Photoacoustic heterodyne breath sensor for real-time measurement of human exhaled carbon monoxide

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\section*{ABSTRACT}

A breath sensor for real-time measurement of human exhaled carbon monoxide is reported. This breath sensor is based on a novel photoacoustic heterodyne gas sensing technique, which combines the conventional photoacoustic spectroscopy with the beat-frequency detection algorithm, thus offering a fast response time and a convenient optical alignment, as well as eliminating the needs for frequency calibration and wavelength locking. The principle of photoacoustic heterodyne gas sensing was explained in detail. The performance of the photoacoustic heterodyne breath sensor was evaluated in terms of minimum detection limit, response time, and linearity. The exhaled carbon monoxide levels of eight volunteers were measured and the results demonstrate the reliability and feasibility of this breath sensor.

1. Introduction

The advancement of science and technology has led to the emergence of an increasing number of non-invasive screening techniques. These techniques were developed to compensate for the shortcomings of conventional diagnostic procedures and serve as an essential foundation for the prevention, diagnosis, treatment, and evaluation of the prognosis of diseases, such as the use of photoacoustic technology to determine the levels of sugar in human blood [1], the diagnosis of human intestinal malabsorption diseases utilizing Raman spectroscopy [2], the measurement of human exhaled ammonia by quartz-enhanced photoacoustic spectroscopy for screening of liver and kidney diseases [3], and the measurement of blood alcohol levels through the skin by gas-phase biosensor [4]. All of these things point to the non-intrusive screen techniques which are the future of clinical diagnosis. The molecules that make up human exhaled gas are diverse, with H\textsubscript{2}O accounting for \(~5\,\%\), N\textsubscript{2} accounting for 78\,\%, O\textsubscript{2} accounting for 13–16\,\%, CO\textsubscript{2} accounting for 4–5.3\,\%, and Ar and other gases (CO, NH\textsubscript{3}, acetone, methanol, and so on) accounting for 1\,\% [5]. The quantity of exhaled gas varies, depending on factors such as food, level of physical activity, and overall health. Ethanol (C\textsubscript{2}H\textsubscript{5}O), hydrogen (H\textsubscript{2}), nitrogen oxides, carbon monoxide (CO), branched-chain hydrocarbons, and \textsuperscript{13}CO\textsubscript{2} are the six types of exhaled gases that are now on the breath test list maintained by the Federal Drug Administration in the United States. It is allowed to utilize the concentration level of one of the six exhaled gases as an indication to assist physicians in screening for and diagnosing illness [6]. When the body breaks down heme to create bilirubin, which is the primary source of endogenous CO synthesis, an equal quantity of CO is produced as a byproduct of the metabolic process. The minute quantity of endogenous CO that is produced by the body is connected to many different types of biological oxidation, such as lipid peroxidation, photooxidation, and auto-oxidation of exogenous oxidants. The process of lipid peroxidation, which is brought on by oxidative stress, is the body’s most significant non-heme breakdown route. Since CO is exhaled as a final waste product, its concentration in exhaled breath may be measured and analyzed to provide valuable information on oxidative stress and the presence of respiratory diseases.

Respiratory monitoring is a non-invasive screening tool that is both quick and safe to use, but its potential has not been fully exploited. With the advent of tunable laser spectroscopy, the tunable laser technique has been widely applied in many fields due to its combination of high sensitivity and excellent selectivity. In recent years, the growing studies on the measurement of exhaled CO were reported. In 2005, Moeskops et al. [7] demonstrated three distinct methods for the CO detection based on the direct absorption technology. Using these methods, they were able to accomplish the breath CO detection. The minimal amount

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of absorption that could be detected was $1.2 \times 10^{-5}$ cm$^{-1}$ at an acquisition rate of 3 Hz. In 2017, a CO breath analyzer was developed by Ramin et al. [8] based on tunable diode laser absorption spectroscopy (TDLAS), whose detection sensitivity reached 9 ppb. The first clinical study of human exhaled CO based on quartz-enhanced photoacoustic spectroscopy (QEPAS) was accomplished by Nicolas et al. [9] in 2019, and the sensitivity of the sensing device reached 20 ppb with an integration time of 1 s. Despite the fact that these sensing systems have attained the required level of sensitivity for detecting the CO content of exhaled breath, they have complex optical systems, which result in tedious calibration procedures. Therefore, there is an immediate need for the development of a superior sensing system that can be applied to clinical practice with a more robust design and a simpler operation. In 2017, Wu et al. [10] proposed beat-frequency quartz-enhanced photoacoustic spectroscopy (BF-QEPAS) technology. BF-QEPAS has a faster response compared to QEPAS and traditional photoacoustic spectroscopy (PAS) [11–30]. Furthermore, the resonance frequency and Q value of the quartz tuning fork can be simultaneously obtained when the detection signal is measured, allowing the sensor to operate without the need for a complex calibration.

