A Novel Handheld Real-time Carbon Dioxide Analyzer for
Health and Environmental Applications

by

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ABSTRACT

The accurate and fast determination of carbon dioxide (CO\textsubscript{2}) levels is critical for many health and environmental applications. For example, the analysis of CO\textsubscript{2} levels in exhaled breath allows for the evaluation of systemic metabolism, perfusion, and ventilation, and provides the doctors and patients with a non-invasive and simple method to predict the presence and severity of asthma, and Chronic Obstructive Pulmonary Disease (COPD). Similarly, the monitoring of CO\textsubscript{2} levels in the atmosphere allows for assessment of indoor air quality (IAQ) as the indoor CO\textsubscript{2} levels have been proved to be associated with increased prevalence of certain mucous membrane and respiratory sick building syndrome (SBS) symptoms.

A pocket-sized CO\textsubscript{2} analyzer has been developed for real-time analysis of breath CO\textsubscript{2} and environmental CO\textsubscript{2}. This CO\textsubscript{2} analyzer is designed to comprise two key components including a fluidic system for efficient gas sample delivery and a colorimetric detection unit integrated into the fluidic system. The CO\textsubscript{2} levels in the gas samples are determined by a disposable colorimetric sensor chip. The sensor chip is a novel composite based sensor that has been optimized to provide fast and reversible response to CO\textsubscript{2} over a wide concentration range, covering the needs of both environmental and health applications. The sensor is immune to the presence of various interfering gases in ambient or expired air. The performance of the sensor in real-time breath-by-breath analysis has also been validated by a commercial CO\textsubscript{2} detector. Furthermore, a 3D model was created to simulate fluid dynamics of breath and chemical reactions for CO\textsubscript{2} assessment to achieve overall understanding of the breath CO\textsubscript{2} detection process and further optimization of the device.
DEDICATION

To my parents and husband
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There are so many people to thank for helping me during the last four years. Ph.D study has never been easy but with so many people’s help and support, I have really enjoyed it and gained valuable experience.

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\[ Absorbance = 0.4 - \frac{0.4386}{1 + \exp \left( \frac{pH - 9.41085}{0.63016} \right)} \]

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\]

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CHAPTER 1
INTRODUCTION

Carbon dioxide (CO$_2$) is a naturally occurring gas which exists in both the Earth’s atmosphere and the exhaled breath of human being and other animals. As part of the carbon cycle, the concentration of CO$_2$ in the atmosphere is around 330 parts per million (ppm). High CO$_2$ levels in the ambient air may cause several environmental problems and health issues. As a metabolic product, the CO$_2$ levels in the exhaled breath of human being typically range from 3.5% to 6%. Abnormal CO$_2$ levels in the exhaled breath can be considered as the indication of several diseases. Therefore, a capability that can detect carbon dioxide (CO$_2$) with high accuracy and with fast response time is critical for many health and environmental applications.

1.1. Applications of Breath CO$_2$ analysis

The monitoring of CO$_2$ concentration in breath has come to play an increasingly important role in research related to personal healthcare applications [1–15]. The measurement of end-tidal carbon dioxide (EtCO$_2$), which is the carbon dioxide levels released at the end of expiration, is a well-known method to evaluate systemic metabolism, perfusion, and ventilation. It provides doctors and patients with a non-invasive and simple method to predict the presence and severity of asthma, chronic obstructive pulmonary disease (COPD) and diabetic ketoacidosis, and evaluate the effectiveness of treatment. In the meantime, the quantification of volume of CO$_2$ in
exhaled breath (VCO₂) provides valuable information for weight management, as VCO₂ is commonly used for the estimation of resting energy expenditure (REE), which typically represents more than 75% of total energy expenditure (TEE) of human body [16].

1.1.1. Respiratory management

Among various chronic diseases, chronic obstructive pulmonary disease (COPD) and asthma are the leading concerns [1-15]. In US, COPD is a major cause of disability, and also the third leading cause of death. Around 10 million Americans are known to have COPD, most of whom are middle-aged or older adults. Typically, COPD includes two main conditions: emphysema and chronic bronchitis. Emphysema is caused by the damage to the walls between alveoli in the lungs, which leads to fewer and larger alveoli instead of many tiny ones, and as a result, the gas exchange in the lungs is hindered. Chronic bronchitis involves the constant inflammation of the airways (bronchial tubes), which leads to the production of extra mucus inside the airways and make the patients hard to breathe. Generally, most of patients with COPD suffer from both emphysema and chronic bronchitis.

Besides COPD, asthma is also a chronic, sometimes life-threatening, disease that inflames and narrows the airways. Different to COPD, asthma may affect people of all ages. In US, more than 25 million people have been diagnosed with asthma. About 7 million of these people are children. For the patients who suffer from asthma, their airways are often swollen and inflamed, which make them very sensitive to some irritants (asthma triggers) such as dust, chemicals and smoke. When having an asthma attack, the
patient’s airways will be badly swollen and produce lots of mucus which make the patient difficult to breathe [17–18].

Currently, there is no cure for asthma and COPD. The damage to airways and lungs cannot be reversed. However, proper treatment and disease management will help patients to slow the progress of the disease and live a normal life [17–18]. Successful management of asthma and COPD requires routine monitoring of the patients’ respiratory status. Therefore, new technologies, such as personal devices that allow patients to monitor their diseases under free-living (or near free-living) conditions, can contribute significantly to respiratory disease management by minimizing doctor visits, lowering healthcare costs, and providing more frequent measurement disease signatures for evidence-based treatment.

Real-time breath carbon dioxide analysis, also called capnography, is a simple and non-invasive method to monitor the state of COPD and asthma and evaluate the effectiveness of treatment. It provides a real-time numeric reading of breath CO₂ concentration (or partial pressure) and graphic display of CO₂ waveform throughout the process of respiration. As shown in Fig. 1.1 (a), before expiration (A – B), the partial pressure of CO₂ in gas sample is close to zero. As expiration starts (B – C), an upstroke in CO₂ level can be observed as alveolar gas, which is rich in CO₂, rapidly mixes with the CO₂-free anatomical dead space gas. With expiration continuing (C – D), the CO₂ level reaches the plateau and the CO₂ level at the end of expiration is called End-tidal CO₂ (EtCO₂). When inspiration begins (D – E), a rapid decrease in CO₂ level can be seen from the waveform, which is caused by the washout of alveolar gas from gas sample. For the
patients with COPD or asthma, their CO\textsubscript{2} waveforms exhibit a characteristic “shark fin” shape that differs from that of subjects with normal lung function. As shown in Fig. 1.1 (b), the take-off angle (α) of the expiratory upstroke phase is decreased and the alveolar plateau elevation angle (β) is increased in CO\textsubscript{2} waveforms, which are the result of bronchospasm (COPD and asthma). Typically, bronchospasm causes a slower and more erratic emptying of CO\textsubscript{2} from the alveoli and results in a delay in the expiratory upstroke [19–24]. It is worthwhile to note that these differences in CO\textsubscript{2} waveforms are characteristic of bronchospasm and the differences increase with disease severity.

![Figure 1.1](image)

**Fig. 1.1.** (a) The breath CO\textsubscript{2} waveform of healthy subject with normal breath. (b) The breath CO\textsubscript{2} waveform of subject with bronchospasm. The differences in CO\textsubscript{2} waveforms can be observed between healthy subject and patient with bronchospasm [1].
In addition, breath CO\textsubscript{2} analysis can also be used for the identification and assessment of ventilation-perfusion mismatch. The End-tidal CO\textsubscript{2} (EtCO\textsubscript{2}) is reflective of CO\textsubscript{2} concentration in the alveolar areas of the lung, and typically used as an important tool of diagnosis. In the meantime, since EtCO\textsubscript{2} level is proportional to the partial pressure of arterial CO\textsubscript{2}, it is also commonly used to evaluate the gas exchange efficiency in the lungs [25–31]. In general, the normal range of EtCO\textsubscript{2} level is between 35 – 45 mmHg. An elevated EtCO\textsubscript{2} level (>= 45 mmHg) and decreased respiratory frequency are typically indications of hypoventilation, which may be caused by fever, sepsis, pain, severe difficulty breathing, depressed respirations, chronic hypercapnia and altered mental status such as overdose, sedation and intoxication. In contrast, a reduced EtCO\textsubscript{2} level (l<= 35 mmHg) and increased respiratory frequency may be the indications of hyperventilation, which may relate to anxiety, bronchospasm, pulmonary embolus and cardiac arrest (Fig. 1.2) [32–34].

![CO\textsubscript{2} waveforms for normal ventilation, hyperventilation and hypoventilation.](image)

Fig. 1.2. The CO\textsubscript{2} waveforms for normal ventilation, hyperventilation and hypoventilation.
1.1.2. Diabetic ketoacidosis (DKA) assessment

Type 1 diabetes mellitus, which is caused by impaired insulin secretion and/or function, is the second most common chronic disease in children. In US, about 1 in every 600 children is diagnosed with Type 1 diabetes. Diabetic ketoacidosis (DKA), which mainly occurs in patients with Type 1 diabetes mellitus, is a life-threatening complication of diabetes mellitus that requires urgent inpatient treatment. The patients with DKA usually have syndromes such as hyperglycemia (blood glucose levels $\geq 250$ mg/dl), ketonuria and metabolic acidosis (blood pH $< 7.3$ or serum bicarbonate levels $< 15$ meq/dl) [35, 36]. In practice, blood glucose levels can be measured at the bedside via a hand-held glucometer. The presence or absence of urine ketones can be rapidly determined with a urine dipstick. However, the assessment of metabolic acid–base status still remains challenging as the severity of acidosis is usually assessed by analyzing venous or arterial blood samples. Those blood tests are painful and invasive, and the blood specimen transport and other delays may impede timely availability of the results to the treating clinician [36, 37]. Therefore, a non-invasive, fast and accurate determination of the metabolic acid-base status is needed. Since EtCO$_2$ closely approximates the arterial CO$_2$ tension (PaCO$_2$) and body serum bicarbonate (HCO$_3^-$) is linearly proportional to PaCO$_2$, EtCO$_2$ values can be used for the estimation of serum bicarbonate levels in patients with healthy lungs (alveolar tissues) [37]. Fearon et al., Gilhotra et al., Garcia et al., and Soleimanpour et al. have found that the EtCO$_2$ values are proportional to the serum bicarbonate levels and significantly lower in patients with DKA
Therefore, EtCO$_2$ can serve as a promising method for prediction of presence and severity of diabetic ketoacidosis.

1.1.3. Weight management

Obesity is one of the most important public health problems in US. Currently, around 35% of adults and 17% of children and adolescents are obese. Obesity can lead to several health problems such as heart disease, stroke, type 2 diabetes and certain types of cancer [40, 41]. An important aspect of the study of obesity is the assessment of energy balance and intake. An accurate assessment and tracking of total energy expenditure (TEE) can guide individuals to achieve proper energy balance [42]. In general, TEE is made up of resting energy expenditure (REE), activity energy expenditure (AEE) and thermic effect of food (TEF). REE is the energy expenditure required to maintain basic body functions in a resting state. AEE is the energy expended by the body in any movement, including both exercises and obligatory activities. TEF is the energy used for digestion of food. Compared to AEE (~ 15% of TEE) and TEF (~ 10% of TEE), REE usually represents the largest percentage (>75%) of TEE [42]. Therefore, an accurate estimation of REE plays an important role in weight management [43]. Various equations have been developed to calculate REE based on physical characteristics of individuals (e.g., gender, weight, height, age), but the accuracy of the equations is questionable, particularly for overweight and obese populations [44], athletes, and patients undergoing weight loss [45–48]. Currently, the most widely accepted method for measuring REE is
indirect calorimetry, which determines REE based on oxygen consumption (VO$_2$) and carbon dioxide production (VCO$_2$) rates using the Weir equation [42, 43]:

$$
\text{REE} = [3.9 \times \text{VO}_2 + 1.1 \times \text{VCO}_2] \times 1.44
$$  

(1.1)

where REE is in kCal/day, and VO$_2$ and VCO$_2$ are the consumed oxygen rate and produced carbon dioxide rate in mL/min. Thus the breath CO$_2$ analyzer, in combination with a breath O$_2$ detection element and an expiratory flow monitoring system, can be used for REE estimation and weight management.

Fig. 1.3. The balance between energy intake and energy expenditure. REE, which accounts for over 75% of TEE, plays an important role in weight management.

1.2. Applications of environmental CO$_2$ analysis
CO₂ is a naturally occurring gas with its normal concentration around 330 parts per million (ppm) in the atmosphere. According to the Occupational Safety and Health Administration (OSHA) standards, CO₂ can be considered safe at levels below 0.5% [49]. High indoor CO₂ levels, which are usually a result of poor building ventilation performance, may cause several health problems such as headaches and poor sleep quality [13, 50]. Erdmann et al. found that elevated CO₂ levels inside buildings are significantly associated with increased prevalence of certain mucous membrane and respiratory sick building syndrome (SBS) symptoms [15]. SBS is typically used to describe a set of symptoms such as headache, fatigue, eye symptoms, nasal symptoms and respiratory tract symptoms. It is usually reported by individuals who spend long time indoors, particularly in office buildings. The prevalence of SBS symptoms will reduce by 85% with decrease in indoor CO₂ levels. In addition, the indoor CO₂ levels can also impair people’s decision-making performance. Satish and Mendell et al. found that elevated indoor CO₂ levels are associated with statistically significant and meaningful reductions in decision-making performance [14]. Therefore, monitoring CO₂ levels to assess indoor air quality (IAQ) plays an increasingly important role in both public and personal health.
1.3. CO₂ sensors – State of the Art

To date, a number of different technologies have been developed for detection of CO₂ in gas and liquids. The most popular ones include: gas chromatography (GC) in combination with mass spectrometry (MS), potentiometry, non-dispersive infrared technology (NDIR) and colorimetry. However, only some of those technologies have been commercialized for breath analysis and environmental monitoring, since the response and or sensitivity of the sensors may not meet the requirements for breath and environmental CO₂ analysis.

1.3.1. Gas chromatography (GC) and mass spectrometry (MS)

Gas chromatography and mass spectrometry (GC-MS) are usually the most reliable technologies for quantification analysis of gases. In general, a gas chromatograph
uses a column to separate different compounds based on their different retention time, which is the time used for each compound to pass through the column. As the compounds exit the end of the column, they are detected and identified electronically by the instrument (Fig. 1.5) [53, 54]. Mass spectrometer, on the other hand, detects the compounds by ionizing the compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios. The compounds are firstly ionized by the instrument, and separated according to their mass-to-charge ratio. Those separated ions are then detected by a mechanism capable of detecting charged particles. The atoms or molecules can finally be identified by correlating known masses to the identified masses [55, 56]. Although GC and MS techniques exhibit high accuracy in measurement of gas concentration, they are very expensive and cannot be used for breath-by-breath analysis of CO₂.

Fig. 1.5. Schematic diagram of a gas chromatograph.
1.3.2. Potentiometry

1.3.2.1. Potentiometry by Severinghaus electrode. The potentiometric method for measuring CO$_2$ was developed by John W. Severinghaus in 1958. Severinghaus electrode is one of the most widely used potentiometric CO$_2$ sensors. The sensor usually includes a thin polymer membrane, a hydrogen carbonate containing electrolyte solution, a thin hydrophilic spacer sheet soaked with the electrolyte solution and a pH probe. Typically, the thin polymer membrane is made of polytetrafluoroethylene (PTFE), polymethylpentene (TPX), silicone or polypropylene (PP). CO$_2$ in gas sample permeates through the permeable membrane into the hydrogen carbonate solution, which leads to a decrease in pH of the system and the concentration of CO$_2$ in gas samples can be determined through measurement of localized changes in ion activity [57]. The Severinghaus - type CO$_2$ electrode is small in size and has low cost. However, the response time of the CO$_2$ electrode, which mainly depends on the permeation of CO$_2$, is relatively slow [58]. It also needs to be calibrated regularly using standard calibration gas as the electrolyte solution inside the sensor is not stable over long periods of time. Furthermore, the sensor is susceptible to interference, which limits its application in both breath CO$_2$ analysis and environmental monitoring.

