vascular regulation, and host defense.¹ Clinical measurements of exhaled nitric oxide (eNO) have been shown to be useful for noninvasively diagnosing inflammatory-related diseases.^{2–5} Chemiluminescence is at present the most commonly used technique for measuring eNO. Reported chemiluminescence eNO levels, however, vary drastically for normal individuals by as much as 80 parts in 10^9 (ppb).¹ Such large variations can be explained by factors that affect chemiluminescence instruments, including unstable temperatures, variations in gas calibration procedures, varying exhalation flow rates, and interference from endogenous or exogenous water vapor and CO₂.^{6–8} Tunable diode laser absorption spectroscopy (TDLAS) by use of mid-IR IV–VI lasers has more recently been used to measure endogenous eNO and to correlate its levels with various inflammatory diseases, including asthma and chronic obstructive pulmonary disease.^{9,10} More-widespread use of mid-IR TDLAS technology in clinical settings will be possible if two problems are solved. First, calibration of the instrument requires skilled operators, expensive consumable gases, and (or) complicated gas dilution systems. Second, most commercial mid-IR TDLAS systems require the use of liquid nitrogen, another consumable that is not readily available in many clinical offices. In this Letter we present an eNO breath-analysis system that overcomes the limiting factors of TDLAS by a novel method of internal calibration and implementation of closed-cycle refrigeration technology. Internal calibration is accomplished by simultaneous measurement of CO₂ with the same laser that is used to measure eNO. This technique

reduces the effects of laser-power fluctuations and allows consistent measurement of eNO concentrations over time.

The experimental setup is shown in Fig. 1. A double-heterostructure mid-IR semiconductor laser was mounted on a temperature-controlled stage inside a sealed cryostat. The cryostat was maintained at 102 K by use of a closed-cycle cryogenic refrigerator that required no liquid nitrogen refills. An integrated heater located just beneath the laser maintained constant operating temperatures with an accuracy of ± 0.01 K. Various optical mirrors and a lens were used to direct and collimate the laser beam through a 107-m White cell and onto a HgCdTe mid-IR photodetector.

Two function generators provided the control waveforms for tuning and modulating the laser across

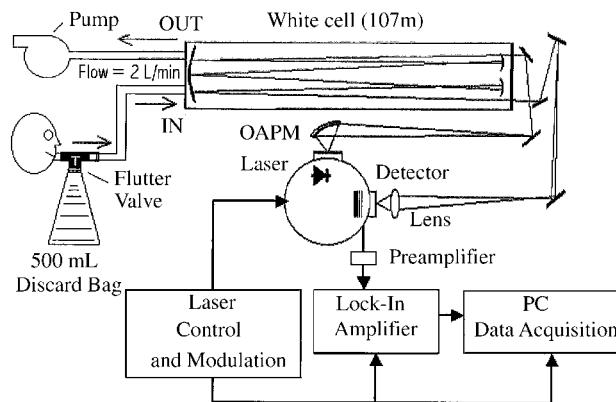


Fig. 1. Experimental setup, including a cryostat (circle) with a laser and detector inside, an off-axis parabolic mirror (OAPM), a White cell, and control and acquisition components.

NO, CO₂, and H₂O absorption features from 1912.4 to 1913.0 cm⁻¹. A 22-kHz triangle waveform was superimposed upon a 40-Hz sawtooth waveform, a technique known as sweep integration coupled with wavelength modulation. The detector signal after preamplification was sampled at twice the modulation frequency by a commercial lock-in amplifier to yield second-harmonic (2*f*) spectra. An analog-digital converter acquired 500 data points per scan from the output of the lock-in amplifier and stored the scan in the memory of a personal computer for analysis.

To reduce high-frequency noise, we ran 75 scans, coaveraged and digitally filtered with a custom graphical user interface software program. Slight thermal variations of the laser can cause shifting of the absorption spectra. Instead of using a second gas cell and detector as a reference, which can greatly add to the cost of a TDLAS system, we used a strong H₂O absorption line with an unambiguous peak at 1912.57 cm⁻¹ to align the same H₂O peak in the acquired spectra before averaging to reduce smear.

