Non-Invasive and Time-Dependent Blood Sugar Monitoring via Breath-Derived CO₂ Correlation using Gas Chromatograph with Milli-Whistle Gas Analyzer

Cheng-Huang LIN, Luo-Xian WU, Kuan-Hao CHEN, Hsu-Feng LO, King-Chuen LIN, Toshio KASAI, Chien-Chung CHEN, Chung-Hung SHIH, Maria Carla MANZANO, Gil Nonato SANTOS, Enrique MANZANO, and Derrick Ethelbert YU

1Department of Chemistry, National Taiwan Normal University, Tingchow Rd., Taipei 10617, Taiwan
2Department of Chemistry, National Taiwan University, Roosevelt Rd., Taipei 10617, Taiwan
3Institute of Scientific and industrial Research, Osaka University, Ibaraki, Osaka 567-0047, Japan
4Graduate Institute of Biomedical Materials and Tissue Engineering, Taipei Medical University, Wu-Hsing St., Taipei, 11031 Taiwan
5School of Respiratory Therapy, Taipei Medical University, Wu-Hsing St., Taipei, 11031 Taiwan
6Division of Pulmonary Medicine, Department of Internal Medicine, Taipei Medical University Hospital, Taipei, 11031 Taiwan
7Physics Department, De La Salle University, 2401 Taft Ave., Manila, 0922 Philippines
8Electronics and Communications Engineering Department, De La Salle University, 2401 Taft Ave., Manila, 0922 Philippines
9Chemistry Department, De La Salle University, 2401 Taft Ave., Manila, 0922 Philippines

*Corresponding author. E-mail: chenglin@ntnu.edu.tw (C.-H. Lin)
Abstract

A clear and positive correlation between the CO₂ concentration and the blood sugar level is observed via a non-invasive and time-dependent monitoring of CO₂ concentration from human breath that is carried out by using a home-made gas chromatography (GC)/milli-whistle compact analyzer. The time-dependent sampling of CO₂ concentration correlated between 5.0 to 5.6 % (1%≈10⁴ ppm) in accordance with blood sugar level variations of 80 to 110 mg/dL. The analytical method results in a rapid, continuous and non-invasive determination of blood sugar level via the measurement of CO₂ concentration exhaled from the lungs.

Keywords: Gas chromatography/milli-whistle compact analyzer, RQ index, CO₂, human breath monitoring, non-invasive blood sugar level measurement, respiration
Introduction

The blood sugar level is the index of concentration of glucose present in the blood of human and other animals. Glucose is stored in skeletal muscle and liver cells in the form of glycogen. Metabolic homeostasis utilized the glucose present in the blood thereby making cellular respiration possible. Living organisms harvest and store energy in molecules such as adenosine triphosphate (ATP).\(^1\) For respiration in plants, the mass spectroscopic study for the accurate determination of the total rate constant of respiration reaction of plant leaves has confirmed the exact 1:1 correspondence between \(O_2\) consumption and \(CO_2\) emission as suggested from the following chemical equation as is well known.\(^2-4\)

\[
C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \tag{1}
\]

The exhaled breath of humans can be a viable indicator of a disease present in the respiratory system. The exhaled breath is composed of inorganic compounds such as carbon dioxide, oxygen, and nitric oxide; non-volatile compounds like isoprostanes, cytokines, leukotrienes and hydrogen peroxide; and volatile organic compounds (VOCs). These VOCs can be saturated hydrocarbons, unsaturated hydrocarbons, oxygen, sulphur, and nitrogen. In most breaths, common VOCs include isoprene, acetone, ethanol, methanol, other alcohols and alkanes.\(^5\)
The production of VOCs in exhaled breath is theorized to be emitted by infected cells or products of metabolic pathways. For example, isoprene is produced along the melavonic acid pathway of cholesterol synthesis. Acetone on the other hand is produced from glucose metabolism and alkanes such as ethane and pentane is produced from oxygen free radical-mediated lipid peroxidation of fatty acid components of cell membranes. The acetone and alkanes can be used as biomarkers for oxidative stress.\(^6\)\(^{10}\) In human respiration, insulin is released from the pancreas to normalize the blood sugar level whenever it is elevated. However, in patients with diabetes mellitus, the absence or insufficient production of insulin results in hyperglycemia. The diagnosis of diabetes mellitus can be established with any of the following criteria: glycohemoglobin A1c (HbA1c) \(\geq\) 6.5%; plasma glucose \(\geq\) 126 mg/dL after an overnight fast; symptoms of diabetes mellitus and a random plasma glucose level of \(\geq\) 200 mg/dL.