1.3.2.2. Solid - state potentiometric sensor. Solid - state sensor, which works potentiometrically according to the Nernst equation [59, 60], is another widely used sensor for gas detection. The sensor typically uses alkali carbonates, such as Li$_2$CO$_3$ and BaCO$_3$, as the electrodes and Li$_3$PO$_4$ or NASICON$^\circledR$ as solid electrolyte for the detection
of CO₂ [59–64]. The electrochemical equilibrium at the sensing electrode is the formation or decomposition of alkali carbonate involving both CO₂ and O₂. At high temperature (T > 300°C), alkaline ions are mobile via alkali ion vacancies inside the electrolyte. Charge compensation occurs by migration of alkali ions from the side having lower CO₂ and O₂ concentrations to the side with higher concentration. As a result, alkali carbonate is formed on the side with higher gas concentration and it disappears on the other side. And the concentration of CO₂ in gas samples can be determined via measuring electromagnetic field (emf) of the system. The sensor is small in size and low cost. It also has good long-term stability and fast response. However, it must operate at high temperature and still suffers from strong interference from humidity, which limits its application in breath CO₂ analysis [59–64].

Fig. 1.6. Schematic illustration of the solid-state carbon dioxide sensor [60].
1.3.3. Non-dispersive infrared (NDIR) sensor

Currently, most of the CO₂ sensors in the market are based on non-dispersive infrared technology. It determines the CO₂ levels by measuring the absorption of electromagnetic radiation in the IR range. In general, the key components of NDIR sensors include an infrared source, a detection chamber, a wavelength filter, and an infrared detector. The gas sample is pumped or diffuses into the detection chamber. Infrared light that emits from the light source is filtered by the optical filter to ensure that only wavelengths in the absorbing spectrum of CO₂ enter the detection chamber. And the light intensity is determined by the infrared detector. Based on Beer – Lambert’s Law, the light intensity measured by the detector is inversely proportional to the CO₂ concentration in the gas sample [65]. NDIR sensors, which provide very fast response and high accuracy in CO₂ measurement, are commonly used in breath CO₂ analysis and environmental monitoring. However, like most of the other CO₂ sensors, NDIR sensors are also susceptible to humidity or require special pretreatment of the gas samples to reduce the humidity level. In addition, the instruments are usually bulky and very expensive, which limit their applications only to hospitals and clinical setting or environmental facilities [66, 67].
1.3.4. Colorimetric sensor

Colorimetry is another method to measure CO$_2$ in both our breath and the external atmosphere [68–73]. The colorimetric sensor typically comprises a light source, a detection chamber, a sensing element with CO$_2$ indicator and a photodetector. The sensing principle behind colorimetric CO$_2$ sensor is based on the chemical reactions between CO$_2$ and the pH-sensitive indicators. Briefly, gaseous CO$_2$ reacts with the sensing chemicals coated on the sensing element, and the pH indicator changes color due to the decrease in pH of the sensing element. Compared to the infrared CO$_2$ sensors, colorimetric CO$_2$ sensors have several potential advantages, such as compactness and low cost, which make them suitable for personal breath analysis and home air quality monitoring. However, the response and recovery time of most current colorimetric CO$_2$ sensors are too slow to be used for real-time breath-by-breath analysis (Fig. 1.8) and the sensitivity and reversibility of the sensing elements are not good enough to ensure accurate detection of environmental CO$_2$ levels. In order to solve this problem, sensor
pre-conditioning procedures have been established, including specific heat treatment to acquire fast response and recovery, which usually results in more complex instrumental systems [73].

![Image of Easy Cap II](image)

**Fig. 1.8** (a) Easy Cap II breath CO₂ detection and (b) its response to real breath. The sensor’s 90% response time ($t_{90}$) is 2.02s, which is too slow to achieve accurate breath-by-breath CO₂ patterns. Also, the sensor is semi-quantitative, which cannot provide accurate EtCO₂ levels.

Thus, there is a need in the development of a small, low-cost and easy-to-use CO₂ sensor that is capable for everyone to use anytime and anywhere for a more complete and accurate assessment of the patient’s disease status at home or a better control of the indoor air quality. During the work present in this thesis, a wireless pocket-sized CO₂ analyzer for real-time analysis of breath CO₂ and environmental CO₂ has been developed.
This CO₂ analyzer is based on colorimetric technology. The disposable sensor chip is used to detect CO₂ levels in both breath and the atmosphere. As CO₂ passes through the detection chamber and reacts with the sensing chemicals coated on the surface of the sensor chip, the sensor changes color and the concentration of CO₂ is determined by measuring the color change of the sensing element. The CO₂ analyzer syncs with a mobile device and an application (app) is run to provide the real-time data display, storage, and transmission of the testing results. In addition, a specific fluidic system has been designed to ensure the efficient gas sample delivery. And a 3D model has also been created to simulate breath delivery fluidics and CO₂ sensing chemical reactions for overall understanding of the breath CO₂ detection process and further optimization of the device.
CHAPTER 2
DEVELOPMENT AND MODELING OF A CARBON DIOXIDE SENSOR

2.1. Introduction

Among various chronic diseases, chronic obstructive pulmonary disease (COPD) and asthma are the leading concerns [1-5]. About 10 million Americans have been diagnosed with COPD and another ~20 million with asthma [5, 6]. Breath carbon dioxide analysis is a well-known method that measures the breath CO₂ level, which is proportional to the partial pressure of CO₂ dissolved in blood [7-12]. The method is popular, effective and widely used to diagnose and evaluate the states of COPD and asthma, however, most of the current CO₂ equipment are based on infrared detection, which requires collecting breath samples with a pump, sample treatment to reduce interference from high breath humidity, and frequent calibration originated from signal drift. The high cost has also limited the use of current CO₂ equipment inside hospitals.

An alternative approach to measure CO₂ is based on colorimetric detection, which has been explored and developed by many groups with promising performance [68-73]. Nonetheless, in order to analyze CO₂ in real time and real breath (high humidity and temperature) without pre-conditioning of the sample, we need not only fast and accurate CO₂ sensor, but also efficient and reliable fluidic design that can deliver the breath sample from the mouth to the sensor, which remains unsolved in the existing colorimetric detections.
In this chapter, we preliminarily designed a low cost and high performance CO₂ analyzer for breath CO₂ analysis. The analyzer features an accurate colorimetric CO₂ sensor that can analyze end tidal CO₂ (EtCO₂) concentration. More importantly, it includes a fluidic system designed for efficient delivery of breath sample to the colorimetric sensor and accurate measurement of the flow rate of expired air. A 3D model of this CO₂ analyzer was also created to simulate the fluid dynamics of breath samples inside the device and chemical reactions between breath samples and the sensing element, such that we are able to achieve reliable analysis of real breath sample with high humidity and varying temperature.

2.2. Experimental

The colorimetric CO₂ sensor described in this chapter is based on the color change of CO₂ sensing probe under different pH conditions. When CO₂ was absorbed by the sensor element, the CO₂ sensing probe, which is a pH indicator, will change color due to the decrease in pH of the sensor element (Fig. 2.1). The concentration of CO₂ in gas sample can be determined by measuring the color change of the sensor element.

![Color change of pH indicator at different pH value](image)
Fig. 2.1. The colorimetric CO$_2$ sensor is based on the color change of pH indicator under different pH conditions. During CO$_2$ detection, pH indicator changes color due to the absorption of CO$_2$.

2.2.1. Reagent and sensor preparation

The CO$_2$ sensor element developed in the present chapter contained a phase transfer catalyst as CO$_2$ capture reagent and thymol blue as CO$_2$ sensing probe [68-73]. All the reagents used were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). The sensor chip was made of a transparent acrylic sheet, on which a sensing and reference areas were created (Fig. 2.2). The sensing area was coated with a solution containing the phase transfer agent and thymol blue, while the reference area was coated with phase transfer agent only. The thicknesses of chemical coating layers for both the sensing and reference areas were determined to be a few hundreds of $\mu$m.

When the warm breath sample was brought into contact with the sensing element, water vapor condensed to the sensor surface, forming a thin film of aqueous solution (see more details in later sections). Initially, the pH of the solution film was found to be ~9.05, but it decreased as CO$_2$ in the breath dissolved in the film. The pH decrease in the sensing area was detected by a color change of thymol blue from blue to yellow due to pKa$_2$ ~ 8.9 [68, 69]. It is important to notice that the condensation of water plays role of hydrating the sensing chemicals and promoting the absorption of CO$_2$. In contrast, the reference
area did not contain thymol blue dye and its color change was negligible, which served as a reference to correct drift in the color detection system.

![CO₂ sensor chip](image)

**Fig. 2.2.** CO₂ sensor chip prepared with phase transfer agent and thymol blue. The sensor element changed color from blue to green after exposure to CO₂.

2.2.2. Device description

The CO₂ analyzer is sketched in Fig. 2.3. It has a detection chamber, which includes a red LED (wavelength = 633 nm, LEDtronics. Inc., CA, USA) at the top of chamber as light source and a photodiode array (OSRM GmbH, Germany) at the bottom as light detector. The LED wavelength was chosen to closely match the absorption peak of thymol blue (Fig. 2.4). The detection chamber has also a sensor chip receiver located above the light detector. The sensor chip is inserted into the sensor chip receiver, and illuminated by the light source. The absorbance of the sensing area on the sensor chip is determined from the measured light intensities of the sensing and reference areas as a function of time, according to
\[ \text{Absorbance}(t) = -\log_{10}\left( \frac{I_{\text{sensing}}(t)}{I_{\text{reference}}(t)} \right), \quad (2.1) \]

where \( I_{\text{sensing}} \) and \( I_{\text{reference}} \) are light intensities for the sensing and reference areas, respectively. Absorbance change is given by

\[ \Delta \text{Abs}(t) = \text{Absorbance}(t) - \text{Absorbance}(0), \quad (2.2) \]

where \( \text{Absorbance}(0) \) is the absorbance prior to the exposure of the sensor surface to breath sample. The measured absorbance change can be further normalized by the initial absorbance value, and it is the normalized absorbance change, Normalized \( \Delta \text{Abs}(t) \), that is used to characterize the color change of thymol blue associated with \( \text{CO}_2 \) concentrations in the present work.
Fig. 2.3. (a) 3D geometry of the colorimetric CO$_2$ analyzer device and main device components. Simulated and real expired air was passed through the device from the mouthpiece. (b) Lateral view of the significant positions of the device indicated as follows: 1- inlet, 2- orifice (device narrowest portion), 3- middle portion between inlet and sensor chamber, 4- sensor chamber, 5- portion between sensor chamber and the outlet, 6- outlet. (c) Schematic representation of sample flow direction, and sensor chip components, showing the position of the sensing and reference areas.
2.2.3. Device characterization - Chemical characterization

Since the CO₂ detection is based on the pH change, which is measured from the color change, a calibration curve between absorbance change and the pH value is required, which was obtained by casting solutions of different pH values (measured with a pH electrode, Extech Instruments, NH, USA) onto the sensing area. The calibration curve, shown in Fig. 2.5, can be fitted with a simple function,

\[
\text{Absorbance} = 0.4 - \frac{0.4386}{1 + \exp\left(\frac{\text{pH} - 9.41085}{0.63016}\right)}. \tag{2.3}
\]
Using the calibration function by Eq. 2.3, one can relate the measured color change to the chemical reaction-induced pH change in the sensing area, which was needed for direct comparison between the measured color change and modeled chemical reaction taking place in the sensing area.

Fig. 2.5. The relationship between the measured normalized absorbance change and the pH of sensing system modified with thymol blue. The absorbance value increased with pH and followed a sigmoidal function:

\[
Absorbance = 0.4 - \frac{0.4386}{1 + \exp\left(\frac{pH - 9.41085}{0.63016}\right)}
\]

Values of pka for thymol blue are indicated in the figure. Detected color changes in the CO₂ analyzer device were due to pka₂ (with a color change from blue (pH > pka₂) to yellow (pH < pka₂)).
Another important calibration is to determine the relationship between the color change and the breath CO$_2$ concentration, which can be achieved through two methods. In the first technique, we prepared artificial expired breath samples by mixing 80% N$_2$ + 20% O$_2$ gas with different concentrations of CO$_2$ (Praxair, Inc), and then pumped (Aqueon, WI, USA) the mixed gases through a sealed water system immersed in a thermostatic water bath (Thermo Scientific, USA) at 37.5 °C to generate 35 °C and 100% relative humidity artificial breath. The CO$_2$ concentration tested in this system ranged from 0.03% to 6.5%. The artificial breath samples were introduced into the breath analyzer at a flow rate between 6 L/min and 18 L/min. In the second method, we directly used real breath samples from volunteers to further validate the performance of our breath analyzer.

2.2.4. Device characterization - Temperature and flow characterization

During the tests, we have measured the temperature profile along the flow pathway for both the artificial and real breath samples by placing temperature probes at different positions in the CO$_2$ analyzer device. The flow rate and the pressure drop along the flow pathway were monitored with a flow meter (Newark, flow sensor 0-20 LPM) and a differential pressure sensor (All Sensors, USA).

2.3 Modeling methods

In order to achieve an overall understanding of the breath CO$_2$ detection process and a further optimization of the device, a 3D model of the CO$_2$ analyzer device was created using Comsol Multiphysics (COMSOL, Inc., MA, USA). This model simulated
fluid dynamics of the breath sample inside the device and chemical reactions between the breath sample and the sensor surface. The 3D geometry of the flow path and detection chamber is shown in Fig. 2.3.

2.3.1. Fluid dynamics simulation

To model the mass transport, the Reynolds numbers under various breathing conditions, e.g. flow rates between 6 to 20 L/min, were determined at the critical narrowest flow path of the system (position 2, Fig. 2.3(b)), which were relatively large (Table 2.1), indicating the presence of turbulent flow [74]. It is also worthy to notice that the presence of a sharp orifice in the geometry of the system introduced a turbulent flow that not only affected position 2 (Fig. 2.3(b)) but also positions located downstream along the device (see below, Fig. 2.6(a)). For this reason, a turbulent flow model was used. In addition, since the pressure change across the analyzer is relatively small (<14 cm H₂O for 6 to 18 L/min), the breath sample was treated as incompressible gas in the model simulation.

<table>
<thead>
<tr>
<th>Flow rate (L/Min)</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re</td>
<td>2697</td>
<td>3596</td>
<td>4495</td>
<td>5393</td>
<td>6292</td>
<td>7191</td>
<td>8090</td>
<td>8989</td>
</tr>
</tbody>
</table>

*Re = \( \rho vD/\mu \), where \( \rho \) is the density of expired air (1.26 kg/m³), \( v \) is the mean velocity of the flow, \( D \) is the diameter of the device (labeled as position 2 in Fig. 2.3(b)), \( \mu \) is the dynamic viscosity of expired air (1.983×10⁻⁵ kg/m/s).
The turbulent flow model used here is based on the Reynolds Averaged Navier-Stokes equation, which includes the turbulent kinetic energy \(k\) and turbulence dissipation rate \(\varepsilon\). This model is known as the \(k-\varepsilon\) turbulence model, and takes the form of

\[
\rho (\dot{u} \cdot \nabla) \dot{u} = \nabla \left[ -p \mathbf{I} + (\mu + \mu_T)(\nabla \dot{u} + (\nabla \dot{u})^T) - \frac{2}{3} \rho \kappa \mathbf{I} \right] + \mathbf{F}
\]

with \(\mu_T = \rho C_\mu \frac{K^2}{\varepsilon}\) and \(\nabla \cdot (\rho \tilde{u}) = 0\), where \(\rho\) is density of expired air (~1.26 kg/m\(^3\)), \(\tilde{u}\) is velocity gradient tensor, \((\nabla \tilde{u})^T\) is “transpose” of the velocity tensor, \(\rho\) is pressure, \(\mu\) is dynamic viscosity of expired air, \(\mu_T\) is turbulent viscosity, \(\mathbf{F}\) is volume force, \(C_\mu\) is a model constant (=0.09), and \(\bar{u}\) is the average velocity [74].

In addition to considering turbulent flow, we took into account heat transfer between the relative warm breath (~35 °C) and the cold surroundings (~25 °C). Since the flow rate is rather fast (6 to 18 L/min), heat transfer due to conduction mechanism was assumed negligible, and mainly driven by forced convection, which is described by

\[
-\bar{n} \cdot (-K \nabla T) = h \cdot (T_{\text{ext}} - T)
\]

where \(\bar{n}\) is the flow direction, \(K\) is the thermal conductivity of airflow, \(\nabla T\) is temperature gradient tensor, \(h\) is the convection heat-transfer coefficient, \(T_{\text{ext}}\) is the device external temperature and \(T\) is the fluid temperature [74].

Equations 2.4 and 2.5 were solved numerically with the following boundary conditions.

