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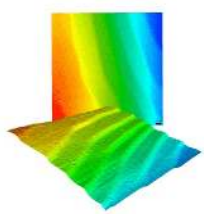


# A fully integrated standalone portable cavity ringdown breath acetone analyzer

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Breath analysis is a promising new technique for nonintrusive disease diagnosis and metabolic status monitoring. One challenging issue in using a breath biomarker for potential particular disease screening is to find a quantitative relationship between the concentration of the breath biomarker and clinical diagnostic parameters of the specific disease. In order to address this issue, we need a new instrument that is capable of conducting real-time, online breath analysis with high data throughput, so that a large scale of clinical test (more subjects) can be achieved in a short period of time. In this work, we report a fully integrated, standalone, portable analyzer based on the cavity ringdown spectroscopy technique for near-real time, online breath acetone measurements. The performance of the portable analyzer in measurements of breath acetone was interrogated and validated by using the certificated gas chromatography-mass spectrometry. The results show that this new analyzer is useful for reliable online (online introduction of a breath sample without pre-treatment) breath acetone analysis with high sensitivity (57 ppb) and high data throughput (one data per second). Subsequently, the validated breath analyzer was employed for acetone measurements in 119 human subjects under various situations. The instrument design, packaging, specifications, and future improvements were also described. From an optical ringdown cavity operated by the lab-set electronics reported previously to this fully integrated standalone new instrument, we have enabled a new scientific tool suited for large scales of breath acetone analysis and created an instrument platform that can even be adopted for study of other breath biomarkers by using different lasers and ringdown mirrors covering corresponding spectral fingerprints.  [http://dx.doi.org/10.1063/1.4930121]

## I. INTRODUCTION

Breath analysis, offering potential for nonintrusive disease diagnosis and metabolic status monitoring via testing exhaled breath components, has become an emerging research field in medicine, human healthcare, and medical instrumentation. However, current technology drawbacks in breath analysis instrumentation or method such as using sample pre-concentration, time-consuming sample preparation, and long data analysis time often result in the limited number of human subjects or samples used in a breath study.[1–4] Consequently, research data on breath analysis are of less statistical significance for addressing a fundamental question in the field such as what is the quantitative relationship between the concentration of a breath biomarker and a clinical diagnostic parameter, because addressing this question is heavily dependent upon availability of large amounts of clinical data. To this end, a real-time online breath analyzer with high data throughput (analyzing a large number of subjects in a short experimental period) would be desired in the field.

Breath analysis requires a highly sensitive and highly selective instrument in order to identify and determine concentrations of specific biomarkers due to their low concentrations and the presence of a large quantity of trace compounds in a single exhaled breath. Gas chromatography-mass spectrometry (GC-MS)[5–10] has been regarded as a gold-standard method for trace gas analysis for its high sensitivity and high accuracy. However, the GC-MS method is not suitable for breath analysis using a large number of samples due to its complicated sample preparation procedure, time-consuming test process, and high instrumentation and operational costs. In addition to the conventional GC-MS method, several relatively new MS-based analytical techniques including proton transfer reaction mass spectrometry (PTR-MS),[11,12] ion mobility spectroscopy (IMS),[13,14] and selected ion flow tube mass spectrometry (SIFT-MS)[15–18] have been developed rapidly for real-time online breath analysis. Breath analysis is also conducted by using electrical sensors,[19–21] which are comparatively inexpensive and smaller in size, but the issues with detection selectivity and requirement of frequent baseline calibration remain to be further addressed.

A laser spectroscopy-based technique, which is highly sensitive, selective, and of real-time response without the

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need of sample pre-process, allows breath analysis to be advanced from laboratory research to potential commercial instrumentation. Laser spectroscopy-based techniques used in breath analysis include, but not limited to, the tunable diode laser absorption spectroscopy,[22,23] cavity ringdown spectroscopy (CRDS),[24–27] integrated cavity output spectroscopy,[28–31] cavity enhanced absorption spectroscopy,[32] cavity leak-out absorption spectroscopy,[33–37] photoacoustic spectroscopy,[38] quartz-enhanced photoacoustic spectroscopy,[39] and optical frequency comb cavity-enhanced absorption spectroscopy.[40,41] Although some of these techniques have been used for breath analysis in a lab-based experimental system or a prototype, none of them have been used to develop a commercial or close-to commercial breath instrument for clinical use, except for the lead-salt laser absorption instrument that has been used commercially for breath NO analysis.[42]

CRDS technique has high sensitivity and high selectivity. CRDS also has the advantages of fast response, relatively low costs, and relatively small instrument geometry. These unique properties make CRDS be a suitable technique for developing a portable instrument for real-time, online breath analysis with high data throughput. Since the first demonstration of the pulsed- and cw-cavity ringdown spectra of acetone in the UV and in the near infrared, respectively, in 2004, the pulsed-CRDS has been used for breath acetone analysis under various situations (human subjects, animals, in a lab or a clinic); however, none of the systems reported before (the lab-setup or a cavity with separated electronics and light source, etc.) were a fully integrated standalone instrument.[43–47]

In this work, we have built a fully integrated instrument based on the pulsed-CRDS technique for near-real time, online breath acetone measurements. This portable instrument can be readily placed in different places such as an office in a clinic to achieve quantitative analysis of breath acetone using a large number of subjects in a short period of time.

