CO-detection from biological tissues using a mid-infrared laser based gas sensor

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The motivation for this work has been to detect and assess the role of certain gases that are important in biomedical systems. In this report we describe the detection of CO at ppb levels from vascular smooth muscle cells (1×10^7) in the basal state using a mid-infrared laser based gas sensor.

Recent work indicates that carbon monoxide (CO) produced by heme oxygenase can play a role as a physiological messenger similar to nitric oxide[1]. Since the CO production from biological tissues is extremely small (~ 100 ppb), the measurement technique of the CO concentration has been limited to gas chromatography. Although this method is sensitive, it cannot measure the CO concentration directly, and requires several time consuming intermediate steps of chemical reactions (about 15 min).
Infrared absorption spectroscopy using a difference frequency generation (DFG) technique[2] is an attractive alternative approach for the detection of trace levels of biological CO. Furthermore, simple absorption measurements can detect CO directly. Unlike in gas chromatography, we avoid the addition of any chemicals which react with CO in order to determine the concentration. This work demonstrates the use of a novel sensitive, selective mid-infrared gas sensor capable of detecting biological CO at the ppb level in real-time.
A periodically-poled lithium niobate (PPLN) crystal is pumped by a convenient pseudo tunable high power diode laser (ie, 1.5 W single-frequency cw titanium: sapphire ring laser) operating at 864.86 nm and a compact diode pumped, non-planar monolithic ring Nd:YAG laser operating at 1064.6 nm with an output power of 750 mW. The output of both lasers is coupled into a single mode fibers using free space couplers with coupling efficiencies of 60%. Fiber polarization controllers were used to set the polarization states for both input beams to be linear and vertical. The two fiber coupled pump sources were combined into a single mode fiber using 850/1080 nm fiber wavelength division multiplexer (WDM) as an effective beam splitter. An AR coated achromatic 10 mm focal length lens was used to image the beam spot from the output fiber end into the 19 mm long PPLN crystal, which contain eight gratings with periods of 22.4 μm to 23.1 μm in increments of 0.1 μm. A grating period of 22.8 μm was required for DFG at 4.61 μm which corresponds to a suitable CO absorption line [R(6)] without interference from other gases. The crystal temperature was controlled to 23°C for maximum spectral quasi-phasematching. The mid-infrared DFG output was collimated by an uncoated 50 mm focal length CaF_2 lens and the residual pump beams were blocked by an AR coated Ge filter. The DFG radiation at 4.61μm was measured to be 15 μW. A part of 20% of the IR output was deflected by a coated ZeSe beamsplitter and monitored by liquid-nitrogen-cooled InSb detector. The beam transmission through the beamsplitter was focused into a multipass absorption cell which provided an 18.3 m optical path length with 90 passes through a compact sample volume of 400 cm^3. The cell was connected to a vacuum manifold that included a pressure gauge and a 21-G needle for collecting the sample gas. After exiting the cell, the IR beam was collected by an off-axis

parabolic mirror and focused onto a second liquid-nitrogen-cooled InSb detector. The detectors had a noise-equivalent power (NEP) of 0.8 pW $(Hz)^{-1/2}$. For balanced detection of CO in ambient air the IR beam path to the first detector was set to be nearly equal to the beam path outside the multipass cell to the second detector.
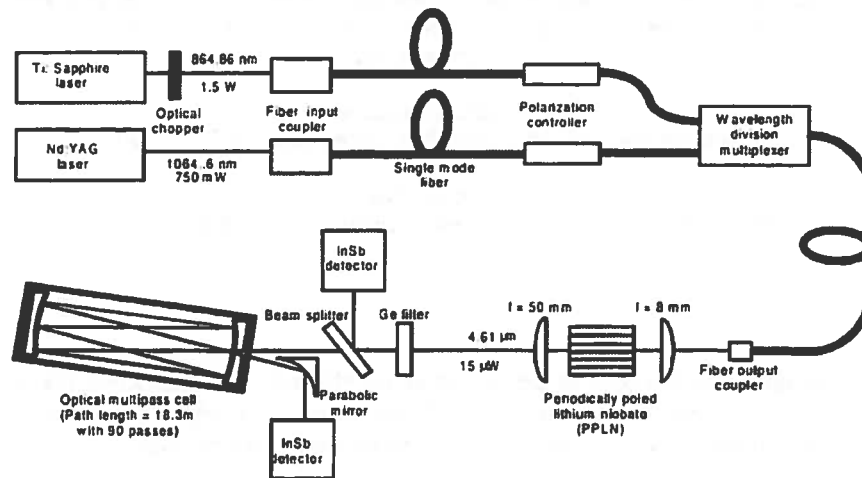
Fig. 1: Optical set-up of the mid-infrared laser based gas sensor for CO detection

High resolution wavelength scans of the Ti: Sapphire laser were performed by tilting a thin etalon by means of a computer controlled galvanometer. For absorption measurements, a scan range of 7 GHz was adequate to monitor a single CO peak at a pressure of 100 Torr. The DFG signal detected after the multipass absorption cell was filtered and amplified with a lock-in amplifier. The data was digitized and transferred to a laptop computer through a 16-bit A-D card. The data was analyzed using LabVIEW 5.0-Software (National Instruments, Inc). Segments of CO absorption were removed from the base line that matches the estimated base width of CO Lorentzian absorption peak. An eighth order polynomial was then fitted to the remaining baseline to approximate 100% transmission at the CO peak. In addition, a Lorentzian lineshape was fitted to the CO absorption line[2].