1) **Flow velocity on the device wall** [74]:

28
\[ \mathbf{\tilde{u}} \cdot \mathbf{n} = 0 \]  
\[ [(\mu + \mu_t)(\nabla \mathbf{u} + (\nabla \mathbf{u})^T) - \frac{2}{3} \rho k \mathbf{I}] \mathbf{n} = -\rho \frac{u}{\delta_w} \mathbf{u}_{tang} \]  
\[ \mathbf{\tilde{u}}_{tang} = \mathbf{\tilde{u}} - (\mathbf{\tilde{u}} \cdot \mathbf{n}) \mathbf{n} \]  
\[ \nabla \kappa \mathbf{n} = 0 \]

where \( \mathbf{n} \) indicates the flow direction, and \( \mathbf{u}_{tang} \) represents the tangential direction of the velocity vector.

2) Flow velocity at the device inlet:

\[ \mathbf{\tilde{u}} = -U_0 f(t) \mathbf{n} \]  

where \( U_0 \) is linear velocity of airflow at the inlet, \( f(t) \) is a time dependent function, which can be used to describe the breath pattern (assumed constant in this work), and \( \mathbf{n} \) is the flow direction.

\[ \kappa = \frac{3}{2} \left( U_0 \mathbf{I}_T \right)^2 \]  

where \( I_T \) is turbulent intensity

\[ \varepsilon = C_{\mu}^{3/4} \frac{K^{3/2}}{L_T} \]  

where \( L_T \) is turbulence length scale

\[ [(\mu + \mu_t)(\nabla \mathbf{u} + (\nabla \mathbf{u})^T) - \frac{2}{3} \rho k \mathbf{I}] \mathbf{n} = 0 \]

where the viscous stress \( (\mu + \mu_t)(\nabla \mathbf{u} + (\nabla \mathbf{u})^T \mathbf{n}) \) and apparent stress \( -\frac{2}{3} \rho k \mathbf{I} \cdot \mathbf{n} \) were considered zero.
3) **Flow velocity at the device outlet:**

\[ p_2 = P_0 = P_{atm} \]  
\[ \nabla \vec{n} = 0 \]  
\[ \nabla \vec{e} = 0 \]

4) **Temperature at the inlet:**

\[ T_m = 306.80K \]

5) **Temperature on the wall and at the outlet:**

\[ T_w = 298.15K \]

### 2.3.2. Chemical reaction kinetics

As described in the Experimental Section, the measurement of CO\(_2\) concentration was based on the absorption of CO\(_2\) by the sensing system, which is formed due to surface modification and water condensation from the sample (see more details below). Therefore, the transformation and chemical reactions of CO\(_2\) in the sensing and reference areas were described by

\[ \text{CO}_2(g) \underset{k_0}{\overset{k_0'}{\rightleftharpoons}} \text{CO}_2(aq) \]  
\[ \text{CO}_2 + H_2O \underset{k_1}{\overset{k_1'}{\rightleftharpoons}} H_2CO_3 \]  
\[ H_2CO_3 \overset{k_2}{\underset{k_2'}{\rightarrow}} H^+ + HCO_3^- \]  
\[ H^+ + OH^- \overset{k_3}{\underset{k_3'}{\rightarrow}} H_2O \]  
\[ HCO_3^- \overset{k_4}{\underset{k_4'}{\rightarrow}} CO_3^{2-} + H^+ \]
Steps 2, 3, 4 and 5 can be combined and simplified as

\[
CO_2 + OH^- \xrightleftharpoons[k_k']{k_k} HCO_3^-
\]

(R2.6)

\[
CO_2 + H_2O \xrightleftharpoons[k_k']{k_k} H^+ + HCO_3^-
\]

(R2.7)

where R2.6 represents CO\(_2\) reaction in the presence of excessive hydroxide anions, and R2.7 represents CO\(_2\) reaction after consumption of hydroxide anions. R2.6 and R2.7 were assumed to take place faster than R2.1 because the latter includes dissolution and internal diffusion of CO\(_2\) within the thin solution films formed by water vapor condensation. This assumption is reasonable because the acid-base reaction takes place in the pico-second range [25], compared to the milli-second range estimated for the diffusion of CO\(_2(aq)\) within the thin film layer on the sensor cartridge.

Thymol blue (TB) was the indicator for \([H^+]\) and \([OH^-]\) resulted from steps R2.6 and R2.7, which are described by

\[
TBH_2^{(red)} + H_2O \xrightleftharpoons[k_k']{k_k} TBH^-^{(yellow)} + H_3O^+
\]

(R2.8)

\[
TBH^-^{(yellow)} + OH^- \xrightleftharpoons[k_k']{k_k} TB^2^-^{(blue)} + H_2O
\]

(R2.9)

\[
TB^2^-^{(blue)} + H^+ \xrightleftharpoons[k_k']{k_k} TBH^-^{(yellow)}
\]

(R2.10)
Note that steps R2.8, R2.9, and R2.10 are also acid-based reactions, and can be combined with R2.6 and R2.7 to reflect the color changes due to the presence of dissolved \( CO_2(aq) \) in the sensing area, which takes the form of,

\[
TB^+ (\text{blue}) + H_2O + CO_2(aq) \rightarrow TBH^- (\text{yellow}) + OH^- + CO_2(aq) \rightarrow TBH^-(\text{yellow}) + HCO_3^-
\]  
(R2.11)

\[
TBH^- (\text{yellow}) + CO_2 + H_2O \rightarrow TBH^-(\text{yellow}) + H^+ + HCO_3^- \rightarrow TBH_2^-(\text{red}) + HCO_3^-
\]  
(R2.12)

Because the acid-base reactions and associated indicator reactions (steps R2.2 to R2.12) are fast, the rate determining step is the dissolution and diffusion of \( CO_2 \) (step R2.1), which can be described by the rate equation,

\[
\frac{d[CO_2]}{dt} = aC^* \sqrt{D(r + s)}
\]  
(2.19)

\[ C^* = \frac{P_{CO_2}}{K_{CO_2}} \]  
(2.20)

where \( a \) and \( s \) are constants, \( D \) is the diffusion coefficient of \( CO_2 \) (~1.42×10^{-5} \text{ cm}^2/\text{s} ), \( P_{CO_2} \) is the partial pressure of \( CO_2 \), \( K_{CO_2} \) is Henry’s constant of \( CO_2 \) (29.4 L·atm/mol) and \( r \) is the apparent reaction constant given by

\[
r \approx k_6 + k_5 \frac{K_w}{K_{a2}} \frac{[CO_3^{2-}]}{[HCO_3^-]} \]

(2.21)

where \( \frac{K_w}{K_{a2}} \frac{[CO_3^{2-}]}{[HCO_3^-]} = [OH^-] \), \( K_{a2} = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \), and \( K_w = [H^+][OH^-] \).
Note that the above equation of CO₂ diffusion rate, was cited from Danckwerts et al. under a similar condition [76, 77].

Based on steps R2.6 and R2.7, the concentration of protons in the thin solution films of the sensing and reference areas is given by

\[ [H^+] = \frac{K_{al} \times [CO_2]}{[HCO_3^-]} \]  \hspace{1cm} (2.22)

where \( K_{al} = 4.6 \times 10^{-7} \).

Based on Eq. 2.22, and the assumption that the concentration change of bicarbonate is negligible compared to the initial concentration ~0.01778 mol/L, the proton production rate can be expressed as

\[ \frac{d[H^+]}{dt} = \frac{K_{al}}{[HCO_3^-]} \times \frac{d[CO_2]}{dt} \]  \hspace{1cm} (2.23)

Eq. 2.23 and the related Eqs. 2.19 – 22 were used to correlate gas phase CO₂ concentration \( P_{CO_2} \) and absorbance changes due to \([H^+]\) changes, using the formerly introduced function in Fig. 2.4 (Eq. 2.3). It is worth to notice that the transport of CO₂ through the sensing layer occurred on the perpendicular direction along the sensor surface. While the condensation of H₂O on the sensor surface was assumed to be instantaneous driven by the gradient difference between the sample temperature and the ambient temperature (which applies to the sensor surface), the absorption of CO₂ was governed by internal diffusion in the sensing layer (given by Eq. 2.19 and 20).
Furthermore, the condensed H$_2$O was limited to a constant amount resulting from a constant sample volume passed through the system. These conditions defined a constant bicarbonate concentration for a given CO$_2$ concentration in the sample. As a result, the concentration of protons across the sensor surface could be simulated through a modified surface reactions model using Eq. 2.23 and the simulated absorbance patterns were obtained from Eq. 2.3 (see below). In addition to Eq. 2.3 and Eqs. 2.19 - 23, the mass transport and heat transfer equations (Eqs. 2.4 and 5) were used to simulate the response of the sensor as a function of CO$_2$ concentrations ranging 0.03% to 6.5%, at 35 °C. These equations were also used to determine the effective surface density of protons (moles/cm$^2$) that was correlated to color changes on the sensor surface (see below).

2.4. Results and Discussion

2.4.1. Characterization of the mass transport

In order to characterize the flow of the breath CO$_2$ sensor, mass transport simulations were carried out under various flow rates. Fig. 2.6(a) shows the velocity profile (upper panel) and streamline (lower panel) at a flow rate of 6L/min. The profile is clearly non-homogeneous and turbulent, especially in the region where the orifice (shown as the narrowest diameter portion) is located, and near the sensor cartridge. This flow profile is different from the velocity profile expected for a flow path with a uniform cross sectional area [78].

As a method to validate the model simulation, volume flow rate vs. pressure difference across the orifice was measured experimentally. The result is compared with the simulation in Fig. 2.6(b). Note that the pressure difference was determined by
connecting the inlet and outlet of a differential pressure sensor at points 2 and 5 of the flow path (Fig. 2.6(b)). The experimental and simulated results are in quantitative agreement, indicating that the turbulent flow model provides accurate description of the fluidic dynamics of the system.

The simulation results were further validated as compared with the Bernoulli equation, which expressed the flow rate vs. pressure difference by

\[
\text{Flow rate} = A_2 \cdot \sqrt{\frac{2}{1 - \frac{A_2^2}{A_1^2}}} \left( \frac{\Delta P}{\rho} \right),
\]

where \( \Delta P \) is the pressure difference, \( \rho \) is the gas density, and \( A_1 \) and \( A_2 \) are the cross-sectional areas at the orifice and at a location behind the orifice, respectively. As shown in Fig. 2.6(b), the flow rate – pressure difference relation by the Bernoulli principle agrees well with both the experimental and simulated results. It is worth to note that although the overall flow rate of the breath CO\textsubscript{2} sensor can be reasonably described by the Bernoulli principle, detailed velocity profile can only be obtained with the numerical simulation.
Fig. 2.6. Mass transport characterization of the CO₂ analyzer device: (a) Velocity profile and streamline of velocity profile for 6L/min flow rate. (b) The pressure drop of the airflow between position 5 and 2 (~ 1 mm away from orifice) at different flow rates. The experimental data (○) fitted the theoretical results (solid line) obtained from Bernoulli’s equation, and showed good agreement with the simulated data (●) from k-ε turbulence model.

2.4.2. Characterization of the temperature profile

Simulations of heat transfer process were carried out to determine the temperature profile at different flow rates. Fig. 2.7(a) shows the temperature profile throughout the CO₂ sensor for a typical flow rate of 6 L/min. It can be observed that the temperature decreases from 35 °C at the inlet to ~28 °C at the outlet with an average temperature of 31°C on the sensor chip. In addition, the heat loss was proportional to the flow velocity and the maximum temperature drop is across the narrowest portion of the device.

In order to corroborate the heat transfer model, the temperature was measured experimentally, and the results were compared to the simulations in Fig. 2.7(b). It shows excellent agreement between the experimental and simulated temperature profiles. The temperature decreases along the flow path from the device inlet to the outlet. This temperature profile has an important implication in water condensation along the flow path and on the sensor cartridge based on the following consideration. Since the initial temperature of expired air is around 35°C and the relative humidity of expired air is considered as constant (RH = 100%), the dew point of expired air reduced as the expired air was cooled down when it flows from the inlet towards the outlet of the device. As a
result, water vapor condenses on the wall of the analyzer and on the sensor cartridge. As mentioned earlier, the detection mechanism of breath CO₂ originates from the chemical reactions of the dissolved CO₂ from the breath samples within the water condensation film formed on the sensor cartridge. Evidence of water condensation from the inlet and throughout the analyzer was gathered by using a Selected Ion Flow Tube – Mass Spectrometry (SIFT-MS), which allow determining in-situ water vapor densities [79, 80]. Accurate vapor densities readings were taken using the SIFT-MS (Profile 3 from Instrument Science) set with H₃O⁺ ion source, and the instrument inlet tip. The readings ranged from breath condensing conditions (100% RH) at the inlet of the analyzer to ambient humidity (22% RH) at a distance of few centimeters away from the outlet. These evidences in connection to the observed water condensation on the sensor cartridge further allowed supporting the reaction mechanisms of the sensor.
Fig. 2.7. Heat transfer characterization: (a) Temperature profile of the CO$_2$ analyzer device corresponding for a flow rate of 6 L/ min with input and output temperature of 35 and 25 °C, respectively. (b) The temperature distribution of the artificial expired air throughout the device. There was a good correlation between real temperature ($x$) and the simulated temperature ($y$) with the following relationship: $y = 2.51 + 0.992x$, $r^2 = 0.9988$.

2.4.3. Characterization of the chemical reaction

The basic sensing principle, as described earlier, is that the CO$_2$ in breath interacts with the sensing element and thus decreases the pH of the system, which is determined via monitoring the color change of thymol blue in the sensing area. The relationship between the concentration of CO$_2$ and the absorbance of thymol blue was investigated by both numerical simulation and experimental measurement.
Figs. 2.8(a) and (b) show the measured and simulated normalized absorbance vs. time at the CO$_2$ concentration of 0.03% and 6.5%, respectively. First, the plots show that the simulated and experimental results are in good agreement, further validating the models used in the simulation. Second, the results show that the color change (of thymol blue from blue to green) at a fixed flow rate of 6 L/min takes place rapidly at the initial stage and then slows down. The rapid response in the early stage is due to reaction step R2.6, which corresponds to the reaction of CO$_2$ with the excess amount of hydroxyl ions. Over time, hydroxyl ions are consumed and step R2.7, corresponding to the dissolution of CO$_2$, takes over, which is denoted by the slower phase at a later stage.

Furthermore, we are able to correlate the maximum color change (or maximum absorbance change) to the CO$_2$ concentration. Fig. 2.8(c) plots experimental and simulated maximum changes in the normalized absorbance vs. CO$_2$ concentration, and the good agreement indicates that our proposed reaction mechanism and kinetics are reasonable.
Fig. 2.8. (a) Measured and (b) simulated normalized absorbance versus time at the CO$_2$ concentration of 0.03% to 6.50%, respectively. The normalized absorbance value decreased as thymol blue changed color from blue to yellow. (c) Calibration of sensor response to CO$_2$. The experimental data (■) are in good agreement with the simulated results (●). The decrease in Normalized ΔAbs followed Langmuir Absorbance model:

$\text{Normalized Absorbance} = \frac{1.13x}{(0.776 + x)}$

We also would like point out that the chemical reaction of the CO$_2$ analyzer was the spatial distribution of the color change across the sensor surface. Non-uniform distribution of the color change is expected because the flow is inhomogeneous and turbulent, which affects the pH or proton profile across the sensor surface, resulting in inhomogeneous color development. Consequently, the spatial distribution of the color change may be used to characterize the actual concentration gradient of protons on the sensor surface. Figs. 2.9(a) and (b) show an experimental image of the sensing area after 6.5 s of exposure to a CO$_2$ sample, and a simulated image obtained under a similar
condition, respectively. Note that the simulated image from the simulated proton concentration profile is shown in Fig. 2.9(d), which also matched the proton distribution pattern calculated from the real image (Fig. 2.9(c)). The simulation not only reproduces the inhomogeneous color change across the sensor surface, but also predicts the reaction time necessary for the accurate CO$_2$ concentration analysis in the sample. For instance, relatively short exposure (6.5 s) of the sensor surface to CO$_2$ concentrations that are relevant to real breath samples produced non-homogeneous color development at the top left part of the sensing area (yellow), while the other areas remain blue. Therefore, sufficient exposure time is needed to minimize the inhomogeneity caused by turbulent flow, and to ensure the reaction sites across the entire sensing area to react with CO$_2$ in the sample. Finally, the experimental and simulated images are in good agreement, which, once again, validates the models.
Fig. 2.9. (a) The real and (b) simulated sensing area at $t = 6.5s$. The simulated sensing surface represented the concentration gradient of protons. (c) The 3D surface plot of proton concentration in the real sensing area and (d) the 3D surface plot of proton concentration in the simulated sensing area. The concentration distribution of protons was non-uniform across the sensing area.
2.4.4. Validation of the model

Based on the simulation and real image processing outcomes, we set a reaction time of 60 s for the CO\textsubscript{2} analyzer device, which is equivalent to 6 L sample volume for a typical breathing rate of 6 L/min. Therefore, 6L volume samples of both real and artificial breath were used for the CO\textsubscript{2} analyzer device calibration. Once the breath CO\textsubscript{2} analyzer was calibrated, 6L-volume samples were injected and processed as unknown samples using the device calibration to assess the CO\textsubscript{2} concentration. In parallel, a commercial CO\textsubscript{2} analyzer (Vacumed, Venture, CA) was used to determine the CO\textsubscript{2} concentration in the samples. In Fig. 2.10, we compare the results assessed from our CO\textsubscript{2} analyzer device and the commercial CO\textsubscript{2} analyzer. Clearly, the CO\textsubscript{2} concentration in both cases correlate at a slope of 1.01, an error of <2\%, and a squared - regression coefficient ($r^2$) of 0.9981, which indicates an accuracy level close to a 100\% between these two devices.
Fig. 2.10. Correlation of CO₂ levels from real and artificial breath samples determined by the CO₂ analyzer device (y) and a commercial CO₂ analyzer instrument (x). Linear correlation analysis of the variables was: \( y = 1.007 \times, r^2 = 0.9981 \) indicating good accuracy level.