## II. INSTRUMENT DESIGN, CONFIGURATION, AND ELECTRONIC PACKAGING

The fully integrated, standalone, portable breath acetone analyzer is illustrated in Fig. 1. The two front panels of the instrument display a typical ringdown decay waveform (top) and real-time ringdown measurement signals (bottom). All displays are controlled via the touch screen. The detailed layout of the mechanical design and the main parts of the analyzer, including a laser source, a ringdown gas cavity, a detector, a computer with embedded data acquisition card, a pressure manometer, a switching power supply, and a control circuit board, is shown in Fig. 2(a). Figure 2(b) shows a schematic diagram of the breath acetone analyzer. All parts were assembled on an optical-electrical base that was housed in the instrument box of 65 cm × 40 cm × 20 cm. Details of the five major components that were integrated in the instrument, the light source, the optical ringdown cavity, the data acquisition, the sampling device, and the electronic control, are described below.

FIG. 1. Photograph of the fully integrated, standalone, portable breath acetone analyzer.

### A. Laser source

A pseudo single-mode ($TEM_{00}$) Q-switched Nd:YAG laser (Changchun New Industries Optoelectronics, China) was used as the light source. It operated at 266 nm with a repetition rate of 1 kHz and single-pulse energy of 4.5 $\mu$J. It was equipped with a compact laser head (20.9 mm × 88 mm × 74 mm). Except for using a circular aperture to shape the laser beam and two plano mirrors (Thorlabs, U.S.) to direct the beam, no mode-matching optical components were used to couple the laser beam into the ringdown cavity.

### B. Ringdown cavity

The ringdown cavity consisted of a 50-cm long stainless steel pipe with an inner diameter of 3.81 cm (CRD Optics, Inc., U.S.), one pair of mirror mounts (CRD Optics, Inc., U.S.), and one pair of high-reflectivity UV mirrors (Los Gatos Research,

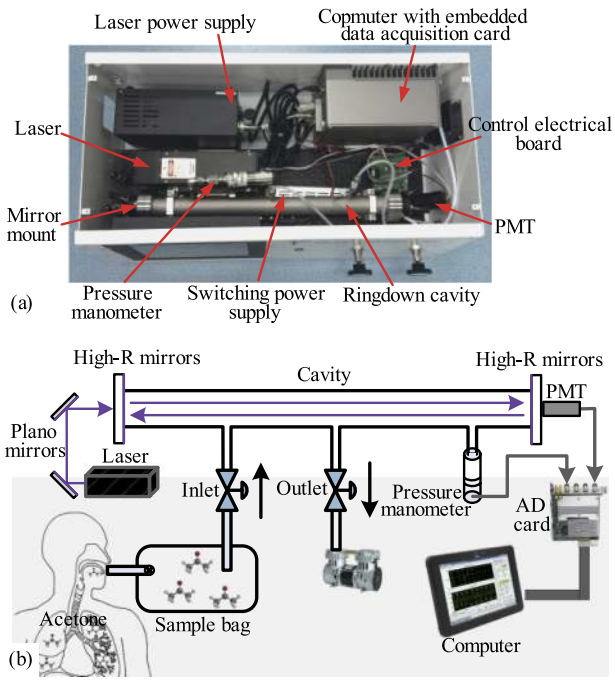


FIG. 2. The mechanical design (a) and schematic diagram (b) of the portable ringdown breath acetone analyzer.

U.S., R = 99.9956%, radius of curvature = 1 m). Each mirror mount held a mirror whose position could be adjusted in multiple dimensions via three alignment screws. The gas cell was equipped with three ports, as shown in Fig. 2, one was connected to a micropressure manometer (MKS870B, U.S.) and the other two were the gas cell's inlet and outlet. The gas inlet of the analyzer was connected to a sampling device, and the gas outlet of the analyzer was connected to a vacuum pump (Oerlikon, SC5D, Germany). In order to make sure the gas cell was not contaminated by breath gas moisture and residuals from the previous breath sample, the gas cell was flushed by air each time after one breath sample was tested.

### C. Sampling method

A subject exhaled a single deep breath via a disposable mouthpiece into a fluorinated ethylene propylene (FEP) breath-gas-collection bag (HaoChenTianCheng, China), which was connected to the gas inlet of the analyzer using a section of quarter-inch tubing. The gas inlet valve of the breath-gas-collection bag was shut off after the breath sample collection. Subsequently, the breath sample collected in the breath gas bag was introduced into the gas cell by opening the gas inlet valve. It was tested that the FEP sampling bag can keep breath gas fresh for up to 6 hours (h). Each new breath bag was cleaned using high-purity nitrogen (>99.99%) prior to use. No additional procedure was used to handle excessive moisture in the exhaled breath since water molecules have no absorption at 266 nm.