In this study, we developed a trace gas sensor for the real-time measurement of human exhaled CO. The new sensor uses a photoacoustic heterodyne technique which combines the conventional PAS with beat-frequency detection algorithm. Compared to the conventional PAS, the photoacoustic heterodyne gas sensor features a faster response time and an easier optical configuration.

2. Theory of photoacoustic heterodyne sensing

Traditional PAS gas sensors generally use a microphone as a sound transducer to retrieve gas concentration by converting sound wave signal into current signal. However, even highly sensitive microphones are difficult to directly detect the weak acoustic wave signal generated by the gas molecules absorbing laser energy. Therefore, an acoustic resonator is often used to amplify the sound wave signal. A low-noise differential photoacoustic cell consisting of two buffers and two resonators is shown in Fig. 1. According to the basic principles of the PAS detection, the sound signal collected by the highly sensitive microphone comes from the pressure change caused by the periodic temperature change of the gas in the resonator. In this way, the modulated laser makes the sound signal resonate with the resonator, thereby enhancing the signal amplitude detected by the highly sensitive microphone.

The acoustic vibration in the resonator is induced by many “power sources”, which are evenly distributed along with the excitation light beam. Since the photoacoustic cell is closed, these “power sources” can be treated as a whole and cause the pressure in the resonator to oscillate. The period of the compression and expansion of the gas in the resonator is much shorter than the time required for heat transfer, so that this process can be considered as an adiabatic process. According to the adiabatic equation of an ideal gas:

$$PV^\gamma = C$$  \hspace{1cm} (1)

where $P$ is the pressure in the resonator, $V$ is the gas volume, $\gamma$ is the ratio of the specific heat at constant pressure to the specific heat at constant volume, and $C$ is a constant. When the gas in the resonator is displaced by vibration, according to the adiabatic equation:

$$(P_0 + p_1)(V_0 - \xi S)^\gamma = P_0 V_0^\gamma$$ \hspace{1cm} (2)

where $P_0$ is the original pressure, $p_1$ is the changed pressure, $V_0$ is the original gas volume, $\xi$ is the displacement generated when the resonator vibrates, and $S$ is the resonator cross-sectional area. After rearranging Eq. (2), it can be expressed as:

$$\frac{P_0 + p_1}{P_0} = \left(\frac{V_0}{V_0 - \xi S}\right)^\gamma = (1 - \frac{\xi S}{V_0})^\gamma$$ \hspace{1cm} (3)

Due to the displacement $\xi \ll V_0$ in the process of acoustic vibration, Eq. (3) can be simplified as:

$$\frac{P_0 + p_1}{P_0} = (1 - \frac{\xi S}{V_0})^\gamma \approx 1 + \frac{\xi S}{V_0}$$ \hspace{1cm} (4)

By solving Eq. (4), $p_1$ can be obtained:

$$p_1 \approx p_0 \frac{\xi S}{V_0}$$ \hspace{1cm} (5)

where $p_0$ is the gas density and $v$ is the speed of sound. When the pressure in the resonator changes, the reaction force on the resonator can be expressed as:

$$F = -p_0 S = -\frac{S^2 p_0 v^2}{V_0 - \xi}$$ \hspace{1cm} (6)

Obviously, the magnitude of the force is proportional to the displacement, and their direction is opposite. Therefore the gas in the resonator can be seen as an "air spring", and its elastic coefficient is $K = \frac{S^2 p_0 v^2}{V_0}$.

According to the above parameters, the equation of motion of the gas in the resonator is derived:

$$M \frac{d^2 \xi}{dt^2} + R \frac{d \xi}{dt} + K \xi = Sp$$ \hspace{1cm} (7)

where $M$ represents the mass, $p$ represents the sound pressure generated by the "power source", and $R$ represents the frictional force on the resonator wall when the gas vibrates. The complex form of $p$ is used to solve Eq. (7) to obtain:

$$\xi = \xi_0 e^{-\frac{t}{\tau}} \cos(2\pi f_0 t - \varphi_0) + \xi_1 e^{-\frac{t}{\xi_1}}$$ \hspace{1cm} (8)

where $\xi_0$ is the maximum amplitude of the damping vibration, $\tau$ is the damping time of the vibration, $f_0$ is the resonant frequency of the resonator, $\varphi_0$ is the initial phase at the start of the vibration, $\xi_1$ is the maximum amplitude of the stable vibration, $f$ is the modulation frequency of the laser inducing $p$. It can be found that the first term of the complete solution is the same as the decay vibration equation of a particle, and it is also the transient solution of the gas motion equation in the resonator, and the second term is the steady-state solution of the gas motion equation in the resonator. When the sound pressure $p$ is a wave packet containing several periods of vibration, the transient solution will dominate in the complete solution.