2.5. Conclusions

In this chapter, we have preliminarily designed a colorimetric-based breath analyzer including a CO₂ sensor and a fluidic system for efficient delivery of high humidity and variable temperature breath sample, and have applied this analyzer to accurately detect and analyze the CO₂ in real breath. Moreover, we have proposed models taking into account mass transport, heat transfer and chemical reactions. The numerical simulation results based on the models and actual geometry of the device are compared with experimental data, showing quantitative agreement. In addition, the simulations provide both spatial and temporal distributions of breath flow velocity, pressure and temperature, and also spatial profiles color development over time. This information is difficult to achieve merely from experiments, but is critical for further optimizing the performance of breath analyzer.

However, due to the specific design of the sample delivery system, which includes a small orifice along the air passageway inside the device to measure the flow rate of exhaled breath, users are not able to inhale through the device. Also, as the engraved gird patterns on the surface of the sensor chip delays the response of sensor to CO₂, only an average CO₂ level in the exhaled breath can be provided by this CO₂
analyzer, which means the performance of the sensor must be further improved to meet the requirements of real-time analysis of CO₂. The 3D model created in this chapter, which accurately simulated the gas sample fluid dynamics and chemical reactions, can be used to predict the sensor response under different conditions and provide valuable information for sensor optimization.
3.1. Introduction

The determination of CO₂ concentration in breath plays an important role in personal health care. For example, the breath CO₂ patterns can be used as a fast and non-invasive method for diagnosis of asthma and COPD [1–13]; the end-tidal CO₂ levels can also be used to evaluate the presence and severity of diabetic ketoacidosis [5, 6]; and the average CO₂ level in expired breath can be used for the estimation of resting energy expenditure [16]. Similarly, the monitoring of CO₂ level in the atmosphere is also critical to indoor air quality control since high indoor CO₂ level is associated with the prevalence of sick building syndrome symptoms and likely to significantly impair people’s decision-making performance [14]. Since the CO₂ level in expired breath typically ranges from 3% to 6% and the CO₂ concentration in the atmosphere is just around a few hundred ppm (parts per million) [9, 14], it is important to develop a CO₂ sensor with high accuracy and large dynamic range, which can be used for both breath CO₂ analysis and indoor CO₂ monitoring.

In Chapter 2, we have developed a pocket-sized colorimetric sensor which can be used for breath CO₂ measurement. The sensor element changes color after exposure to CO₂ and shows good sensitivity at relatively low CO₂ level. However, the sensor shows low sensitivity and slow response at high CO₂ level, which is not suitable for real-time breath-by-breath CO₂ analysis since it may bring high measurement error when the expired CO₂ level and/or breathing frequency is relatively high. Therefore, it is very
important to improve the performance of the sensor element to meet the requirements of real-time breath analysis.

In this chapter, we optimized the performance of the sensor element by increasing its sensitivity, enlarging its dynamic range and improving its response time.

3.2. Experimental

3.2.1. Optimization of sensitivity and dynamic range

Since the concentration of CO$_2$ is determined by measuring the color change of the CO$_2$ sensing probe, which is caused by the change of pH in the sensor element, the sensor sensitivity and dynamic range can be improved by choosing a sensing probe with significant color change after exposure to CO$_2$ and a phase transfer agent with high CO$_2$ capture ability.

3.2.1.1. Selection of CO$_2$ sensing probe: As described in Chapter 2, during the CO$_2$ detection process, the pH of the sensor element typically varies between 6 and 9. Therefore, a CO$_2$ sensing probe with pKa value around 7 or 8 is probable to provide high sensor sensitivity. In this study, two sensing probes, thymol blue and $m$-cresol purple, were selected to prepare the CO$_2$ sensor elements using the method described in Chapter 2. The sensor response was measured to investigate the effect of sensing probe on sensor sensitivity and dynamic range.

3.2.1.2. Selection of phase transfer agent: In the sensing system, phase transfer catalyst, which is typically a quaternary ammonium hydroxide, was used as the CO$_2$ capture reagent to provide the reversible response of the sensor element since this sensing system can be considered as a HCO$_3^-$/CO$_3^{2-}$ buffer system after the OH$^-$ ions from the
phase transfer agent reacted with CO$_2$ in gas sample. Also, this quaternary ammonium base can interact with the CO$_2$ sensing probes, which is thymol blue or $m$-cresol purple in this case, and form a hydrated ion-pair (Fig. 3.1) to increase the solubility of the sensing probes in the system [68, 74]. Two quaternary ammonium hydroxides, tetramethylammonium hydroxide (TMAH) and hexadecyltrimethylammonium hydroxide (HDAH), were selected to prepare the sensor elements. The sensor response was measured to investigate the effect of phase transfer agents on sensor sensitivity and dynamic range (Fig. 3.2). In this case, in order to evaluate the reversibility of the sensor element, simulated breath sample and dry air into the sensor device were pumped into the device alternately to simulate the process of respiration. The detailed experimental process will be described later in Chapter 5.

![Fig. 3.1. The sensing mechanism of the CO$_2$ sensor element prepared with quaternary ammonium hydroxide and pH indicator. The quaternary ammonium hydroxide interacted](image)

Before test

After test
with pH indicator and formed a hydrated ion-pair. The ion-pair changed color after interacting with CO₂.

![Molecular structures of (a) hexadecyltrimethylammonium hydroxide (HDAH) and (b) tetramethylammonium hydroxide (TMAH).]

3.2.2. Optimization of sensor response and recovery time

When the sensor is used for breath-by-breath CO₂ analysis, the sensor changes color during expiration and recovers during inspiration. The response time of the CO₂ sensor depends on both the chemical reactions and the mass transport of CO₂ molecules between gas sample and the sensor element. The efficiency of mass transport of CO₂ between the sensor element and gas sample can be optimized by selecting an ultra-hydrophobic sensor substrate and adjusting the device configurations. The reversibility of chemical reactions can be tuned by adding a pH stabilizer into the sensing system.

3.2.2.1. Selection of sensor substrate: As discussed in Chapter 2, during the sampling process, humid breath was introduced into the sensor device and water vapor was condensed on the sensor surface forming an alkaline sensing solution. CO₂ molecules in breath were absorbed by the sensing solution and decreased the pH of the system, which caused the color change of the sensing probe. While during the purging process,
dry air was introduced into the device. The sensor surface was dried up and CO₂ molecules were released from the sensor element. The pH of the sensing system recovered to its initial state and the color of the sensing probe changed back correspondingly. Since the CO₂ stripping depends on the drying of the sensor element, the improvement in hydrophobicity of the sensor substrate may play an important role in the optimization of sensor response. In this study, four hydrophobic materials including an acrylic sheet engraved with grids, a flat acrylic sheet, photo paper coated with urethane and a polytetrafluoroethylene (PTFE) membrane, were selected as the sensor substrates. HDAH and m-cresol purple were coated on the surface of the sensor substrate as the sensing chemicals. The response of CO₂ sensor elements exposed to simulated breath samples was compared to investigate the effect of surface hydrophobicity on sensor response.

3.2.2.2. Effect of pH stabilizer: As described above, due to the presence of the phase transfer agent, the sensing system can be considered as a buffer system after OH⁻ ions from the phase transfer agent reacted with CO₂ in gas sample. The reversibility of the CO₂ sensor is dependent on the buffer capacity of the sensing system. Thus, a strong buffer system with its pH ranging from 6 to 9 may provide the sensor element with a high reversibility. In this study, the amine buffer system was selected as the pH stabilizer of the sensing system to investigate the effect of buffer solution on the optimization of sensor response time.

3.3. Results and discussion

3.3.1. Sensitivity and dynamic range

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Since the pH of the sensing system varies between 6 and 9 during the CO$_2$ detection, a CO$_2$ sensing probe with the pKa value around 8 may provide the CO$_2$ sensor with a high sensitivity and large dynamic range. Thymol blue and $m$-cresol purple were selected as the CO$_2$ sensing probes in this study as their pKa values are 8.9 and 8.3, respectively. The color change and corresponding reactions of thymol blue and $m$-cresol purple are shown in Fig. 3.3. After interaction with CO$_2$, thymol blue changes color from blue to yellow while $m$-cresol purple changes color from purple to yellow.
Fig. 3.3. The color change and corresponding reactions of thymol blue and \( m \)-cresol purple.

The titration plots of thymol blue and \( m \)-cresol purple are shown in Fig. 3.4 (a). It can be observed that \( m \)-cresol purple provides a wider dynamic range of \( \text{CO}_2 \) \% concentration due to its lower pKa. Compared with thymol blue [110], \( m \)-cresol purple changes color more sensitively under weakly alkaline to neutral conditions (pH value: 9.0 – 6.0), which typically corresponds to the range of normal breath \( \text{CO}_2 \) levels (1.0\% - 11.5\%) [111–113]. Similarly, the calibration curves of the \( \text{CO}_2 \) sensor elements prepared with thymol blue and \( m \)-cresol purple, respectively, indicate that the sensor prepared with \( m \)-cresol purple has better sensitivity with the concentration of \( \text{CO}_2 \) higher than 4\% [Fig. 3.4 (b)].
Fig. 3.4. (a) Titration curves for the sensing system prepared with thymol blue and m-
cresol purple. The color changes in the CO$_2$ sensor were due to the pKa value (for thymol
blue: color changes from blue (pH $>$ pKa) to yellow (pH $<$ pKa); for m-cresol purple:
color changes from purple (pH $>$ pKa) to yellow (pH $<$ pKa)). (b) Calibration curves of
sensors prepared with thymol blue and m-cresol purple, respectively. Compared with
thymol blue, m-cresol purple changes color more sensitively under neutral to weakly
alkaline conditions, which provides a larger dynamic range for the breath CO$_2$ detection.

The effect of phase transfer agent on sensor response was also investigated. The
CO$_2$ sensor element prepared with hexadecyltrimethylammonium hydroxide (HDAH)
shows stable and high response. When exposed to 4% CO$_2$ gas sample, the sensor
response is around 0.47 (V) and there is almost no baseline shift. In contrast, the signal obtained from the sensor prepared with tetramethylammonium hydroxide (TMAH) is just around 0.22 (V) and an obvious baseline shift can be observed. Therefore, a quaternary ammonium hydroxide with long carbon chain may provide better sensitivity as the long carbon chain can increase the hydrophobicity of the sensing system.

In addition, the sensitivity of the CO₂ sensor can be further optimized by adjusting the concentration of phase transfer agent in the sensing system. The response of sensor elements prepared with sensing solutions containing different concentrations of HDAH. The dynamic range and sensitivity of CO₂ sensor can be improved by increasing the concentration of phase transfer agent in the sensing system since the high concentration of phase transfer agent can increase the CO₂ capture ability and buffer capacity of the sensing system.

3.3.2. Sensor response and recovery time

As described in the Experimental Section, the sensor response and recovery time, which depends on both chemical reactions and mass transport of CO₂ between gas sample and the sensor element, can be optimized by selecting a hydrophobic material as the sensor substrate and using a buffer solution as the reaction rate enhancer.

In this study, four hydrophobic sensor substrates were selected to prepare the CO₂ sensor chips. Compared with the commercial colorimetric CO₂ sensor (Easy Cap II, Fig. 1.8), the sensor elements prepared with hydrophobic sensor substrates show faster response time (Table 3.1). It can be observed that, the sensor made from the hydrophobic
acrylic sheet shows faster response and recovery compared to the commercial CO$_2$ sensor made from ethyl cellulose. However, the grids engraved on the sensor surface delay the mass transport of CO$_2$ and thus hinder the further improvement of the sensor response and recovery time. The sensors prepared with flat acrylic sheet and photo paper both show faster response time of around 1130ms due to their flat and hydrophobic surfaces, which indicate that the sensor response time can be further optimized by using a sensor substrate with flat surface. Among all the sensor elements, the sensor prepared with PTFE membrane shows best breath-by-breath signal and fastest 90% response ($t_{\uparrow 90}$) and recovery ($t_{\downarrow 90}$) time, which is due to its specific porous structure (Fig. 3.6) and ultra-hydrophobicity. As described in Chapter 2, during expiration, water vapor from breath sample condensed on the sensor surface and formed a specific buffer solution for CO$_2$ absorption. The porous structure of the PTFE membrane enhanced the condensation of water vapor due to the capillary action. During inspiration, dry air passed through the device and dried up the sensor element. The sensor element was dried up very quickly due to its ultra-hydrophobicity. The CO$_2$ molecules were rapidly released from the sensor element as the sensing system was dried up. The ability of the PTFE membrane to quickly absorb and strip off CO$_2$ enables the fast sensor response.
**Exhalation:**

\[
\text{CO}_2, \text{H}_2\text{O} \xrightarrow{\text{Humid breath}} \text{Sensor} \quad \text{pH} \downarrow
\]

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \rightarrow \text{H}_2\text{CO}_3 \\
\text{H}_2\text{CO}_3 & \rightarrow \text{H}^+ + \text{HCO}_3^-
\end{align*}
\]

**Inhalation:**

\[
\text{CO}_2, \text{H}_2\text{O} \xleftarrow{\text{Dry air}} \text{Sensor} \quad \text{pH} \uparrow
\]

\[
\begin{align*}
\text{H}^+ + \text{HCO}_3^- & \rightarrow \text{H}_2\text{CO}_3 \\
\text{H}_2\text{CO}_3 & \rightarrow \text{CO}_2 + \text{H}_2\text{O}
\end{align*}
\]

Fig. 3.5. The color change and chemical reactions of CO\textsubscript{2} sensor during breath-by-breath CO\textsubscript{2} analysis.

**Table 3.1. 90\% response and recovery time of CO\textsubscript{2} sensors.**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( t_{150} ) (ms)</th>
<th>( t_{350} ) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy Cap II (Commercial product)</td>
<td>2500</td>
<td>4500</td>
</tr>
<tr>
<td>Acrylic sheet engraved with grids</td>
<td>1750</td>
<td>2750</td>
</tr>
<tr>
<td>Flat acrylic sheet</td>
<td>1125</td>
<td>875</td>
</tr>
<tr>
<td>Photo paper coated with urethane</td>
<td>1130</td>
<td>1500</td>
</tr>
<tr>
<td>PTFE membrane</td>
<td>437</td>
<td>1000</td>
</tr>
</tbody>
</table>
Fig. 3.6. SEM image of PTFE membrane coated with sensing chemicals. The porous structure of the PTFE membrane enables fast and reversible sensor response.