### D. Electronic control data acquisition system

The design of the electronic control for the portable ringdown breath acetone analyzer is shown in Fig. 3. The connection interface between each device was identified in the figure. The red line represents the power supply. The green line represents communications or signals. The vacuum pump, laser, and electrical switch were powered by a 110-220 V AC supply.

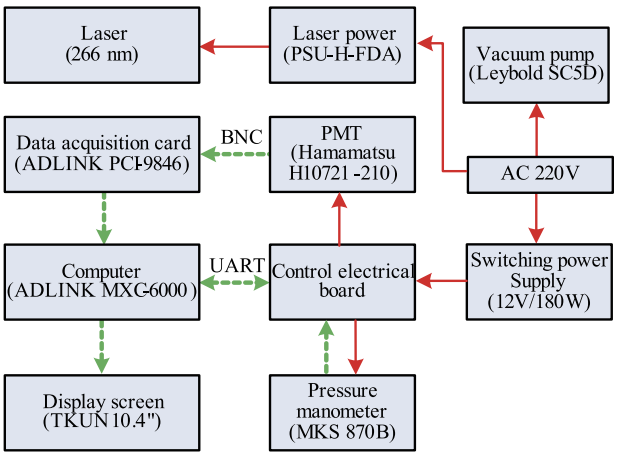


FIG. 3. Diagram of the electronic control for the portable ringdown breath acetone analyzer.

In this electronic control data acquisition system, the in-house electrical circuit board has integrated multi-functions. At first, it supplies DC power to the pressure sensor and the photomultiplier tube (PMT) by converting the output of the city power supply. Meanwhile, the gain of the PMT was controlled by the electrical circuit board through generating a reference voltage using a 16-bit digital-analog converter (DAC) AD5563. The reference voltage of AD5663 was 1.100 V that was the maximum gain control voltage of the PMT module. The calculated accuracy of the gain control signal was ±0.2 mV, which ensured the accuracy and stability of the PMT's gain. Furthermore, the analog signals from the platinum resistance thermometer sensor (PT1000) and from the pressure sensor (MKS870B) were acquired and converted by a 24-bit Sigma-Delta analog-digital converter (ADC) and then were calculated to corresponding temperature and pressure values by the microcontroller unit (MCU, MSP430F149). Subsequently, the results were transmitted to the computer via a serial port and a modbus communication protocol.

A ringdown signal consisted of a series of pulsed spikes with an exponentially decayed intensity, whose temporal behavior requires a high bandwidth to resolve. As shown in Fig. 4, the decay waveform consisted of a rising time and an exponential decay. The rising time was shorter than 1% of the 10 $\mu$s width of the signal. According to the experiential formula, BW = $0.35/T_r$, where BW denotes the bandwidth of the detector and $T_r$ means the rising time of the signal. The minimum bandwidth of the detector and the data acquiring system should be 3.5 MHz. Based on this estimation, the ringdown signal was captured by a fast response PMT with a typical response time of 0.57 ns, and then digitalized by a high speed data acquisition card (PCIe9846D), which had 3 dB bandwidth of 20 MHz for acquiring the rising of the ringdown signal.

Each ringdown decay waveform was input to an embedded data acquisition card (ADLINK PCI-9846, Taiwan) that digitalized a ringdown waveform to 10 000 data points. A ringdown event was triggered by the sync signal output from the laser control unit. The digitalized ringdown waveform was transferred to a computer and displayed on a 10.4-in. touch screen embedded in the front panel of the breath analyzer (Fig. 1). The in-house developed ringdown software was installed in the computer to obtain a ringdown time by fitting

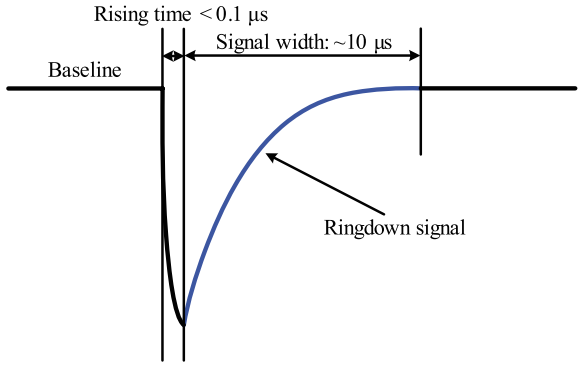


FIG. 4. Estimation of data acquiring system's bandwidth.

the ringdown data points to a single exponential decay. The current analyzer does not display the measured concentration of acetone on the display panel. The concentration was calculated off-line using the measured ringdown times that were automatically saved in the instrument.

