# Breath Testing with a Mid-IR Laser Spectrometer

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## ABSTRACT

A mid-IR tunable diode laser absorption spectrometer (TDLAS) equipped with a multiple-pass gas cell was use to measure breath samples from a number of student volunteers at the University of Oklahoma. Test subjects included one to two pack-a-day cigarette smokers and non-smokers. The concentrations of four different molecules, $N_2O$, $^{12}CO_2$, $^{13}CO_2$ and CO, were measured by each laser scan in the 2206.1 $cm^{-1}$ to 2207 $cm^{-1}$ spectral range. The average concentration of nitrous oxide ($N_2O$) increased slightly for smokers versus non-smokers and was generally higher (12%) than the ~255 ppm concentration measured in ambient air. Carbon monoxide concentrations, however, were much higher in breath samples from cigarette smokers. Ambient concentrations of carbon monoxide, ~0.4 ppm, increased from ~1.0 ppm in non-smokers to levels over 13.4 ppm in smokers. These measurements provide clear evidence of the well-known effect that cigarette smoking has on replacing oxygen with carbon monoxide in human hemoglobin. Carbon dioxide concentrations of smokers were generally decreased by ~12%. Mid-IR laser measurements also provided $^{13}CO_2/^{12}CO_2$ isotope ratio values, and smokers had a ~30% greater concentration of isotopic $^{13}C$ in their breath. The possible mechanisms for $^{13}CO_2$ isotopic increases are at present unknown. Overall, long-path TDL spectroscopy of exhalation products is a uniquely powerful tool. The TDL systems can be used for noninvasive diagnosis of a wide range of metabolisms and pathologies.

**Keywords:** mid-IR laser, absorption spectroscopy, breath testing, carbon monoxide (CO), carbon dioxide ($CO_2$), nitrous oxide ($N_2O$), isotopes ratio, cigarette smoke, ambient air.

## 1. INDRODUCTION

There is considerable interest for developing noninvasive diagnostic breath monitoring procedures for assessing metabolic reactions using tunable diode lasers. Tunable diode laser absorption spectroscopy is one of the most capable and sensitive techniques for high-resolution multicomponent gas detection. Lead-salt tunable diode lasers (TDL) fabricated mostly from columns IVA and VIA of the periodic table, emit in the 3-20 μm range. This spectral range covers fundamental ro-vibrational bands with strong absorption coefficients of many important biological exhalation products such as NO, CO, $CO_2$, $N_2O$, $NH_3$, $C_2H_6$, $H_2O$, $H_2O_2$.

In general, noninvasive exhalation monitoring is based on measuring either specific metabolites or selective sets of non-radioactive isotopes, which correspond to specific metabolic processes. For example, isotopic ratios of $^{13}CO_2/^{12}CO_2$ can be measured after the ingestion and metabolism of $^{13}C$ labeled urea, fructose, aminopyrine, etc. Alternatively, specific by-products related to distinct metabolic processes can be observed directly, such as acetone with diabetes or carbon disulfide with schizophrenia. Overall, there is significant interest throughout medicine for the development of rapid, noninvasive monitors for oxidative stress. At present, several different classes of exhalation products have been shown to correlate to oxidative stress. These reporter molecules include hydrocarbons, as for example ethane and pentane derived from omega 3 and omega 6 fatty acid oxidation, hydrogen peroxide, hypochlorous acid, carbon monoxide and nitrous oxide. In this study,



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$N_2O$ and CO are observed along with $^{13}CO_2/^{13}CO_2$ isotopic ratios. In general, there is an extremely large range of volatile exhalation compounds that may be of use. In essence, TDL systems have the sensitivity to be a very unique diagnostic tool.

Traditionally, direct absorption spectroscopy has been used extensively for monitoring of trace species concentrations in gaseous samples. However, this technique frequently lacks the required sensitivity for many applications. Parts-per-billion sensitivity is required for monitoring trace gases and less abundant isotopic species important to exhaled chemistry. To achieve such a high sensitivity range in laser absorption spectrometers a long optical absorption path up to several tens of meters is required. In this work, we have employed the recently developed multipass cell configuration or astigmatic variant of the Herriott cell. This configuration makes feasible the folding of a long path length into a small volume while allowing the circulating laser beam to more fully fill the whole volume of the cell.[1] Our compact TDL spectrometer equipped with the multipass cell provides excellent analytical characteristics of high sensitivity, high response speed and very good line selectivity due to the narrow-linewidth (<100 MHz) of lead-salt diode lasers. Moreover, mid-IR TDL spectroscopic monitoring offers a simple and effective way for performing real-time isotopic measurements in gaseous compounds.