During CO\textsubscript{2} detection, a buffer system was formed in the sensor element. CO\textsubscript{2} interacted with the buffer system and decreased the pH of the sensor element. The chemical reactions between CO\textsubscript{2} and the buffer system, which are reversible, provided the sensor with reversible response. A pH stabilizer with strong buffer capacity can enhance the reversibility of the chemical reactions and thus shorten the sensor response time. In this study, an amine buffer was selected as the pH stabilizer since the pH of this buffer system typically varies between 7 and 9, which is close to the pKa of \textit{m}-cresol purple. The response of the CO\textsubscript{2} sensor prepared with an amine buffer shows fast and reversible response and its \textit{t\textsubscript{190}} and \textit{t\textsubscript{490}} are as fast as 250 ms and 475 ms, respectively (Table 3.2).
Table 3.2 The 90% response and recovery time of CO$_2$ sensors prepared with and without buffer

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$t_{90}$ (ms)</th>
<th>$t_{50}$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without buffer</td>
<td>450</td>
<td>1050</td>
</tr>
<tr>
<td>With buffer</td>
<td>250</td>
<td>475</td>
</tr>
</tbody>
</table>

3.4. Conclusions

This chapter described the optimization of the CO$_2$ sensor element. The sensor dynamic range and sensitivity can be improved by selecting a pH indicator with relatively low pKa and a phase transfer agent with long carbon chain. The low pKa provides the pH indicator with significant color change under high CO$_2$ level conditions. The phase transfer agent with long carbon chain increases the hydrophobicity and CO$_2$ capture capacity of the sensing system. The sensor prepared with $m$-cresol purple and HDAH shows good sensitivity and a large dynamic detection range from ~50 ppm to 11.5%. The sensor response time can be optimized by selecting an ultra-hydrophobic material as the sensor substrate and a strong buffer solution as the pH stabilizer. The hydrophobic sensor substrate improves the efficiency of mass transport of CO$_2$ and the strong buffer system enhances the reversibility of chemical reactions. The sensor prepared with PTFE membrane and amine buffer shows fast and reversible response with its 90% response time less than 250 ms.
CHAPTER 4
FURTHER OPTIMIZATION OF SENSOR PERFORMANCE BY MODELING

4.1. Introduction

A pocket-sized CO\textsubscript{2} analyzer with high accuracy and fast response time plays an important role in personal healthcare and environmental monitoring [1–15]. As described in Chapter 1, the determination of end-tidal CO\textsubscript{2} (EtCO\textsubscript{2}) provides the doctors and patients with a non-invasive method to diagnose asthma, Chronic Obstructive Pulmonary Disease (COPD), and cardiovascular diseases [5, 6]. And the monitoring of indoor CO\textsubscript{2} levels provides valuable information for assessment of indoor air quality (IAQ) since higher levels of indoor CO\textsubscript{2} are associated with increased prevalence of certain mucous membrane and sick building syndrome (SBS) symptoms [15].

In Chapter 2 and 3, we have successfully developed and optimized a pocket-sized CO\textsubscript{2} analyzer which can be used for real-time analysis of breath CO\textsubscript{2}. The CO\textsubscript{2} analyzer features a detection unit and a sample delivery system. The detection unit includes an optoelectronic detection system consisting of a red LED (wavelength = 633 nm, LEDtronics. Inc., CA, USA) as a light source and a photodiode (OSRM GmbH, Germany) as a light detector (Fig. 5.1(a)). During the test, a sensor element was inserted into a detection unit. The response of the sensor was characterized by measuring the change in the intensity of transmitted light caused by the interaction of CO\textsubscript{2} with the sensing element. Gas samples are delivered to the detection unit through the sample delivery system which includes a pressure transducer to monitor the flow rate of gas sample.
After optimization, the CO₂ sensor showed reversible response to CO₂ and a dynamic detection range from 0.3% to 11.5%. Also, the CO₂ analyzer showed fast response with a 90% response time of less than 250ms, which indicated that the CO₂ analyzer is suitable for breath-by-breath analysis. However, the CO₂ sensor still needs to be optimized as sensor recovery is slower than sensor response during the tests (Fig. 3.7 and 3.8). To achieve an overall understanding of the breath CO₂ detection process and improve the performance of the CO₂ analyzer, a 3D model was created in Chapter 2 using Comsol Multiphysics (COMSOL, Inc., MA, USA). By simulating the fluid dynamics of gas sample inside the device and chemical reactions between gas sample and the sensing element, the sensor response under different conditions can be predicted.

This chapter outlines an effort to improve the performance of the CO₂ analyzer by simulating the response of sensor under different device configurations. Based on the simulation results, a better device configuration will be achieved for further optimization of the sensor response.

4.2. Modeling of sensor response

As the response of the CO₂ sensor relates to the delivery of gas sample during the test, the performance of this CO₂ analyzer can be further optimized by improving the efficiency of the gas sample delivery system, which typically depends on the device configurations. A new 3D model of the CO₂ analyzer was created using Comsol Multiphysics (COMSOL, Inc., MA, USA) based on the existing model described in Chapter 2 (Fig. 2.1) and the requirements for breath-by-breath analysis. This model
simulated the fluid dynamics of gas sample inside the device and the chemical reactions between gas sample and the sensing element. The 3D geometry of the flow path and detection chamber is shown in Fig. 4.1.

Fig. 4.1. 3D geometry of the CO$_2$ analyzer. A red LED is used as the light source. During the test, a sensor chip was inserted into the detection chamber. The subject inhaled fresh air and exhaled CO$_2$ containing gas sample through the device. The CO$_2$ level in gas sample was determined by measuring the color change of the sensing element in the sensor chip.

4.2.1. Fluid dynamics

To simplify this problem, a fixed flow rate of 6L/min was used to simulate the fluid dynamics of gas sample inside the device, which is a normal respiratory (inspiratory and expiratory) flow rate for healthy people. According to the device geometry shown in Fig. 4.1, the gas flow inside the device can be considered as a laminar flow and the delivery of gas sample was simulated using a Single-phase Laminar Flow Model, which takes the form of
\[
\rho (\vec{u} \cdot \nabla) \vec{u} = \nabla \cdot \left[ -p \vec{I} + \mu (\nabla \vec{u} + (\nabla \vec{u})^T) \right] + \vec{F}
\]  
(4.1)

with \( \rho \nabla \cdot \vec{u} = 0 \), where \( \rho \) is density of gas sample (~1.26 kg/m\(^3\)), \( \nabla \vec{u} \) is velocity gradient tensor, \((\nabla \vec{u})^T\) is “transpose” of the velocity tensor, \( p \) is pressure, \( \mu \) is dynamic viscosity of gas sample, \( \vec{F} \) is volume force, and \( \vec{u} \) is the average velocity [74].

Equation 5.1 was solved numerically with the following boundary conditions.

1) **Flow velocity on the device wall** [74]:

\[ \vec{u} = 0 \]  
(4.2)

2) **Flow velocity at the device inlet**:

\[ \vec{u} = -U_0 f(t) \vec{n} \]  
(4.3)

where \( U_0 \) is linear velocity of airflow at the inlet, \( f(t) \) is a time dependent function, which can be used to describe the breath pattern (assumed constant in this work), and \( \vec{n} \) is the flow direction.

3) **Flow velocity at the device outlet**:

\[ p = p_{am} \]  
(4.4)

\[ [\mu (\nabla \vec{u} + (\nabla \vec{u})^T)] = 0 \]  
(4.5)

4.2.2. Mass transport

Since the concentration of CO\(_2\) in both breath and the ambient air is typically lower than 6%, the mass transport of CO\(_2\) inside the device was simulated using a Transport of Diluted Species Model, which can be described by the diffusion-convection equation,
\[
\frac{\partial c_i}{\partial t} + \bar{u} \cdot \nabla c_i = \nabla \cdot (D \nabla c_i) + R
\]  
(4.6)

where \(c_i\) denotes the concentration of CO\(_2\) in gas sample, \(D\) is the diffusion coefficient of CO\(_2\), \(\bar{u}\) is the average velocity and \(R\) refers to the rates of chemical reactions between CO\(_2\) and the sensing element. In this case, the diffusion coefficient of CO\(_2\) was set to be \(1.6 \times 10^{-5}\) m\(^2\)/s \[116\].

The boundary conditions were set as follows,

1) Concentration of CO\(_2\) at the device inlet

\[c_i = c_0 \times g(t)\]  
(4.7)

Where \(c_i\) is the concentration of CO\(_2\) at the device inlet, \(c_0\) denotes the maximum concentration of CO\(_2\) (end-tidal CO\(_2\)) in gas sample, which was set to be 4% in this model, \(g(t)\) is a time dependent function, which is used to describe the variation of CO\(_2\) level in gas sample to simulate the inspiration-expiration process. The variation of CO\(_2\) level vs. time was plotted in Fig. 4.2.

2) Concentration of CO\(_2\) at the device outlet

\[-\bar{n} \cdot D \nabla c_i = 0\]  
(4.8)

3) CO\(_2\) flux into the sensing element

\[-\bar{n} \cdot \vec{N}_i = N_{CO_2}\]  
(4.9)

with \(N_{CO_2} = -R_{ads} + R_{des}\), where \(\vec{N}_i\) is the molar flux of CO\(_2\), \(R_{ads}\) denotes the rate of adsorption of CO\(_2\) and \(R_{des}\) refers to the rate of desorption of CO\(_2\).
Fig. 4.2. Variation of CO$_2$ level in gas sample vs. time in the 3D model. It described the variation of CO$_2$ level inside the device during test to simulate the inspiration-expiration process: during inspiration, fresh air was introduced into the device, the CO$_2$ level was close to zero; during expiration, exhaled breath was introduced into the device, the CO$_2$ level increased to 4% within a couple of seconds.

4.2.3. Sensor response

The color of the sensor depends on the pH level of the sensing system, which varies with the adsorption and desorption of CO$_2$ in the sensing element. During expiration, humidity from expired air condensed on the sensor and formed a CO$_3^{2-}$/HCO$_3^-$ buffer solution in the sensor. CO$_2$ in expired air was adsorbed by the buffer solution which decreased the pH level of the sensing system, and changed the color of the pH sensing probe from purple to yellow (Fig. 3.1). This color change was correlated to the CO$_2$ concentration in the gas sample. Conversely, during inspiration, when CO$_2$-free dry
air was introduced into the device, CO$_2$ was stripped from the sensor and the color changed back from yellow to purple. In this work, a Surface Reaction Model which can simulate the variation of CO$_2$ level in the sensing system during the process of respiration was used to simulate the response of CO$_2$ sensor. The CO$_2$ level in the sensor can be described by the following equations,

\[
\frac{\partial c_{s,i}}{\partial t} + \nabla \cdot (-D_i \nabla c_i) = R_{s,i} 
\]  
(4.10)

\[
\bar{N}_{s,i} = -D_i \nabla c_{s,i} 
\]  
(4.11)

\[
\theta_i = \frac{c_{s,i} \sigma_i}{\Gamma_s} 
\]  
(4.12)

where $N_{s,i}$ is the surface molar flux of CO$_2$, $c_{s,i}$ represents the surface concentration of CO$_2$ in the sensor, $D_i$ denotes the diffusion coefficient of CO$_2$ in the sensing element ($1.6 \times 10^{-10}$ m$^2$/s [117]), $R_{s,i}$ is the rate of surface reactions including both adsorption and desorption, $\theta_i$ is a dimensionless number which describes the fractional surface coverage of CO$_2$, $\Gamma_s$ is the density of sites that can adsorb CO$_2$ on the sensor surface, which was set to be $4.0279 \times 10^{-5}$ mol/m$^2$ and $\sigma_i$ is the occupancy number of CO$_2$, which was assumed to be 1 in this model.

4.3. Device configurations

The measurement of CO$_2$ concentration was based on the adsorption-desorption of CO$_2$ in the sensing element. According to Chapter 2, since the rates of chemical reactions between CO$_2$ and the buffer system in the sensing element are much faster than that of dissolution and diffusion of CO$_2$ into the buffer system, the response time of CO$_2$ sensor is mostly dependent on the gas dissolution and diffusion process. Therefore, the
efficiency of the gas sample delivery system is critical to the performance of the CO$_2$ analyzer. In order to achieve a more efficient gas sample delivery system for further optimization of the CO$_2$ analyzer, the response of sensor under different device configurations were simulated by using the 3D model described in Section 4.2.

4.3.1. Dimensions of the gas sample delivery system

With a fixed volumetric flow rate, the velocity of gas sample inside the device depends on the diameter of the gas sample delivery system. In order to study the influence of device dimensions on the performance of the CO$_2$ analyzer, three diameter values (14, 18 and 22mm) were selected to create the 3D model described in Section 4.2 (Fig. 4.3). The response of CO$_2$ sensor under different conditions was then simulated.
Fig. 4.3. 3D geometries of the CO$_2$ analyzer with different device dimensions. Three diameter values (14, 18 and 22mm) were selected to create the 3D model to study the effect of device dimensions on the response of CO$_2$ sensor.

4.3.2. Depth of sensor chip receiver

The efficiency of CO$_2$ diffusion into the sensing element also depends on the position of sensor chip inside the device. In order to evaluate the influence of depth of sensor chip receiver on the sensor response, three depth values (1, 2 and 4mm) were selected (Fig. 4.4) and the response of CO$_2$ sensor was simulated by using the 3D model.

Fig. 4.4. 3D geometries of the CO$_2$ analyzer with different sensor chip receiver depth. Three depth values (1, 2 and 4mm) were selected to create the 3D model to evaluate the influence of depth of sensor chip receiver on sensor response.
4.4. Results and Discussion

The response of sensor exposed to 4% CO₂ gas sample was simulated by using the 3D model described above and the results are shown in Fig.4.5. As discussed in Chapters 2 and 3, the color change of the CO₂ sensor represents the pH level of the sensing system which is determined by the concentration of CO₂ in the sensing system. Hence in this simulation, the sensor response can be described by the variation of concentration of CO₂ in the sensing element. Three diameters of the sample delivery system (D₁ = 14 mm, D₂ = 18 mm and D₃ = 22 mm) were selected to create this 3D model to evaluate the influence of device dimensions on the sensor response. It can be observed that the simulated sensor response doesn’t change with the diameter of the sample delivery system, which indicates that the device dimensions have no effect on the sensor response. Since the pH level of the sensing element varies with the adsorption-desorption of CO₂ in the sensor, the sensor response just depends on the diffusion and chemical reactions and will not be affected by the velocity of gas sample.
Fig. 4.5. Simulated response of sensor exposed to 4% CO$_2$. The volumetric flow rate was set to be 6L/min and three diameters of the sample delivery system ($D_1 = 14$ mm, $D_2 = 18$ mm and $D_3 = 22$ mm) were selected to create this 3D model. The simulated sensor response doesn’t change with the diameter of the sample delivery system, which indicates that the device dimensions have no effect on the sensor response.

The influence of depth of sensor chip receiver on sensor response was also studied. Three depth values (1, 2 and 4mm) were selected to create this 3D model (Fig.4.4) and the simulated sensor response is shown in Fig. 4.6. It can be observed that the 90% response time of the CO$_2$ sensor increased with the increasing depth of sensor chip receiver. As we know, the efficiency of adsorption-desorption of CO$_2$ in the sensing element is determined by the diffusion and chemical reactions. The rates of chemical
reactions depend on reaction kinetics and the efficiency of diffusion of CO\(_2\) relates to the concentration gradient of CO\(_2\) and the position of the sensor chip inside the device. Therefore, in order to achieve a fast and reversible response, the CO\(_2\) sensor chip must be directly in contact with the laminar flow of CO\(_2\) gas sample.

Fig. 4.6. Simulated response of sensor exposed to 4% CO\(_2\) under different sensor configurations. The volumetric flow rate of CO\(_2\) gas sample was set to be 6L/min. The depth of the sensor chip receiver was set to be 1, 2 and 4mm. The simulated 90% response time of the CO\(_2\) sensor decreased with the increasing depth of sensor chip receiver.
4.5. Conclusions

In this chapter, we have simulated the sensor response under different device configurations based on the 3D model created in Chapter 2 and breath-by-breath test requirements. The numerical simulation results indicated that the response of CO$_2$ sensor is not affected by the diameter of the sample delivery system but closely related to the position of the sensor chip receiver inside the device. This information is difficult to achieve merely from experiments, but is critical for further optimizing the performance of the CO$_2$ analyzer.
5.1. Introduction

The balance between energy intake and energy expenditure is key to weight management and obesity prevention in adults. An accurate assessment and tracking of total energy expenditure (TEE) can guide individuals to achieve proper energy balance [16]. To date, most end-consumer commercial devices monitor energy expenditure related to physical activity by using physical sensors, such as accelerometers and GPS-distance tracking [81, 82]. While important for long-term health outcomes, physical activities typically count for less than 15% of TEE [16]. TEE also includes a small portion of energy expenditure related to food-induced thermogenesis, which is ~10%. In contrast to physical-activity energy expenditure and thermogenesis, resting energy expenditure (REE), represents the largest percentage (>75%) of TEE [16]. REE is the energy expenditure required to maintain basic body functions in a resting state, which cannot be measured by the physical sensors mentioned above. Furthermore, the physical-activity sensors are inadequate for monitoring low-energy physical activities, such as office work [83, 84]. For these reasons, determining REE may be critically important for weight management programs.