## III. DATA PROCESSING ALGORITHM AND METHOD

The background subtraction method was used to determine acetone concentration in breath samples. The rationale of the background subtraction method includes an assumption that the absorbance difference between the atmosphere and a breath gas at 266 nm is attributed to the absorption of acetone alone. The reliability of this assumption was vigorously evaluated by investigating the possible absorbance from other atmospheric molecules and high abundance breath volatile organic compounds (VOCs) that include several atmospheric molecules ($H_2O$, $N_2$, $O_2$, and $CO_2$), high concentration breath gases, which include ammonia ($NH_3$) (0–1 ppm), methane ($CH_4$) (2–10 ppm), nitric oxide (NO) (1–20 ppb), nitrous oxide ($N_2O$) (1–20 ppb), and carbon monoxide (CO) (1–10 ppm), and high abundance breath VOCs that include isoprene ($C_5H_8$), methanol ($CH_3OH$), ethanol ($C_2H_5OH$), propanols ($C_3H_8O$), ethane ($C_2H_6$), and pentane ($C_5H_{12}$).[43] The highest abundance VOC isoprene in human breath has absorbance of $1.23 \times 10^{-7}$ at 266 nm. The isoprene's contribution to the absorption at 266 nm is about 200 times smaller than that of acetone in normal human breath. Other breath VOCs mentioned above have no or negligible absorption as compared with the absorption of acetone at 266 nm.

This method was described in detail in our previous studies.[43–46] In this method, absorbance of the atmosphere is used as the background baseline. $\tau_{atm}$, $\tau_0$, and $\tau_{breath}$ are the ringdown times when the gas cell is filled with the laboratory atmosphere to 1 atm, the cell is vacuumed, and the cell is filled with a breath gas to 1 atm, respectively. The absorbances of the atmosphere and the breath gas are expressed by

$$A_{atm} = \sigma_{atm}(\nu) n_{atm} d = \frac{d}{c}\left(\frac{1}{\tau_{atm}} - \frac{1}{\tau_0}\right), \quad (1)$$

$$A_{breath} = \sigma_{breath}(\nu) n_{breath} d = \frac{d}{c}\left(\frac{1}{\tau_{breath}} - \frac{1}{\tau_0}\right), \quad (2)$$

where $\sigma_{atm}(\nu)$, $\sigma_{breath}(\nu)$ are the absorption cross sections of the transition line at frequency ν at 266 nm when the gas cell is filled with the laboratory atmosphere and a breath gas to 1 atm, respectively. $n_{atm}$, $n_{breath}$ are the sample concentrations when the gas cell is filled with the laboratory atmosphere and a breath gas to 1 atm, respectively, d is the distance between the two mirrors, which is 50 cm in this work, and c is the speed of light.

Then, the absolute concentration of breath acetone (the upper limit) in the breath gas is obtained by[43,45]

$$\Delta A = A_{breath} - A_{atm} = \sigma_{acetone}(\upsilon)\, n_{acetone} d, \quad (3)$$

where $\Delta A$ is the absorbance difference, $\sigma_{acetone}(\nu)$ is the absorption cross section of the transition line at frequency ν for acetone at 266 nm, and $n_{acetone}$ is the acetone concentration.

## IV. RESULTS OF THE INSTRUMENT PERFORMANCE

### A. Signal stability and the limit of detection (LoD)

A typical baseline scan of this portable breath acetone analyzer is shown in Fig. 5, in which each data point was generated by averaging over 100 ringdown events. The ringdown time baseline stability is defined as $\sigma/\bar{\tau}$, where σ and $\bar{\tau}$ are the standard deviation and the averaged ringdown time, respectively. The best baseline stability was 0.16% in this study. The mirror reflectivity can be calculated by measuring a ringdown time in an empty gas cell and it was 99.93%. Therefore, a theoretical LoD of the portable analyzer for acetone was estimated to be 57 ppb based on the 3-σ criteria[43] by using the ringdown baseline stability, 0.16%, mirror reflectivity, 99.93%, cavity length of 50 cm, and the acetone absorption cross section at 266 nm, $4.5 \times 10^{-20}$cm$^2$/molecule[48] at the atmospheric pressure and room temperature.

### B. Linear response behavior

Acetone samples in different concentrations in nitrogen were tested to investigate the linear response behavior of the breath analyzer. Figure 6 shows the measured acetone concentrations versus known sample concentrations, which had a range of 0–20 ppm that covered possible acetone concentrations in all subjects used in this study. The linear fitting curve shows good linearity (R = 0.999). It suggests that the ringdown breath acetone analyzer has a good linear response to acetone in the entire concentration range for the human subjects used in this study.