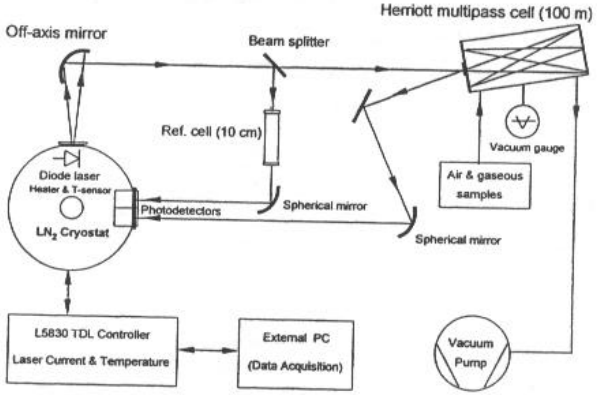


**Figure 1.** Experimental setup of the tunable diode laser (TDL) absorption spectrometer for the detection of trace gaseous species in human exhaled breath.

We demonstrated the capabilities of our system by monitoring the physiological concentrations of CO, $^{12}CO_2$, $^{13}CO_2$, $N_2O$, $H_2O$ present in exhaled breath of smokers and non-smokers in real-time with no sample preparation. Although our working spectral range (2206 to 2208 $cm^{-1}$) is not the optimum range for these molecules, it was possible to measure the molecular concentrations with a precision of about 4%. Trace gas monitoring in human breath by this technique can open new opportunities for medical diagnostics.

## 2. Experimental Techniques

A simplified experimental layout employed in this work is illustrated in Figure 1. The optical system consists essentially of three components that are integrated on a common small optical bench (17"x33") to give a very compact design. A liquid nitrogen cooled dewar (T >78K) which contains lead salt diode lasers and MCT photodetectors, optical components for directing the laser beam, and a Herriott multipass cell with 100-m-long optical path (New Focus 5612) are mounted on the optical table. In the present work, Laser Photonics Inc. supplied the TDL optical design (Model L5310), the laser current and temperature controller (Model L5830) and the laser source dewar (Model L5736) containing the L5600 series tunable diode lasers.

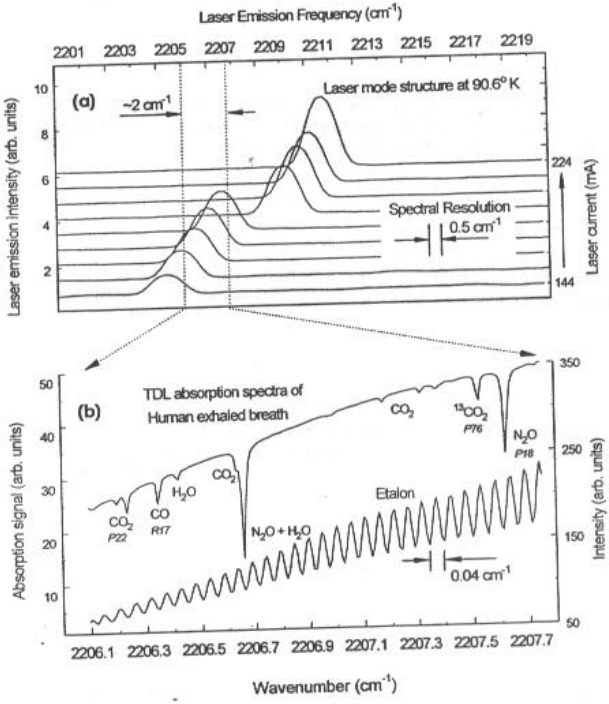


**Figure 2.** **(a)** Mid-IR laser emission spectra at 90.6 K for injection currents of 144 to 224 mA in 10 mA increments as measured by a Fourier transform infrared spectrometer with a 0.5 $cm^{-1}$ resolution. **(b)** Absorption spectrum of a non-smoker human breath samples (40 Torr, 100-m-path-length) around 2206.9 $cm^{-1}$ (top) and Ge etalon calibration spectrum induced by the single mode laser beam with a free spectral range of 0.048 $cm^{-1}$ (bottom).