Rat aortic smooth muscle cells were cultured serially in minimum essential medium and were plated in T75 culture flasks with a volume of $250 cm^3$. The cells served as a specimen when they grow up to confluent monolayers were containing about 1×10^7 cells. Two groups of the flasks were prepared. One group of flasks contained cells which was not treated with any chemicals (Control group), while the other contained cells treated with 20 μM hemin 8 hours prior to the CO measurement (Hemin group). Hemin is one kind of hemes which is a substrate of CO intervened with heme oxygenase (HO), described by the following reaction :

$$Heme \overset{HO}{\Longrightarrow} CO + Biliverdin$$
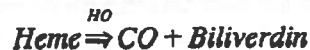
The HO-mediated heme degradation is the primary mechanism for production of cellular CO.

The flask including cells and medium was sealed and placed on a heating pad to keep the medium temperature constant to 37°C. Two 21-G needles connected to the flask were attached to the multipass cell and to a gas cylinder with pure nitrogen gas. Then the following procedures were repeated every 30 minutes to measure the cultured-cell-produced CO continuously: (1) A vacuum inside the multipass cell was generated by a vacuum pump. (2) The flask containing gas, which should be mixture of pure nitrogen and a certain amount of the produced CO from the cells was pumped into the multipass absorption cell until inside pressure of the cell reached a level of 100 Torr. (3) The loss of gas within the flask, approximate $54 cm^3$, was refilled with pure nitrogen to maintain the inside pressure of the flask constant to ambient level. (4) CO in the multipass cell was measured using the DFG based IR absorption spectroscopy.

A CO spectrum of R(6) absorption line at 2169.198 cm^{-1} at room-temperature and 100 Torr is shown in Fig. 2. The measurement time is 2 min. The width of the fitted Lorentzian curve was estimated using the theoretically predicted Lorentzian width from HITRAN 96. The Lorentzian-lineshape fit to the data has a FWHM of 1.77×10^{-2} cm^{-1}, and then yields a fitted magnitude of the peak absorption of 2.33×10^{-2}. The CO mole fraction computed from the absorption peak is 236 ± 10 ppb. The error corresponds to the root mean squared fit residuals. This corresponds to a detection sensitivity of 20 ppb CO for a signal-to-noise ratio (S/N) of 2.
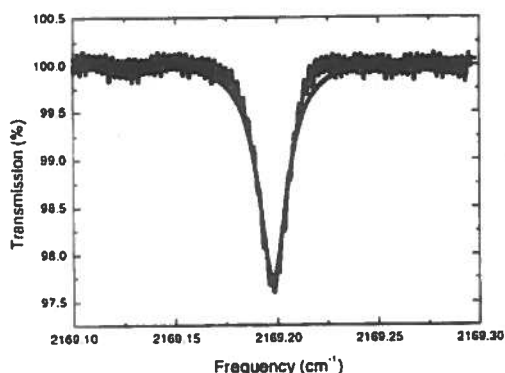
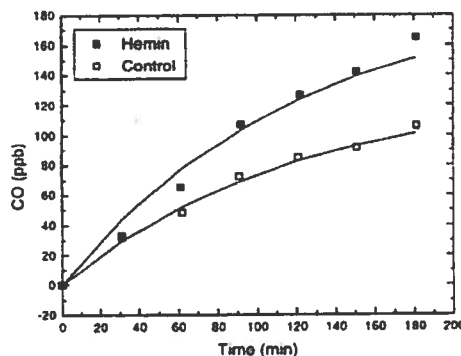Fig. 2: Spectrum of the R(6) absorption line in room-temperature at 100 Torr pressure

Fig. 3: CO production from vascular smooth muscle cells

A time trace of the measured CO within the flask containing the vascular smooth muscle cells is shown in Fig. 3 for a duration of 180 minutes. A CO production was observed even in control cells, but the CO production from the hemin treated cells was larger. If it is assumed that the CO generation rate; r (ppb/ 30 min) is constant during the entire time period of 180 min, we can describe CO concentration (C) within the flask as a function of time C(t), that satisfies the following equation:

$$C(t) = r + C(t-30)(V_{flask}-V_{loss})/V_{flask} \quad (t \geq 30 \text{ min}, C(0) = 0 \text{ ppb}) \qquad (1)$$

where V_{flask} represents the flask volume of 250 cm^3 and V_{loss} a gas volume which is delivered to the multipass cell during every filling and procedure, estimated to be 54 cm^3. Substituting these figures into Eq.(1) results in:

$$C(t) = 4.63 \, r \, (1-0.784^{t/30}) \qquad (2)$$

We applied Eq.(2) as a fitting line to the data shown in Fig. 3 obtain each value of r. As a result, the r of the control cells was 28.5 ± 4. (ppb/30 min) and r of the hemin treated cells was 42.6 ± 8.8 (ppb/30 min). Substituting these values into gas equation; n = PV/RT where n is the number of moles; P is the pressure inside the flask (1.01×10^5 N/m^2); V is the volume of the flask (2.50×10^{-4} m^3); R is the gas constant ($8.31 \times$ J•K^{-1}•mol); T is temperature in the flask (310K), we obtain 280 ± 40 (pmol/ 30 min) and 418 ± 86 (pmol/ 30 min), respectively.

The laser based gas sensor technique should be applicable to other biomedical relevant gases in particular NO[3,4].

References :

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