### C. Response time and reproducibility

Figure 7 shows a typical response of the analyzer to the laboratory air and the breath samples from three Type 2 diabetic (T2D) subjects. The graph shown in Fig. 7 is directly displayed on the front panel (bottom) of the instrument (see Fig. 1). The ringdown time difference can be obviously observed when the gas cell is filled with a breath sample or the laboratory air. This test served as the background

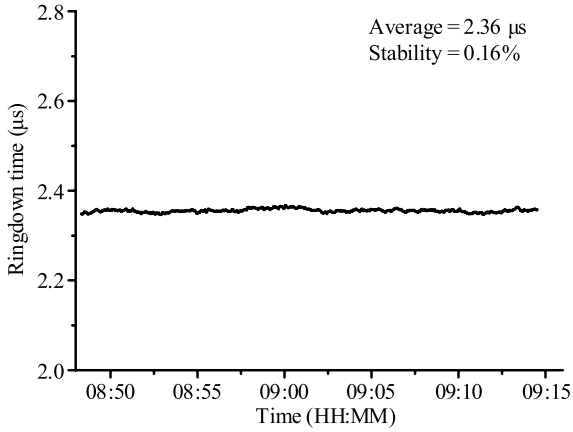


FIG. 5. The evaluation of the ringdown breath analyzer baseline stability.

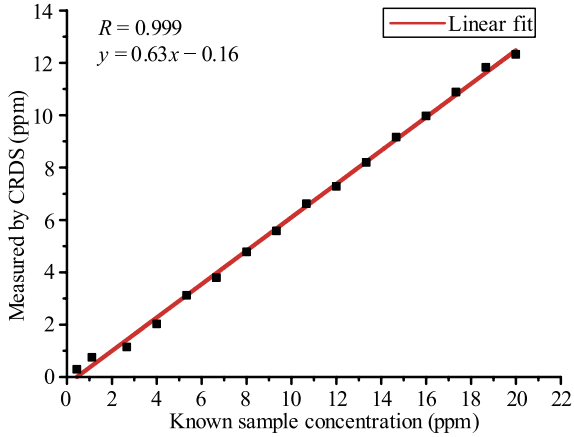


FIG. 6. Linear response to various acetone concentrations measured by the portable breath analyzer.

measurement. The result shows good reproducibility and fast response of the ringdown breath analyzer. The sharp rising and falling edges of each block indicate the rapid-response (~1 s) of the analyzer. At the end of each test, the ringdown time went back to the same baseline level when the gas cell was evacuated. These tests demonstrate that the portable analyzer has the features of high sensitivity, good reproducibility, and fast and linear response.

### D. Measurement accuracy

In order to investigate the accuracy of the instrument, the concentrations of breath acetone obtained from the analyzer and from the certified GC-MS system were compared. Acetone concentrations from 9 breath samples (5 T2D subjects and 4 healthy individuals) were measured using both the ringdown breath acetone analyzer and the certified GC-MS facility. In this study, the GC-MS analysis was conducted in the State Key Laboratory on Odor Pollution Control, Tianjin Academy of Environmental Science, which is a certified facility for trace chemical species analysis. The used analytical method is referred to as the Compendium Method TO-15.[49] A cryogenic pre-concentrator (ENTECH Instruments, Inc., U.S., 7100A) was employed to remove $N_2$, $CO_2$, and $H_2O$ after a 3-stage pre-concentration procedure.

Subsequently, the VOCs-enriched sample was introduced into the GC-MS system (Agilent Technologies, U.S., 7890A/5975C) under the analytical conditions. The operating conditions and procedures of the pre-concentration and analytical conditions of the GC-MS system can be seen elsewhere.[50] The MS detector profiled breath VOCs in the full scan mode and detected those specific compounds in the selected ion monitoring mode with a higher sensitivity and better signal-noise ratio. An internal standard was applied when performing quantification using bromochloromethane.[9,51]

Figure 8 shows a comparison of the measured acetone concentrations using the two individual methods in the range of 0.5–2.5 ppm. The slope (1.06) in the fitting equation suggests that the obtained acetone concentrations using both methods are consistent. This GC-MS validation test confirms that the ringdown breath acetone analyzer is a reliable instrument for fast (~1 s), highly sensitive (>57 ppb), and quantitative measurement of breath acetone.

### E. Summary of the specifications of the breath analyzer

The specifications, experimental parameters, and performance of the portable near-real time online ringdown breath acetone analyzer were tabulated in Table I.