As a non-invasive glucose monitoring technique, the analysis of breath samples has the potential for the monitoring of early stages of diabetes mellitus.\(^{11}\)\(^{14}\) Symptoms of diabetic ketoacidosis can be detected by the smell on a person's breath because acetone is produced as a direct byproduct of the spontaneous decomposition of acetoacetic acid. In our previous study, we have reported on applications of the Hadamard transform-gas chromatography/mass spectrometry method that permits the rapid and sensitive detection of acetone in the breath at the sub-ppmv levels. The use of headspace SPME (solid-phase microextraction)-GC/MS for the analysis of acetone in human breath was also performed and compared.\(^{15}\) Although it is a non-invasive
monitoring method, the mass spectrometer is bulky and complicated to use thereby compromising the accessibility and speed of analysis. In this study, a rather simple and compact gas chromatograph with home-made milli-whistle system\textsuperscript{16} is utilized for a non-invasive and time-dependent (continuous and rapid) blood sugar monitoring through the measurement of CO\textsubscript{2} in exhaled breath. Although acetone was selected as the main target compound in our former report,\textsuperscript{15} we found that the concentration levels of CO\textsubscript{2} from human breath could be selected as another target to avoid false positive. There are many CO\textsubscript{2} analytical techniques are currently used. However, any electronic detector has a lifetime for use and each detector has its own specific detectable targets, such as organic or inorganic gases. GC/milli-whistle gas analyzer is a kind of universal detector since the sample gases are detected by physical principle, that is depending on frequency shifts. Thus, it can be used for detecting any kind of gases, even for noble gases. Especially, exhaled breath contains a lot of moisture, which is interference to electronic instruments. When those electronic devices were used, water removal becomes an important task. However, when the GC/milli-whistle gas analyzer was used, the step of water removal can be skipped and this is the reason for why we used GC/milli-whistle. Herein, we report the direct and time-dependent correlation between CO\textsubscript{2} levels from human breath and blood sugar concentrations.
**Experimental**

A previously reported GC/milli-whistle system for the quantitative determination of hydrogen was used for this study. In this system, the milli-whistle is connected to the outlet of the GC capillary, thus, when the GC-eluants and carrier gas pass through the whistle, a sound wave is generated and picked up by a microphone and then rapidly subjected to a Fourier transformation. Along the process, the size of the milli-whistle and its physical characteristics and details of its construction are also investigated and optimized.

**Figure 1**

Figure 1 shows a schematic diagram of the GC/milli-whistle detection system with the inset of a typical chromatography spectrum of O₂ and CO₂ peaks. From our previous study, it was shown that the limits of detection were found to be ~3 µL each injection (in the case of gas samples), including hydrogen, helium, argon, and carbon dioxide; in the case of liquid samples, including methanol, cyclohexane, tetrahydrofuran, hexane, and acetone, the limits of detection were determined to be ~10 µg/each injection, respectively. In this study, the whistle and microphone were set-up in a sound-proofed box to avoid surrounding noise. The breath gas samples were collected in a Tedlar bag (SKC) from nondiabetic healthy volunteers (Volunteer A and B) and injected into the GC column by a syringe injector (#1710; Hamilton). Nitrogen was used as carrier.
gas and the pressure was maintained at 14 psi. The pressure of makeup N₂ gas was kept at 2 kg/cm² resulted in a frequency of ~8400 Hz in the milli-whistle (ID, 1 mm; effective length, 10 mm). The experiments were performed at room temperature (~25°C), thus, the non-inclusion of GC oven in the experimental set-up. As the microphone generates sound wave in time domain, an on-line programmed PC was used to rapidly transform time domain into a frequency domain as shown in the inset. The gas chromatograph (GC 5890; Hewlett-Packard, Avondale, PA, USA) was equipped with an HP-PLOT Q column (30 m × 0.53 mm × 40 µm; Agilent Technologies). The milli-whistle was made from brass to acquire the sound frequency. The sampling rate of the microphone (AT9942; Audio-Technica) was set at 20,000 Hz. A LabVIEW program (National Instruments, USA) with built-in Fourier transform function was used for real-time frequency monitoring. Ultra-purified (>99.99%) oxygen, nitrogen and CO₂ gases were purchased from Fong-Ming Industrial (Taiwan).