In fact, the American Dietetic Association has strongly recommended the use of REE measurement for adult weight management [85]. Various equations have been developed to calculate REE, but the accuracy of the equations is questionable,
particularly for overweight and obese populations [86], athletes, and patients undergoing weight loss [42, 87–89]. The most widely accepted method for measuring REE is indirect calorimetry, which determines REE based on oxygen consumption (VO₂) and carbon dioxide production (VCO₂) rates using the Weir equation [16, 85]. A simplified approach is to detect VO₂ or VCO₂ alone and estimate REE by assuming a fixed ratio of VCO₂/VO₂ (0.85). The ratio between VCO₂/VO₂ is defined as the respiratory quotient (RQ), which can vary over a wide range (e.g., 0.7-1.0). Therefore, it is desirable to perform indirect calorimetry by measuring both the VO₂ consumption and VCO₂ production rates [16]. Indirect calorimetry can be performed using several methods, which include room calorimeters, metabolic carts, and the Douglas bag method [16, 90], but these methods are unsuitable for personal use at home. A handheld device and other small analyzers have been developed [91, 92], but they determine REE based on the detection of consumed VO₂ only, which is subject to the limitation discussed above [16].

To address these limitations, the purpose of the present study in this chapter is to evaluate the accuracy of a new pocket-sized metabolic analyzer based on the CO₂ analyzer we developed in the last chapter. In the present work, we also developed a novel colorimetric O₂ sensor for the determination of O₂ level in exhaled breath (details of sensor preparation cannot be disclosed in the thesis). This pocket-sized metabolic analyzer integrated both the CO₂ and O₂ analyzer, which can be used for simultaneous detection of VO₂ and VCO₂, and has the ability to determine both REE, as well as energy expenditure (EE) of low-level physical activity. The device, in combination with existing commercial physical-activity energy-expenditure trackers, creates the opportunity for a
more accurate assessment of TEE in free-living conditions, and therefore, individual’s caloric needs.

5.2. Materials and methods

5.2.1. Subjects

Seventeen adult subjects (10 males, 7 females) from Arizona State University (ASU) voluntarily participated in the study. The study included healthy individuals and women who were not pregnant or nursing. The number of subjects was chosen based on a power calculation [93] estimated from typical mean and standard deviation values for REE [94]. Assuming a typical mean value for REE of 1800 kCal/day, with a standard deviation of 200 kCal/day (10% error), a sample size of 16 subjects allows detection of a difference in REE values (e.g. 1800 and 1600 kCal/day) with a power of 0.80. In the present study, the 17 subjects contributed with a total of 31 on-line measurements of energy expenditure (described below). Physical characteristics of the subjects, including fat, lean body, and muscle mass, were assessed (Table 5.1). The physical characteristics represented a relatively broad range, from underweight to obese: body mass index (BMI) ranged from 15.7 to 36.9 kg/m² [94]. The study followed a protocol approved by the Institutional Review Board of Arizona State University (IRB protocol #1012005855). All subjects provided written informed consent prior to participation. The study was carried out at ASU from January 2011 to July 2011. In addition, measurements related to off-line testing involved 17 subjects and were performed at ASU from July 2011 to July 2012.
Table 5.1. Physical characteristics of the study’s subjects (n = 17)

<table>
<thead>
<tr>
<th>Number</th>
<th>Age (y)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>BMI (kg/m²)</th>
<th>Waist-to-hip ratio</th>
<th>Fat mass (kg)</th>
<th>Lean body mass (kg)</th>
<th>Muscular mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>7</td>
<td>27±5</td>
<td>52.8±1.9</td>
<td>162.2±5.3</td>
<td>0.73±0.02</td>
<td>12.1±3.4</td>
<td>40.7±2.0</td>
<td>13.6±1.0</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>27±6</td>
<td>77.5±20.2</td>
<td>179.2±5.2</td>
<td>23.9±5.3</td>
<td>18.4±13.8</td>
<td>59.1±8.6</td>
<td>21.3±6.9</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>27±6</td>
<td>68.8±20.1</td>
<td>173.2±9.7</td>
<td>22.6±4.7</td>
<td>16.2±11.7</td>
<td>52.6±11.3</td>
<td>18.6±6.7</td>
</tr>
</tbody>
</table>

5.2.2. Study overview

The subjects of the study participated in the measurements are as follows. Fifteen subjects participated in the REE measurement group, which refrained from structured physical activity for 24 hrs prior to the REE measurement, and fasted (with no beverages or caffeine) for 12 hours before the REE test to avoid the diet-induced thermic effect [95]. The subjects were permitted to drink water before the test. On the other hand, sixteen subjects participated in the EE group, which fasted for at least 4 hours, but had no restrictions on caffeine or beverage intake and structured physical activity. This group was assessed for Energy Expenditure (EE) during routine activities, which included office and bench work. The office work included paperwork and computer work, and the bench work included assembly of electronic components and lab bench work. The test was performed after 2 consecutive hours of work, and the subjects stopped their activities right before the assessments. Both REE and EE measurement groups were assessed using the metabolic analyzer and Douglas bag method (see details below). It is worth noting that from the total study group of 17 subjects, REE and EE measurements for two subjects and one subject, respectively, were excluded due to non-compliance with the preparatory conditions at the time of measurement. In addition, EE measurements were not corrected by caffeine intake.
In order to classify the physical activity level of the study group, the subjects reported frequency, duration, and type of physical activities from the *Compendium of Physical Activities* published by Ainsworth, et al. [96]. In addition, the subjects completed the International Physical Activity Questionnaire (IPAQ- 7 day, self-administered format), which classifies the population as “Low,” “Moderate,” or “High” physical activity level [97]. The subjects in this study were categorized as “Low” physical activity level.

5.2.3. Body composition and anthropometric measurements

Body weight, height, as well as waist and hip circumferences were measured before the assessment of VO$_2$ and VCO$_2$, following the procedure specified by the American College of Sports Medicine's Guidelines for exercise testing and prescription. BMI was calculated as Weight/Height$^2$ (kg/m$^2$). Seven-site skinfold test [98] was performed on all subjects using a Harpenden skin fold caliper (CE 0120, Baty, British indicators) to calculate body density, and Siri equation was applied to calculate fat percentage [87]. The skinfold and circumference measurements were taken 3 times each and the mean value of the 3 measurements was determined. The data was recorded and met the technical measurement error allowed by the International Society for the Advancement of Kinanthropometry [99]. In addition, the muscle mass of each subject was estimated using Lee’s equation [100]. It is worth noting that the skinfold method was preferred over the bioimpedance analysis method. This is because the body fat percentage values by bioimpedance showed a variability higher than 20% when measured consecutively in the same subject under the same conditions [101, 102].
5.2.4. Metabolic analyzer device

The pocket-sized metabolic analyzer in this study is designed to be used with a smartphone and does not require professional calibration to operate. The metabolic analyzer consists of a mouthpiece built with a non-rebreathing Hans-Rudolph valve, and an integrated O$_2$ and CO$_2$ sensor (Fig. 5.1). The integrated O$_2$ and CO$_2$ sensor is a patented technology developed at ASU [103], and has been thoroughly tested and validated for specificity, sensitivity, stability and lifetime against high humidity and variable temperature conditions of breath. A related application (app) is run on a smartphone to provide a user-friendly interface and real-time data display, storage, and transmission of the metabolic parameters. The smartphone offers signal processing capability to the device, and introduces a user interface that is familiar to most users. An Android-based smartphone (HTC Evo 4G) was used for the present study. After the app is launched, the user is prompted to log in with their user name and password. Each user creates an account, inputs personal information, such as date of birth, height, and current weight. Once the user is logged in, the app guides the user to perform the test, and displays the measured energy expenditure.

To obtain REE measurements, subjects were asked to sit quietly for at least 15 mins before each measurement. In contrast, to measure EE related sedentary activities, the energy expenditure measurements were carried out during the sedentary activities. During the REE and EE measurements, the room temperature was maintained at 23 ºC. Before the metabolic analyzer was used, each subject was instructed to breathe using a mouthpiece
not connected to the device for two minutes, while wearing a nose clip. Next, the integrated O\textsubscript{2} and CO\textsubscript{2} sensor of the metabolic analyzer was connected to the exhalation port of the mouthpiece, and the sensor analyzed a 4 L volume of exhaled breath. The exhaled breath was collected in a 4-L bag attached to the outlet of the device (see validation of collected exhaled breath volume). The bag has an error of < 2%, which was determined by use of a calibration syringe (Vacumed, Ventura, CA). The time duration for collecting 4 L exhaled breath was recorded, which was used for determining the exhalation flow rate, and associated VO\textsubscript{2} and VCO\textsubscript{2} (Fig. 4.1). The energy expenditures of the subjects in the REE and EE groups were calculated from VO\textsubscript{2} and VCO\textsubscript{2} using the Weir equation [104] given by

\[
\text{REE} = [3.9 \times \text{VO}_2 + 1.1 \times \text{VCO}_2] \times 1.44
\]  

(5.1)

where REE is in kCal/day, and VO\textsubscript{2} and VCO\textsubscript{2} are the consumed oxygen rate and produced carbon dioxide rate in mL/min. VO\textsubscript{2} and VCO\textsubscript{2} were determined by the exhalation rate (V\textsubscript{E}), and exhaled oxygen and carbon dioxide concentration from the integrated O\textsubscript{2} and CO\textsubscript{2} sensor, with V\textsubscript{E} corrected by ambient pressure and temperature, and vapor pressure of water.
Fig. 5.1. (a) Schematic diagram of the metabolic analyzer for assessment of REE. (b) Measurement of REE using the metabolic analyzer. The sensor element includes an O₂ sensor and a CO₂ sensor. The O₂ and CO₂ levels in exhaled breath can be determined by measuring the color change of the sensing elements.
5.2.5. Validation of collected exhaled breath volume

The exhaled volume of 4L collected under the conditions described above was validated by comparing the results with the exhaled volumes determined with the Douglas bag method. 4 L exhaled breath volume was collected between 0.5 – 1 min, and 40 L exhaled breath volume was collected between 5 – 10 min, respectively, for each of 11 subjects. From the volumes and time durations, as well as measured O₂ and CO₂ concentrations of the collected breath samples, V_e, and exhaled O₂ and CO₂ concentrations were determined. Differences of V_e for both collection conditions were smaller or equal to 3.5 %, with Diff. % defined as: ([4L collection – 40L collection]/[mean value of both collection methods]). This procedure validated the exhaled breath volume condition, and suggested that a 4 L breath sample collected after a 2-minute breathing period can provide REE results that are indistinguishable from those obtained in larger-volume (e.g., 40 L) breath samples.

5.2.6. Modified Douglas bag method

A modified Douglas bag method, simply called the Douglas bag method here, was carried out using breath samples collected in the 4-L bags during the energy expenditure test with the metabolic analyzer. Note that the adsorption of O₂ and CO₂ by the sensors in the analyzer was found to be < 0.01%, which is negligible compared to the relatively high O₂ and CO₂ concentrations in exhaled breath. A galvanic fuel cell O₂ analyzer (Vascular
Technology, Nashua, NH) and an infrared CO$_2$ analyzer (GE, Goleta, CA) were used to measure the O$_2$ and CO$_2$ concentrations, respectively, from which the flow rate of the consumed VO$_2$ and produced VCO$_2$ rates were determined. Both sensors were adapted for breath measurements using Nafion tubing (Permapure, LLC), and mini pump (Parker Hannifin Corp). The Nafion tubing was equilibrated with 20 to 30% relative humidity, which (according to the manufacturer) guarantees 10% humidity in the Nafion tubing outlet. The commercial analyzers were calibrated using dilutions prepared from pure air (Praxair) and medical-grade standard calibration gas containing 16.6% O$_2$ and 4.0% CO$_2$ (Vacumetrics Inc., Ventura, CA). Based on the measured VO$_2$ and VCO$_2$, energy expenditure (REE or EE) was calculated using the Weir equation [104]. Finally, V$_E$ was corrected by ambient pressure and temperature, and vapor pressure of water.

5.2.7. Statistical analysis

To analyze the accuracy of the new pocket-sized metabolic analyzer, the values of VO$_2$, VCO$_2$, REE, and EE measured with the device were compared with those by the Douglas bag method, and the correlation between the two methods was analyzed using linear regression method. In addition, the values of VO$_2$, VCO$_2$, REE, and EE from the two methods were analyzed from paired t-tests to determine the statistical difference of the readings by the metabolic analyzer and the Douglas bag method. Furthermore, resting energy expenditure (REE) values were analyzed via Bland-Altman plots.

Linear correlation analysis between REE group measurements and the physical characteristics of the subjects (body weight, BMI, lean body mass and muscle mass) was
performed to determine how REE values from the metabolic analyzer fit with existing trends between REE and the physical characteristics of the subjects.

As a note, the linear regression analysis with null intercept was chosen for the correlation method, following the recommended statistical procedure to assess worst-case correlation between variables that are not expected to render null meaningful values [105]. In addition, when null intercept correlation analysis is applied to two methods (tested method vs. reference method), the correlation slope is directly indicative of the accuracy of the tested method vs. the reference method [106].

5.3. Results

The analytical accuracy of VO$_2$, VCO$_2$ and energy expenditure measurements from the metabolic analyzer device was compared with that of the Douglas bag method for both study groups, REE (n=15 measurements) and EE (n=16 measurements), covering a range of energy expenditure values from ~1000 to 3500 kCal/day. Linear regression analysis, paired t-tests, and Bland-Altman plots were performed to achieve this task as follows:

a) Linear regression analysis: Scatter plots of VO$_2$, VCO$_2$, and energy expenditure measured by the metabolic analyzer device and the Douglas bag method are presented in Fig 5.2(a)-2(c). The results from the linear regression analysis show the following slope, LRS$_0$, and R-squared coefficient, $r^2$ with $p = 0$: LRS$_0$ ± SD = 1.00 ± 0.01, $r^2 = 0.9933$ for VO$_2$; LRS$_0$ ± SD = 1.00 ± 0.01, $r^2 = 0.9929$ for VCO$_2$; and LRS$_0$ (SD) = 1.00 ± 0.01, $r^2 = 0.9942$ for energy expenditure, for both resting and sedentary activity parameters.
Fig. 5.2. Linear regression analysis (Pearson correlation) between the new metabolic analyzer and the Douglas bag method for measuring (a) VO\(_2\), (b) VCO\(_2\) and (c) energy expenditure (n=31). VO\(_2\): consumed oxygen rate, VCO\(_2\): produced carbon dioxide rate; REE: resting energy expenditure. Solid squares correspond to the REE group (n=15), and solid circles correspond to the EE group (n=16). All solid lines show the resulting linear regression fitting accomplished with \(p = 0\).
b) Paired t-tests: The mean values for VO₂, VCO₂, and energy expenditure, including REE and EE for sedentary activities, from the metabolic analyzer and from the Douglas bag method are presented in Table 5.2. The differences for VO₂, VCO₂, REE and EE determined by the two methods are 1.4 mL/min, -0.8 mL/min, -59 kCal/day, and 40 kCal/day, respectively, which are only 0.6, 0.4, 3.2 and 2.5 % of percentage difference (Diff. %), respectively, with Diff. % defined as: ([Metabolic analyzer device parameter - Douglas bag method parameter]/[mean value of both methods]). In addition, no significant difference between the two methods’ values was found for a significance level = 0.05 for VO₂, VCO₂, REE and EE.
Table 5.2. Paired t-test comparison of metabolic analyzer device and Douglas bag method.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>Mean difference</th>
<th>SD</th>
<th>p</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO\textsubscript{2} (mL/min)\textsuperscript{1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Device</td>
<td>247.6</td>
<td>85.4</td>
<td>15.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Douglas bag</td>
<td>246.2</td>
<td>85.2</td>
<td>15.3</td>
<td>1.4</td>
<td>3.8</td>
<td>0.70</td>
<td>0.39</td>
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<tr>
<td>VCO\textsubscript{2} (mL/min)\textsuperscript{1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Device</td>
<td>217.3</td>
<td>82.3</td>
<td>14.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Douglas bag</td>
<td>218.1</td>
<td>78.2</td>
<td>14.0</td>
<td>-0.8</td>
<td>3.5</td>
<td>0.84</td>
<td>0.21</td>
</tr>
<tr>
<td>REE (kCal/day)\textsuperscript{2}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Device</td>
<td>1897</td>
<td>627</td>
<td>162</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Douglas bag</td>
<td>1838</td>
<td>577</td>
<td>149</td>
<td>-59</td>
<td>30.9</td>
<td>0.081</td>
<td>1.88</td>
</tr>
<tr>
<td>EE (kCal/day)\textsuperscript{3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Device</td>
<td>1584</td>
<td>571</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Douglas bag</td>
<td>1624</td>
<td>622</td>
<td>155</td>
<td>-40</td>
<td>35</td>
<td>0.271</td>
<td>1.144</td>
</tr>
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</table>

\textsuperscript{1}n=31, \textsuperscript{2}n=15, \textsuperscript{3}n=16. Abreviations: SD: standard deviation; SEM: standard error of the mean, Mean difference: (Device mean value – Douglas bag mean value); p: p value from the paired t-test, t: t value from paired t-test, VO\textsubscript{2}: oxygen consumption rate, VCO\textsubscript{2}: carbon dioxide consumption rate, REE: Resting Energy Expenditure, EE: Energy Expenditure during sedentary activities.
c) *Bland-Altman plot:* Fig. 5.3 shows a Bland-Altman plot built for REE results to closely evaluate the differences between the metabolic analyzer device and the Douglas bag method for diagnosis of resting energy expenditure. Applying a linear fit onto the plot data results in a null slope and a random averaged difference (metabolic analyzer device - Douglas bag method) ≤10%.