In order to shift the displacement signal $\xi$ from high frequency to low frequency region, a frequency down-conversion of the transient signal requires to be carried out. The transient signal is first mixed with the local modulation signal and then passes through a low pass filter, the photoacoustic heterodyne signal can be obtained:
\[ s = \frac{1}{2} \xi_0 e^{-\Delta t} \cos[2\pi(f_0 - \tau) t - \phi_0] \]  

(9)

This is a beat frequency signal where \( \xi_0 \) is proportional to the target gas concentration, \( \tau \) corresponds to the \( Q \) value of the resonator. Since the modulation frequency of the laser \( f \) is known, the resonant frequency of the resonator \( f_0 \) can be obtained from the beat frequency signal. Therefore, a faster response time with the resonator information including the \( Q \) value and resonant frequency can be achieved simultaneously if the transient solution is measured instead of the steady-state solution in the conventional PAS.

3. Experiment

3.1. Breath sensor design

Fig. 2 depicts the schematic diagram of the photoacoustic heterodyne breath sensor. A mid-infrared distributed feedback quantum cascade laser (DFB-QCL) (AdTech Optics, model HHL-17-62) with a central wavelength of 4.61 \( \mu \text{m} \) and a power of 21 mW was employed as the excitation light source. The laser driver consists of a temperature controller and a current source. The temperature controller was used to set the temperature of the DFB-QCL to 38.5 \( ^\circ \text{C} \). The current source was used to provide a driving current for the DFB-QCL. A sinusoidal wave and a fast-scan right-angle trapezoid-like waveform were generated by a dual-channel function generator [10], and then were sent to the laser driver after passing an electronic adder. The reference signal from the function generator was sent to a lock-in amplifier (Stanford Research Systems, model SR830). A low-noise differential photoacoustic cell equipped with two \( \Psi 25.4 \text{ mm} \times \text{ mm} \) CaF\(_2\) windows having a transmissivity efficiency of > 95\%, was employed. The photoacoustic cell has one air inlet, one air outlet, and two highly sensitive microphones (Primo Microphones Inc., Japan, model EM258). Two needle valves were located upstream and downstream of the gas path of the photoacoustic cell. The output signal from the photoacoustic cell was demodulated by the lock-in amplifier and then directed to a personal computer equipped with a data acquisition card.

3.2. Measurement of photoacoustic heterodyne signal

A 20 ppm CO:N\(_2\) gas mixture was introduced into the photoacoustic heterodyne sensor. The pressure and the gas flow rate in the photoacoustic cell were set to be ambient pressure and 70 standard cubic centimeter per minute (sccm), respectively. The sinusoidal waveform as the modulation signal had a frequency of 1934 Hz and a modulation depth of 5 mA. The right-angled trapezoid-like waveform as the sweep waveform had a period of 230 ms, corresponding to a laser current range of 204 mA~224 mA. The addition of two waveforms produced a wave packet containing several periods of vibration. The mixer and the low pass filter were realized through the lock-in amplifier, whose time constant and filter slope were 1 ms and 12 dB, respectively [10]. The equivalent bandwidth of the low pass filter is 125 Hz. A photoacoustic heterodyne signal is shown in Fig. 3, from which the resonant frequency and \( Q \) value of the photoacoustic cell can be retrieved. According to the beat frequency theory:

\[ |f_0 - f| = \frac{1}{\Delta t} = \Delta f \]  

(9)

where \( \Delta t \) is the time difference between two adjacent peaks or valleys of the beat signal, which can be obtained from the beat signal curve, \( \Delta f \) is the absolute value of the difference between \( f_0 \) and \( f \). Since \( f \) and \( \Delta t \) are known, \( f_0 \) can be calculated according to Eq. (9). Based on the classical harmonic oscillator theory, the quality factor can be described as:

\[ Q = \pi f_0 \]  

(10)
The measurement results of the heterodyne signal are shown in Fig. 3. \( \Delta t \) was measured to be 0.008743 s, \( \tau \) was obtained by an exponential fitting to be 0.00636 s, and the resonant frequency and \( Q \) value of the photoacoustic cell obtained from the heterodyne signal were 1819.6 Hz and 36.3, respectively. For a 20 ppm CO: \( \text{N}_2 \) mixture, the maximum amplitude of the beat signal is 16.6 mV and the standard deviation \( \sigma \) of the background noise is 25.1 \( \mu \text{V} \).