An initial experiment was performed using an Oriel MIR 8000 Fourier transform infrared (FTIR) spectrometer with 0.5 $cm^{-1}$ resolution in the laser beam to identify regions of single-mode operation and to evaluate laser-tuning characteristics.

Those regions which coincide with absorption lines of interest were selected for the experiment. Fig. 2a shows the mode structure and tuning characteristics of our 4.5 μm diode laser (L5621) from 2200 to 2220 $cm^{-1}$ for different injection currents from 144 to 224 mA in steps of 10 mA. Also shown, the single mode laser emission region with ~2 $cm^{-1}$ spectral width that we have used for the experiments. In the spectral region from 2206 $cm^{-1}$ to 2208 $cm^{-1}$, corresponding to the laser heat-sink temperature 90.6 K, we performed absorption measurements by programming the TDL spectrometer to scan the laser wavelength rapidly (~0.7 msec) and repetitively over the absorption lines of interest. The absorption signal of the detector output is then recorded and averaged using digital electronics. As shown in Fig. 2b, there are five major spectral features. To identify these lines we compared their position and strength with calculated absorption lines from the HITRAN-96 database. The spectral absorption lines are: carbon monoxide transition in the R branch of the fundamental band (1-0) at 2206.35 $cm^{-1}$ *R(17)*, $^{12}CO_2$ *P(22)* transition at 2206.24 $cm^{-1}$, isotopic line $^{13}CO_2$ *P(76)* transition at 2207.52 $cm^{-1}$ and $N_2O$ *P(18)* transition at 2207.62 $cm^{-1}$.

In tunable diode laser absorption spectroscopy the determination of mole fractions of gaseous species $c$ is based on application of the Beer-Lambert's relation.

$$c = \frac{\ln(I_o/I)}{\alpha_\nu . l . p_t}, \tag{1}$$

Where $I_o$ and $I$ are incident (zero absorption) and peak absorbed laser intensities, $l$ the optical path length, $\alpha_\nu$ the absorption coefficient at frequency $\nu$, and $p_t$ the total cell pressure. The absorption coefficient $\alpha_\nu$ is a function of molecular line intensity and the spectral line profile. In TDL isotopic analysis the relative abundance of two isotopic molecules (1 and 2) may be written as follows:

$$\frac{c_1}{c_2} = \frac{\ln(I_o/I)_1}{\ln(I_o/I)_2} \frac{s_2}{s_1} \frac{r_1}{r_2} k_i, \tag{2}$$

Where $c_1$ and $c_2$ are the concentrations of the two isotopic molecules, $s_1$ and $s_2$ are the line strengths of the probed transitions and $r_1/r_2$ is the natural isotopic abundance ratio of the two molecules. At low working pressure (up to several Torr) where the Doppler broadening is dominant, $k_i = k_D$. Where $k_D$ is,

$$k_D = \frac{\nu_{o1}}{\nu_{o2}} \sqrt{\frac{m_2}{m_1}}, \tag{3}$$

$\nu_{o1}$ and $\nu_{o2}$ are the center frequencies of the absorption lines, and $m_1$ and $m_2$ are the molecular weights of the molecules. In the higher working pressure region where the spectral line is assumed to have the Lorentzian profile, $k_i$ would be the ratio of line broadening parameters ($k_i = k_L$). Where $k_L$ is,

$$k_L = \frac{g_2}{g_1}, \tag{4}$$

$g_1$ and $g_2$ are the line broadening coefficients of the molecules at frequencies $\nu_{o1}$ and $\nu_{o2}$. In either case $k_i$ is around 1.

## 3. Results and Discussions

Breath samples from a number of smoking and non-smoking volunteers were collected into 500 $cm^3$ impermeable Tedlar gas-sampling bags (Jensen Inert Products). Breath, ambient air and cigarette smoke samples were introduced separately into the evacuated (<1 Torr) Herriott cell immediately following collection at room temperature and observed at 40 Torr total pressure. The upper trace in Fig. 2b shows typical results obtained with the 100 meter multipass cell containing non-smoker human breath in the 2206 to 2208 $cm^{-1}$ spectral region. The lower trace in Fig. 2b represents the 25-mm Ge etalon fringes induced by the single mode laser beam and serves as a calibration spectrum. The overall absorption spectra of TDL breath monitoring for smoking and non-smoking individuals and ambient air are shown in Fig. 3. Quantitative analyses of breath, cigarette smoke, and ambient air samples are listed in Table 1.