![Bland-Altman plot](image)

Fig. 5.3. Bland-Altman plot of the metabolic analyzer device *vs.* Douglas Method (null slope observed). Note that the solid horizontal line in the plot indicates the mean difference between results obtained with the different methods and the dotted line represents two times of the standard deviation (SD) from this mean. Note: Number of subjects was 15, and 1 subject of the group was evaluated in two different days, with REE measurements resulting in small differences.
In order to further evaluate the capability of the metabolic analyzer to differentiate between different metabolic rates, an additional regression analysis was performed with the REE values obtained from the metabolic analyzer and the body composition of the subjects. Fig. 5.4(a-d) shows a relationship of the REE measured by the metabolic analyzer to weight, BMI, lean body mass, and muscle mass assessed via anthropometric measurements. R-squared coefficients ($r^2$) between REE values and the physical characteristics of the subject larger than 0.96 with $p = 10^{-13}$ - $10^{-11}$ were found. R-squared coefficient was especially high ($r^2 \sim 0.98$) for muscle mass. This finding is consistent with previous results reported in literature [16].
Fig. 5.4. REE values (n=15) measured with the metabolic analyzer vs. physical characteristics of the subject group. (a) REE vs. Weight; (b) REE vs. Body Mass Index; (c) REE vs. Lean Body Mass; and (d) REE vs. Muscle Mass. All solid lines show the resulting linear regression fitting accomplished with $p = 10^{-13} - 10^{-11}$. 
5.4. Discussion

This study evaluated the application of the CO$_2$ analyzer in combination with an O$_2$ analyzer in assessment of REE and compared the testing results to those from the Douglas bag method, a reference method for measurements of human metabolism. The linear correlation analysis from measurements performed with the metabolic analyzer resulted in VO$_2$, VCO$_2$ and energy expenditure (REE and EE) assessments of nearly 100% accuracy when compared to the Douglas bag method (Fig. 4.2). Furthermore, paired t-tests performed on VO$_2$, VCO$_2$, and energy expenditure (REE, EE) results for both methods did not show any statistical significant difference for $a=0.05$. The Bland-Altman plot of REE from this study indicates agreement between results on the metabolic analyzer device and the Douglas bag method with difference values included within ± 2SD.

Previous observations have addressed the importance of REE as a parameter for calorie intake recommendations in weight loss plans [107]. During weight loss programs, there is a metabolic compensatory response that results in the decline of REE [107, 108]. This energy gap is estimated to be about -8 kCal/lb lost/day, and indicates, after initial weight loss, a need for lowered energy intake or a combination of lowered energy intake and increased physical activity energy expenditure to sustain additional weight loss [87, 108]. It has also been suggested that equations such as Harris-Benedict or MET formula to estimate REE [92, 107, 109] are insufficient in obese and overweight populations, athletes, and weight-loss groups, further underscoring the importance of measuring (not calculating) REE for accurate analysis of energy expenditure.
Finally, REE values assessed from the metabolic analyzer correlate well ($r^2 > 0.96$, $p = 10^{-13} - 10^{-11}$) with the physical parameters, such as weight, BMI, lean body mass and muscle mass of the subjects, indicating the capability of the device for discriminating different resting metabolic rates with physical parameters.

Using the Douglas bag method as a reference method requires several measurements from separate instruments (e.g. oxygen and carbon dioxide, separately). Other metabolic instruments based on oxygen and carbon dioxide detection have been developed (e.g. Oxycon Mobile by CareFusion, Yorba Linda, CA) to facilitate the measurement of energy expenditure. The metabolic analyzer presented here has demonstrated analytical performance comparable to these instruments (not shown). The traditional commercial instruments are relatively high in price and require calibration and operation by professionals. Along this line, several features make the metabolic analyzer advantageous for more widespread use: 1) calibration-free, 2) easy to operate, 2) high portability, and 3) seamless pairing with smartphones. All subjects of the study were able to independently use the metabolic analyzer to achieve accurate results without supervision of professionals.

5.5. Conclusions

A systematic study has been carried out to test and evaluate a pocket-sized metabolic analyzer which integrated both the CO$_2$ analyzer described in Chapter 2 and a novel O$_2$ analyzer. The study shows that the metabolic analyzer device provides accurate measurements of VO$_2$ and VCO$_2$, which enables accurate determination of energy
expenditure. The CO₂ analyzer can be applied in weight management. This capability is especially relevant for overweight or obese populations under weight loss programs. Compared to the traditional technology, the pocket-sized metabolic analyzer allows for accurate energy expenditure assessment at home, which is suitable for weight management.
6.1. Introduction

A device that can detect carbon dioxide (CO\textsubscript{2}) with high accuracy and with fast response time is critical for many health and environmental applications [1–15]. For example, measuring CO\textsubscript{2} levels in breath at the end of expiration, known as end-tidal CO\textsubscript{2} (EtCO\textsubscript{2}), allows for non-invasive evaluation of systemic metabolism, perfusion, ventilation, and cardiac output, which provides doctors and patients with a non-invasive method to diagnose asthma, Chronic Obstructive Pulmonary Disease (COPD), and cardiovascular diseases [5, 6]. Similarly, monitoring of indoor CO\textsubscript{2} levels allows for assessment of indoor air quality (IAQ). Higher levels of indoor CO\textsubscript{2} are associated with increased prevalence of certain mucous membrane and sick building syndrome (SBS) symptoms [15]. Infrared detection technology is currently utilized for measuring CO\textsubscript{2} in breath and in air. While useful, this technology experiences strong interference from humidity that is present both in breath and in air. Moreover, the infrared approach requires special sample pretreatments in order to reduce the humidity, which further adds to the cost of the device technology and limits its usefulness for applications in clinical settings. In the case of indoor environmental CO\textsubscript{2} sensing, the use of infrared technology is hampered by the interference of environmental humidity making detection of CO\textsubscript{2} levels inaccurate. There is a need, therefore, for developing a compact, low-cost, easy-to-use, and accurate CO\textsubscript{2} sensor for tracking CO\textsubscript{2} in human breath and for monitoring indoor air quality.
An alternative to infrared sensing is a detecting method based on colorimetry, which identifies CO\textsubscript{2} based on the change of color of a pH-sensitive indicator [68–73]. Compared to the infrared CO\textsubscript{2} sensors, the colorimetric approach has several potential advantages, including simplicity, miniaturization, low cost, and immunity to humidity changes, thereby making colorometric sensors an attractive technology. While these sensors may show great promise, their response and recovery time are too slow for breath-by-breath analysis, and their detection limits and reversibility are insufficient to ensure accurate detection of CO\textsubscript{2} in the environment. In order to solve these problems, many attempts, including pre-treatment of the sensing materials have been undertaken to reduce cost and ensure high performance of colorimetric CO\textsubscript{2} sensors. These improvement activities, however, often result in more complex instruments and sensor preparation methods.

In Chapters 2, 3 and 4, we introduced a preliminary designed breath analyzer for the determination of the expired CO\textsubscript{2} and evaluated its application in the assessment of resting energy expenditure. The device features a colorimetric sensor that could analyze breath CO\textsubscript{2} concentration accurately, and a fluidic system for efficient delivery of breath sample to the sensing element. A 3D model was created to simulate the sample flow and reaction of CO\textsubscript{2} with the sensing materials and color changes associated with the chemical reactions [110]. When used as a part of a metabolic analyzer, the CO\textsubscript{2} sensor provides an accurate measurement of VCO\textsubscript{2} which is critical for the estimation of resting energy expenditure (Chapter 4). Despite the success, the sensor response was slow and semi-reversible, which is not suitable for breath-by-breath CO\textsubscript{2} analysis as needed for capnography, and moreover, which is insensitive to real-time monitoring of CO\textsubscript{2} in air. In
this chapter, we optimized the CO\textsubscript{2} analyzer by decreasing the response time to around 0.1 sec., which enables breath-by-breath analysis (see more details in Experimental and Results Section). Additionally, the sensor has a wide dynamic range (with the CO\textsubscript{2} concentration up to 11.5\%), and low detection limit of a few tens of ppm, which are suitable for indoor air quality monitoring.

6.2. Experimental

6.2.1. Reagents and sensor preparation

The colorimetric CO\textsubscript{2} sensor presented in this chapter was prepared by coating a sensor chip with a solution containing CO\textsubscript{3}\textsuperscript{2-}/HCO\textsubscript{3}\textsuperscript{-} buffer and \textit{m}-cresol purple as the sensing element [69, 110]. All the reagents used in this work were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). As described in the last chapter, when an ambient air sample or warm breath sample was brought into contact with the sensor chip, the pH of the sensing material decreased, which led to a color change [110]. This color change was detected with an optoelectronic detection system (see details below).

Note that, in order to meet the requirements of real-time CO\textsubscript{2} analysis in both exhaled breath and the ambient air, several differences are stated in the present work with respect to previous work [110]. Regarding the sensor: \textbf{a}- The sensor substrate uses a fluorinated hydrophobic membrane instead of transparent polyethylene-based plastic sheet; \textbf{b}- The hydrophobic membrane has microstructures and surface properties that provide faster water desorption and significantly improved sensor time response; \textbf{c}- The
new indicator, m-cresol purple, provides a wider dynamic range of CO₂ % concentration due to its lower pKa. Compared with thymol blue [110], m-cresol purple changes color more sensitively under weakly alkaline to neutral conditions (pH value: 9.0 – 6.0), which typically corresponds to the range of normal breath CO₂ levels (1.0% - 11.5%) [111–113]

Regarding the device: a- For breath applications, the gas flow is designed that both exhalation of breath samples, as well as inhalation of clean air are enabled through the device. The later enables in-situ sensor regeneration; b- The device’s gas flow component has larger diameter (~20 mm) than the device published in the last chapter (few mm), which significantly reduces the resistance to breathing, enabling breath-by-breath analysis.

6.2.2. Device description

The CO₂ sensor chip was inserted into a detection chamber, which included an optoelectronic detection system consisting of a red LED (wavelength = 633 nm, LEDtronics. Inc., CA, USA) as a light source and a photodiode (OSRM GmbH, Germany) as a light detector (Fig. 6.1(a)). The response of the sensor was characterized by measuring the change in the intensity of transmitted light caused by the interaction of CO₂ with the sensing element (Fig. 6.1(b)). Gas sample, from either the ambient air or a flow containing simulated breath was directed to the sensor detection chamber with a pump. Real breath test was also conducted by asking a volunteer to breathe into the device via a mouthpiece. The device also contains an electronic circuit, which collects
and processes the data from the optoelectronic detection system, and then wirelessly sends the data to a smartphone via a Bluetooth chip.

Fig. 6.1. (a) Pocket-sized CO$_2$ sensor device. It has inlets and outlets that allow the detection and analysis of both breath and environmental air samples. The key-sensing element is a CO$_2$ sensor chip, which can be inserted into or remove from the device. The device also features an electronic circuit for signal processing and Bluetooth for wireless transmission of data. (b) The sensor chip changes color from purple to yellow after exposure to CO$_2$ and the concentration of CO$_2$ is determined by measuring the change in intensity of transmitted light for the sensing element.
6.2.3. Device characterization and validation

*Simulated breath samples:* In order to investigate the response of the CO₂ sensor to real breath, a humidified CO₂ gas mixture was employed to simulate the expired air. The simulated expired breath samples were prepared by first mixing 80% N₂ + 20% O₂ air with different amounts of CO₂ (Praxair. Inc.), ranging from 0.03% to 11.5%. The CO₂ mixtures were then pumped through a sealed water system immersed in a thermostatic water bath (Thermo Scientific, USA) at 37.5°C to generate 35°C and 100% relative humidity. The simulated expired gas samples and ambient air were then introduced into the breath analyzer alternately at a flow rate of 6L/min to simulate expiration and inspiration processes as shown in Fig. 6.2(a).

*Simulated environmental samples:* For environmental CO₂ analysis, the CO₂ gas mixtures were prepared by mixing ultrahigh purity air with CO₂ to simulate indoor air with CO₂ concentration ranging from 0 ppm to 1350 ppm. The simulated indoor air samples and ultrahigh purity air were then alternately introduced into the CO₂ analyzer at a flow rate of 6 L/min as shown in Fig. 6.2(b).

*Sensor signal measurement:* The change in light intensity of the CO₂ sensor was used to characterize the color change upon exposure to alternating sampling and purging periods of fixed time. The change in light intensity of the CO₂ sensor is evaluated as sensor signal from the baseline signal. This means that baseline shifts are corrected, which renders relatively small sensor response dispersion error at a given CO₂ concentration (see below for more details). The change in transmitted light of the CO₂ sensor is:
\[ \Delta I(t) = I(t) - I(0), \]

where \( I(0) \) is the light intensity prior to the exposure of the sensor surface to the gas samples, \( I(t) \) is the light intensity at time \( t \) during the sampling process. In addition, the performance of this device was further validated using a commercial breath \( \text{CO}_2 \) analyzer (capnography analyzer from VacuMed, CA) and real breath samples from volunteers (Fig. 6.2(c)).

**Cross-sensitivity analysis:** The interference of other chemicals present in expired air and atmosphere, such as ethanol, acetone, acetonitrile, and \( \text{NH}_3 \) was investigated by introducing the humidified gas mixtures containing pure \( \text{N}_2 \) and the interfering gases into the \( \text{CO}_2 \) analyzer. The response of the \( \text{CO}_2 \) sensor exposed to the interfering gases was compared with the response of the sensor exposed to 1% \( \text{CO}_2 \) gas mixture.

Sensor response under different humidity conditions: The performance of the sensor under different ambient humidity conditions was investigated by introducing the simulated expired air containing 3.5% \( \text{CO}_2 \) (prepared as mentioned in Section 6.2.3) and ambient air with different humidity levels (30% - 90% relative humidity, \( T = 25^\circ\text{C} \)) into the breath analyzer alternately at a flow rate of 6L/min. The sensor signal was plotted versus the ambient humidity level.

Shelf-life analysis: Once the sensor chips were prepared, they were sealed in small sample packages and stored under the ambient condition (25\(^\circ\text{C}, 30\% \text{ RH})\). The shelf-life of sensor chip was evaluated by examining the response of a freshly-opened sensor chip to simulated breath samples (5\% \( \text{CO}_2 \) in simulated expired air) versus storage time.
Fig. 6.2. Schematic diagram of calibration setup for investigating the response of the CO$_2$ sensor to (a) breath samples and (b) trace CO$_2$ in air gas samples. (c) Schematic diagram of the laboratory setup for validating the performance of the CO$_2$ sensor using real breath samples from volunteers and a commercial breath CO$_2$ analyzer.