### 3.3. Modulation frequency optimization

The resonant frequency and \( Q \) value of the photoacoustic cell were measured by the traditional PAS method, and the results are shown in Fig. 4, which are in good agreement with those of the photoacoustic heterodyne signal. In fact, in the measurement of the photoacoustic heterodyne signal, the difference \( \Delta f \) between \( f_0 \) and \( f \) is a crucial parameter due to the fact that the amplitude of the heterodyne signal varies with the different \( \Delta f \). With an optimal modulation depth of 5 mA, the peak amplitude of the heterodyne signal as a function of the modulation frequency is presented in Fig. 4. When \( \Delta f \) is 114 Hz, the corresponding modulation frequencies are 1934 Hz and 1706 Hz, which are two optimum frequencies for the sensing system. For each measurement, the system can automatically adjust the modulation frequency according to the resonance frequency obtained last time, making the system work at the optimal modulation frequency. In this way, the measurement requirement of the resonant frequency in the traditional PAS can be removed.

### 3.4. Sensor system linearity

The linearity of the photoacoustic heterodyne sensor was evaluated at atmospheric pressure and the gas flow rate of 70 sccm. The CO: \( \text{N}_2 \) mixtures with different concentration levels of 0.85 ppm, 2.275 ppm, 2.988 ppm, 3.145 ppm, and 3.7 ppm were directed into the sensor in turn. 200 data points for each concentration level were measured at an interval of 230 ms, and the results of the measurements are shown in Fig. 5(a). The amplitude of the photoacoustic heterodyne signal of 0.85 ppm CO is 0.82 mV, and the \( \sigma \) of the background noise is 0.0251 mV, corresponding to a signal-to-noise ratio of 32. The detection sensitivity and normalized equivalent noise absorption coefficient are 26 ppb and \( 2.82 \times 10^{-9} \text{ cm}^{-1} \text{WHz}^{1/2} \). The mean values of the signal amplitudes at different concentration levels are shown in Fig. 5(b). The linearity of the sensor response to the CO concentration levels was confirmed by the high R-square value (0.9989) of the linear fitting.

### 4. Real-time CO measurement in human exhaled breath

Real-time CO measurement of exhaled human breath was demonstrated using the developed photoacoustic heterodyne sensor. The breathing data of eight volunteers were collected, three of which were smokers and five of which were non-smokers. Fig. 6 depicts a complete breath testing of one of the smoking subjects, which takes \( \sim 80 \) s. The volunteer was asked to totally relax, take a deep breath and then blow through the breath sampler until the air in the lung was exhausted. The exhalation process took \( \sim 30 \) s as shown in the blue rectangle of Fig. 6. The exhaled breath took 10 s to arrive in the photoacoustic cell from the breath sampler due to a long gas tubing between them. Such a long gas tubing was used to isolate the sound noise from the airflow disturbance. The CO concentration reached the maximum of 7.5 ppm at 40 s and then gradually went down. Eventually, the signal amplitude returned to the baseline at 80 s. Due to the presence of CO in the air, the baseline of the sensor was not zero in the beginning and in the end, as shown in Fig. 6. Since the CO concentration level in the air is relatively stable, the measurement results will not be affected.

Fig. 7 displays the test results from the eight volunteers, which reveals that the exhaled CO concentration levels of the three smokers were significantly higher than those of the nonsmokers. Since smoking
produces carbon monoxide, the data collected after smoking is not useful for assessing the severity level of the disease. The smokers in the experiment did not smoke in the two hours prior to the measurement. Based on the research [31,32], the CO concentration level of a smoker is > 6 ppm. Three smokers are easily identified in Fig. 7. The remaining five non-smokers breathed much less CO than smokers.

5. Conclusions

A photoacoustic heterodyne breath sensor for real-time CO measurement was developed by combining the conventional PAS with beat-frequency detection algorithm. This breath sensor removes the need for calibration of resonant frequency and wavelength locking in the traditional PAS, and offers a faster response time and ability to analyze trace gas samples. A detection sensitivity of 26 ppb was achieved with an integration time of 1 ms, corresponding to an NNEA of 2.82 × 10^{-9} cm^{-1/2}. The possibility of self-testing for diseases in a non-invasive manner, without the need for complex professional laboratory facilities and/or chemical reagents, is particularly appealing in view of the current global pandemic. Simultaneous identification and quantification of breath CO can be utilized as a clinical reference for the non-invasive diagnosis of oxidative stress and respiratory diseases. Deployment of the photoacoustic heterodyne breath sensor in hospitals for field tests of the patients to assess its performance will be conducted in the near future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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