**Table 1.** Quantitative analyses of the breath samples, cigarette smoke and ambient air.

| Subjects | $^{12}CO_2$ | $^{12}CO$ | $^{13}CO_2$ | $N_2O$ | $^{13}CO_2/^{12}CO_2$ |
|---|---|---|---|---|---|
| smoker #1 | 2.12 % | 8.26 ppm | 300 ppm | 290 ppb | 1.42 % |
| smoker #2 | 2.0 % | 13.45 ppm | 260 ppm | 306 ppb | 1.32 % |
| smoker #3 | 2.7 % | 12.47 ppm | 350 ppm | 276 ppb | 1.3 % |
| light-smoker | 1.53 % | 3.8 ppm | 187 ppm | 293 ppb | 1.2 % |
| non-smoker #1 | 2.5% | 1.3 ppm | 247 ppm | 274 ppb | 1 % |
| non-smoker #2 | 2.5 % | 1 ppm | 253 ppm | 280 ppb | 1 % |
| non-smoker #3 | 2.2 % | 0.82 ppm | 210 ppm | 277 ppb | 1 % |
| non-smoker #4 | 2.3 % | 0.85 ppm | 225 ppm | 304 ppb | 1 % |
| cigarette smoke | 1.8 % | 135 ppm | 200 ppm | 1.5 ppm | 1.1 % |
| ambient air | * | 0.32 – 0.5 ppm | * | 230 - 280 ppb | - |

* Not detectable at the specified spectral range.

TDL breath monitoring shows the concentrations of carbon monoxide (CO) in cigarette smoke is about 135 ppm, which is almost 270 times greater than CO level in ambient air. Ambient concentrations of carbon monoxide, ~0.4 ppm, increased from ~1.0 ppm in non-smokers to levels over 13.4 ppm in smokers (with ~2 packs / day). These measurements provide clear evidence of the well-known effect that cigarette smoking has on replacing oxygen with carbon monoxide in human hemoglobin. Carbon monoxide has ~200 times greater affinity for hemoglobin (Hb) than oxygen, and thus smokers typically exhibit between 1% to 3% greater circulating HbCO levels. Carbon monoxide is also a competitive inhibitor for $O_2$ binding to hemoglobin, myoglobin, cytochrome c oxidase and the variety of cytochrome p450 systems.

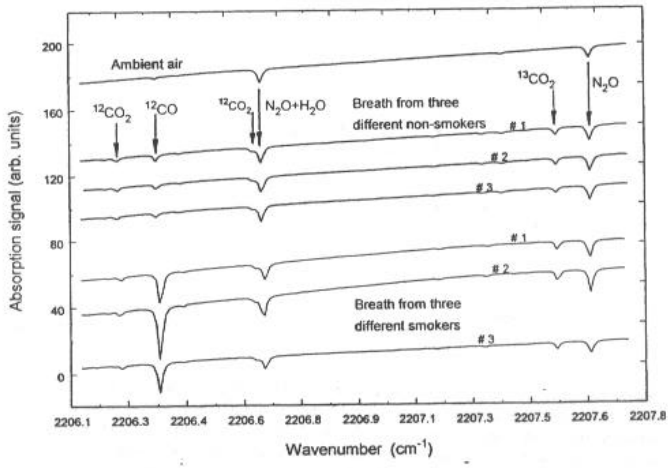


**Figure 3.** Absorption spectra of TDL breath testing from two smoking and non-smoking groups and ambient air.

Metabolically, endogenous CO can also be produced as the initial breakdown product during heme oxidation. Heme oxidation occurs during the natural heme protein turnover but can be enhanced under a variety of oxidative stresses and hemolytic anemia conditions. Endogenous CO removal via breath is sensitive to blood pH variations and to the extent of blood saturation with oxygen and thus could characterize gas transportation properties of blood, its acid-base balance and its biochemistry.[2] Monitoring of endogenously produced CO in non-smoking subjects should be a good indicator for a number

of blood disorders.[3] It is expected that the higher level of CO in smokers observed here is predominately due only to the increased exposure to CO in smoke.