### F. Test of healthy and diabetic subjects using the validated ringdown breath acetone analyzer

49 nondiabetic healthy subjects, 13 Type 1 diabetic (T1D), and 57 T2D patients participated in this study over a study period of two weeks. The research procedures and activities followed the research protocols approved by the Institutional Review Board (IRB approval number: IRB2013-053-01) of human subject research in Tianjin. The more detailed information including process and experimental results was shown in the supplementary material.[52]

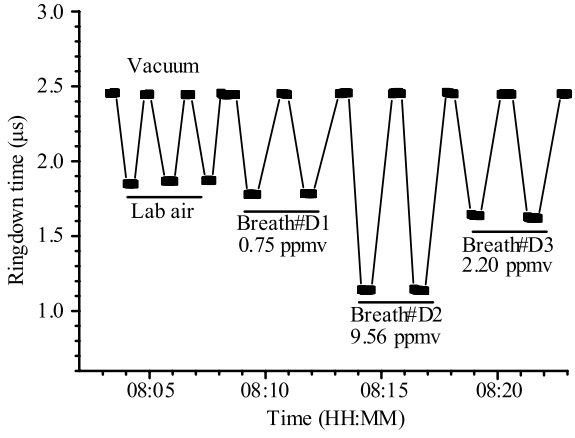


FIG. 7. Fast response of the breath analyzer to the change of breath gas.

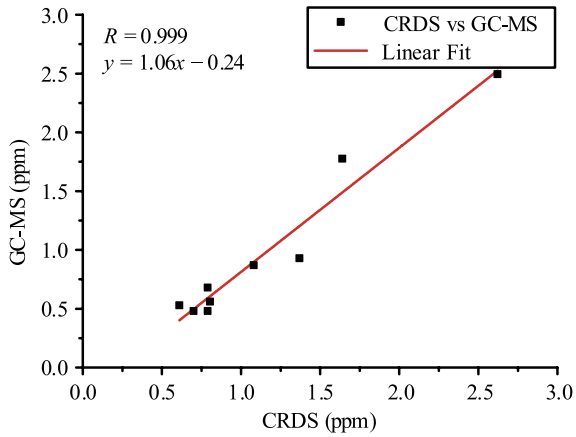


FIG. 8. The performance of the ringdown breath analyzer validated by the certified GC-MS facility.

TABLE I. Specifications, experimental parameters, and performance of the acetone breath analyzer.

| Portable near-real time online ringdown breath acetone analyzer | |
|---|---|
| Specifications | Dimension: 65×40×20 $cm^3$<br>Weight: ~15 kg<br>Display/storage: Auto/internet |
| **Experimental parameters** | |
| Laser wavelength | 266 nm |
| Single-pulse energy | 4.5 $\mu$J |
| Repetition rate | 1 kHz |
| Dimension of the laser head | 20.9×8.8×7.4 $cm^3$ |
| Dimension of the laser power supply | 23.8×14.6×10.2 $cm^3$ |
| Mirror reflectivity | R > 99.9956% |
| Cavity length | 0.5 m |
| Cavity pressure | Zero Torr or atmospheric pressure |
| Sampling method | Online |
| Response spectral range of PMT | 230 ~ 700 nm |
| Dimensions of PMT | 5.0×2.2×2.2 $cm^3$ |
| Average number of sampling | 128 |
| **Performance parameters** | |
| Average ringdown time of a vacuum cell | 2.36 $\mu$s |
| Measured mirror reflectivity | 99.93% |
| The best baseline stability | 0.16% |
| Detection limit | 57 ppb |
| Response time of the analyzer | ~1 s |
| Linear response to acetone in the range of 0–20 ppm | $R = 0.999$ |
| The slope of the validation curve by GC-MS | R = 1.06 |

Four breath samples from each individual subject were collected and tested under four different conditions: fasting, 2 h post-breakfast, 2 h post-lunch, and 2 h post-dinner in this study. The mean value of breath acetone concentration for the diabetic subjects (T1D and T2D) under both fasting and 2 h post-meals is all higher than that in the nondiabetic healthy human subjects correspondingly, as shown in Fig. 9.

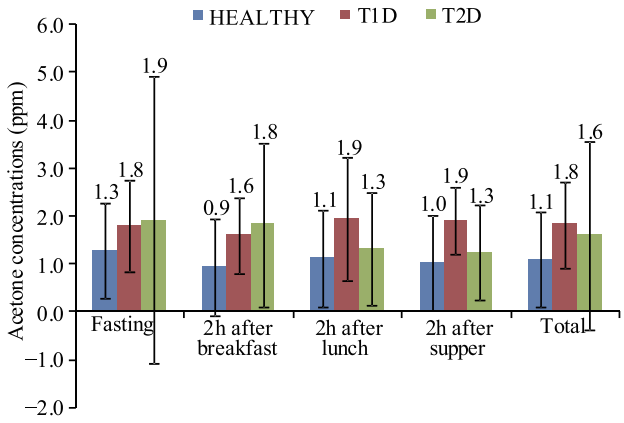


FIG. 9. Mean breath acetone concentrations in the 49 nondiabetic subjects, 13 T1D subjects, and 57 T2D subjects under both fasting and 2 h post-meals. The error bar corresponds to one standard deviation.