A mechanical pump reduced the gas-cell pressure to 20 Torr and induced a 2 L/min flow that was held constant with mass flow controllers. Teflon tubing was used for all connections in the breath-collection apparatus to reduce NO reaction with the sidewalls. A disposable mouthpiece was connected to a 500-mL discard bag and Teflon tubing by a custom-designed T piece. A discard bag collected the first half of the exhaled breath, which contained mainly headspace breath. Once the discard bag was full, a one-way flutter valve prevented discarded breath from reentering the T piece. The remaining exhaled breath, or enriched alveolar breath, was collected for 20 s at a constant flow rate of 2 L/min. Subjects were instructed to force exhalation, which helps to close the vellum and limit nasal NO contamination.⁶ Institutional Review Board approval for human subject research was granted by the University of Oklahoma, Norman Campus, and each volunteer signed a consent form before breath donation.

Spectra acquired during exhalation were compared with a reference spectrum of nonasthmatic human breath, shown in Fig. 2. The NO absorption feature at 1912.79 cm⁻¹ and either the CO₂ absorption feature at (A) 1912.69 cm⁻¹ or the one at (B) 1912.97 cm⁻¹ were measured simultaneously. We subtracted the peak signal voltages for NO and CO₂ (*V*_{NO} and *V*_{CO₂}, respectively) in the reference spectrum from the average baseline voltage (*V*_{BL}) to form a voltage ratio, (*V*_{NO} - *V*_{BL})/(*V*_{CO₂} - *V*_{BL}). A least-squares-fitting routine compared the absorption features of NO and CO₂ in the acquired spectra with the equivalent absorption features in the reference spectrum to compute relative magnitudes of absorption (*a*_{NO} and *a*_{CO₂}).¹¹ Example *a*_{NO} and *a*_{CO₂} trends over time during single exhalations from asthmatic and nonasthmatic volunteers are shown in Fig. 3. The maximum values of *a*_{NO}(*t*) and *a*_{CO₂}(*t*) over the duration of exhalation that deviated most from the prebaseline and postbaseline values were used in calculating exhaled levels. Tests were accepted if *a*_{CO₂} was sufficiently large to indicate a proper breath donation. This method accounts for

when a subject exhales NO concentrations that are less than the ambient NO concentrations. An overall absorption ratio, *A*_{NO}/*A*_{CO₂}, was then computed with Eq. (1):

$$\frac{A_{NO}}{A_{CO_2}} = \frac{a_{NO_{MAX}}}{a_{CO_2_{MAX}}} \frac{(V_{NO} - V_{BL})}{(V_{CO_2} - V_{BL})}. \quad (1)$$

The *A*_{NO}/*A*_{CO₂} ratio was used to estimate eNO levels, assuming that a normal human being exhales a peak CO₂ concentration (*C*_{CO₂}) of approximately 4%.¹² Equation (2) was used to calculate the approximate concentration of eNO (*C*_{eNO}) and was derived from Beer's law:

$$C_{eNO} = \left(\frac{A_{NO}}{A_{CO_2}} \right) \left(\frac{g_{NO}}{g_{CO_2}} \right) \left(\frac{S(\nu)_{CO_2}}{S(\nu)_{NO}} \right) C_{CO_2} \quad (2)$$

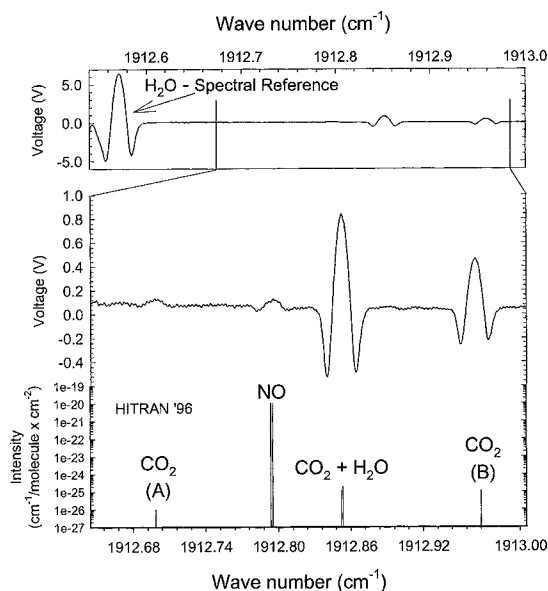


Fig. 2. Second-harmonic spectrum of measured-breath NO, CO₂, and H₂O near 1912.79, 1912.69, and 1912.55 cm⁻¹, respectively, with corresponding HITRAN data plotted below.