There are approximately 100~150 mL air space from the mouth to the esophagus. To account for this effect, gas samples were acquired after the subject held breath for 40 s and the injection volume was 100 µL in each case. Nitrogen was used as the makeup and carrier gases and the fundamental frequency observed was at 8400.1 Hz. The frequency changes for CO₂ and O₂ were found to be -1.8 Hz and -3.8 Hz, respectively. The negative values show that the frequency observed was lower than the fundamental frequency due to the molecular weights of the GC-eluants which are lower than the carrier/makeup gas, and that the observed frequencies are higher than that of the carrier gas, whereas GC-eluants with molecular weights higher than that would produce lower frequencies.
Figure 2

Figure 2 shows the calibration line between a CO\textsubscript{2} peak that typically appears in a chromatography spectrum as shown in the inset of Figure 1, and the CO\textsubscript{2} concentration in the unit of % (1\% = 10\textsuperscript{4} ppm). There is a good linear correlation between the two when we use the fitting line $y = 1.707x - 0.16$ within the standard deviation of $R^2 = 0.996$. Relative standard deviation (RSD) was found to be 2.2%. A blood sugar meter (Accu-Chek Mobile), equipped with a sampling tip (Accu-Chek fastclix) was used for obtaining reference data of the blood sugar value.

Results and discussion

The average percentage of CO\textsubscript{2} in conventional human breath is around 4\% and this value increases slightly when the breath is held for a few seconds and so on. After holding the breath for 40 seconds, the frequencies for the CO\textsubscript{2} and O\textsubscript{2} peaks change to -1.8 Hz and -3.8 Hz, for example, as we see in the inset chromatogram of Figure 1. The amount of CO\textsubscript{2} and O\textsubscript{2} gases can be calculated to be 5 \% and 13.7 \%, respectively, by using the calibration curve of Figure 2. In this study, we are particularly interested in the time dependence of the amount of the exhaled CO\textsubscript{2} concentration, so that we focused on the CO\textsubscript{2} peak obtained from two volunteers (A and B). The volunteers woke up at 6 am in the morning, then took 350 mL of rice milk at 10 am. The breath samples were collected.
every fifteen minutes from the volunteers with Tedlar bag and were immediately analyzed by injection with the syringe injector as shown in Figure 1. In parallel, the blood sugar value was measured with the blood sugar meter from the same volunteers during the procedure.