Carbon dioxide ($^{12}CO_2$) concentrations of smokers were generally decreased by ~12% whereas $^{13}CO_2$ isotopic levels were increased ~30% compared to non-smokers. $CO_2$ is the final oxidation product during mitochondrial respiration but can also be generated during cytoplasmic oxidation processes. $CO_2$ is an $O_2$ metabolite and non-smokers have more $O_2$ in their blood stream. It is interesting to speculate that the reduced $CO_2$ level in smokers reflects the general inhibition of the Hb-dependent transport of $O_2$. In Figures 2b and 3, the spectral feature at location 2207.52 $cm^{-1}$ is due to the $^{13}C$ labeled carbon dioxide molecules that are naturally present in the human breath. The measured $^{13}CO_2/^{12}CO_2$ ratio in smokers (~1.3%) were found to be about ~20% to ~30% greater than the natural $^{13}CO_2/^{12}CO_2$ isotopic ratio (1.12%). The reason for this general enrichment of the heavier $^{13}CO_2$ isotope is not known, but the difference in isotopic ratio is unlikely due to dietary habits associated with C4 plants, which are rich in $^{13}C$, versus C3 plants, which have lower $^{13}C$ concentrations.

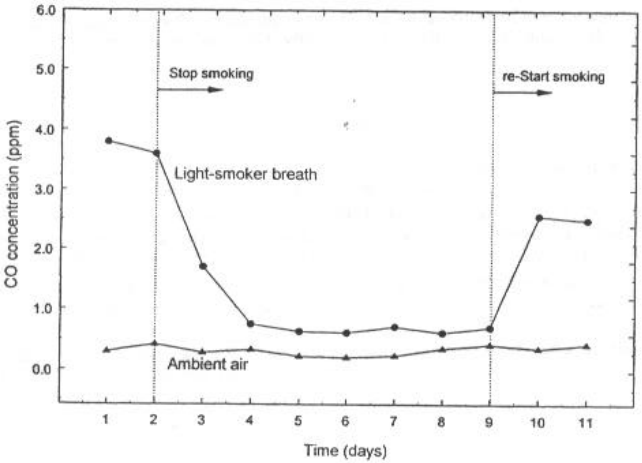


**Figure 4.** Carbon monoxide variation in breath of a light-smoker before after and during one-week non-smoking (upper trace) compared to the variation of CO concentration in ambient air (lower trace).

Our TDL breath-monitoring showed only slight differences in nitrous oxide levels between the breath samples of smoking and non-smoking groups. Both levels were slightly higher than the averaged ambient air concentrations. Based on the TDL results, cigarette smoke contains 1.5 ppm nitrous oxide which is ~5 times greater than that in ambient air. The average concentration of nitrous oxide increased slightly for smokers versus non-smokers and was generally higher (12%) than the ambient $N_2O$ concentrations of ~255 ppm. $N_2O$ is typically considered to be produced through anaerobic microbial metabolism during denitrification processes. Recently, however, it has been observed that $N_2O$ can be produced endogenously in humans by reduction of nitric oxide, a messenger molecule that promotes vasodilatation at low levels but enhances programmed cell death at high levels. The rate of $N_2O$ production in young children and in the aged is higher than that in healthy adults.[4]

In a related experiment, a light smoker volunteer (~1 pack / day) stopped smoking for one week. Fig. 4 shows the shift in carbon monoxide (CO) level in his breath before, during and after the not-smoking period. As represented in Fig. 4, CO concentrations in a light smoker's breath takes about 2 days to drop from ~3.8 ppm to the non-smoking CO levels.

## 4. Summary and Conclusion

In summary, we have demonstrated that a lead-salt diode laser absorption spectrometer combined with a long optical path cell can be conveniently applied to human breath testing. The gas species analyzed in human breath included CO, $N_2O$ and $CO_2$ isotopic ratios. The technique is noninvasive and insensitive to the presence of water vapor (up to 3%) in human breath, which can cause troubles in techniques such as mass spectrometry. In TDL absorption spectroscopy, analyzing gases in a complex mixture like a breath sample is free from spectroscopic interference from other molecules and isotopes with the same nominal mass. Future improvements can be made by choosing stronger absorption lines, using second harmonic detection technique to increase signal to noise ratios and using dynamic subtraction routines to determine exogenous versus endogenous gas production. The use of multiple laser systems can be expected to be extremely useful for covering the full range of metabolic reporter molecules.


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