## V. ADVANTAGES AND LIMITATION OF THE BREATH ANALYZER COMPARED WITH OTHER INSTRUMENTS

There are several types of breath acetone instruments currently available.[53–58] Here, the "instrument" designates only "a portable, standalone, integrated, and auto- or semi-automated instrument" and excludes those that are just transportable and loosely connected systems even though they are tested in a clinic. For example, in our previous report, a compact atmospheric ringdown cavity with a cavity length of 10 cm was constructed and implemented to explore the potential instrument portability (optical configuration and detection sensitivity).[44] That device was not integrated with electronics, a light source, a detector, pressure control, and sample introduction. The detection limit of the system was only 1.5 ppm due to the short cavity length. Later on, the first portable acetone detection device using CRDS at 266 nm for human breath analysis was demonstrated and used for breath acetone measurements in a clinic.[46] With a longer cavity, a better detection limit of 0.49 ppm was achieved. However, this "portable" device was still not integrated; it included the cavity mounted in an optical plate, an oscilloscope displaying ringdown waveform, a detector powered by a bulky high voltage power supply, and a laptop for data processing. The cavity had no pressure control, which had adverse effects on reproducibility and difficulty in cavity cleaning. Those systems or prototypes are, therefore, not referred to as an instrument defined in this work.

The breath acetone instruments listed in Table II are based on several different techniques or their combinations, such as GC combined with semiconductor sensors, MS combined with different gas specification methods (GC, PRT, and SIFT), laser spectroscopy, or semiconductor electronic noses. The Biogas Acetone Analyzer (BAS-2000) for breath acetone and isoprene determination was developed by Kinoyama *et al.*[53] A calibration procedure is required and the chemical separation process is relatively time-consuming. The analyzer is a GC-equipped semiconductor gas detector, in which synthetic air was used as the carrier gas and the calibration using mixed gas was performed every 50 samples. The BAS-2000 has a LoD of 0.1 ppm for acetone with reproducibility of ±1% and linearity up to 5 ppm. It has been used in 451 college students for the annual medical checkup. Breath samples were collected into breath-sampling bags and introduced into BAS-2000 using a syringe. It requires 2.5 ml breath gas and takes more than 3 min to analyze one sample. But this instrument is still much faster and less time-consuming than the conventional GC-MS.

Compared to the conventional MS-based (GS-MS) method, PTR-MS and SIFT-MS are the two most promising MS-based techniques used for on-site breath acetone analysis. The main advantages of PTR-MS and SIFT-MS are their fast response (hundreds of milliseconds) and capability of analyzing a single breath. Systems based on the two techniques have long been used for clinical study, and their size and weight have evolved to be transportable to date. For example, the latest SIFT-based system (Profile 3) has been engineered to be 120 kg in weight [e.g., Refs. 54, 11, 12, and 15–18. The challenge to make this third generation SIFT-

TABLE II. Current breath acetone instruments and their specifications of the technique, response time, sampling approach, calibration, portability, and cost estimate.

| Name/technique | Response time | Sampling approach | Calibration requirement | Portability | Costs | References |
|---|---|---|---|---|---|---|
| Biogas acetone analyzer (BAS-2000)/GC equipped semiconductor gas detector | 3–4 m | Bag collection and syringe introduction | Calibration performed on every 50 samples | Yes | Low | 53 |
| Conventional GC-MS | ~1 h | Sophisticated and time consuming | Yes | No | Very high | 5–10 |
| PTR-MS and SIFT-MS | Real time | Complicated | Yes | SIFT-MS Profile 3 weighs 120 kg | Middle | 54 |
| Breath acetone analyzer/ semiconductor-based gas sensor | ~1 m; recovery time: ~1 m | Straw-based method | Use calibration curves obtained with simulated breath | Hand-held 6.5 cm × 10 cm × 2.5 cm, 125 g | Low | 55–57 |
| Portable FT-IR multicomponent analyzer/FT-IR spectroscopy | 10 spectral scans/s | Mouthpiece or facemask-based method | Use certified gases and liquids as reference | Portable 18 kg | Middle | 58 |
| Cavity ringdown breath acetone analyzer | Near-real time | Direct breathing into gas cell or into a bag | No | Portable, 65 cm × 40 cm × 20 cm, 15 kg | Low | This work |

MS instrument system (Profile 3) more transportable may be compounded by the requirements of the vacuum/pressure control systems and the associated parts for the ion generation, injection, and detection. Due to the limited tube length, e.g., 5 cm, the full scan operation mode is less practical because of the short fly time (low selectivity). On the other hand, the gated mode for a selected mass-charge ratio, e.g., $m/z = 59$, is more useful for breath acetone analysis if the isobaric interference is effectively taken care of.