6.3. Results and Discussion

6.3.1. Sensing mechanism of the CO$_2$ sensor

The basic sensing principle of the new CO$_2$ sensor is based on the adsorption and desorption processes of CO$_2$ in the sensor during the test [110]. Briefly, during the sensing process, as gas samples (either expired breath or environmental air) were introduced into the device, CO$_2$ was absorbed by the CO$_3^{2-}$/HCO$_3^-$ buffer system in the sensor, and the pH of the sensing system decreased. However, during the purging process, the clean air flowed over the sensor, and the CO$_2$ molecules were dissociated
from the sensor, resulting in the recovery of pH to the initial value. As described in Section 4.2.1, m-cresol purple was used as the pH indicator of the sensing system. Since the variation of pH in the sensor was correlated to the concentration of CO₂ in the gas flow, the concentration of CO₂ in either the expired breath or environmental air can be determined via monitoring the color change of m-cresol purple (mCP). The chemical reaction process is as follows [75, 76]:

\[
CO_2(g) \xrightleftharpoons[k]\ k_{-1} CO_2(aq)
\]

(R6.1)

\[
mCP^{2-}(purple) + H_2O + CO_2(aq) \xrightleftharpoons{k} mCP^{2-}(purple) + H_2CO_3 \xrightleftharpoons{k} mCpH^{-}(yellow) + HCO_3^{-}
\]

(R6.2)

where \( CO_2(aq) \) is the dissolved CO₂ in the sensor, \( mCP^{2-} \) is the deprotonated form of m-cresol purple and \( mCpH^{-} \) is the protonated form of m-cresol purple in the \( CO_3^{2-}/HCO_3^{-} \) buffer solution.

6.3.2. Characterization of the CO₂ sensor

**Breath CO₂ analysis calibration:** Fig. 6.3(a) shows the response of the CO₂ sensor exposed to the alternating atmospheres of dry air and humidified 5% CO₂ gas mixture. As described earlier, when the CO₂ gas sample was introduced into the device, water vapor condensed onto the sensor surface and CO₂ was absorbed by the \( CO_3^{2-}/HCO_3^{-} \) buffer solution in the sensor, which decreased the pH level of the sensing system, and changed the color of the pH sensing probe from purple to yellow (Fig. 6.1(b)). This color change was measured as a change in the transmitted light intensity, which was correlated to the CO₂ concentration in the gas sample. Conversely, when CO₂-free dry air was introduced
into the device to simulate the inspiration process, CO₂ was stripped from the sensor and the color changed back from yellow to purple, which was also recorded by the light intensity change. The CO₂ concentration in the simulated breath sample can be determined from the maximum intensity change in each simulated breathing cycle. It is worthy to notice, the sensor signal noise at the maximum response level is generated by a current inability of the analog-to-digital converter of the microcontroller used in the detection system to smoothly manage small voltage signal changes. The problem is currently being corrected from hardware design standpoint. In addition, Fig. 6.3(b) shows the maximum light intensity change vs. the CO₂ concentration in the gas samples. It can be observed that the maximum change of light intensity increases with CO₂ concentration from 0.03% to 11.5% with a Langmuir-like behavior (R² = 0.9986). This concentration range sufficiently covers the need of breath CO₂ analysis for medical applications, which typically ranges between 2.5 and 5.0% in healthy individuals [114].
Fig. 6.3. (a) Response of the CO₂ sensor exposed to the alternating atmospheres of dry air and artificial expired air containing 5 % CO₂. The light intensity increased rapidly when the sensor was exposed to CO₂-containing atmosphere and returned to the initial value rapidly when the exposed to CO₂-free atmosphere. (b) The relationship between the maximum intensity change (indicated by ΔV, the output of the photodiode) and CO₂ concentration. The light intensity increased with the CO₂ concentration. Response vs. concentration to Langmuir-like equation is shown in the figure insert.

*Environmental CO₂ analysis calibration:* Fig. 6.4(a) shows the response of the CO₂ sensor exposed to the alternating atmospheres of ultrahigh purity air and simulated environmental air samples. The light intensity increased as CO₂ interacted with the sensing system and reduced gradually as pure air passed through the device. Fig. 6.4(b)
shows the relationship between the maximum change of light intensity in each sampling-purging cycle and CO₂ concentration. The intensity change increased with CO₂ concentration with a Langmuir-like behavior ($R^2 = 0.9965$). The detectable range of the sensor to the ambient CO₂ was found from few tens of ppm to few thousand ppm levels, which covers the needs for environmental CO₂ detection [115].
Fig. 6.4. Application of CO₂ sensor for environmental sensing: (a) Response of the CO₂ sensor exposed to the alternating atmospheres of simulated environmental air samples. (b) The light intensity changes increases with CO₂ concentration in the simulated environmental air sample. Response vs. concentration fitting to Langmuir-like equation is shown in the figure insert.

6.3.3. Cross-sensitivity study

In order to investigate the interference effects of other chemicals that might exist in the expired breath and ambient air, humidified gas mixtures containing pure N₂ and different interfering gases were introduced into the CO₂ device at a flow rate of 6L/min.
for 30s. As shown in Fig. 6.5, compared to the response of the sensor exposed to a 2% CO\textsubscript{2} gas sample, the interference of the other gases is within 6%, which can be considered negligible in breath CO\textsubscript{2} analysis as the CO\textsubscript{2} levels in breath typically ranges from 3.5 to 5.5 %. However, interference from chemicals such as NH\textsubscript{3} needs to be corrected when the CO\textsubscript{2} sensor is used for environmental CO\textsubscript{2} monitoring.

![Fig. 6.5. Cross-sensitivity of CO\textsubscript{2} sensor for other gases in the expired and environmental air. Compared to the response of the sensor exposed to a 2% CO\textsubscript{2} gas sample, the interference of the other gases is within 6%.](image)

6.3.4. Interference from ambient humidity and shelf-life study

In addition to the above-mentioned studies, the effect of ambient humidity on breath CO\textsubscript{2} analysis was evaluated. Some interference effect was detected for the relative humidity levels higher than 80%. We found that the sensor signal decreased by around 10% as the ambient humidity increased to 90%. However, the responses of the sensor to
humid ambient air and humid air with CO₂ mixture (e.g. simulated breath at 35°C, and 100% humidity) are different. The sensor showed fast and reversible response with the ambient relative humidity ranging between 46 and 90%. Therefore the effect of extremely high ambient humidity could be corrected by use of an internal humidity sensor in the CO₂ analyzer.

The long-term stability of the sensor chip was also studied. We found that over five-month storage under the ambient condition, the variation of sensor response to simulated breath sample containing 5% CO₂ was within 10%, which indicated a shelf-life of at least 5 months without any particular refrigeration or storage conditions.

6.3.5. Real breath analysis

In order to investigate the application of the CO₂ sensor to real breath analysis, including breath patterns and ventilation rate, the CO₂ sensor was calibrated using breath samples in combination with flow rate measurements from an in-situ flow meter. The breath patterns of two volunteers were also analyzed. The volunteers included one healthy person and one asthma patient from the VA Medical Center, Phoenix, AZ. They were asked to breathe normally through the mouthpiece. The end-tidal CO₂ values (the maximal concentration of CO₂ at the end of expiration) were calculated and compared with the values assessed from a capnogram recorded with a commercial breath CO₂ analyzer (Fig. 6.6). The “capnogram” refers to a real-time waveform of CO₂ concentration in the process of respiration with gas samples taken from a sidestream port located a few millimeters away from the colorimetric sensor as shown in Fig. 6.2(c). From Fig. 6.6(a) and (b), it can be seen that the end-tidal CO₂ values measured by the
new CO₂ analyzer were comparable to the values assessed by the commercial analyzer for both the healthy person and asthma patient. In addition, the capnogram obtained from the new CO₂ analyzer also showed good agreement with the commercial device. The 90% response time of the CO₂ analyzer was less than ~150 ms, which meets the requirements for breath-by-breath CO₂ analysis (< 200 – 300 ms). Furthermore, the new CO₂ analyzer showed faster response than the commercial analyzer, which had a fraction of a second delay in gas detection due to the aspiration of the gas sample from the sample site through the sampling tube (around 3 ft. long) and into its detection unit (infrared detection chamber) (Fig. 6.2(c)). It was evident that time response improvements were achieved in the new CO₂ analyzer due to the *in-situ* location of the sensor chip in the mainstream of the breath sample flow.
Fig. 6.6. Comparison of the CO\(_2\) sensor by this work and a commercial infrared CO\(_2\) analyzer. Both sensors were exposed to real breath samples from (a) a healthy person and (b) an asthma patient. The capnograms obtained from the present CO\(_2\) sensor and the commercial device are in good agreement with each other.

In addition, the end-tidal CO\(_2\) levels measured by the CO\(_2\) analyzer were compared with those obtained from the commercial capnogram (Fig. 6.7) Clearly, the CO\(_2\) concentration in both cases correlated at a slope of 1.00, an error of 2.6\%, and a squared - regression coefficient (r\(^2\)) of 0.875, which indicated an accuracy level around 90\% between these two devices. However, the performance of the CO\(_2\) analyzer still needs to be further optimized to achieve a higher accuracy.
Fig. 6.7. Correlation of the end-tidal CO$_2$ levels of volunteers determined by the CO$_2$ analyzer device ($y$) and a commercial capnogram instrument ($x$). Linear correlation analysis of the variables was: $y = 1.00x$, $r^2 = 0.875$ indicating good accuracy level.

6.4. Conclusions

This chapter describes an optimized CO$_2$ sensor for personal healthcare and environmental monitoring. The device has an integrated circuit and an optical-based detection chamber, which includes a colorimetric CO$_2$ sensor. The CO$_2$ sensor shows reversible response to CO$_2$ and a dynamic detection range from ~50 ppm to 11.5%. When compared to a commercial infrared capnography detector, the CO$_2$ analyzer shows fast response with a 90% response time of less than 150ms, which indicates that the CO$_2$ analyzer is suitable for breath-by-breath analysis. In addition, the CO$_2$ sensor shows accurate detection of CO$_2$ levels after non-linear calibration of the sensor response. The
interference study indicates that other chemicals typically present in the expired breath and ambient air have negligible effects on the response of the CO₂ sensor. The shelf-life of the CO₂ sensor can be at least five month and the interference of ambient humidity to sensor response is within 10%. Compared with traditional CO₂ measurement technology, the new pocket-sized CO₂ analyzer allows for simple, fast, and accurate assessment of breath CO₂ levels, and breath CO₂ profiles for patients with COPD and asthma, as well as CO₂ levels in ambient air. Despite the success, the performance of the CO₂ sensor still needs to be improved since the sensor recovery during inspiration process is slower than the sensor response during expiration process.
CHAPTER 7
CONCLUSIONS

We have designed a colorimetric-based CO₂ analyzer for real-time breath analysis and environmental monitoring. This CO₂ analyzer, which includes a CO₂ sensor and a fluidic system for efficient delivery of gas samples, provides accurate measurement of CO₂ level. The performance of this CO₂ analyzer depends on both the chemical reactions and mass transport of CO₂ molecules between gas sample and the sensing element. A CO₂ sensing probe with relatively low pKa and a phase transfer agent with long carbon chain were selected to provide the sensor with a higher sensitivity and a large dynamic range. The response time of this CO₂ analyzer was improved by using ultra-hydrophobic PTFE membrane as the sensor substrate and using the amine buffer as the pH stabilizer.

In order to evaluate the performance of the CO₂ analyzer, we have applied this CO₂ sensor in combination with an O₂ sensor as a metabolic analyzer for assessment of resting energy expenditure. The metabolic analyzer device provides accurate measurements of VO₂ and VCO₂, which enables accurate determination of energy expenditure. This capability is especially relevant for overweight or obese populations under weight loss programs. Moreover, we have created models taking into account of mass transport and heat transfer of breath samples inside the CO₂ analyzer device as well as chemical reactions for CO₂ measurement. The numerical simulation results based on the models and actual geometry of the device are compared with experimental data, showing quantitative agreement. Based on the simulation and experimental results, we have optimized the CO₂ analyzer for real-time analysis of breath CO₂ and environmental
CO₂. The new CO₂ analyzer showed reversible response to CO₂ and a dynamic detection range from few tens of ppm to 11.5%. When compared to a commercial infrared capnography detector, the CO₂ analyzer showed faster response with a 90% response time of less than 150ms, which indicates that the CO₂ analyzer is suitable for breath-by-breath analysis. In addition, the CO₂ sensor showed accurate detection of CO₂ levels after non-linear calibration of the sensor response. The cross-sensitivity study indicated that other gases, which can typically be present in the expired or ambient air, had negligible interference to the response of CO₂ sensor. Compared with the traditional CO₂ measurement technology, the new pocket-sized CO₂ analyzer allows simple and accurate assessment of breath CO₂ levels, and breath CO₂ profiles for patients with COPD and asthma, as well as CO₂ levels in ambient air.
CHAPTER 8

FUTURE WORK

The future work will focus on the further optimization of the CO₂ analyzer according to the simulation results in Chapter 5. Device configurations will be improved and the sensor response will be tested under the new configurations.

Since the response of the CO₂ sensor may also depend on the physical and/or chemical properties of the sensor substrate material, such as porosity, thickness and hydrophobicity, more simulations will be performed to predict the sensor response based on the 3D model created in Chapter 5. Experiments will also be performed to validate the simulation results.

The reproducibility and long-term stability (> 1 year) of the CO₂ sensor chip will be evaluated and more real breath validation with COPD or asthma patients will be performed.
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APPENDIX A

OPTICAL DETECTION SYSTEMS
In this work, we developed a colorimetric CO$_2$ sensor for real-time breath and environmental monitoring. The sensor element changed color after exposure to CO$_2$ and the color change was detected by the optical detection system. Based on the specific design of the sensor devices, the two optical detection systems were selected to detect the color change of the sensor element.

(1) Silicon photodiode array

One optical detection system is based on the silicon photodiode array (Fig. A1). The photodiode is a semiconductor component which can convert light into current. It will produce a flow of current in response to the absorption of photons or charged particles. Since the current generated by the photodiode is proportional to incident light and there is an almost linear relationship between the photodiode response and wavelength of visible light spectrum (Fig. A2), the photodiode can be used to detect the color change of the sensor element.

Fig. A1. Silicon photodiode used for optical detection.
Fig. A2. Response curve of silicon photodiode. There is an almost linear relationship between photodiode response and visible light wavelengths [A1].

As shown in Fig. 4.1, a photodiode array was integrated in the sensor device and located at the bottom of the detection chamber. Since the color change of the sensor element is most sensitive under red light, a red LED was selected as the light source. When sensor was exposed to CO₂, the sensor element changed color from blue (or purple) to yellow. The absorption of red light by the sensor element decreased and thus more red light was absorbed by the photodiode array. As a result, the output of the photodiode array increased.
(2) Cell phone camera

Another optical detection system is based on the cell phone camera. In this case, we designed a sensor device that can fit to the cell phone camera. A white LED was integrated into the device and used as the light source. The sensor chip was inserted into the detection chamber and pictures of the sensor element before and after exposure to gas sample were taken by the camera to analyze the color change. Since each color can be expressed in RGB (Red, Green and Blue), the color change of the sensor element can be determined by measuring the changes in RGB values of the sensor element through the pictures.

In order to evaluate the performance of cell phone camera in recording and analysis of sensor color change, we tested the output of the cell phone camera under different conditions. Briefly, filter papers printed with different colors were selected as the samples. The filter paper was inserted into the detection chamber and a picture was taken by the cell phone camera. In each picture, a sensing and reference regions were selected, and the absorbance of RGB was determined from the image file. The camera outputs (RGB transmittance (%)) of filter paper samples with varying grey scales are shown in Fig. A3. It can be observed that, the cell phone camera output (RGB transmittance (%)) decreases linearly with the increasing sample opacity, which indicates that the cell phone camera can be used to characterize the opacity of the sensor element. In this case, the samples with white and black color were assigned to 100% and 0% in RGB transmittance, respectively.
Fig. A3. (a) The pictures of filter paper samples with varying grey scales and (b) the cell-phone camera output (RGB transmittance (%)) vs. the sample opacity (transmittance (%)). Samples with white and black color were assigned to 100% and 0%, respectively. The linear relationship between the output signal and the samples opacity indicates a high quality of the cell-phone camera as a detector of light intensity. Pictures at the top show some examples of the samples (with 99, 90, 60, and nearly 0 opacity).
In addition, the capability of the cell phone camera to quantify color change of sensor element after exposure to gas sample was evaluated by analyzing the RGB values of filter papers printed with different colors. Six filter paper samples printed with specific colors (red, green, blue, yellow, orange and black) were selected and the transmittance of the samples was measured by a commercial UV-vis spectrophotometer (BioSpec-mini, Shimadzu). The filter paper samples were then inserted into the sensor device and pictures were taken by the cell phone camera. The RGB values were obtained from the pictures and shown in Fig. A5. It can be observed that, each filter paper sample shows specific transmission spectrum and RGB values, which demonstrates the capability of the cell-phone camera to quantify colors accurately.

Fig. A4. Transmission spectra of filter paper samples and the corresponding RGB color profiles obtained with a cell phone camera. Results are the average of multiple
measurements performed on the same sample. Picture of the samples taken with the camera are shown as insets.

REFERENCE