**FIGURE 3**

Figures 3A shows the time-dependence plots of the CO₂ concentration and the corresponding blood sugar values obtained from one of volunteers (5 volunteers were examined). The plots with the blue solid line show the changes of CO₂ concentration levels as the function of time; the error bars show the deviation of samples corrected within three different days. The bottom plots with the red broken line show the changes of the blood sugar levels obtained with a blood sugar meter. For Volunteer A, the CO₂ concentration level changed from 5.0% before ingesting rice milk, to 5.6% 30 minutes after ingesting rice milk, while the blood sugar level of the same volunteer changed from 80 mg/dL to 110 mg/dL before and after the intake, respectively. For another volunteer (data not shown), the CO₂ concentration changed from 5.5% prior to rice milk intake, to 6.5% 30 minutes after the intake, while the corresponding blood sugar level changed from 90 mg/dL to 125 mg/dL. Normally, a typical blood sugar level without eating for a few hours is known to be less than 100 mg/dL and it is less than 140 mg/dL two hours later after eating. The other volunteers were also shown similar tendencies.
Figure 3B shows the time-dependence of the breath CO₂ concentration and the blood sugar values for volunteers, without rice milk intake. In this case, the time-dependence of CO₂ detection remained constant (flat) throughout the same time span, specifically, around 80 mg/dL for Volunteer A and 85 mg/dL for Volunteer B. It is known in medical science that blood sugar cannot be metabolized when there is insulin disorder, thus the increase in blood sugar levels. Therefore, it can be explicitly deduced from the present result that the volunteers A and B are not diabetic patients. Furthermore, the experimental results reveal time-sensitive positive correlation between the breath CO₂ concentration and the corresponding blood sugar levels, thus indicating that the breath CO₂ measurement is a reliable alternative method to monitor the blood sugar level. Respiratory quotient (RQ) is a dimensionless number used in the calculation of basal metabolic rate estimated from carbon dioxide production. It is derived based on the ratio of CO₂ produced by the body with the O₂ consumed by the body. For practical purpose, the term CO₂/O₂ ratio based on the peak ratio of CO₂ and O₂ from human breath is used due to the difficulty of ascertaining the O₂ consumption by the body.

Figure 4 shows the results of CO₂/O₂ ratio obtained from the volunteer. As can be seen, a positive correlation as two major peaks were observed 30 minutes after breakfast and lunch, respectively. It is known that blood sugar (C₆H₁₂O₆) react with O₂ to produce CO₂ and H₂O, thus it can be inferred that blood sugar level can directly correlate with CO₂ production. However, there may be other factors or chemical reactions in the body that may influence the direct correlation...
between blood sugar and CO₂, such as different types of lung diseases, thus the need for initial calibration (initial fasting) as demonstrated. To address the issue, control experiments were done in which the volunteers were tested without ingesting anything or fasting. As shown in Figure 3B, both the blood sugar level and CO₂ in the breath all keep almost at constant during the whole experimental period.

Overall, the results preliminarily prove that the blood sugar level has a certain correlation with the CO₂ concentration in exhaled breath. For future prospects, as the increase in sample size and conditions/parameters are achieved, more detailed mechanisms of the intercorrelations of food digestion, absorption and respiration can lead not only to a more accurate, reliable, rapid non-invasive and time-dependent blood sugar analysis, but to the determination of other respiratory diseases and conditions as well.

**Conclusion**

The correlated results between CO₂ concentration in exhaled breath with the blood sugar level indicate that blood sugar determination and monitoring can sufficiently be accomplished by means of a rapid and non-invasive breath CO₂ concentration analysis using the milli-whistle coupled GC technique. The analytical procedure can be an alternative to the conventional blood sampling (extraction) method for the determination of blood sugar level.
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Figure Captions

Figure 1  Schematic diagram of the GC/milli-whistle detection system used, in which the whistle and a microphone were soundproofed in a box to avoided noise from surround.  Inset, typical GC chromatogram for a breath sample obtained from a volunteer.

Figure 2. The calibration line between a CO₂ peak that typically appears in a chromatography spectrum as shown in the inset of Figure 1; the CO₂ concentration in the unit of % (1% = 10⁴ ppm).

Figure 3. Frame 3A shows the time-dependence plots of the CO₂ concentration and the corresponding blood sugar values obtained from the volunteer, respectively, after taking bean milk 30 min later.  The plots with the blue solid line show the changes of CO₂ concentration levels as the function of time; the error bars show the deviation of samples corrected within three different days. The bottom plots with the red broken line show the changes of the blood sugar levels obtained with a blood sugar meter.  Frame 3B, same experiment as described above, but without taking bean milk.

Figure 4. The relationship between CO₂/O₂ ratio and blood sugar levels.
Fig. 1 C. -H. Lin, et al.
Fig. 2 C. -H. Lin, et al.
Fig. 3 C. -H. Lin, et al.
Fig. 4. -H. Lin, et al.