The semiconductor-based portable breath acetone analyzer introduced by Toyooka *et al.* uses two types of gas sensors with different sensitivities.[55] The design offers breath acetone measurement to be in the range of 0.2–50 ppm with a resolution of 0.1 ppm, even taking into account the presence of ethanol, hydrogen, and humidity. The analyzer has the dimensions of 6.5 cm × 10 cm × 2.5 cm, weighs 125 g, and is powered by two AA batteries. It had been used in the investigation on daily diet managements for over 14 days in 17 healthy adult subjects with body-mass indices (BMIs) above the Japanese standard. Other types of small size electronic noses have also been available for breath acetone analysis. For instance, a hand-held alcohol analyzer calibrated by GC was used for breath acetone analysis in children with refractory seizures who were consuming ketogenic diet.[56,57] In general, an electronic nose with their smaller size and low instrumental cost is good for detection of an individual gas once it is calibrated. However, the challenges of specificity, baseline drifting, and frequent recalibration remain in breath analysis.

Different from the MS and electronic nose based breath acetone instruments, a laser spectroscopic acetone instrument has its own advantages.

(1) The selectivity is based on spectral fingerprints, not reliant upon chemical reaction products or an external reference, and free from isobaric interference.
(2) With known absorption cross sections, the spectroscopic instrument gives absolute measurement of concentration.
(3) Because of the simple measuring principle that allows for a simple instrument configuration, the instrumentation cost is low and the instrument can be portable.

For example, the portable Fourier transform infrared (FT-IR) spectrometer (Gasmet™, Temet Instruments Oy, Finland) developed by Laakso *et al.* was used as a point-of-care analyzer for breath screening in patients.[58] The analyzer could obtain spectrum in the wavenumber range of 4000-900 $cm^{-1}$ with a resolution of 8 $cm^{-1}$ at a rate of 10 scans/s. For breath acetone measurement, it requires strict calibration using certified liquids to obtain reference spectra. Otherwise, the low spectral resolution (8 $cm^{-1}$) may not give an accurate determination of acetone due to the spectral overlap. As compared with the FT-IR spectrometer, the present cavity ringdown breath acetone analyzer uses a narrow linewidth (<0.1 $cm^{-1}$) laser source at 266 nm and the absorption path-length is effectively enhanced by the ringdown multiple-pass approach. Therefore, these two factors offer a combined feature of high selectivity and high sensitivity, besides the near-real time response and online breath sample introduction.

As compared with the inexpensive electronic noses, the instrument cost of the ringdown analyzer is much higher due to the expensive laser source and ringdown mirrors, but the operating cost is lower as the ringdown analyzer does not generate chemical wastes and requires no calibration. One limitation of the current ringdown breath acetone analyzer is the upper-limit measurement that includes collective absorption from any other trace absorption of other compounds at 266 nm.[43] Given abundant spectral structures of acetone in a reduced pressure in the UV spectral region,[44] more accurate measurement (not the upper limit of acetone concentration) of acetone using a narrow linewidth UV laser can be achieved.

For the cavity ringdown system, the contamination of the cavity mirrors caused by using a large number of breath samples will result in minor degradation of LoD because the

contamination will lower the mirror reflectivity that affects LoD. However, the minor change in mirror reflectivity, e.g., a 5% decrease in the measured ringdown time of a vacuumed cavity, does not affect the measurement accuracy of the instrument, according to the ringdown measuring principle (Equations (1) and (2)). The current instrument can have a decrease of less than 5% in the ringdown time of a vacuumed cavity when it is continuously used over 3 months at a usage rate of 40 breath tests per day; yet that small change has negligible effect on the instrument normal operation (accuracy and precision). With an improved cavity design in the next version of the instrument, we expect that the mirror degradation caused by the long-term use of breath gas samples will not be a concern at all.

## VI. CONCLUSIONS

The CRDS based experimental setups, bulky transportable systems, and prototypes have been used in breath acetone measurements in the previous efforts; however, a standalone, fully packaged, portable breath acetone analyzer based on the CRDS technique for near-real time, online breath acetone measurement has not been achieved before. Here, we reported the first of its kind instrument's design, configuration, specifications, validation, and tests in detail. The performance of the instrument for quantitative measurements of breath acetone was investigated and validated using the certificated GC-MS. The results show this portable analyzer is ready for reliable near-real time (~1 s), online (online introduction of breath sample without pre-treatment) breath acetone analysis with high sensitivity (>57 ppb), high selectivity, high accuracy, and high data throughput. The validated portable breath analyzer was demonstrated via measuring 351 breath acetone samples collected from 49 nondiabetic healthy subjects in the CRDS lab and 70 diabetic patients (13 T1D subjects and 57 T2D subjects) under various situations (fasting or post-meals) in a clinic. This portable breath analyzer can be readily placed in different places in a clinic to perform real-time and online breath acetone analysis using a large number of subjects (human or animal) in a short period of time. The current acetone breath analyzer uses an off-line process to yield the absolute acetone concentration from the recorded ringdown times. A future upgraded version of the instrument will integrate a software package to allow absorption cross section of acetone at given conditions, such as wavelength, pressure, and temperature, to be input through a parameter-setting panel, and an absolute acetone concentration will be displayed from each measurement. The current instrument platform can be extended for the development of a line of breath gas instruments using corresponding laser sources and ringdown mirrors.

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