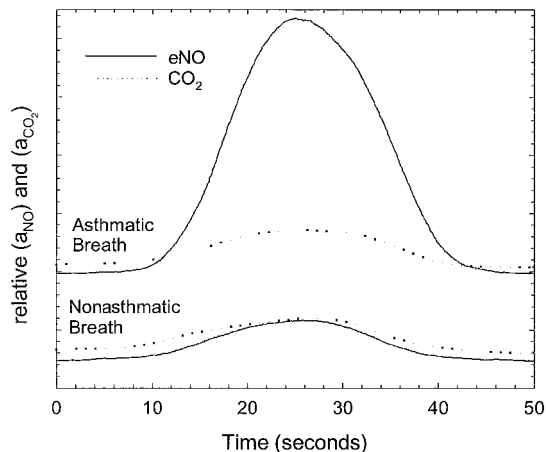


Fig. 3. Trends in recorded magnitude absorption, *a*(*t*), plotted for eNO and CO₂ during exhalation from a steroid-naive asthmatic volunteer and a normal volunteer.

Table 1. Measured eNO Concentrations [in ppb] from Seven Subjects

CO ₂ ^a	Day	Asthmatics		Nonasthmatics				
		1	2	3	4	5	6	7
A	1	25	40	14	14	4	11	8
A	6	25	28	11	17	6	12	4
B	28	22	27	5	13	6	12	6
B	29	22	31	8	12	4	9	6
Mean ± standard deviation		24 ± 2	32 ± 6	10 ± 4	14 ± 2	5 ± 1	11 ± 1	6 ± 2

^aA, CO₂ at 1912.69 cm⁻¹; B, CO₂ at 1912.97 cm⁻¹.

The line strengths, $S(\nu)$, and pressure-broadening coefficients, g , for NO and CO₂ are found in the HITRAN database.¹³ Equation (2) assumes that laser power is approximately equivalent across the NO and both CO₂ absorption features, since they are in close spectral vicinity to each other, <0.2 cm⁻¹.

Concentrations of eNO from seven volunteers, two steroid-naive asthmatics and five nonasthmatics, were measured four times each over a period of one month. The results are shown in Table 1. The first two measurements used the CO₂ absorption feature at 1912.69 cm⁻¹ (reference A), and the last two used the CO₂ absorption feature at 1912.96 cm⁻¹ (reference B). Nonasthmatics had a calculated mean eNO concentration of 9.1 ± 4 ppb, consistent with normal eNO levels reported by Yates.¹⁴ Compared with the nonasthmatics, the asthmatic volunteers exhaled increased eNO and had a mean eNO concentration of 28 ± 6 ppb. Other medical inflammatory-related conditions can affect eNO concentrations. For example, elevated eNO levels from a nonasthmatic subject with an upper respiratory tract infection (26.3 ppb) and low eNO levels from a severe asthmatic treated with corticosteroids twice daily (7.4 ppb) were observed; both are consistent with the medical literature.¹

The novel method described in this Letter utilizes the ability of a single IV–VI semiconductor mid-IR laser to measure eNO and CO₂ simultaneously in exhaled breath and calculate eNO levels without the need for calibration. Although this study contained a small sample size, repeated eNO measurements provided consistent results and correlated well with a diagnosis of asthma or other inflammatory conditions such as a respiratory tract infection. We demonstrated the spectral versatility of the method by obtaining measurements by use of two CO₂-absorption features with different line strengths. Variations in exhalation flow, a major factor affecting the determination of accurate eNO concentrations by use of the chemiluminescence technique, are not a problem with this TDLAS technique, since simultaneous CO₂ measurement provides an internal calibration parameter that accounts for any variations in flow. Internal calibration, as described in this Letter, could

be utilized in various mid-IR technologies to eliminate the need for calibration and improve the reliability of measuring NO and other molecules in exhaled breath.

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